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Section (i) Marine Science

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Section (ii) Botany

REPRODUCTIVE BIOLOGY OF *GAZZA MINUTA* FROM MYEIK COASTAL WATERS*

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Abstract

Reproductive biology of *Gazza minuta* was studied by using the samples collected from the catches of trawl fisheries in Myeik coastal waters during January to December 2014. Spawning takes place throughout the year, with peak in June-January. The observed length at first maturity was 9.5 cm total length in males and 10.0 cm in females. The gonadosomatic index value was more prominent in females than in males. Sex ratio also indicates the general dominant of females over males. Fecundity varied from 10,002 to 69,421 eggs. Fecundity has positive relationship between fish length, fish weight and ovary weight of fish.

Keywords: Fecundity, gonadosomatic index, length at first maturity, spawning, *Gazza minuta*

Introduction

Species of *Gazza minuta* belonging to the Family Leiognathidae is a small sized (<300mm in standard length) bottom living fish and diagnosed by snout blunt, forward protracted mouth part, the presence of caniniform teeth on both jaws and body color silver with irregular marks or vertical wavy lines. The common name of this species is toothpony and also locally known as Ni-shaw or San-sat in Myeik and Nga-din-gar (or) Nga-waing in Myanmar. They are caught as by catch in variety of gears but the major contribution comes from the trawls fisheries. Large sized fishes are eaten as fresh and small sized ones are used as raw materials in the fish meal plants of Myeik.

The size at first maturity, spawning season, fecundity, sex ratio, histological examination of gonads and gonadosomatic index value are the essential parameters to determine the reproductive potential of individual fishes. These parameters are widely applied to formulate capture fisheries management strategies such as enforcement of minimum catch at size restriction and to close the fishing season during peak breeding period.

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Moreover the understanding of reproductive biology of species can provide scientific advice for fisheries management. Information on the maturation and spawning of *Gazza minuta* species is available from the seas around India (Jayabalan 1988, James and Badrudeen 1986). Although Thet Lyar Win (2012) stated the identification of *Gazza minuta* from Myeik, there is no information on the reproductive biology of this species. Thus, the present study aims to determine the size at first maturity and spawning period, to find out gonadosomatic index value and to get a better understanding of the fecundity in relation to fish length, fish weight and gonad weight of fish.

Materials and Methods

Samples were collected from the catches of trawl fisheries operated in Myeik coastal waters (Fig 1), from January to December 2014. A total of 422 specimens were examined during the study period. Total length and weight of each individual was measured to the nearest 0.1cm and 0.01 g respectively. Maturity stages were determined based on the external appearance like color, size, and the proportion of the gonad area occupied by them in the body cavity followed the system used by Rao *et al*. (2015). And then, the percentage occurrence of different maturity stages of fish in every month was recorded to estimate the spawning season of fish.

In histological analysis, the ovary was fixed in formalin, dehydrated in upgraded series of ethanol and cleared in xyline. After that, the fixed ovary was embedded in paraffin and cut section of 5μm thickness by rotary microtone. These sections were deparaffinised in xyline, hydrated in downgraded series of ethanol, subsequently double-stained with hematoxylin and eosin and examined under the microscope. The identification of oocytes is mainly based on the appearance of nucleus, nucleolus, vacuoles and yolk vesicle.

Sex was recorded by careful examination of the gonad. Sex ratio was calculated and tested for the expected ratio of 1: 1 by chi- square (χ^2) analysis according to the formula:

$$\chi^2 = \frac{\sum(O-E)^2}{E}$$

where O= observed frequency of males or females, E= expected frequency of males or females

For estimation on the size at first maturity, the lengths of fish were grouped into 0.5 cm interval size groups. Fishes with stages IV and above maturation were considered as mature fishes. The length at which 50% of the individuals attain sexual maturity L50 was estimated by fitting the point where the total length of fish (X axis) and 50% level of maturity (Y axis) are met.

Gonadosomatic index (GSI=gonad weight/fish weight*100) was calculated separately for males and females. Fecundity estimation based on the ripe ovaries was calculated according to the formula: Fecundity= total weight of ovary/sub-sample weight of ovary* no of ova in the sub-sample. Fecundity in relation to fish total length, fish weight and gonad weight were calculated by applying the method of least square based on the equation:

Log F= Log a+ b LogX

where F= Fecundity, a= constant, b= exponent and X= fish length (or) fish weight (or) gonad weight

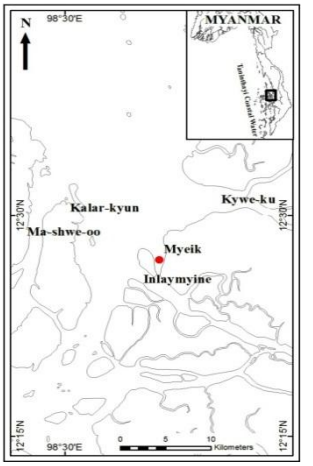


Figure 1. Map showing the Myeik coastal area

Result

Maturity stages

The gonad of *Gazza minuta* is rounded, unpaired structure lying in the middle of the body cavity attached to its dorsal wall. The maturity classification followed the system used by Rao *et al*. (2015) (Table 1). Six maturity stages were recognized as immature (stage I), early maturing (stage II), maturing (stage III), mature (stage IV), ripe (stage V) and spent (stage VI) (Figs. 2 and 3).

Table 1: Maturity classification of *Gazza minuta*

Stage	Characteristics
Stage I - Immature	The immature ovaries are characteristically small, transparent, pale in color and occupy a very portion of body cavity. Ova are invisible to naked eye. The immature testes are small, transparent, pale in color and occupy the posterior part of body cavity.
Stage II - Early maturing	The early maturing ovary is pale-yellow in color, translucent in appearance and occupies less than 1/3rd of the body cavity. Ova are invisible to naked eye. The early maturing testes are pale whitish in color, semitransparent and occupy nearly 1/3 of body cavity.

Stage	Characteristics
Stage III - Maturing	The maturing ovary is yellow in color and occupies ½ of the body cavity. Blood capillaries visible. Granular ova are clearly visible with naked eye. The maturing testes are creamy white in color, translucent in appearance and occupy nearly ½ of the body cavity.
Stage IV - Mature	The mature ovary compact and occupy more than half of the body cavity. They are yellow in color with numerous blood capillaries over the entire ovary. Granular ova are clearly visible with the naked eye. The mature testes are creamy white, soft and occupy about ¾ of the body cavity.
Stage V - Ripe	The ripe ovaries are bright yellow in color with numerous blood capillaries and occupy about ¾ to nearly entire length of body cavity. Translucent eggs clearly visible in the ovary. Ripe testes are soft, creamy white in color, occupy entire length of body cavity and exude milt under slight pressure.
Stage VI - Spent	The ovaries are flabby and loose, pale yellow in color and occupy not more than half of the body cavity. Spent testes are flabby and occupy nearly ½ of body cavity.

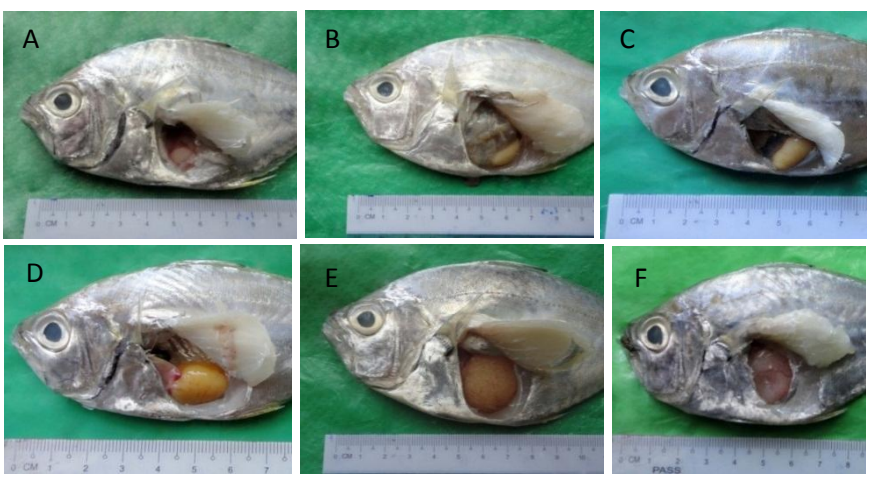


Figure 2. Different maturity stages of ovaries in *Gazza minuta* A) immature; B) early maturing; C) maturing; D) mature; E) ripe and F) spent.

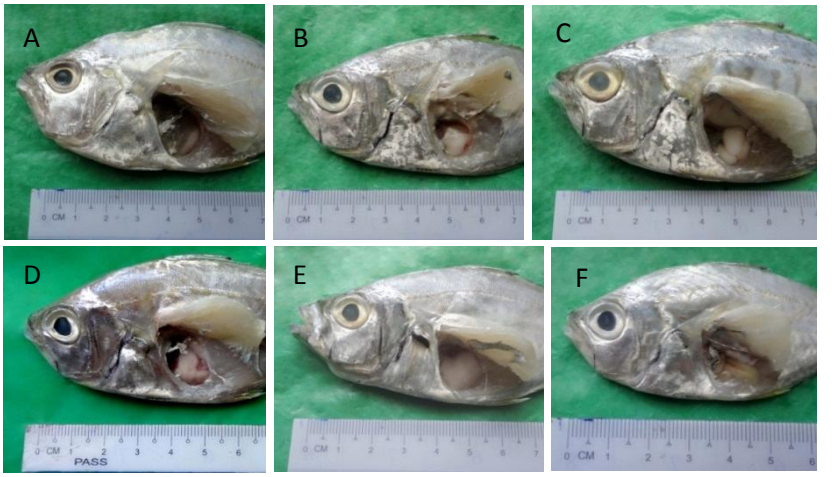


Figure 3. Different maturity stages of testes in *Gazza minuta* A) immature; B) early maturing; C) maturing; D) mature; E) ripe and F) spent.

Histological analysis of oocytes

In histological analysis, six stage of oocytes development (Fig. 4) were observed in the ovaries of silverbellies.

1. Chromatin nucleolus stage: Oocyte is transparent and rounded or more or less polygonal shape with the large nucleus visible at the centre.

2. Perinucleolar stage: A large number of nucleoli of different sizes are arranged along the periphery of the nucleus. Oocyte is surrounded with single layer of follicle cells.

3. Yolk vesicle formation stage (or) cortical alveoli formation: Yolk vesicles started to appear at the periphery of the oocyte, nucleus irregular in shape. They increased in size and number to form several peripheral rows and give rise to cortical alveoli.

4. Vitellogenic stage (or) yolk stage: The development of yolk globules is observed in this stage. The nucleus migrates toward the periphery. The unique oil droplet was clearly seen at central part of the oocyte. The cortical alveoli are further pushed toward the periphery and become arranged in two to three successive layers.

5. Ripe stage: Oocyte is transparent and completely packed with yolk mass. Oocyte increased in size by hydration. The layers of cortical alveoli are clearly observed. The nucleus disappeared due to the condense yolk accumulation.

6. Spent stage or atretic stage: Oocytes are irregular in shape. Various types of postovulatory follicles present. The yolk contents were completely disappeared.

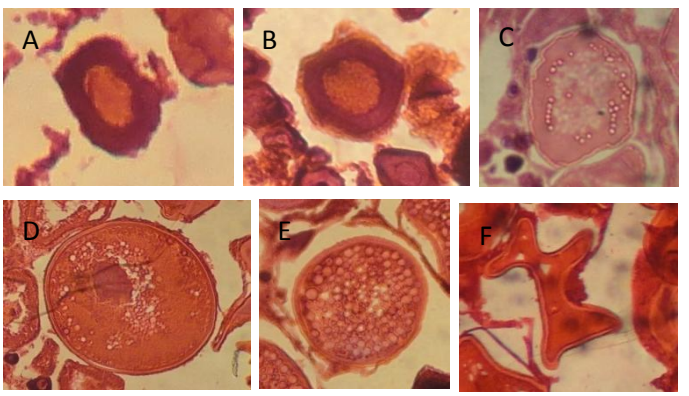


Figure 4. Different stages of oocytes A) Chromatin nucleolus stage; B) Perinucleolar stage; C) Yolk vesicle formation stage (or) cortical alveoli formation; D) Vitellogenic stage (or) yolk stage; E) Ripe stage and F) Spent stage or atretic stage.

Occurrence of different maturity stages

The occurrence of mature males and females was recorded to determine the spawning season of fish. *Gazza minuta* in stage I and II

(immature and early maturing males) occurred in almost all months except November (Fig 5). Maturing males (stage III) were encountered in almost all months except January and February, comprising with the maximum percentage of 38.7% (May) and minimum percentage of 13.4% (June). The percentage of males was found to be highest in the month of June until January in mature stage IV. Ripe males in stage V was not found in June, October, November and December. Fish with spent stage was only observed in April, June, July, November and December with small percentage.

Females in stage I (immature) was recorded in almost all months except January, September and October (Fig 6). Monthly percentage occurrence of female ranged from 3.3% (October) to 43.3% (April) in early maturing stage. Fish with maturing ovary occurred in almost all months except April and May. Mature females were observed in all months and its percentage reached to minimum in February and March (20%) and maximum in January (75%). Except March and April, ripe females were observed in all months. For spent stage, it was only recorded in January, August, September, November and December respectively.

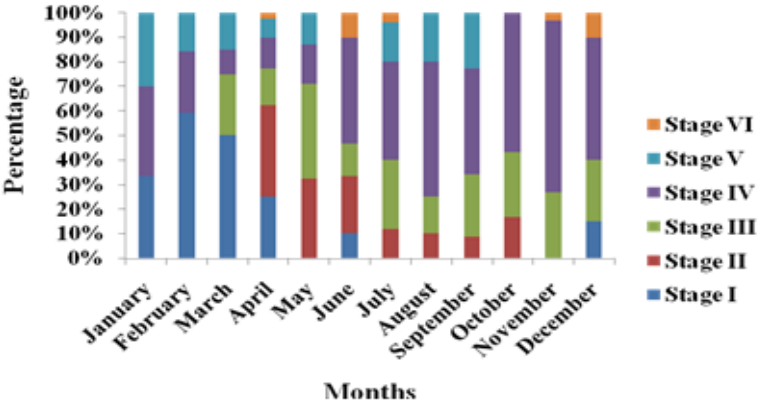


Figure 5. Monthly percentage occurrence of maturity stages of *Gazza minuta* (Males).

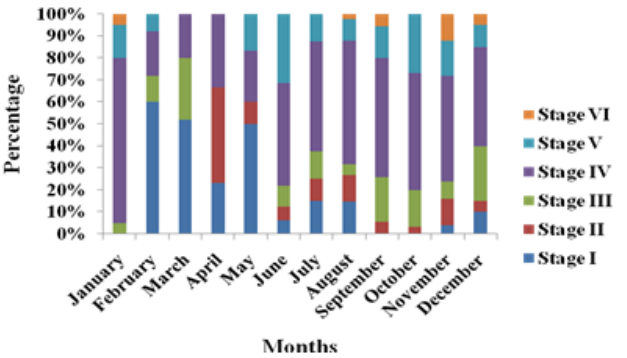


Figure 6. Monthly percentage occurrence of maturity stages of *Gazza minuta* (Females).

Length at first maturity

The percentage occurrence of mature fish in different length group of males and females of *Gazza minuta* was illustrated in Fig. 7. No mature male occurred in 7.3-7.7 cm length group. Percentage of mature fish increased with the increase of length for both male and females. The mean size at first maturity (50%) was about total length of 9.5 cm in males and 10.0 cm in females.

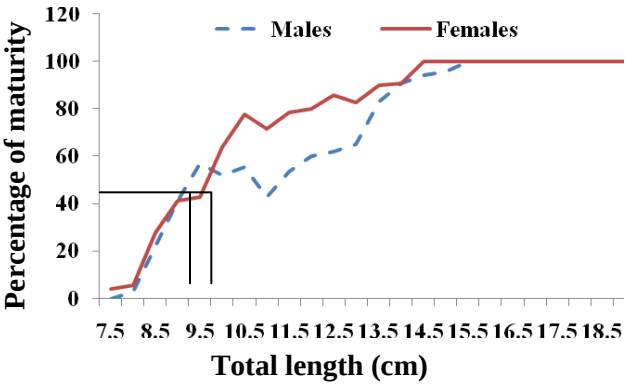


Figure 7. Length at first sexual maturity of males and females

Sex ratio

The monthly sex ratios of *Gazza minuta* were estimated and tested for the expected ratio of 1:1 by chi-square ($\chi 2$) analysis. The resulted average ratio was 1 male: 1.1 females ($\chi 2$=1.9). Females were more abundant in almost all months except February and April (Table 2). The range of chi-square values (0.02 to 1.6) showed that there was no significant difference in number of males and females in all months from the expected 1: 1 ratio.

Table 2. Monthly sex ratio of *Gazza minuta*

Months	M:F	$\chi 2$	Months	M:F	$\chi 2$
Jan	1:1.2	0.4	July	1:1.1	0.1
Feb	1:0.9	0.03	Aug	1:1.3	0.5
March	1:1.1	0.1	Sept	1:1.1	0.03
April	1:0.9	0.1	Oct	1:1.2	0.3
May	1:1.3	0.4	Nov	1:1.1	0.02
June	1:1.2	0.3	Dec	1:1.5	1.6

Gonadosomatic index (GSI)

The monthly GSI values ranged from 1.2 to 2.9 for males and from 3.0 to 6.5 for females. The average GSI values of females were always higher than those of males in all months (Fig. 8). The average GSI values obtained for males and females were 2.1 and 4.6 respectively.

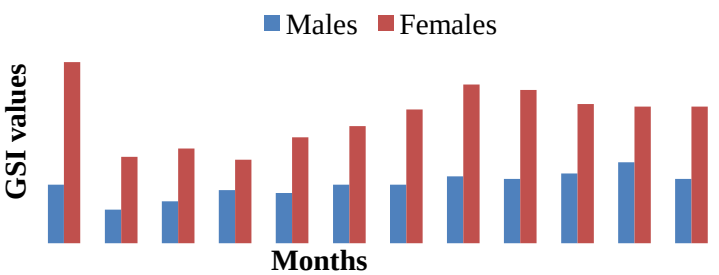


Figure 8. Monthly average GSI values of males and females

Fecundity

Fecundity estimation was based on 18 ripe females ranging in fish size between 8.5 cm and 16.5 cm TL and weight between 15.2 g and 50.1 g. The number of ova varied from 10,002 to 69,421 with average fecundity of 28,281 ova. The regression analysis of fecundity on fish length, fish weight and gonad weight can be expressed as

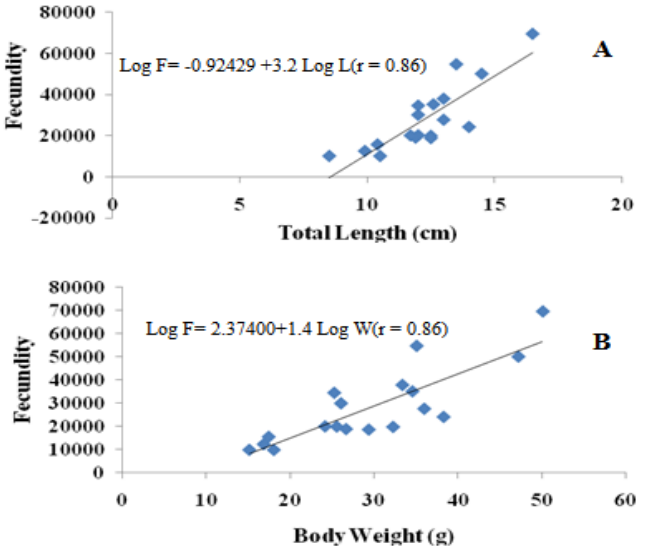
Log F= 0.92429 + 3.2 Log L (r = 0.86) (Fig. 9A)

Log F = 2.37400+ 1.4 Log W (r = 0.86) (Fig. 9B)

Log F = 3.55889 + 1.8 Log Wg (r = 0.94) (Fig. 9C)

where F= fecundity, L= total length of fish, W= weight of fish and Wg= weight of gonad

The resultant correlation coefficient r values indicated that the correlation was significant.



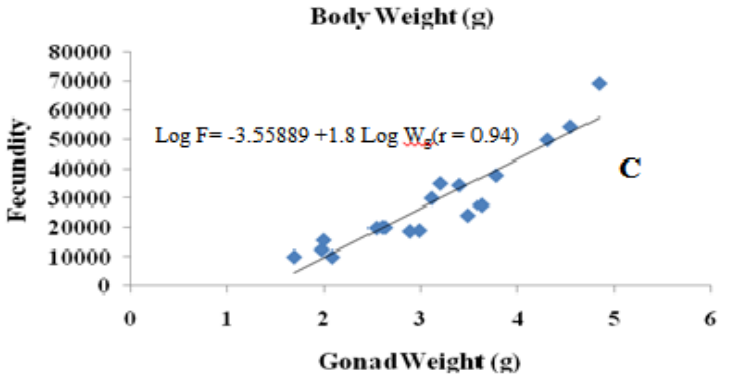


Figure 9. Fecundity in relation to A) total length; B) body weight and C) gonad weight of *Gazza minuta.*

Discussion

Species of *Gazza minuta* regularly contributes to the catches of trawl fisheries of Myeik coastal waters. Maturity stages of silverbellies were classified as three stages based on ova characteristics (Arora, 1952), five stages in female and three stages in male based on the external appearance of ovaries (Abraham *et al*. 2011) and six stages (Rao *et al*. 2015). The scale of maturation stages is different in different groups of species and in different regions (Abraham *et al*. 2011). In the present study, six stages of maturity (Immature, Early maturing, Maturing, Mature, Ripe and Spent) were classified in the gonads of *Gazza minuta* (Figs. 2 and 3).

Reproduction also involves changes in growth and development of oocytes during the process of gonad maturation (Priyadharsini *et al*. 2013). Histological examination is accurate method to determine the oocyte development. The general pattern of histological development of the oocytes of the present study is similar to that of the most teleosts. The six oocyte stages were identified in the ovaries of silverbellies in the present study according to the scales modified by Priyadharsini *et al.* (2013) and Agarwal (1996).

The spawning season of fish has been determined by the percentage of mature fishes present in the catch. In the present study, mature gonads of

males and females occurred in all months and its percentage was high from the months of June till January (Figs. 5 and 6). So *Gazza minuta* spawns throughout the year, with peak during June to January. Similarly, the population of *Gazza minuta* from Porto Novo coast of India spawns during July to January (Jayabalan, 1988). James and Badrudeen (1986) also stated species of *Gazza minuta* from the seas around India spawn over a prolonged period. In general, spawning activity varied according to geography.

Studies on the size at first maturity is essential to ensure a sustained yield by regulating the mesh size of the net, to make sure that the smaller fish also gets an opportunity to spawn at least once in their life time. *Gazza minuta* mature first at an average total length of 9.5 cm in males and 10.0 cm in females in the present study (Fig. 7). This size at first maturity of present study was slightly smaller than that of Jayabalan (1988) in which he estimated the length at first maturity of *G. minuta* from Porto Novo coast as 99 mm in males and 102 mm in females.

Sex ratio studies provide information on the proportion of male to female fish in a population and are expected to be 1: 1 in nature. Table 2 showed that the number of females were more dominant in the catches than the males. However, the analysis of chi-square method showed that there was no significant difference at 5% probability level. Jayabalan (1988) also indicated the predominance of females in the silverbellies catches of Porto Novo coast.

Gonadosomatic index (ratio of gonad weight to body weight) is an indirect method for estimating spawning season of species. The monthly GSI value of the present study ranged from 1.2 to 2.9 in males and from 3.0 to 6.5 in females. The average values obtained for males and females were 2.1 and 4.6. Seah *et al.* (2009) reported that the mean value of GSI for *Gazza minuta* from the coastal waters of South China Sea was 0.382 in females and 0.093 in males. The resultant values of present study were observed to be higher than

the mean value of GSI for of South China Sea that was reported by Seah *et al*. (2009). The monthly average GSI values of females were always higher than those of males in all months of present study period. Similarly, GSI value of silverbellies was higher in females than in males (Jayabalan 1988).

Fecundity (total number of eggs per fish) is the most common measure of reproductive potential in fish. Pillai (1972) stated the fecundity of *Gazza minuta* as ranging from about 7950 to 28430 eggs in the Gulf of Mannar. From Porto Novo, Jayabalan (1988) estimated that the number of eggs in *G. minuta* varied from 11650 to 26750. Thus, the estimation of fecundity on *G. minuta* (10,002 - 69,421 eggs) of the present study was higher than those of Pillai (1972) and Jayabalan (1988).

The observed correlation coefficient 'r' values of the present study indicated that fecundity is more related to the gonad weight than total length and body weight of *Gazza minuta*. Thus, it is indicated that the weight of gonad is more suitable indices for estimating the fecundity than length and weight of fish.

Conclusion

According to the observation, it could be concluded that *Gazza minuta* spawn throughout the year, with peak in June-January. Thus, prohibition on fishing should be put during these months. Although species of *G. minuta* are caught as by catch in various fishing gears, they are mainly contributors of trawl that is multispecies fisheries. The optimum mesh size for each species may affect the other species taken in this gear. Therefore, many more researches on stock structure for fisheries management should be carried out to decrease fishing effort for sustainable utilization on this species.

Acknowledgement

The author acknowledges Dr. Htay Aung, Rector (Retd), Mawlamyine University and Dr. Nyo Nyo Tun, Professor (Head) of Marine Science Department, Myeik University for giving opportunity to do research on this species and valuable advice.

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THE COMMERCIAL FISHES OF THANLWIN RIVER MOUTH AND ADJACENT WATERS, MON STATE, MYANMAR

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Abstract

A study on the commercial fishes from Thanlwin River Mouth and Adjacent Waters, Mon State, Myanmar was conducted in 2009 and 2014. A total of 96 species of commercial fishes was recorded. Of which, 29 species (9 species of fresh water and 20 species of marine fishes), 30.2%, were exported to foreign countries and other regions. The classification system, the station wise species distribution and the compositions of the species compared with the previous record, the family-wise composition of commercial species, their role of economic importance and the main trend of commercial fish distribution channels were presented. Among the commercial species, the species of Thread fin (Polynemidae), Croaker (Sciaenidae), Hilsa (Clupeidae), Bombay duck (Harpadontidae) and Anchovy (Engraulididae) were the most economically important species. The Thread fin, Croaker and Hilsa were more abundant at the Mouth of Thanlwin River (Mawlamyine, Moattama, Bilugyun). Bombay duck and Anchovy (especially *Coilia dussumieri*) were more abundant at Setse`and Kyaikkhami. The highest abundant (by weight) commercial species from the catch of offshore fishing vessels were *Congresox* spp., *Ilisha* spp., *Polynemus* spp., *Pampus argenteus*, *Chirocentrus nudus*, *Arius* spp., *Tenualosa* spp., *Chrysochir aureus*, *Cynoglossus* spp. and *Lepturacanthus* spp., respectively. The IUCN assessed Red List species (LR/nt), *Scoliodon laticudus* (spadenose shark), was recorded and observed this species was more abundant at Setse` and Kyaikkhami.

Keywords: Commercial fishes, distribution, IUCN Red List species, *Scoliodon laticudus*, Thanlwin River Mouth, Myanmar.

Introduction

The Thanlwin River, also called the Salween River, is one of the main rivers of Myanmar, lies between 15º and 16 º 30' N and 97 º 21' to 97 º 36' E. It is the world's 26 th longest river (with a length of nearly 3,000 km) and

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Southeast Asia's last great river to remain free-flowing (http://www. Wikipedia.org/wiki/Salween-River). There are also a number of smaller rivers discharging a freshwater load into the Gulf of Martaban. The southwest monsoon brings rain from the Bay of Bengal and the rainy season in Myanmar (June-October). As a consequence, this is a good for support freshwater, brackish and marine fisheries. Some species of fishes migrate to find good breeding-ground into the Thanlwin River such as the species of Thread fin, Croaker, Hilsa and Anchovy. The array of ichthyofauna met in its upper, middle and lower reaches present an interesting variety of fish species (Myint Myint Than, 1983).

Mon state in Myanmar is one of the regions famous for its inland, inshore and offshore fisheries. The major account responsible for this is the Thanlwin River, its associated estuaries and Adjacent Waters. It receives many torrential streams, water-falls and tributaries of rugged mountains and receives the larger tributaries such as the Attaran, Gyaing Rivers, etc.

Studies on the ichthyofauna of the Thanlwin River Mouth was formerly reported in 1983 by Myint Myint Than and recorded 50 species of commercial and zoological value of the fish species. Although many local researchers were presented on the value of the Thanlwin River . At the regional conference on "Value of Thanlwin/Salween River", focusing on the information and research on the commercial fish species of Thanlwin River Mouth were still rare. So, the present study attempted to find out the commercially important fish species of Thanlwin River Mouth and Adjacent Waters, to know their species distribution and to record the most commercially important species of the study area. The study also expects to become a base line data providing for further study.

Materials and Methods

The specimens identified in the study were collected from five stations of Thanlwin River Mouth and Adjacent Waters; Mawlamyine (Lat.16° 29' N, Long. 97° 37' E), Moattama (Lat.16° 31' N, Long. 97° 36' E), Bilugyun, lied between (Lat.16° 12' and 16° 32' N, Long. 79° 35' and 79° 52 ' E), Kyaikkhami (Lat. 16° 03' N, Long. 97° 33' E) and Setse` (Lat. 15° 56' N, Long. 97° 37' E), Mon State, Myanmar in 2009 and 2014. In the study, the commercial fishes from both the fresh water and marine species were analyzed because the water from Thanlwin River Mouth not only relates with the Gulf of Moattama (Martaban), but also with some fresh water rivers, mainly Gyaing and Attran Rivers (Fig.1). The total catch of fishes from offshore fishing vessels was calculated on the data form the May 2009 to April 2010. Identifications were largely based upon their distinctive morphology. The classifications system of fresh water species were mainly followed to Munro (1955), Jayaram (1981), Mohsin and Azmi (1983) and for marine species were mainly followed to Day (1878) and Carpenter, *et al*. (1999).The role of economic importance (highly commercial, commercial and minor in commercial) and economic species were considered in terms of local demand, usage, value, abundance, and exportable potential of the species informed by the Department of Fishery, Mon State and also followed to Mya Than Tun, (2001), Sann Aung (2003) and Hla Win, *et al*., (2008).

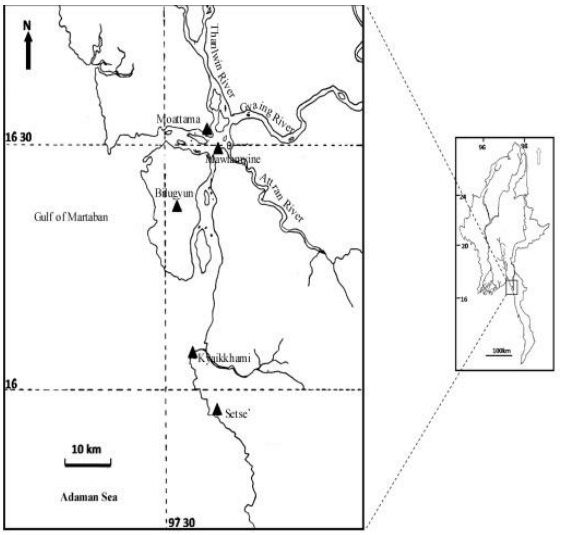


Figure 1. Mapshowing the sample collection sites

Results

In the study, a total of 96 species of commercial fishes belonging to 71 genera of 48 families from 17 orders under 2 classes were identified (Table 1). Of which, 3 species (*Scoliodon laticaudus, Dasyatis imbricatus and Narcine brunnea*) were cartilaginous and the rest 93 were bony fishes. Among the recorded species, 20 (20.8%) were fresh water species and 76 (79.2%) were marine species (Figure.3).

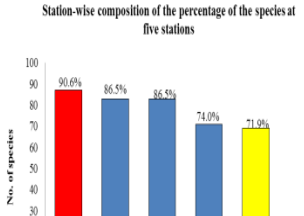
The station wise species distribution and the compositions of the species compared with the previous record by Myint Myint Than, 1983 were reported in Table 2. The number of species collected from five stations were not significantly differ; Mawlamyine; 87, Moattama; 83; Bilugyun; 83, Kyaikkhami; 71 and Setse`; 69, respectively (Table 2 and figure 2). The highest number of species composition was found at station Mawlamyine (87 species) and the lowest was at Stations Setse` (69 species).

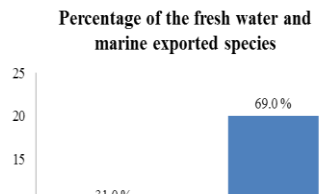
The family-wise species composition of the commercial fish species from Thanlwin River Mouth and Adjacent Waters was depicted in figure 8. The highest number of species composition was found at the families, Engraulidae (13 species), then followed Sciaenidae (7 species), Clupeidae (5 species) and Polynemidae (5 species), respectively.

Besides all species were locally consumed, 29 species (30.2%) were exported to other regions and foreign countries (Table 3). Among the exported specis, 9 species (31.0%) were fresh water species and 20 species (69.0%) were marine species. The role of economic important exhibiting highly commercial, commercial and minor in commercial species together with local consumption and/or exported species were highlited in the Table 3 and figure 5.

Figure 9 represents the total catch of all landed fishes caught from offshore fishing vessels during May 2009 to April 2010. The most abundance caught species from offshore fishing vessels were *Congresox* spp., *Ilisha* spp., *Polynemus* spp., *Pampus argenteus*, *Chirocentrus nudus*, *Arius* spp., *Tenualosa* spp., *Chrysochir aureus*, *Cynoglossus* spp. and *Lepturacanthus* spp., respectively.

Figure 10 pointed the main trend of fish distribution channels caught from Thanlwin River Mouth and Adjacent Waters landed to Mawlamyine District.





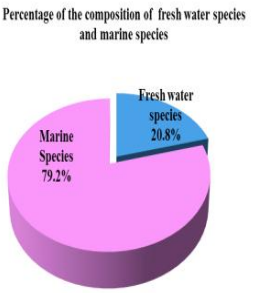


Figure 3. Percentage of the composition of fresh and marine species of commercial fishes from Thanlwin River Mouth and Adjacent Waters

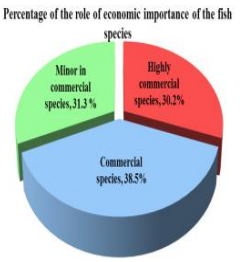


Figure 5. Percentage of the role of economic important species from Thanlwin River Mouth and Adjacent Waters

Table 1. Classify list of the collected commercial fish species of the Thanlwin River Mouth and Adjacent Waters during the study

Order	Family	Genus	Sr. No	Species	Local Name
Carcharhiniformes	Carcharhinidae	*Scoliodon*	1	*Scoliodon laticaudus* Müller & Henle, 1838	Nga-mann
Rajiformes	Dasyatidae	*Dasyatis*	2	*Dasyatis imbricatus* (Bloch & Schneider, 1801)	Nga-lake-kyauk
Torpediniformes	Narcinidae	*Narcine*	3	*Narcine brunnea* Annandale, 1909	Nga-latt-htone
Anguilliformes	Muraenesocidae	*Congresox*	4	*Congresox talabonoides* (Bleeker, 1853)	Nga-shwe
Aulopiformes	Synodontidae	*Saurida*	5	*Saurida undosquamis* (Richardson, 1848)	Nga-pa-lway
Batrachoidiformes	Batrachoididae	*Batrichthys*	6	*Batrichthys grunniens* (Linnaeus, 1758)	Nga-oat-pher
Channiformes	Channidae	*Channa*	7	*Channa striata* (Bloch, 1793)	Nga-yant
Clupeiformes	Chirocentridae	*Chirocentrus*	8	*Chirocentrus nudus* Swainson, 1839	Nga-da-lwel
	Clupeidae	*Anodontosoma*	9	*Anodontostoma chacunda* (Hamilton, 1822)	Nga-wun-pu, Bar-thi
		Escualosa	10	*Escualosa thoracata* (Valenciennes, 1847)	Yae-Kyi-ngar
		Sardinella	11	*Sardinella gibbosa* (Bleeker, 1849)	Nga-kown-nyo
		Tenualosa	12	*Tenualosa ilisha* (Hamilton, 1822)	Nga-tha-lauk
			13	*T. toil* (Valencinnes, 1847)	Nga-tha-lauk-yout-pha
	Pristigasteridae	*pellona*	14	*Pellona ditchela* Valenciennes, 1847	Nga-zin-pyer, Myat-san-kyal
		Raconda	15	*Raconda russeliana* Gray, 1831	Nga-da-lar
	Engraulididae	*Coilia*	16	*Coilia dussumieri* Valenciennes, 1848	Mee-tan-thwe, Nga-kyan-ywat
			17	*C. ramcarati* (Hamilton, 1822)	Mee-tan-thwe, Nga-kyann-ywuat
			18	*C. reynaldi* Valenciennes, 1848	Mee-tan-thwe, Nga-kyann-ywuat
		Setipinna	19	*Setipinna taty* (Valenciennes, 1848)	Nga-byar
			20	*S.tenuifilis* (Valenciennes, 1848)	Nga-byar, Nga-pa-sharr
			21	*S. wheeleri* Wongratana, 1983	Nga-byar, Nga-taung-pyar
		Stolephorus	22	*Stolephorus baganensis* Hardenberg, 1933	Nga-ni-tu
			23	*S. commersonii* Lacepede, 1803	Nga-ni-tu
			24	*S. indicus* (van Hasselt, 1823)	Nga-ni-tu
		Thryssa	25	*Thryssa dussumieri* (Valenciennes, 1848)	Nga-byar
			26	*T. kammalensis* (Bleeker, 1849)	Nga-byar
			27	*T. mystax* (Bloch & Schneider, 1801)	Nga-byar
			28	*T. stenosoma* Wongratana, 1983	Nga-ae-book, nga-phout-htime
Cypriniformes	Cyprinidae	*Labeo*	29	*Labeo calbasu* (Hamilton, 1822)	Nga-net-pyar
		Osteobrama	30	*Osteobrama alfredianus* (Valenciennes, 1844)	Nga-phan-ma
Elopiformes	Megalopidae	*Megalops*	31	*Megalops cyprinoides* (Broussonet, 1782)	Ka- lor-lae`

Order	Family	Genus	Sr. No	Species	Local Name
		Otolithoides	64	*Otolithoides pama* (Hamilton, 1822)	Nga-poat-thain, Nga-byat
		Pennahia	65	*Pennahia anea* (Bloch, 1793)	Nga-byat
		Pterotolithus	66	*Pterotolithus maculatus* (Cuvier, 1830)	Nat-ga-daw
	Scombridae	*Rastrelliger*	67	*Rastrelliger kanagurta* (Cuvier, 1816)	Pa-lar-tu
		Scomberomorus	68	*Scomberomorus commerson* (Lacepède, 1800)	Nga-kon-shat
			69	*S. guttatus* (Bloch & Schneider, 1801)	Nga-kon-shat
	Siganidae	*Siganus*	70	*Siganus canaliculatus* (Park, 1797)	Mal-taw-lat-thae
	Sillaginidae	*Sillago*	71	*Sillago sihama* (Forsskål, 1775)	Nga-pal-lway
	Stromateidae	*Pampus*	72	*Pampus argenteus* (Euphrasen, 1788)	Nga-moat-phyu
	Theraponidae	*Therapon*	73	*Therapon jarbua* (Forsskål, 1775)	Nga-goan-kyarr
			74	*Terapon puta* Cuvier, 1829	Nga-goan-kyarr
	Trichiuridae	*Trichiurus*	75	*Lepturacanthus savala* (Cuvier, 1829)	Nga-ta-khon
Pleuronectiformes	Cynoglossidae	*Paraplagusia*	76	*Paraplagusia blochi* (Bleeker, 1851)	Nga-kway-shar
	Soleidae	*Euryglossa*	77	*Euryglossa harmandi* (Sauvage, 1878)	Nga-kway-shar
Scorpaeniformes	Platycephalidae	*Platycephalus*	78	*Platycephalus indicus* (Linnaeus, 1758)	Nga-sin-nain
Siluriformes	Ariidae	*Arius*	79	*Arius burmanicus* (Day, 1870)	Nga-yaung, Nga-yaung-kyar
			80	*A. caelatus* Valenciennes, 1840	Nga-yaung, Shwe-nga- yaung
			81	*A. maculatus* (Thunberg, 1792)	Nga-yaung
	Bagridae	*Aorichtys*	82	*Aorichtys seenghala* (Sykes, 1839)	Nga-goung
		Mystus	83	*Mystus vittatus* (Bloch, 1794)	Nga-zin-yaine
			84	*M. wolffii* (Bleeker, 1851)	Nga-zin-yaine
	Clariidae	*Clarias*	85	*Clarias batrachus* (Linnaeus, 1758)	Nga-khu
	Plotosidae	*Plotosus*	86	*Plotosus canius* Hamilton, 1822	Ka-byaown, Pin-lail-nga-khu
	Schilbeidae	*Silonia*	87	*Silonia silondia* (Hamilton, 1822)	Nga-myuin
	Siluridae	*Ompok*	88	*Ompok bimaculatus* (Bloch, 1794)	Nga-nau-thann
		Wallago	89	*Wallago attu* (Bloch & Schneider, 1801)	Nga-bat
Synbranchiformes	Mastacembelidae	*Macrognathus*	90	*Macrognathus siamensis*(Günther, 1861)	Nga-mway-htoe
			91	*M. zebrinus*(Blyth, 1858)	Nga-mway-htoe
	Synbranchidae	*Monopterus*	92	*Monopterus albus* (Zuiew, 1793)	Nga-shint-ne
			93	*M. cuchia* (Hamilton, 1822)	Nga-shint-mawe
Tetraodontiformes	Tetraodontidae	*Lagocephalus*	94	*Lagocephalus lunaris* (Bloch & Schneider, 1801)	Nga-pu-tinn
		Monotretus	95	*Monotretus cutcutia* (Hamilton-Buchanan, 1822)	Nga-pu-tinn
		Xenopterus	96	*Xenopterus naritus* (Richardson, 1848)	Nga-pu-tinn

Table 2. The station-wise species distribution compared with the previous record at Salween (Thanlwin) River Mouth by Myint Myint Than (1983)

Sr No	species	Present record Stations					Previous record*
		Mawlamyine	Moattama	Bilugyun	Kyaikkhami	Setse	Salween River Mouth
1	*Scoliodon laticaudus*	+	+	+	+	+	+
2	*Dasyatis imbricatus*	+	+	+	-	-	+
3	*Narcine brunnea*	+	+	+	+	+	-
4	*Congresox talabonoides*	+	+	+	+	+	-
5	*Saurida undosquamis*	+	+	+	+	+	+
6	*Batrichthys grunniens*	+	+	+	+	+	+
7	*Channa striata*	+	+	+	+	+	+
8	*Chirocentrus nudus*	+	+	+	+	+	-
9	*Anodontostoma chacunda*	+	+	+	+	+	-
10	*Escualosa thoracata*	+	+	+	+	+	+
11	*Sardinella gibbosa*	-	-	-	+	-	+
12	*Tenualosa ilisha*	+	+	+	+	+	-
13	*T. toli*	+	+	+	+	+	+
14	*Pellona ditchela*	+	+	+	+	+	-
15	*Raconda russeliana*	+	+	+	+	+	-
16	*Coilia dussumieri*	+	+	+	+	+	-
17	*C. ramcarati*	+	+	+	+	+	+
18	*C. reynaldi*	+	+	+	-	-	-
19	*Setipinna taty*	+	+	+	+	+	-
20	*S. tenuifilis*	-	-	-	+	-	-
21	*S. wheeleri*	+	+	+	+	+	+
22	*Stolephorus baganensis*	+	+	+	+	+	+
23	*S. commersonii*	-	-	-	+	+	-
24	*S. indicus*	+	+	+	+	+	-
25	*Thryssa dussumieri*	+	+	+	+	+	+
26	*T. kammalensis*	+	+	+	+	+	+
27	*T. Mystax*	+	+	+	+	+	+
28	*T. stenosoma*	+	+	+	+	+	-
29	*Labeo calbasu*	-	-	-	+	+	-
30	*Osteobrama alfredianus*	+	-	-	+	+	-
31	*Megalops cyprinoides*	+	+	+	+	+	-
32	*Notopterus notopterus*	-	-	-	+	+	-
33	*Anbas testudineus*	-	-	-	+	+	+

Sr No	species	Present record Stations					Previous record*
		Mawlamyine	Moattama	Bilugyun	Kyaikkhami	Setse	Salween River Mouth
34	*Atropus atropus*	+	+	+	+	+	-
35	*Megalaspis cordyla*	+	+	+	-	-	-
36	*Scomberoides tol*	+	+	+	-	-	-
37	*Selar crumenophthalmus*	+	+	+	+	+	-
38	*Lates calcarifer*	+	+	+	-	-	-
39	*Drepane longimana*	+	+	+	+	+	-
40	*Formio niger*	+	+	+	-	-	-
41	*Gerres filamentosus*	+	+	+	-	-	-
42	*Pentaprion longimanus*	+	+	+	-	-	-
43	*Apocryptes lanceolatus*	+	+	+	-	-	-
44	*Baleophthalmus boddarti*	+	+	+	-	-	-
45	*Glossogobius giuris*	-	-	-	+	+	+
46	*Harpadon nehereus*	-	-	-	+	+	+
47	*Datnioides quadrifasciatus*	+	-	-	+	+	+
48	*Rhinomugil corsula*	+	+	+	+	+	+
49	*Valamugil speigleri*	+	+	+	-	-	+
50	*Nemipterus japonicus*	+	+	+	+	+	-
51	*N. nematophorus*	+	+	+	+	+	-
52	*Eleutheronema tetradactylum*	+	+	+	+	+	+
53	*Polynemus indicus*	+	+	+	+	+	+
54	*P. paradise*	+	+	+	+	+	+
55	*P. plebeius*	+	+	+	+	+	
56	*P. sextarius*	+	+	+	+	+	-
57	*Pomadasys maculatus*	+	+	+	+	+	+
58	*Rachycentron canadum*	-	-	-	+	-	+
59	*Scatophagus argus*	+	+	+	+	+	-
60	*Chrysochir aureus*	+	+	+	+	+	+
61	*Johnieops vogleri*	+	+	+	+	+	-
62	*Johinus coitor*	+	+	+	+	+	-
63	*Nibea soldado*	+	+	+	+	+	-
64	*Otolithoides pama*	+	+	+	+	+	+
65	*Pennahia anea*	+	+	+	-	-	-
66	*Pterotolithus maculatus*	+	+	+	+	+	+

Sr No	species	Present record Stations					Previous record *
		Mawlamyine	Moattama	Bilugyun	Kyaikkhami	Setse`	Salween River Mouth
67	*Rastrelliger kanagurta*	+	+	+	+	+	+
68	*Scomberomorus commerson*	+	+	+	+	+	+
69	*S. guttatus*	+	+	+	+	+	-
70	*Siganus canaliculatus*	-	-	-	+	+	-
71	*Sillago sihama*	+	+	+	+	+	-
72	*Pampus argenteus*	+	+	+	+	+	+
73	*Therapon jarbua*	+	+	+	+	+	+
74	*Therapon puta*	+	+	+	+	+	+
75	*Lepturacanthus savala*	+	+	+	+	+	-
76	*Paraplagusia blochi*	-	-	-	+	+	-
77	*Euryglossa harmandi*	+	-	-	+	+	-
78	*Platycephalus indicus*	+	+	+	+	+	+
79	*Arius burmanicus*	-	-	-	+	+	-
80	*A. caelatus*	-	-	-	+	+	+
81	*A. maculatus*	+	+	+	+	+	
82	*Aorichtys seenghala*	+	+	+	-	-	-
83	*Mystus vittatus*	+	+	+	-	-	-
84	*M.wolffii*	+	+	+	+	+	
85	*Clarias batrachus*	+	+	+	-	-	-
86	*Plotosus canius*	+	+	+	+	+	-
87	*Silonia silondia*	+	+	+	-	-	-
88	*Ompok bimaculatus*	+	+	+	-	-	-
89	*Wallago attu*	+	+	+	-	-	-
90	*Macrognathus siamensis*	+	+	+	-	-	-
91	*M. zebrinus*	+	+	+	-	-	-
92	*Monopterus albus*	+	+	+	-	-	-
93	*M. cuchia*	+	+	+	-	-	-
94	*Lagocephalus lunaris*	-	-	-	+	+	+
95	*Monotretus cutcutia*	-	-	-	+	+	+
96	*Xenopterus naritus*	+	-	-	+	+	+
Total number of species by station		87	83	83	71	69	
Total number of species				**96**			**26**

Symbols: +, Presence; -, Absence. * Source: Myint Myint Than (1983)

Table 3. The list of the role of economic importance (local consumption and exported) of fish species from the Thanlwin River Mouth and Adjacent Waters

Sr No	Species	Role of economic importance			
		Highly commercial	Commercial	Minor in commercial	Local consumption/ Exported species
1	*Scoliodon laticaudus*		½		Local
2	*Dasyatis imbricatus*		½		Local
3	*Narcine brunnea*			½	Local
4	*Congresox talabonoides*	½			Local / Export
5	*Saurida undosquamis*		½		Local
6	*Batrichthys grunniens*			½	Local
7	*Channa striata*	½			Local / Export
8	*Chirocentrus nudus*	½			Local / Export
9	*Anodontostoma chacunda*		½		Local
10	*Escualosa thoracata*			½	Local
11	*Sardinella gibbosa*		½		Local
12	*Tenualosa ilisha*	½			Local / Export
13	*T. toli*		½		Local
14	*Pellona ditchela*			½	Local
15	*Raconda russeliana*			½	Local
16	*Coilia dussumieri*			½	Local
17	*C. ramcarati*			½	Local
18	*C. reynaldi*			½	Local
19	*Setipinna taty*			½	Local
20	*S. tenuifilis*			½	Local
21	*S. wheeleri*			½	Local
22	*Stolephorus baganensis*			½	Local
23	*S. commersonii*			½	Local
24	*S. indicus*			½	Local
25	*Thryssa dussumieri*			½	Local

Sr	Species	Role of economic importance

		Highly commercial	**Commercial**	**Minor in commercial**	**Local consumption/ Exported species**
26	*T. kammalensis*			½	Local
27	*T. Mystax*			½	Local
28	*T. stenosoma*			½	Local
29	*Labeo calbasu*	½			Local / Export
30	*Osteobrama alfredianus*		½		Local
31	*Megalops cyprinoides*			½	Local
32	*Notopterus notopterus*	½			Local / Export
33	*Anbas testudineus*		½		Local
34	*Atropus atropus*		½		Local
35	*Megalaspis cordyla*	½			Local/ Export
36	*Scomberoides tol*	½			Local/ Export
37	*Selar crumenophthalmus*		½		Local
38	*Lates calcarifer*	½			Local / Export
39	*Drepane longimana*			½	Local
40	*Formio niger*	½			Local / Export
41	*Gerres filamentosus*		½		Local
42	*Pentaprion longimanus*		½		Local
43	*Apocryptes lanceolatus*			½	Local
44	*Baleophthalmus boddarti*			½	Local
45	*Glossogobius giuris*		½		Local
46	*Harpadon nehereus*		½		Local
47	*Datnioides quadrifasciatus*			½	Local
48	*Rhinomugil corsula*		½		Local
49	*Valamugil speigleri*		½		Local
50	*Nemipterus japonicus*		½		Local
51	*N. nematophorus*		½		Local
52	*Eleutheronema tetradactylum*	½			Local/ Export
53	*Polynemus indicus*	½			Local / Export
54	*P. paradise*	½			Local / Export

Sr No	Species	Role of economic importance			
		Highly commercial	**Commercial**	**Minor in commercial**	**Local consumption/ Exported species**
55	*P. plebeius*	½			Local / Export
56	*P. sextarius*		½		Local
57	*Pomadasys maculatus*			½	Local
58	*Rachycentron canadum*	½			Local / Export
59	*Scatophagus argus*			½	Local
60	*Chrysochir aureus*	½			Local/ Export
61	*Johnieops vogleri*	½			Local/ Export
62	*Johinus coitor*		½		Local
63	*Nibea soldado*		½		Local
64	*Otolithoides pama*	½			Local/ Export
65	*Pennahia anea*	½			Local/ Export
66	*Pterotolithus maculatus*	½			Local/ Export
67	*Rastrelliger kanagurta*	½			Local/ Export
68	*Scomberomorus commerson*	½			Local/ Export
69	*S. guttatus*	½			Local/ Export
70	*Siganus canaliculatus*		½		Local
71	*Sillago sihama*		½		Local
72	*Pampus argenteus*	½			Local/ Export
73	*Therapon jarbua*		½		Local
74	*Therapon puta*		½		Local
75	*Lepturacanthus savala*		½		Local
76	*Paraplagusia blochi*			½	Local
77	*Euryglossa harmandi*			½	Local

(Source: Indicative Price of Export Fish and Fishery Products, Fish Inspection Quality Control Division, and Department of Fisheries, Mon State, 2009-2010)

Sr No	Species	Role of economic importance			
		Highly commercial	**Commercial**	**Minor in commercial**	**Local consumption/ Exported species**
78	*Platycephalus indicus*		½		Local
79	*Arius burmanicus*			½	Local
80	*A. caelatus*		½		Local
81	*A. maculatus*	½			Local/ Export
82	*Aorichtys seenghala*		½		Local
83	*Mystus vittatus*		½		Local
84	*M.wolffii*		½		Local
85	*Clarias batrachus*	½			Local/ Export
86	*Plotosus canius*		½		Local
87	*Silonia silondia*	½			Local/ Export
88	*Ompok bimaculatus*		½		Local
89	*Wallago attu*		½		Local
90	*Macrognathus siamensis*		½		Local
91	*M. zebrinus*		½		Local
92	*Monopterus albus*	½			Local/ Export
93	*M. cuchia*	½			Local/ Export
94	*Lagocephalus lunaris*			½	Local
95	*Monotretus cutcutia*			½	Local
96	*Xenopterus naritus*		½		Local
	Total	**29**	**37**	**30**	**96/29**

(Source: Indicative Price of Export Fish and Fishery Products, Fish Inspection Quality Control Division, and Department of Fisheries, Mon State, 2009-2010)

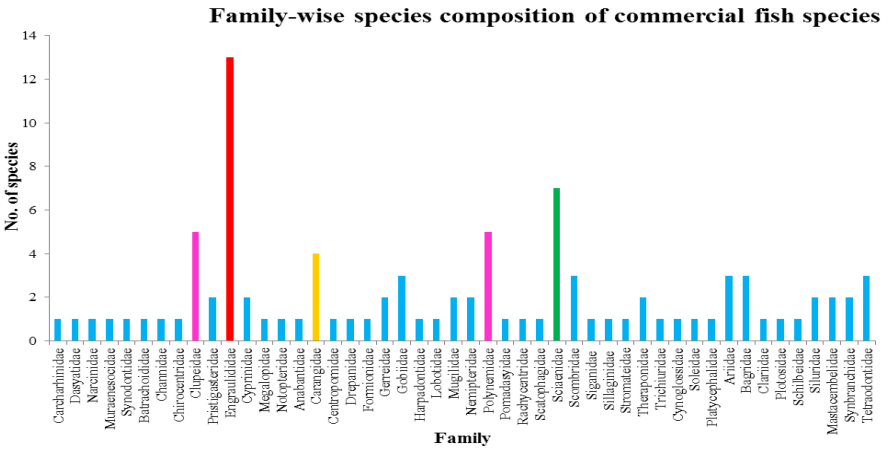


Figure 8. Family-wise species composition of the commercial fish species from Thanlwin River Mouth and Adjacent Waters

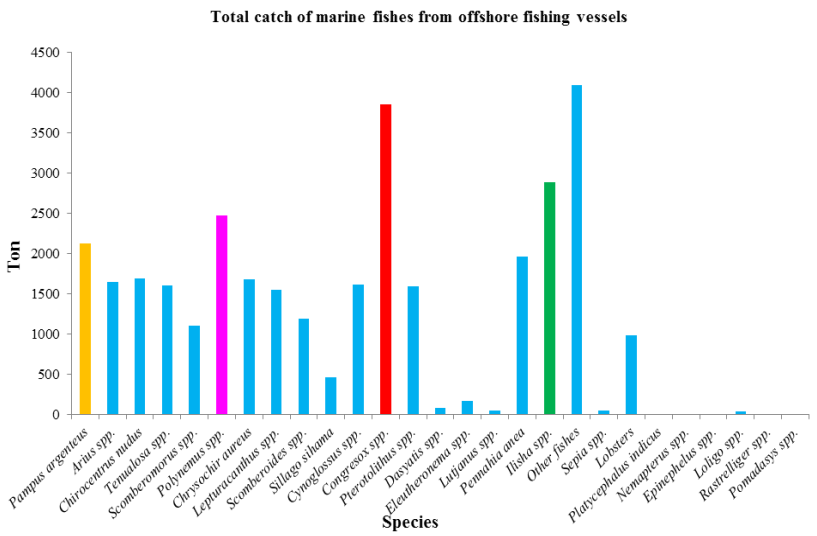


Figure 9. The total catch of fishes from offshore fishing vessels (Source: Department of Fishery, Mon State, 2010)

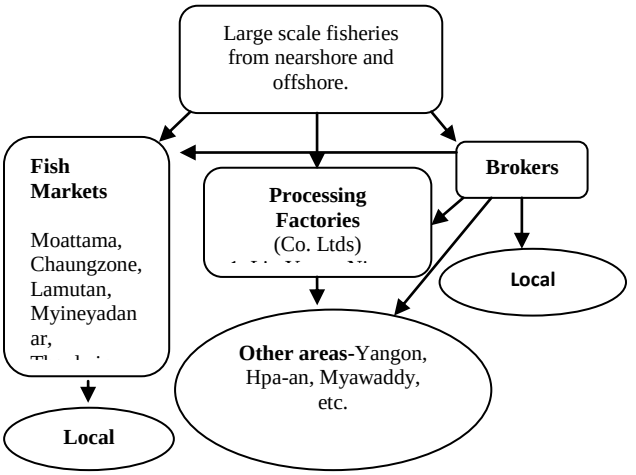


Figure 10. Flowing chart showing the main trend of commercial fish distribution channels caught from Thanlwin River mouth and Adjacent Waters landed to Mawlamyine District
(Source: Department of Fishery, Mon State)

Discussion

Myanmar possesses a long coastline approximately 2,832 km, a continental shelf of 228,781 km ^{2} and Exclusive Economic Zone (EEZ) of 486,000 km ^{2}, 8.1 million ha of inland freshwater bodies, many rivers, creeks, streams, natural ponds, lakes and puddles (Khin Maung Aye *et al*. 2006). The extensive river systems and the monsoon rainfall contribute the richness of inland fisheries and its long coastal regions have great variety of aquatic life. Marine fishes account for about 75 % of the total fish production in Myanmar, and 25 % coming from fresh water (Ministry of Livestock and Fisheries, 2009). Fish and shrimp constitute higher groups of aquatic animals, having great commercial value (Sann Aung, 2003).

Fish is one of the most important main animal protein resources in Myanmar. They can be utilized as food in many ways such as dried, salted, smoked, paste, sauce, fresh state for locally and also export to many other

countries to earn foreign currency. Fishery sector is considered as the most important one after the agriculture sector to fulfill the protein requirement of the people of Myanmar and to provide the food security as well as to get the opportunity for the employment to a large number of fishry communities and rual dwellers. Fishes with high protein contents are available with more or less reasonable price and hence they are of great demand by most people of Myanmar. Economically, the fish constitute a very important group of animals (Department of fisheries, Myanmar, 2009).

The early reports on the fresh water fishes of Myanmar was by Major Berdmore who made a fairly representative collection of fishes from the Sittaung River System and published by Blyth in 1860; fishes from the Kachin Hills, especially the tributary streams of the Malikha River was published by Mukerji in 1933-1934 and the upper Chindwin collection of the American Museum of Natural History was reported in part by Hora and Misra in 1940. Research on marine resources of Myanmar was first carried out by Ba Kyaw in 1965, followed by Hilda and Pereya in 1969 and Druzhinin in 1972 (as cited in Mya Than Tun 2001). The systematic survey undertaken in Myanmar was “Marine Fishery Resources Survey and Exploratory Fishing Project” with the assistance of Food and Agriculture Organization of the United Nations during 1979 to 1983. The classification of fishes by economic class was reported by Stromme, *et al*., in 1981 at the survey "the marine fish resources of Burma" with Dr. Fridtjof Nansen ship (Sann Aung, 2003). Sea Fishery Resource Survey and Research Unit, Department of Fishery sureyed on the commercial marine fishes of 58 species in 1999, Mya Than Tun (2001) reported 351 species of the pelagic and demersal marine fishes of Myanmar, Sann Aung (2003) exhibits economically important 70 species of fishes from Myanmar Seas and Hla Win *et al*., (2008) exhibited commercial fishes form Myanmar Water including of 40 fresh water and 172 marine species.

In this study, 96 species of fresh water and marine fishes inhabiting vicinity of Thanlwin River Mouth and Adjacent Sea were identified. Although

many records and systematic of both fresh water and marine fisheries were made by various ichthyologists of the Academic Departments of Universities and Colleges, and also occasionally in the Fishery Departments and other Research Centers, there have been relatively few or very little ichthyological studies on Thanlwin River Mouth, although a report on the fishes of the Salween (Thanlwin) River Mouth was surveyed by Myint Myint Than in 1983. She surveyed the ichthyofauna of the Salween River (Thanlwin River) Mouth, recorded 50 species of commercial and zoological value of fishes. In comparing with her observation, 26 species (27.1%) of the samples were same with her recorded species. This might be due to the differences of the stations, sample collection time, and the species that she omitted to report. She only selectively exhibited commercially and zoologically valuable species from her collected species (about 100 species). Her eight sampling sites are the largest fishing centers; Amherst, Kadonpaw, Kyauktan, Martaban, Kalwe, Ahlat, Seplar, and Kamake. From which, only two stations are the same with the present study; the Martaban (Moattama) and Kyauktan (included in Mawlamyine station).

The number of species collected from five stations was slightly variation. The highest number of species composition was found at Mawlamyine and the lowest was at Setse.

The rarest species of the specimens collected were only one or two or less than five in number, during sampling (except *Arius burmanicus*). These species were *Narcine brunnea, Megalops cyprinoides, Pentaprion longimanus, Datnioides quadrifasciatus, Pomadasys maculatus, Nibe soldado, Pterotolithus maculatus, Euryglossa harmandi, A. burmanicus* and *Aorichtys seenghala*. The exceptional species *A. burmanicus*, called Nga-yaung-kyar in Myanmar name, was only recorded at Stations Setse` and Kyaikkhami, but not in the rest three stations, and *Lagocephalus lunaris, Monotretus cutcutia* and *Xenopterus naritus* are more abundance at stations Setse` and Kyaikkhami.

Among the commercial species, the species of Thread fin (Polynemidae), Croaker (Sciaenidae), Hilsa (Clupeidae), Bombay duck (Harpadontidae) and Anchovy (Engraulidae) were the most economically important species. The Thread fin, Croaker and Hilsa were more abundance at the Mouth of Thanlwin River (Mawlamyine, Moattama, Bilugyun). Bombay duck and Anchovy (especially *Coilia dussumieri*) were more abundance at the station Setse`and Kyaikkhami.

Scoliodon laticudus, was more abundance at Setse` and Kyaikkhami. The International Union for Conservation of Nature (IUCN) has assessed this species as Red List of threatended species; Lower Risk, Near Threatened (LR/nt) species.

In the study area, the fishes are utilized as food in various ways such as fresh, dried, salted, smoked, and even some trash fishes can be made as fish paste (Nga-pi) and fish sauce (Ngan-pyar-yae). So, classified the samples as the three grates based upon their locally demand, usage, value, abundance, and exportable potential. Twenty nine species were highly in commercial, 37 were commercial species and 30 were minor in commercial species.

According to the observations, Thread fin (Family- Polynemidae), Croaker (Family-Sciaenidae), Hilsa (Family-Clupeidae), Bombay duck (Family-Harpadontidae) and Anchovy (Family-Engraulididae) were represented as the most important among the commercial species in the study area. These findings are well agreed with the findings of Tint Swe (2011), Khine Myat Myat Htwe, (2012), Mi Mya Mya Thet (2013) and Ohmar min (2013). Tint Swe (2011) reported that the Bombay duck, anchovies, croakers, ribbon fish and small shrimps were major resources and economically important along the coast of Mon State. Fishery and biology of herring fishes were studied along the Mon State Coastal Waters and its adjacent waters by Khine Myat Myat Htwe (2012), and she described the two species of *Tenualosa*, *T. ilisha* and *T. toil*, are economically important and this result is similar in the present study. Ohmar min (2013) studied the fishery and biology

of sciaenid fish and she stated that the two species, *Chrysochir aureus* and *Otolithoides pama*, are economically important along Mon Coastal Waters. It was found that the two species of polynemid fishes, *Polynemus paradiseus and P. indicus* are economically important and commonly found along the Mon State Coastal Waters and its adjacent waters by Mi Mya Mya Thet (2013).

In the present study, Bombay duck and Anchovy were more abundance at the stations Kyaikkhami and Setse`. Among the anchovies species, *Coilia dussumieri* was the most abundant species in the catches of bag net fishery and popularly consumed in Mon State and also exported to other regions as dried item. Thread fin, Croaker and Hilsa species were more abundant at Mawlamyine, Moattama and Bilugyun.

Conclusion

In the study, a total of 96 species of commercial fishes were recorded from Thanlwin River Mouth and Adjacent Waters. The IUCN assessed Red List species (LR/nt), *Scoliodon laticudus* (spadenose shark), was recorded at all stations. The number of species collected from five stations did not vary very much. Besides 29 species were exported to other regions and foreign countries, all of the fishes were popularly consumed by local people. Among the commercial species, the species of Thread fin (Polynemidae), Croaker (Sciaenidae), Hilsa (Clupeidae), Bombay duck (Harpadontidae) and Anchovy (Engraulididae) were the most economically important species. During the study, diverse species of commercial fishes were observed. They are not only important for local people as food but also support finance by exporting them to other areas and foreign countries. The economic and livelihood of many local people relies upon the commercial fishes of the Thanlwin River Mouth and Adjacent Waters. Thus, further studies are still needed and this should be made to know their biology, fishery, ecology and economical studies to support the socioeconomic development for the local people.

Acknowledgements

I wish to express my sincere gratitude to Dr. Htay Aung, Rector (Retd.), Mawlamyine Univesity, for his suggestions and giving guidlines to carry out this research work. My special thanks are also to Dr. Nyo Nyo Tun, Professor, Head of Department of Marine Science, Myeik University, for her encouragement in preparing this manuscript. Thanks are also to U Kyaw Htun Nyo, Deputy Assistant Officer, Department of Fishery, Mon State, for providing the fishery data and information concerning the Thanlwin River Mouth and Adjacent Waters.

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SOCIO-ECONOMIC CONDITIONS, MARINE RESOURCES UTILIZATION AND DEMOGRAPHY OF SMALL-SCALE FISHING COMMUNITIES IN PALAW TOWNSHIP, TANINTHAYI REGION

Zin Lin Khine [1] and Pann Mo Mo [2]

Abstract

A socio-economic assessment was conducted in fishing communities of Palaw Township. Key informant interviews (KII) data indicated that the infrastructure at community level was limited in these communities. Most respondents were identified as Burmese and Buddhist. Education attainment levels were higher in household head between the ages of 21-40 years than others and they also had degrees. Fishing was the main source of income for most respondents in these communities but their incomes were not enough for their livelihoods. They faced most difficulties in the rainy season (June-September). The primary occupations of some respondents were inshore fishing, collecting bivalves, fish (buying and selling), agriculture and shop keeping. Groupers, spanish mackerels, silver grunts, rays, sardinella, ponyfish, croaker and anchovy were caught around Anyin-pho, Anyin-ma, Ma-li, Kyunn-hla, Tha-mi-hla, Kyauk-kar, Khan-ti and Lit-ku throughout the year. The high dependence on marine resources at the household and community level perpetuated the threats on marine resources. Most household heads had low awareness of rules and regulations of marine resources. Most households did not want to participate in decision-making to conserve marine resources. Fishermen who had fishing experience answered that there was a decrease in the catch of marine resources at the present period. These small-scale fishing communities are required conservation plans to improve public awareness and to enforce fishery and forestry laws and to select marine protect area (MPA).

Key words: Fishing, households, livelihoods, marine resources, Palaw, socioeconomic.

Introduction

Palaw fishing communities are located in Tanintharyi Region, the southern part of Myanmar. In Palaw, fishing is the main economic activity

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with emphasis on different kinds of fish, shrimps, bivalves, crabs, fish paste, dried fish, salted-fish, nypa sap, fermented nypa sap and nypa sugar production. Most people who live in fishing communities of Palaw are depending on the marine resources. Malleret (2004) observed that the most dependent villages on marine resources were seafront and mangrove villages.

A few years ago, marine resources including fish, shrimps, squids, crabs and marine bivalves (*Meretrix meretrix*) were common in Palaw fishing communities. Most local people collected the bivalves and exported dry products to other towns. However, marine resources have been decreasing year after year. As illegal fishing such as trawling and trap boats had entered inshore waters, the habitats where the bivalves lived were destroyed. Holmes *et al.* (2014) observed that fishermen were using inappropriate technologies in certain areas and this can lead to destructive fishing. Trawling was banned within 10 nautical miles of the coastline. Under the 1990 Marine Fisheries Law (amended in 1993), Department of fishery has banned destructive fishing gear, including pair trawling, push-net, electrofishing, and fishing using poisons, chemicals or explosives.

According to socio-economic surveys, semi-structured interview or focus group discussions (FGD) was important to assess coastal habitats and fisheries support systems, human activities and benefits associated with coastal and marine resources in the community. Key informant interviews (KII) data could support community-level demographics, infrastructure, coastal and marine activities and management plans. Socioeconomic information could be used to ensure that the concerns and interests of local communities were taken into account in the management process and to plan and direct education and awareness programmes (Kronen, *et al*.2007). Socio-economic analysis of fisheries is used to support the conservation and management of fisheries industries, aquatic ecosystems and the people who base their livelihood on the exploitation of aquatic resources (Pinello, *et al.* 2017).

The objectives of the present study were: (1) to investigate the socio-economic context of Palaw fishing communities, (2) to determine the respondents' livelihood strategies, marine resource use, knowledge, attitudes and perceptions, (3) to assess the awareness of respondents on rules and regulations of marine resources and (4) to identify appropriate recommendations for the development of programme strategies and management advice.

Materials and Methods

The socio-economic survey was conducted in two fishing communities of Palaw Township, Shat-pone village (12º 34" Lat, 98° 38" Long) and Palaw (13º 07" Lat, 98° 41" Long) from May, 2017 to May, 2018 (Figure 1). Interview questionnaires were used to obtain socio-economic data about this township. According to random sampling method, 50 households from Palaw and 30 households from Shat-pone village were selected to participate in the survey as respondents. It was important to select the most appropriate sources to obtain the required information. Therefore, interviews were conducted with heads of households HH, or informed household members, fishermen, key informants interviews KIIs (village leaders or seniors and informed members of the community) and groups of community members (men, women, young people, etc.). A data code sheet was developed by the team, and used to code the data uniformly for data entry purposes. Data were gathered using a combination of semi-structured interviews, secondary data collection and field observation notes were used to understand basic demographics (population size, ethnic composition, language, religion, education level, and adult literacy rates), primary and secondary occupation, basic services and infrastructure. Focus Group Discussions were conducted at the community level mainly with people who depended largely on marine resources. The data was entered into MS Excel and analyzed using the SPSS v.19 program.

Figure 1. Map showing the study area

Results

Socio-demographic assessment of households

In the present survey area, almost all respondents spoke in Myanmar. The ethnic groups of household members in these communities were Burmese (about 90%) and Kayin (less than 10%). Mean household size was 5.7 in this area according to the records of the present survey. Almost all respondents of this area were Buddhists. Education attainment levels were higher in respondents between the ages of 21-40 years than others and they also had degrees. Household members above the age of 40 did not pass grades 10-11. According to the overall percentages of school attendees, the formal education levels of the different age groups of household members were not very high (Figure 2).

Economic base and livelihood activities

According to the household occupation and livelihood data from these communities, fishing inshore was the main occupation of the inhabitants and the sole source of income. The primary occupation of household heads included commercial fishing inshore (67%), agriculture (8.5%), fish (buying and selling) (7.6 %), dried fish and fermented fish productions (2.8%),

commercial fishing hook-lines (2.3%), collecting bivalves (10.4%) and shop keeping (1.9%) (Figure 3). When respondents were asked about the main sources of old household heads' income, much of their earnings came from inshore fishing, collecting bivalves, fish (buying and selling) and agriculture. Hook-lines fishing, commercial fishing inshore, fish buying centers, dry fish and fermented fish production were the primary occupations for young household heads. The age profile and primary occupation of household heads are shown in Figure 4.

In these fishing communities, both men and women involved in fishing. Men caught fish while females dried the small fish or the left-over fish from the nets. The primary occupations of women were fish processing for salted-fish or dried-fish, dried bivalves production, fermented nypa sap, nypa sugar production, agriculture and shop keeping. While fishing was a major part of their livelihood, agriculture was also a secondary component as few families had access to some land, on which they cultivated on a subsistence basis.

The household survey provided the level of dependence on marine resources at the household level. Most fishermen spent 5 to 6 days of inshore fishing per week. A few days were taken off per month at the beginning of neap tide which was not considered a productive period. Most fishermen who owned small boats could not go fishing during the rainy season (June-September). There was a seasonal variation in income with low earnings during the rainy season with rough weather conditions. In the rainy season, about 70% of respondents found it very difficult to make their livelihoods. The results of socio-economic data showed that most fishing households did not get the major source of income from their fishing. This was in part due to the depletion of marine resources.

Material style of life (MSL) data was used in this study to give an indication of wealth across these communities. This indicator used household

assets as indicators of wealth or poverty. The assessment of the survey team found: poor housing status, artisanal fishing boats, lack of sanitary facilities, malnutrition in children, inability to earn enough for food and clothes. The wealth of individual households was determined according to 3 broad wealth classes – well off, moderate and poor. According to the criteria set in this study for wealth indication, the majority of the surveyed households in these communities were classified as poor (60%), moderately well off (25%) and very few households (15%) were qualified as well off.

Marine resource use

The coastal and marine resources data (Coastal habitats and fisheries support systems), fishery resources data (fishing activities, fishing gears, fishing grounds and volume of catch) and marketing orientation were recorded from key informant interviews (KIIs) and focus group discussion (FGD). Fishing methods used in these communities included nets, traps and hook-lines. Fishing was carried out inshore day and night with small motorized boats. The fishing of Indian mackerels, silver pomfrets, tongue sole, shrimps and sand crabs were very common in Palaw waters during the rainy season (May-August). Groupers, spanish mackerels, silver grunts, rays, sardinella, ponyfish, croakers and anchovys were caught around Anyin-pho, Anyin-ma, Ma-li, Kyunn-hla, Tha-mi-hla, Kyauk-kar, Khan-ti and Lit-ku throughout the year (Figure 5). Ponyfish, croakers and anchovys were dried and salted in processing and the final products were exported to other towns. Among commercial species, lobster that was the highest value was caught by nets in deep water. The main fishing area of lobster was around Taung-thon-lon, Tha-mi-hla and Ma-li Islands. Shrimps were caught by three layers gillnet around Anyin-pho, Anyin-ma, Ma-li, Kyunn-hla, Tha- mi-hla, Kyauk-kar, Khan-ti and Lit-ku the whole year (Figure 5). The main fishing areas of sand crabs were Anyin-pho and Anyin-ma and they were caught by bottom net. Squids were caught light-fishing and trapping between October-April. The bivalves (*Meretrix meretrix*) were collected in the sandy bottom of Nan-eain-kan and

they were sold in the market of village. Some fishermen dried them under the sun to get dry products. The dried bivalve products were very popular in Taninthayi region. There were about seven fish buying centers, six dried fish production and eight dried bivalves production in these communities. Fish from fish buying centers were exported to cooling processing companies in Myeik or Thailand. Some fishermen contributed to the regional income by exporting fish, shrimps and lobsters.

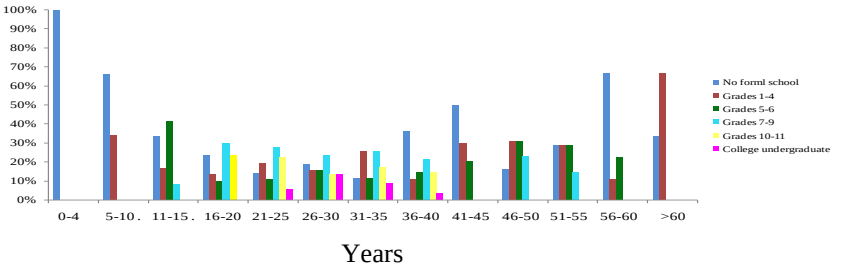

Figure 2. Household members' age and education levels in Palaw fishing communities

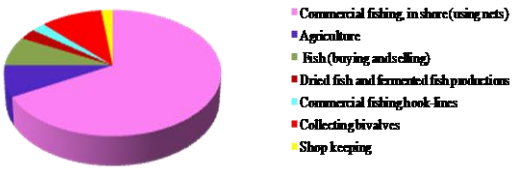

Figure 3. Household heads' primary occupation in Palaw fishing communities

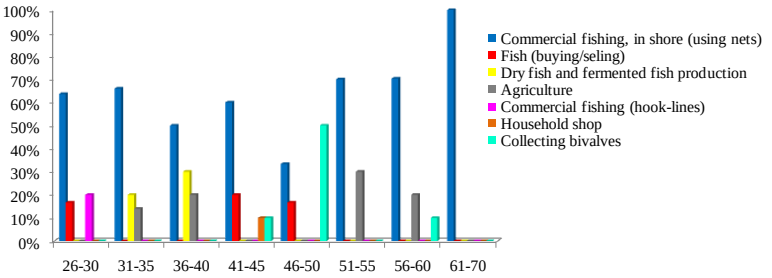

Figure 4. Household heads' primary occupation and age groups in Palaw fishing

Perception of resources conditions and perceived threats to coastal resources

Most local fishermen and trader groups had perceived a decline in the coastal and marine resources. Dependence on marine resources was an indicator of potential threats to marine resources. The relatively high dependence on marine resources at the community level as well as at the household level perpetuated the threats on marine resources. Fifty-nine percentage of respondents perceived that the conditions of mangrove forests were moderate (Figure 6). Some local people converted mangrove to settlement. About 26% of respondents believed the coral ecology was decreased and 63% of respondents considered it was destroyed by illegal fishing activities (trawling or traps fishing) (Figure 7).Most respondents considered the beach condition to be good. There was no beach erosion caused by currents and waves and no pollution in Pho-Shan beach. Upland forests around these communities were cut for residential use in these communities. As different types of sediments deposited in the Palaw River, the depth and width of this river were low and narrow. Many boats faced the difficulties in transportation due to sedimentation in the river. Few local people did sand mining for the use of household. The condition of ground water supplies (rivers and streams) in these communities was considered to be good by the majority of interviewed residents. Some local people encountered the lack of fresh water in summer.

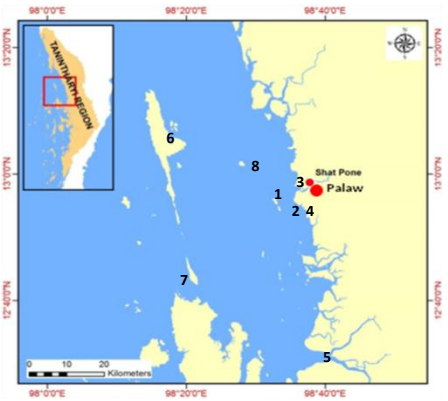

Figure 5. Map showing fishing areas in the waters off islands (1) Anyin-pho (2) Anyin-ma, (3) Nan-eain-kan, (4) Lit-ku, (5) Kyauk-kar (6) Ma-li (7) Tha-mi-hla and (8) Kyunn-hla

Awareness of rules and regulations

Respondents of these communities were asked about their awareness of the rules and regulations of marine resources. The awareness of fishing rules was poor. The 38% of respondents were aware of fishing rules and regulations while 62% of respondents did not have this awareness (Figure 8). Awareness of the uses of mangrove was moderate among respondents of these communities. However, 75% of the respondents were unaware of forestry rules and regulations. It could be concluded that the awareness concerning environmental rules of resort/hotel development, residential development, tourism and marine transportation were low in these communities.

Attitudes to non-market value of resources/Environmental awareness

To assess perceptions of non-market value of resources, as well as environmental awareness, respondents were read a series of eight statements, and asked if they "strongly agree", "agree", "neither agree nor disagree", "disagree" and "strongly disagree" or "don't know" (Figure 9). Attitudes to

the important of reefs for protecting land from storm waves was considerably lower with only 49% of respondents stating either "disagree" or "strongly disagree" and 14% stating neither agree nor disagree. The 67% of households disagreed strongly or slightly to restrict fishing in certain areas for fish and coral to grow and 65% of households also disagreed strongly or slightly to restrict development in some coastal areas as natural environments for future generations. The 60% of households did not have awareness of the importance of mangroves to be protected for fishing. In general, most household heads had low attitudes to environmental awareness.

Participation in decision-making to manage marine resources

According to household interview data, most households didn't want to participate in decision-making to manage marine resources. The 35% of household participated in decision-making on fishery resource but the 65% of household didn't participate (Figure 10). So, most people were not willing for participation in decision-making to manage fishery resources. Currently, most respondents didn't desire to participate in mangrove management.

Catch trends of marine resources

Most fisher groups and traders interviewed had perceived that most resources were in a state of decline in the last 5 years. The numbers of fishermen perceived a drop in catches and income despite an increase of fish prices. The 90% of fishermen who caught bivalves, inshore and pelagic fish estimated that their catches had dropped by half in the last 5 years. More than half fishermen perceived that the catches of sand crabs, prawns and lobsters had dropped. Fishermen sold lobsters live or dead. If lobsters were live lobsters, they fetched a better price. Rays and sharks catches were perceived to have dropped in the last five years. So, these above information showed that marine resources were under high pressure. It was said that the numbers of traders/collectors of bivalves had decreased due to the illegal fishing boats or trawling. So, most households did not depend on it sufficiently to consider it

as a livelihood activity. Old fishermen who had fishing experience answered that there was a decrease in the catch of marine resources at the present time (2017-18). (Figure 11).

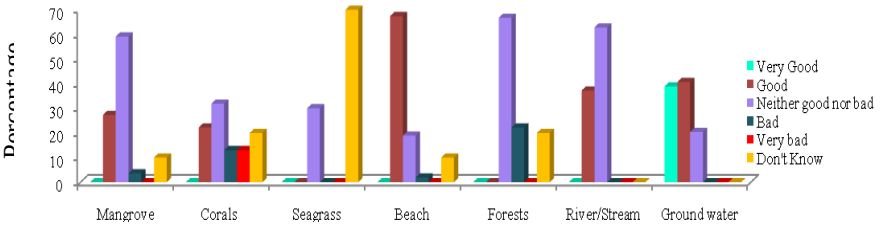

Figure 6. Perception of coastal and marine resources conditions in Palaw fishing communities

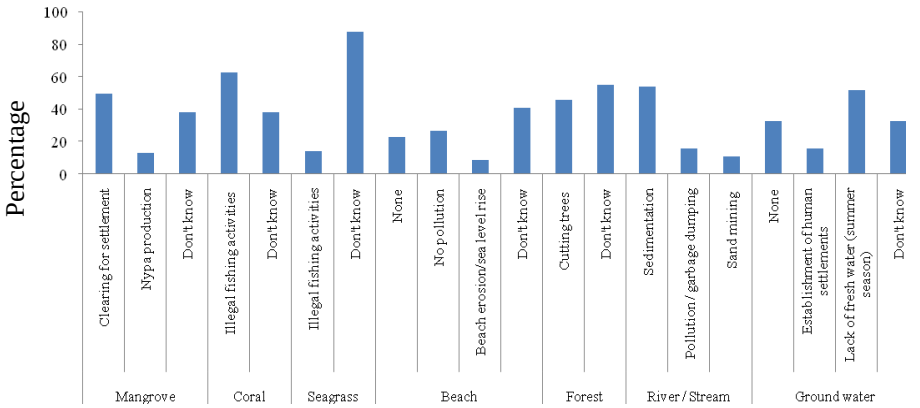

Figure 7. Perceived threats to coastal resources in Palaw fishing communities

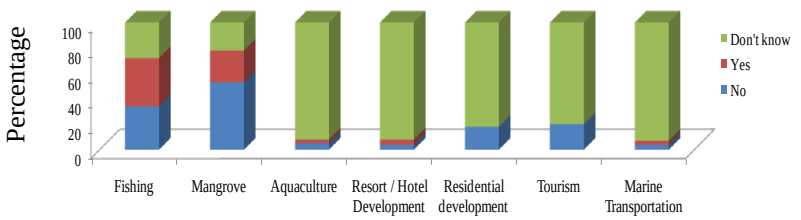

Figure 8. Awareness of rules and regulations of marine resources

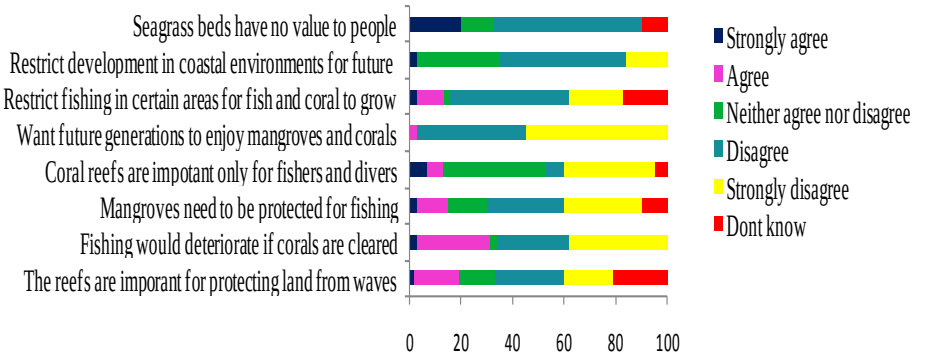

Figure 9. Perceived threats to coastal resources in Palaw fishing communities

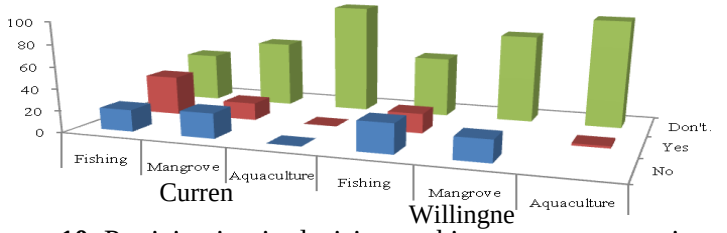

Figure 10. Participation in decision-making to manage marine resources in Palaw fishing communities

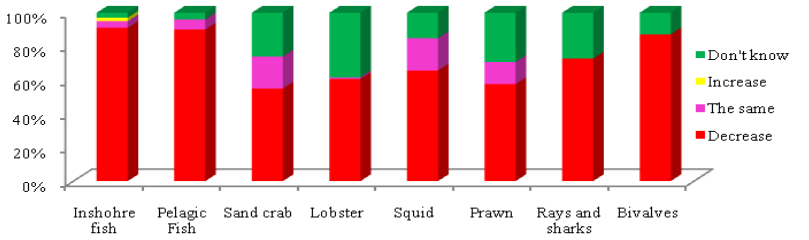

Figure 11. Catch trends of marine resources in Palaw fishing communities

Discussion

The present results from the socio-economic baseline assessment pointed out how to use the marine resources, which resources were facing high pressure, and what threatens the marine resources in these communities.

Most fishing households depended solely on marine resources for their livelihoods. Their primary occupations were fishing, agriculture, shop keeping and collecting bivalves in Palaw. Likewise, the people in Kyauk-Phyar and Thit-Yar-Wa villages depended on the marine resources for their livelihood (Zin Lin Khine and Hnin Hnin Maw, 2016).

According to the present survey, more than half of fishing households faced difficulties in earning livelihoods during the rainy season. Their boats were not able to go fishing due to the rough weather conditions. As they could get low incomes in the rainy season, their incomes were varied seasonally in these communities. Similarly, most fishing households of Myeik Archipelago found that it was very difficult to make their livelihoods in the rainy seasons. According to previous and present data, most fishing households did not consider fishing could provide a sufficient source of income to sustain them, despite ranking fishing as their main source of income. Most fishermen reported the catches of marine resources and fish were depleting year after year in Tanintharyi region (Saw Han Shein *et al*. 2013, Schneider *et al*. 2014, BOBLME 2015, Zin Lin Khine and Hnin Hnin Maw, 2017).

According to the present data, fishermen caught Indian mackerels, silver pomfrets, tongue sole, shrimps, sand crabs, groupers, spanish mackerels, silver grunts, rays, sardinella, ponyfish, croakers, anchovy, etc. by different nets in Palaw waters. From the previous data (Saw Han Shein *et al.* 2013; Schneider *et al.* 2014; BOBLME 2015), the major fishing activities of fishermen included artisanal fishing by stationary nets, driftnets, cage fishing and spear fishing using compressors to catch different marine species. According to the present survey, fishermen who fished by compressor diving and spear fishing were not found and some fishermen collected bivalves. The present observation showed that mangroves were converted to human settlement, corals and seagrass ecology were destroyed by illegal fishing activities (trawling or fishing traps). Holmes *et al.* (2014) reported that Illegal,

Unregulated and Unreported (IUU) fishing was widespread in Myanmar. Watersheds, mangroves, corals and seagrass could be degraded through poorly regulated and planned coastal and riverside activities.

In the present study, the respondents' awareness of rules and regulations indicated that they had low level of awareness in fishing laws. However, the awareness of household members on forestry was moderate level in these communities. The marine fishery law (1990) and the forestry law (1992) were enacted to preserve and protect marine environment (Trachtman, 1997). From the present results, most local people did not know or agree with the importance of corals and mangroves for fishing. Most respondents had low attitudes to conserve marine resources. Environmental awareness decreased among local people. According to the previous observations, a high proportion of respondents did not have knowledge about resource conditions and awareness of rules and regulations (Schneider *et al.* 2014)

Many fishing groups and traders from the present survey area perceived that there was a decrease in the catches of marine resources at the present time (2018) than the last five years. Marine bivalves declined drastically in numbers and their sizes were very small in the current period (2018) as the results of overfishing or illegal fishing methods. As the catch trends of marine resources dropped, most fishermen faced with a lot difficulty in earning livelihoods in the present survey area. According to the previous surveys, marine resources in Myanmar had a dramatic decline in over the past 30 years (BOBLME, 2015 and Howard, 2018).

Conclusion

In socioeconomic survey, household demographic data, economic and livelihoods, coastal and marine activities were assessed and analyzed. Most local people had low attitudes to manage marine resources and they had perceived a decline in coastal and marine resources in the present period

(2018). But most households did not want to participate in decision-making to manage marine resources in both present and future. Marine and coastal resources management and conservation training is needed to increase public awareness about marine and coastal resources rules and regulations. If so, they can establish no take zone (NTZ) and locally managed marine area (LMMA) in the first step. Finally, they can create marine protect areas (MPA) in these communities.

Acknowledgements

We are indebted to Dr Ni Ni Oo and Dr Win Win Than, Pro-rectors of Myeik University, for their support in preparing this work. We would like to express my gratitude to Professor Dr Nyo Nyo Tun, Head of Marine Science Department, Myeik University, for her help. Thanks are also due to my colleagues for their help. We wish to express our heartfelt thanks to interviewee of fishing communities in Palaw.

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SPECIES SUCCESSION OF PHYTOPLANKTON IN RELATION TO SOME PHYSICO-CHEMICAL PARAMETERS IN MYEIK ARCHIPELAGO, SOUTHERN MYANMAR

Yin Yin Htay [1]

Abstract

Phytoplankton samples were collected from ten designated sampling stations from Kywe Ku bridge (Lat. 12º 31´ N and Long. 98º 47´ E) to Done Pale Aw (Lat. 12º 21´ N and Long. 98º 02´ E) covering inshore, nearshore and offshore, Myeik coastal waters from June 2011 to February 2013. A total of 135 phytoplankton species were identified. In the inshore water (Station 1 and Station 2), the species succession was evident by 8 species of diatoms. In the nearshore water (Station 3, Station 4, Station 5, Station 6 and Station 7), fifteen species of diatoms and one species of dinoflagellate were recorded as species succession. In the offshore water (Station 8, Station 9 and Station 10), there were twenty species of diatoms in the species succession. The quantity of physico-chemical parameters were: water temperature 25-30ºC, salinity 4-34‰, pH 7.1-8.2, phosphate 0.01-1.33 mg/l, nitrate 0.01-0.99 mg/l and transparency 0.51-9.0 m in the Myeik coastal waters. The diatoms were more dominant than the dinoflagellate in any season and their densities were always near 90%. The composition of dominant diatoms showed the different situations from the inshore, nearshore to offshore coastal waters. The succession of phytoplankton was positively correlated with some environmental factors such as transparency and temperature, however, the negative correlation was found with nitrate, phosphate, salinity, pH and rainfall in Myeik Archipelago.

Keywords: Offshore, inshore, nearshore, phytoplankton, succession.

Introduction

Myanmar coastline stretches about 2400 km from the Naff River mouth to Kawthaung city, facing the Bay of Bengal and the Andaman Sea in the west which is a southeastern part of the Bay of Bengal. This Bay is a semi-enclosed tropical basin located in the northern Indian Ocean. Since Myanmar has tropical monsoon climate, Myanmar coastal area is influenced by strong

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monsoon regimes. Myeik, in the southern part of Myanmar is an archipelagic region with an area that has 34,340 sq km and consist of over 800 islands. The coasts facing the Andaman Sea are especially noteworthy in terms of marine production. Around these regions, rich terrestrial nutrients are supplied from numerous rivers and there are extensive mangrove forests, which cover 425,000 hectares, the third largest mangrove extent in Southeast Asia. Phytoplankton also needs nutrients to grow. They need a wide variety of chemical elements but the two critical ones are nitrogen and phosphorous since they are needed in quite large amounts but are present in low concentrations in seawater. Nitrogen and phosphorous are like the fertilizers to land plants and are used to make proteins, nucleic acids and other cell parts the phytoplankton need to survive and reproduce. Therefore phytoplankton needs nutrients in well defined ratios. The physico-chemical parameters and quantity of nutrients in water play significant role in the distributional patterns and species composition of plankton. Coastal and near-shore waters are more productive regions in the marine environment due to nutrient add by means of regeneration, upwelling and land run-off.

In aquatic habitats, the environmental factors include various physical properties of water such as solubility of gases and solids, the penetration of light, temperature, and density. The chemical factors such as salinity, pH, hardness, phosphates and nitrates are very important for growth and density of phytoplankton on which zooplankton and some higher consumer depend on their existence. The turbidity may exert a further control on phytoplankton. The inorganic micro-nutrients considered as limited factors affecting the growth of phytoplankton. Natural phytoplankton populations respond to environmental changes in various ways. Variation in phytoplankton community composition depends on the availability of nutrients, temperature and light intensity.

Phytoplankton species succession undergoes the changes due to change in the physical, chemical and biological factors. The influence of

environmental factors on species succession varies significantly with physical factors like temperature and light intensity being the most important and chemical factors like pH, salinity, and nutrient level being the most important. The relative abundance of species succession groups vary seasonally and geographically.

Nitrogen and phosphorus may all well limit species succession. Variation in phytoplankton community composition depends on the availability of nutrients, temperature and light intensity. The seasonal stratification of water columns determines the general availability of the resources light and nutrients for species succession. Succession shifts in phytoplankton community structure are mainly due to change in environmental variables such as nutrients and other physico-chemical variables which influence the distribution and abundance of plankton communities. The phytoplankton is the direct indicators of human intervention in the marine environment. Any extreme changes in their population or composition can be taken as an alarm signal to check the source of pollution in the system. Phytoplankton species undergo the changes in their distribution due to change in the physical, chemical and biological factors.

The objectives of this study are: 1) to identify the taxonomic and nomenclatural knowledge of diatoms and dinoflagellate species from Myeik coastal waters; 2) to recognize the dominant species in Myeik coastal waters; 3) to know the changes of the physico-chemical characters in relation to species succession in Myeik coastal waters; and 4) to understand phytoplankton communities in relation to some environmental parameters in Myeik coastal waters.

Materials and Methods

Myeik coastal waters are situated in the southern part of Taninthayi (Tenasserim) strip fronted by the Andaman Sea, in the northeastern part of the

Bay of Bengal. This study area comprises 10 Stations: 1) The Kywe Ku Bridge Station, 2) Kyauk Phyar River mouth, 3) Kywe Ku-Pahtaw, 4) Pahtaw-Pahtet, 5) A Saung Kaung 6) Masan-pa, 7) East of Ka Lar Kyun, 8) Kattalu, 9) Sha Aw and 10) Done Pale Aw (Figure. 1).

The Kywe Ku bridge Station is located in the Northeast of Myeik at Latitude 12° 30′ N and Longitude 98° 45′ E. Kyauk Phyar river mouth lies at Latitude 12° 31′ N and Longitude 98° 42′ E in the West of Kywe Ku bridge and Northeast of Myeik. Kywe Ku-Pahtaw is situated the Northern part of Myeik at Latitude 12° 31′ N and Longitude 98° 35′ E. Pahtaw-Pahtet is located in the West of Myeik at Latitude 12° 27′ N and Longitude 98° 36′ E. A Saung Kaung lies in the South of Myeik at Latitude 12° 24′ N and Longitude 98° 37′ E. Moreover Masan-pa is situated in the Southwest of Myeik at Latitude 12° 24′ N and Longitude 98° 31′ E. East of Ka Lar Kyun is located in the West of Myeik from Latitude 12° 27′ N and Longitude 98° 31′ E. Kattalu lies North of Myeik at Latitude 12° 30′ N and Longitude 98° 28′ E. Sha Aw is situated in the West of Myeik and North of Thayawthadargyi Kyun at Latitude 12° 25′ N and Longitude 98° 05′ E. Done Pale Aw is located in the West of Myeik, the North of Daung Kyun and East of Thayawthadangyi Kyun at Latitude 12° 21′ N and Longitude 98° 02′ E.

The samples were collected monthly (June 2011–February 2013) from the waters of Kywe Ku Bridge (Latitude 12° 30′ N and Longitude 98° 45′ E) to Done Pale Aw (Latitude 12° 21′ N and Longitude 98° 02′ E) comprising of ten stations (Fig. 1). These samples were collected with a 20 µm mesh size standard plankton net (2 feet long and 25 cm wide) and then towed from the horizontal water (10 m) from an anchored boat for 5 minutes. Sampling was carried out between 10:00 am and 1:30 pm during the neap tide. Each sample was preserved into 2 % formalin/sea water mixture and stored in the Department of Marine Science, Myeik University. The materials used were either examined fresh or preserved in formalin. The data represented salinity,

temperature, transparency, pH, rainfall, nitrate and phosphate of each location.

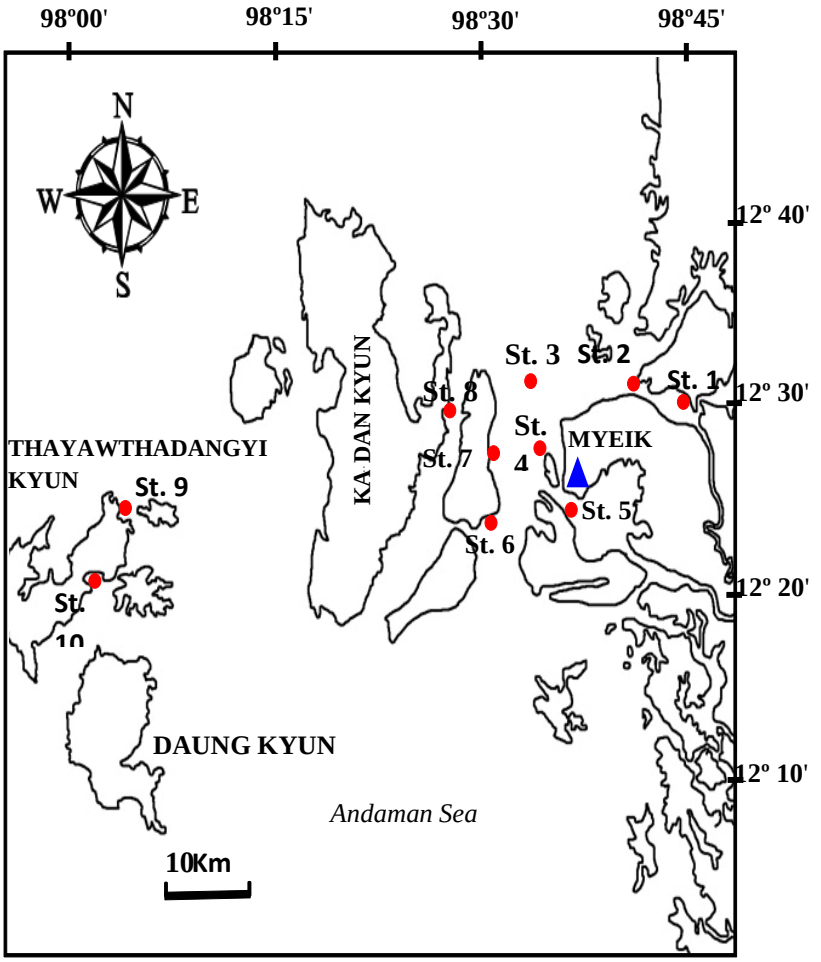


Figure. 1. Map showing the location of Myeik coastal areas and sample collection sites: Station 1 (Kywe Ku Bridge), Station 2 (Kyauk Phyar River mouth), Station 3 (Kywe Ku-Pahtaw), Station 4 (Pahtaw-Pahtet), Station 5 (A Saung Kaung), Station 6 (Masan-pa), Station 7 (East of Ka Lar Kyun), Station 8 (Kattalu), Station 9 (Sha Aw) and Station 10 (Done Pale Aw).

Identification of phytoplankton collected from Myeik coastal waters was done according to the following reference books; Heurck (1896), Allen and Cupp (1930), Hendey (1964), Patrick and Reimer (1966), Shirota (1966), Weber (1966), Sournia (1968), Yamaji (1971), Chandy *et al*. (1992), Hasle and Syvertsen (1997), Steidinger and Tangen (1997), Botes (2001) and Han Shein and Kyi Win (2012). This study basically followed the classification system used by Guiry (2010, 2011, 2012, 2013 and 2014). The phytoplankton species were counted under Nikon light microscope for the measurements of phytoplankton abundance. The formula is simply the basic geometry formula. Results were expressed in the number of cells/m^3. The filtered volume of water entering the phytoplankton can be calculated as follows:

$$V= \pi r^2 l$$

Where,V= the volume of water that passes through the net

r= the radius of the hoop at the front of the net,

l= the distance through which the net was hauled, that was 10 m.

Results

Species succession in relation to some environmental factors by stations of the inshore, nearshore and offshore waters

Phytoplankton, species succession relevant to some physic-chemical characters of some water bodies at Myeik coastal waters was studied for three years (2011-2013). A total of 135 taxa belonging to 66 genera from 40 families, 26 orders, 4 classes and 2 phyla of phytoplankton were recorded from the study areas. Among them, 116 species and 55 genera, under Bacillariophyta (diatoms) and 19 species consisting of 12 genera, under Dinoflagellata (dinoflagellates) were observed. Some physic-chemical factors such as mixing of water mass, temperature, salinity, pH, phosphate, nitrate, transparency and rainfall affect the succession of phytoplankton in the study period.

Species succession in the inshore water (Station 1 and Station 2)

The seasonal variation of ecological response of phytoplankton in the Myeik coastal waters was presented. The species succession was evident by 8 species of diatoms, namely, *Cyclotella striata, Pleurosigma normanii, Odontella sinensis, Odontella mobiliensis, Thalassiosira excentrica, Thalassionema nitzschioides, Thalassionema frauenfeldii* and *Melosira nummuloides*. The succession of *Pleurosigma normanii* was recorded when temperature was 28ºC and salinity 29‰, during the post monsoon season (February 2012). This species decreased from premosoon to monsoon season (June to July). *Melosira nummuloides* 1500 cell/m^3 was observed when temperature was 27ºC and salinity 28‰ during the postmonsoon season (December 2011). The greatest abundance of *P. normanii* 2219 cell/m^3 was recorded in (February 2012) (Table. 1) (Figure. 2).

The most dominant *C. striata* was recorded only in the postmonsoon (January to February). The species succession of *T. excentrica* was found in December during 2011, however, October in 2012. *Odontella mobiliensis* mostly occurred during premonsoon to monsoon season. The species appeared to adapt to the salinity 4-26‰ and temperature 25-28ºC during the study period. The succession of *Odontella sinensis* was found only in the monsoon season. The main species *Thalassionema nitzschioides* and *Thalassionema frauenfeldii* did not show any seasonal fluctuation.

The amount of physico-chemical parameters were: water temperature 25-28ºC, salinity 4-29‰, pH 7.1-7.3, phosphate 0.01-1.33 mg/l, nitrate 0.01-0.99 mg/l and transparency 0.68-1.00m in the inshore water. A noteworthy feature in the observations was that nutrient concentration in the inshore water during months of June and July were higher than other months.

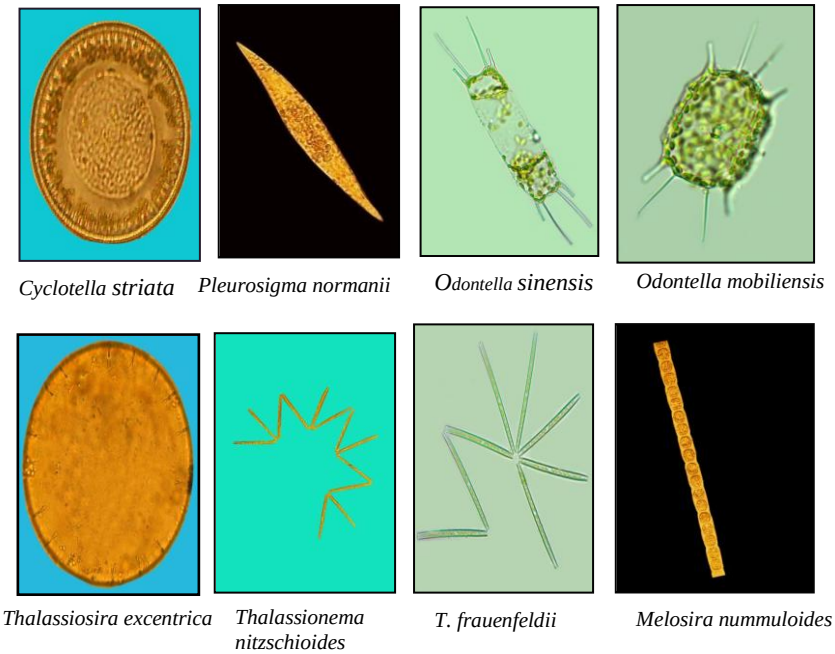


Figure 2. The dominant species in the inshore water during the study period.

Species succession in the nearshore water (Station 3, Station 4, Station 5, Station 6 and Station 7)

Sixteen species of phytoplankton was occurred as the species succession in the near shore of Myeik coastal waters such as *Rhizosolenia imbricata* together with *Guinardia flaccida* and *Proboscia alata* in the postmonsoon season while there was high temperature (26-28°C); and salinity (30-32‰) and then low rainfall (0-160 mm). The species *Chaetoceros curvisetus* was common together with *Thalassionema nitzschioides*, *Thalassionema frauenfeldii* and *Ditylum sol* in the all seasons. They were denser in the postmonsoon season than the other. A dinoflagellate, *Ceratium furca* was dominantly observed from June to November. The high level of

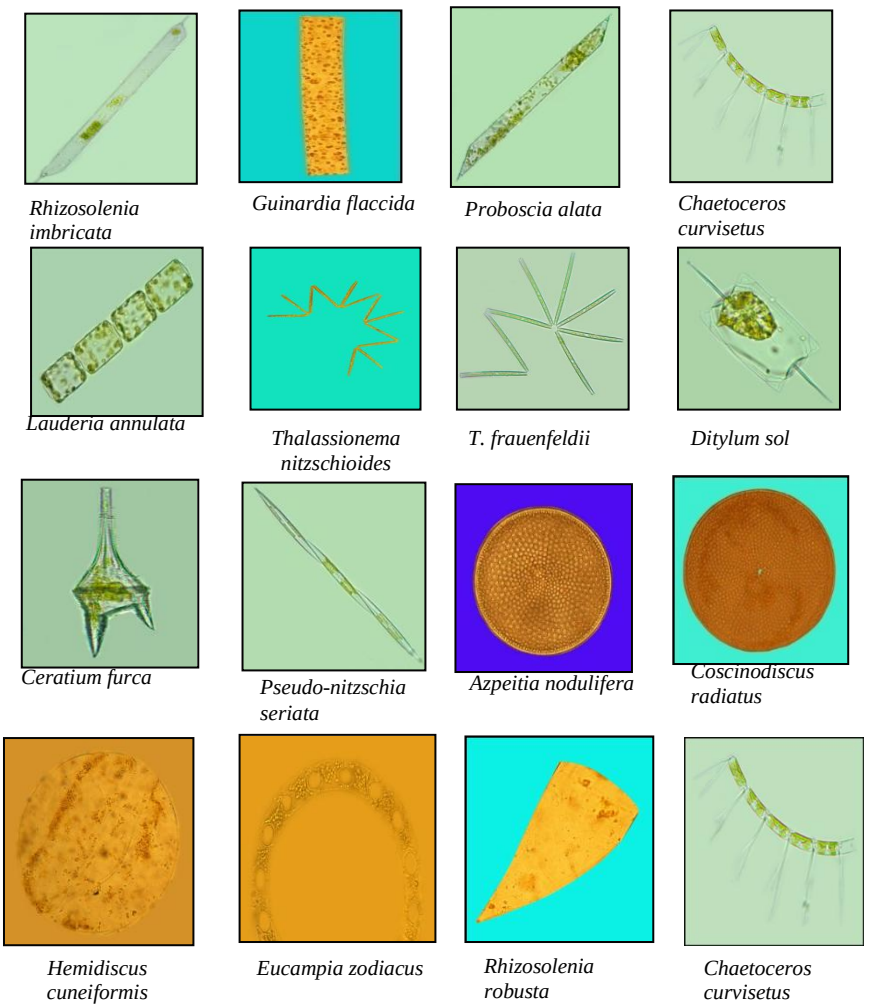


Figure 3. The dominant species in the nearshore water during the study period.

Azpeitia Nodulifera Coscinodiscus radiatus, Odontella sinensis and *Odontella mobiliensis* were common recorded in postmonsoon season. *C. radiatus* (21389 cell/m^3) was the most dominant species in (December, 2011) (Table. 2) (Figs. 3,6,7). Moreover *Pseudo-nitzschia seriata* was common found from premonsoon to monsoon season while there were high nutrient (maximum phosphate 0.31 mg/l and nitrate 0.05 mg/l). In addition, *Hemidiscus cuneiformis* and *Eucampia zodiacus* were abundant in the postmonsoon while the maximum water transparency was 1.56 m.

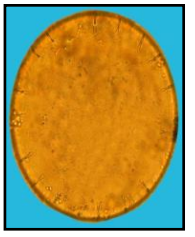
On the other hand, *Rhizosolenia robusta* was also important, occurring in only December, 2012 during high salinity and low rainfall. The quantity of physico-chemical parameters were: water temperature 25-28ºC, salinity 10-33‰, pH 7.1-7.9, phosphate 0.01-0.41 mg/l, nitrate 0.01-0.06 mg/l and transparency 0.51-1.99 m in the nearshore water. The species succession changes were recognized as associated with changing environmental conditions.

Species succession in the offshore water (Station 8, Station 9 and Station 10)

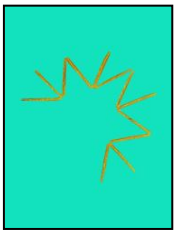
A total 12 species of phytoplankton were dominant according to the monsoon. *Chaetoceros curvisetus* and *Odontella sinensis* were major alga to be succeeded the whole year round but the most dominant in the postmonsoon season at high salinity, (30-34‰) and high temperature (26-30ºC).

Chaetoceros curvisetus

Coscinodiscus radiatus



Thalassiosira excentrica



Thalassionema a nitzschioides

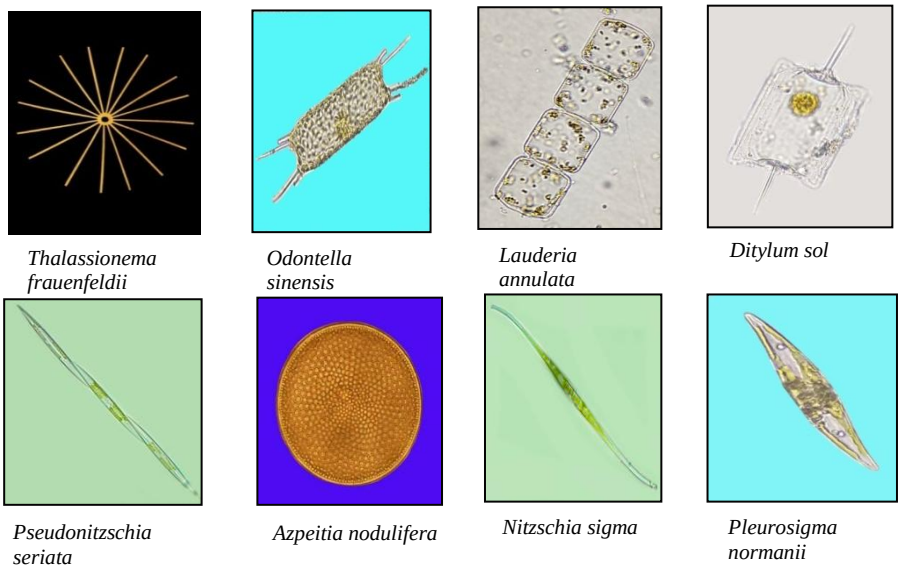


Figure 4. The dominant species in the offshore water during the study

The massive diatoms bloom with high density of *Coscinodiscus radiatus*, *Thalassiosira excentrica*, *Thalassionema frauenfeldii* and *Thalassionema nitzschioides* were detected under conditions of raised nutrient concentration from the premonsoon to monsoon season while temperature 26-29ºC and salinity18-32% .

Moreover, *Lauderia annulata* together with *Ditylum sol* and *Pseudonitzschia* were dominantly found only in the postmonsoon season while temperature was 26-29ºC and salinity, 32-34‰. The maximum dominance of *Azpeitia nodulifera* together with *Nitzschia sigma* appeared in November and they had a toleration of temperature 28-30ºC and salinity 30-31‰. In addition, *Chaetoceros curvisetus* showed the succession in

postmonsoon season. This species was the most dominant species (5623 cell/m^3) in February, 2012 (Table. 3) (Figure. 4).

The amount of physico-chemical parameters were: water temperature 26-30ºC, salinity 18-34‰, pH 7.1-8.2, phosphate 0.01-0.87 mg/l, nitrate 0.01-0.18 mg/l and transparency 0.76-9.0 m in the offshore water. The diatoms were more dominant in any season and their densities were always near 90%. The composition of dominant diatoms showed the different situations from the inshore, nearshore to offshore coastal waters (Tables. 1-3).

Discussion

The phytoplankton community of the study area's water inhabited 90 species of Bacillariophyceae, 25 species of Coscinodiscophyceae, 19 species of Dinophyceae, and 1 species of Dictyochophyceae.

The total phytoplankton community during this proliferation was composed mainly of diatoms (21 species) and dinoflagellate (1 species) dominated by namely, *Azpeitia nodulifera*, *Cyclotella striata*, *Chaetoceros curvisetus*, *Ceratium furca*, *Ditylum sol*, *Eucampia zoodiacus*, *Guinardia flaccida*, *Hemidiscus cuneiformis*, *Lauderia annulata*, *Coscinodiscus radiatus*, *Melosira nummuloides*, *Nitzschia sigma*, *Odontella sinensis*, *Odontella mobiliensis*, *Pleurosigma normanii*, *Proboscia alata*, *Pseudonitzschia seriata*, *Rhizosolenia imbricata*, *R. robusta*, *Thalassiosira excentrica*, *Thalassionema frauenfeldii* and *T. nitzschioides* were recorded during the study period (Tables. 1-3) (Figure.2).

Table. 1. The abundance (cell/m^3) of the species succession at inshore water from June (2011) to February 2013.

Sr. No.	Species	2011 year							2012 year									2013 year	
		J	J	A	S	O	N	D	J	F	J	J	A	S	O	N	D	J	F
1	*Cyclotella striata*	459	520	611	458	518	489	540	750	850	220	450	350	200	510	380	450	650	750
2	*Melosira nummuloides*	1000	1050	1206	1054	1135	1056	1500	1300	1450	300	600	950	1000	900	1000	1000	1250	1420
3	*Odontella mobiliensis*	750	850	951	850	900	785	350	250	350	550	800	500	750	450	650	630	300	360
4	*O. sinensis*	680	650	764	720	678	640	500	450	650	400	350	650	650	500	600	450	338	450
5	*Pleurosigma normanii*	1587	1650	1824	1634	1458	1367	2000	2100	2219	1200	1000	950	1200	1268	1200	1200	1800	1800
6	*Thalassionema frauenfeldii*	1067	1150	1223	1036	1009	986	1000	900	1000	850	750	1000	750	980	750	980	750	1202
7	*T. nitzschioides*	985	1000	1206	984	896	950	1200	1100	980	800	750	850	600	750	800	900	1090	1000
8	*Thalassiosira excentrica*	467	577	577	569	469	510	650	550	670	550	500	350	350	700	350	350	550	600

Table. 2. The abundance (cell/m^3) of the species succession at nearshore water from June (2011) to February 2013.

Sr. No.	Species	2011 year							2012 year									2013 year	
		J	J	A	S	O	N	D	J	F	J	J	A	S	O	N	D	J	F
1	*Rhizosolenia imbricata*	0	0	0	0	0	0	6000	7764	5800	1	1	1	0	1	1	5600	7500	5691
2	*R. robusta*	18	13	15	12	10	15	540	774	650	15	9	8	13	8	13	550	650	600
3	*Guinardia flaccida*	0	0	0	0	0	0	2000	2517	2401	1	1	1	1	0	1	1560	2240	1890
4	*Proboscia alata*	4	3	10	8	10	4	950	1004	850	3	5	8	9	9	6	860	980	790
5	*Azpeitia nodulifera*	1465	1580	2097	2010	1900	1850	200	203	180	1309	1356	1289	1980	1750	1670	190	198	167
6	*Chaetoceros curvisetus*	1950	2412	2000	2500	2000	2450	2200	2450	2500	1896	1982	1780	2350	1850	2365	1950	2243	2300
7	*Thalassionema frauenfeldii*	650	798	760	550	489	800	200	458	430	635	765	720	490	420	783	190	435	410
8	*T. nitzschioides*	1050	1117	950	800	759	1000	400	506	450	1034	1005	935	750	723	980	350	456	432
9	*Ditylum sol*	370	450	560	600	450	389	1292	1308	1200	250	380	400	560	520	270	1100	1254	1256
10	*Ceratium furca*	750	900	850	650	750	825	200	50	10	650	759	755	550	650	750	170	44	8
11	*Coscinodiscus radiatus*	118	100	98	100	98	120	21389	8834	9000	101	87	90	89	80	110	15000	7500	8500
12	*Odontella sinensis*	84	50	65	75	85	75	594	2279	2500	79	45	55	65	89	69	502	1890	2389
13	*Odontella mobiliensis*	50	65	84	65	50	60	1650	1732	1236	35	58	80	65	60	56	1509	1567	1400
14	*Pseudo-nitzschia seriata*	800	910	750	560	450	850	100	193	200	750	850	686	450	380	750	89	170	160
15	*Hemidiscus cuneiformis*	10	3	5	3	5	3	492	1189	1000	9	5	7	5	7	5	389	1097	987
16	*Eucampia zodiacus*	5	6	7	8	5	8	11	856	750	4	5	8	6	5	7	9	750	650

rom June (2011) to February 2013.

	2012 year									2013 year	
	J	F	J	J	A	S	O	N	D	J	F
	560	5623	350	453	360	869	950	1100	3946	4380	5300
	0	0	980	1000	1350	1220	1316	1200	7	2	1
	3	0	570	560	790	690	850	790	2	5	1
	380	441	320	180	470	350	367	226	350	350	380
	500	662	260	330	390	370	259	175	430	402	560
	450	2100	104	139	250	195	180	165	1490	1500	1950
	000	1284	133	122	124	110	98	235	890	990	1100
	790	2008	27	22	37	20	32	29	1800	1750	1870
	020	1077	18	18	15	8	8	3	900	1005	1067
	70	100	1	98	99	85	68	548	87	63	98
	250	350	85	45	65	55	45	569	230	220	330
	380	500	100	46	39	30	27	22	389	400	459

dard deviation of some waters quality parameters from ten stations in Myeik coastal

Mean and Standard deviation

In the present investigation, some environmental factors such as temperature 25-30ºC, salinity 4-34‰, pH 7.1-8.2, phosphate 0.01-1.33 mg/l, nitrate 0.01-0.99 mg/l, transparency 0.38-9 m and rainfall 0-1082 mm occurred in Myeik coastal waters. The physico-chemical factors and their values, affect the succession of phytoplankton in the study areas. Raymont (1963) described that some species have fairly specific requirements for temperature, salinity and nutrients. So the present study seemed to agree with the observation of Raymont (1963). That author recorded that *T. nitzschioides*

was extremely euryhaline with a salinity range from 4 to 34‰. That was agreement with *T. nitzschioides* in this study.

Kyi Win and Han Shein (1987) mentioned that phytoplankton such as *Biddulphia mobiliensis, B. sinensis* and *Pleurosigma spp.* were fairly abundant in Setse, Thanbyuzayat, Mon State. Their result more or less matched with the result from Myeik coastal waters (Present study).

Boonyapiwat (1997) described that the greatest phytoplankton bloom occurred by the highest cell density of *Skeletonema costatum* in the postmonsoon season near the end of Peninsular Malaysia. However *Chaetoceros curvisetus, Coscinodiscus radiatus, Pleurosigma normanii,* and *Rhizosolenia imbricata* were abundantly found in this season from Myeik coastal waters.

Paul *et al*. (2001) observed that *T. frauenfeldii, T. nitzschioides, Chaetoceros lorenzianum* and *C. curvisetus* were abundant in the months of September-October. This result agreed with the result of the present study (nearshore and offshore waters).

The effect of temperature on phytoplankton growth seemed to be direct. It seems that certain phytoplankton was stenothermal. Other species seemed to be eurythermal and were tolerant of a wide temperature range. Temperature was the most important factor affecting diatom and dinoflagellate growth, even more than nutrients, and that phytoplankton abundance directly varied with it. The optimum temperature for diatom development was dependent on the type of flora present. The surface temperature was to some extent responsible for the change in the phytoplankton, species succession.

Paul *et al*. (2001) mentioned that *Skeletonema costatum* preferred the water temperature of 28-30ºC. Therefore that observation was quite similar to the finding of the present study. They also reported that the highly diverse centric diatoms such as *Coscinodiscus sp*., *Chaetoceros sp*. and

Thalassionema frauenfeldii were recorded. So this observation matched with the present study (nearshore water).

Matondkar *et al*. (2002) pointed out that *Rhizosolenia sp.* and *Chaetoceros sp.* were related to their preference for higher salinity from Mandovi and Zuari estuaries. Therefore that result was similar to the observation of the present study (nearshore water). They also described that *Thalassiosira sp*. reflected their preference for the saline region but Selvaraj *et al*. 2003 stated that *Thalassiosira sp*. preferred the intertidal waters of Cochin. This result somewhat agreed with the observation of the present study at Station 4 during this period.

Boonyapiwat *et al*. (2007) reported that the succession of diatom species were mainly in the southern part of Myanmar waters. This author described that the dinoflagellate species, *Ceratium furca* was dominant in Station 2 near the Bay of Bengal. In the present study, this species was also dominant at Station 1 and Station 6 too. Touliabah *et al.* (2010) described that phytoplankton succession was relevant to some physico-chemical characters of some water bodies at Jeddah (Saudi Arabia). Ozbay (2011) suggested that nitrate was the limiting factor for phytoplankton growth. Their observations agreed to the present study.

Palleyi *et al*. (2011) reported that the dominant species recorded at different sampling stations belonged to the genera *Coscinodiscus, Rhizosolenia, Thalasionema, Chaetocerous, Melosira* and *Pleurosigma* from Dharmra river estuary of Odisha coast, Bay of Bengal. That result was similar to some result at some stations in the present study. Rathod (2011) reported that *Thalassionema nitzschioides* was the predominant species in Indian Ocean. It was found that this statement was the same for Myeik coastal waters (Present study). Fonge *et al*. (2012) described that the most frequent species was *Pseudo-nitzschia seriata* from Cameroon. This result more or less matched the result of the present study.

Sahu *et al*. (2012) reported that the productivity and community composition of dinoflagellates were expected to be very scanty in most of the coastal waters from Southeast coast of India. That report somewhat agreed with the observation of the present study. Su Myat (2013) described that *Coscinodiscus radiatus, Cyclotella striata, Lauderia annulata, Guinardia striata* and *Pleurosigma normanii* were commonly detected in the postmonsoon season (December) from the southern part of Myanmar. Therefore her observations more or less coincided with the result of the present study in some stations. Thida Nyunt (2013) showed that *Coscinodiscus sp*. and *Pleurosigma sp*. were the most abundant diatoms species in Kyaikkhami Station. This result was similar to that of the present study at the stations of 1, 3, 4 and 9 Station.

A succession of species was evident with *Cyclotella striata, Melosira nummuloides, Odontella sinensis, Odontella mobiliensis, Pleurosigma normanii, Thalassiosira excentrica, Thalassionema frauenfeldii* and *T. nitzschioides* were generally most abundant diatoms of the inshore water (Table. 1) (Figs. 2,5).

Moreover, *Azpeitia nodulifera, Chaetoceros curvisetus, Ceratium furca, Ditylum sol, Eucampia zoodiacus, Guinardia flaccida, Hemidiscus cuneiformis, Coscinodiscus radiatus, Odontella sinensis, Odontella mobiliensis, Proboscia alata, Pseudonitzschia seriata, Rhizosolenia imbricata, R. robusta, Thalassionema frauenfeldii* and *T. nitzschioides* were the important species in the nearshore water (Table. 2) (Fig.3).

However, *Azpeitia nodulifera, Chaetoceros curvisetus, Ditylum sol, Lauderia annulata, Coscinodiscus radiatus, Nitzschia sigma, Odontella sinensis, Pleurosigma normanii, Pseudonitzschia seriata, Thalassiosira excentrica, Thalassionema frauenfeldii* and *T. nitzschioides* were abundantly occurred in the offshore water during the study period (Table. 3) (Fig. 4).

The maximum standard deviation of temperature at 1.498 was recorded at Station 3 whereas the minimum deviation 0.577 was recorded at Station 8.

As regard the maximum and minimum of salinity were as follows: maximum at Station 1: 9.633 and minimum at Station 10: 3.964. For pH the maximum and minimum were: 0.299 at Station 9 and 0.048 at Station 4 (Table. 4).

The maximum standard deviation of phosphate at 0.456 was recorded at Station 1 whereas the minimum deviation 0.022 was recorded at Station 2. Standard deviations from maximum to minimum for nitrate at different stations were at follow: maximum deviation at Station 1: 0.342 and minimum deviation at Station 6: 0.009. For water transparency the maximum and minimum were: 0.998 at Station 9 and 0.091 at Station 1 (Table. 4).

The nearshore upwelling zone not only had a high yield of nutrients, but also was a high primary production area for phytoplankton and inversely related to zooplankton. The quantities of phytoplankton may be related to the concentrations of nutrients available, consequent on vertical mixing. Ozbay (2011) suggested that nitrate was the limiting factor for phytoplankton growth from the Kars River, Turkey.

At the present study, the peaks of the phytoplankton were mainly due to flourishing of Bacillariophyceae, which contributed 67% of the total phytoplankton community during the postmonsoon season. Gopinathan (1972) described the peak of phytoplankton abundance observed during the postmonsoon season from Cochin backwater. This result was similar to the present study. The structure of phytoplankton communities depended not only on grazing pressure but also on nutrient according to Ozbay 2011.

Patrick and Remier (1966) described that nutrients in the sea were received by drainage from land or were brought to the surface from deep water. In present investigation, the high nutrient concentration in the nearshore waters was due to rainfall and drainage from rivers and streams. The changes and fluctuation in the rainfall occurring within the seasons and from season to season were also chiefly responsible for the blooming and abundance of phytoplankton especially during premonsoon and postmonsoon

periods. Seasonal variations in temperature, salinity, rainfall and nutrients levels all play a major part in phytoplankton species succession.

Conclusions

A study on species succession of phytoplankton in ten different stations of Myeik coastal waters was conducted. Among 135 phytoplankton species, 22 species of diatoms and 1 dinoflagellate were occurred as species succession. The diatoms represented as the dominant group followed by the dinoflagellates as the second group. Therefore Myeik coastal waters are rich waters for the marine organisms to survive and create a productive area.

The different species of plankton revealed various degrees of tolerance to the fluctuations of physical and chemical parameters. In general, the sequential change of the dominant species of phytoplankton occurred in 2-3 times per year in the Myeik coastal waters.

The phytoplankton abundance was positively related to some environmental factors such as transparency and temperature, however, the negative correlation was found when correlated with nitrate, phosphate, salinity, pH and rainfall in the Myeik coastal waters. The peculiar water transparency (9 m) was found at offshore water (Station 9) in February, 2013. The heavy rainfall (1082 mm) was recorded in July, 2011 in Myeik.

The significant seasonal variations of phytoplankton succession were found to be regulated by the changes of seawater characteristics related to the monsoon phenomenon. The abundance and community structure of phytoplankton species seemed to be affected directly or indirectly by environmental factors of Myeik coastal waters. The influences of drastic seasonal change on the diversities of diatoms and dinoflagellates off Myeik coastal waters were recognized. Moreover, the occurrence of high diversities of phytoplankton species in Myeik coastal waters seems to be related by highly enriched organic and inorganic nutrients from various marine environments such as mangrove forests and the runoff of numerous rivers

along the coastlines. This study contributes a baseline result for the sustainability of fisheries in Myeik coastal waters.

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DIVERSITY OF TURRET SHELLS (GASTROPODA: TURRITELLIDAE) IN MON COASTAL AREAS

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Abstract

Studies on diversity of turret shells were conducted at Kawdut (Lat. 15° 49′ N, Long. 97° 23' E), Sitaw (Lat. 15° 11' N, Long. 97° 48' E) and Kyungyi Island (Lat. 15° 04' N, Long. 97° 45' E) in Mon coastal areas from June 2016 to May 2017. A total of 135 individuals were collected from three different stations. Duplicate turret, *Turritella duplicata* and Screw turret, *Turritella terebra* were dominance. Higher density of turret shells were recorded in station 1 with 0.027 ind/m^2 and station 2 was recorded lower density with 0.004 ind/m^2 at Kawdut. Univariate analysis at Kawdut subtidal population recorded higher values for diversity and richness indices compared to Sitaw sandy bottom and Kyungyi Island but the value of evenness index was quite similar between the three stations. Fifty eight individuals of turret shell were collected from study areas for taxonomy identification. Seven groups of turret shell were identified base on the external shell characteristics. Seven species were clearly identified which *Turritella* and *Haustator* were dominant from the intertidal water of Mon coastal shoreline.

Keywords: Gastropoda, Turritellidae, Turret shells, Diversity, Mon coastal areas.

Introduction

The Turritellidae or Turret shells are widely distributed in the Indo-Pacific from East Africa, including Red Sea to Melanesia and New Zealand, north to Taiwan Province of China and south to central Queensland. Turret shells are also found in Southeast Asia and Indian Ocean Region (Abbott, 1991). *Haustator* and *Turritella* species exist as metapopulations, composed of small groups or patches of individuals.

Turret shells are generally elongate gastropods (13-18 cm long), sharply conical in shape, thick, with numerous whorls and a small, square to rounded aperture. Umbilicus is usually absent. Whorls are strongly sculptured

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with spiral ribs or keels. Growth lines arched to sinuous. Outer lip of the aperture is thin, often concave. Inner lip is smooth. Anterior siphonal canal is absent. Operculum is corneous and rounded, with many spiral coils and a central nucleus; border of the operculum very thin, often with flexible bristles. Head is large and prominent, with a short snout and long, tapering tentacles bearing eyes on slight swellings at their outer bases. Foot is rather short, truncate anteriorly, obtusely attenuated posteriorly and grooved beneath. Shells are light to dark brown externally and internally in color. Turret shells live on soft bottoms, from shallow sublittoral zones to a depth of about 30 m (Poutiers, 1998). Turret shells are relatively common at the sandy or muddy substrate of the soft bottoms.

According to Subba Rao (2003), *Turritella* and *Haustator* species exhibit internal fertilization and the chances of successful fertilization are deturretdent on the proximity of other spawning individuals as well as other factors, including water movement. Turret shells reach sexual maturity at a shell length of not less than 15 cm. The reproductive cycle of turret shells appeared to be an annual event. Little is known of turret shells population in Mon coastal areas. Turret shells are also one of the important fisheries resources and it can easily be established as important shellcraft industries to make decorative items. This study was started from June 2016 to May 2017. The line quadrat transect method is a popular method used to estimate the distribution and abundance of benthic organisms present in particular area (English *et al*. 1994). The emphasis was given to identification of turret shell species at first. The aim of the study is to generate some information on the ecological habitat, taxonomy, distribution and abundance of the turret shells in Mon coastal areas.

Materials and Methods

Study areas: Studies on distribution and diversity of turret shells were conducted at Kawdut (Lat. 15° 49′ N, Long. 97° 23' E), Sitaw (Lat. 15° 11' N,

Long. 97° 48' E) and Kyungyi Island (Lat. 15° 04' N, Long. 97° 45' E) in Mon coastal areas from June 2016 to May 2017 (Figure. 1).

Figure 1. Map showing the sampling sites of Turret shells in Mon coastal areas

Quadrat transect technique: A quadrat of 1 m^2 size that was sub-divided to 16 sectors was used. Five replicates of line quadrate were used during the sampling time. The quadrates were set at every 5 m along the transect line set perpendicular to the shoreline. The turret shell assemblage in each quadrat was recorded. After the number of turret shells has been completely counted and recorded, the associated flora in the surround same quadrate were recorded in percentage cover. Estimates of coverage of can be made using a technique developed by Saito and Atobe (1970). This technique uses classes of dominance, which are converted to frequency and percentage cover. Identification was made from the specimen and photographs according to WoRMS (World Register of Marine Species, 2018).

Species identification: A total of 58 individuals were collected from the study areas during low tide and were transferred to the laboratory for species identification, labeled specimens were stored and images were taken and recorded. Shells were measured using digital vernier caliper for total length and other shell morphometric characteristics. Measurement was emphasize the following parameters: length of shell (1), number of whorl (2), type of operculum (3), sculpture of shell (4), suture (5) and aperture (6). For the identification of the different morphological structure on turret shell species, the works of Marwick (1957), Garrard (1972), Abbott (1991), and Oliver (2004) were followed.

Data analysis

Species diversity: Diversity indices were calculated using PRIMER (Plymouth Routines In Multivariate Ecological Research) v5 statistical program. The three diversity indices of the univariate analysis were Shannon's diversity index, Margalef's richness index and Pielou's evenness index.

Multidimensional scaling (MDS) analysis: The multidimensional scaling (MDS) were used to find the distribution of species according to the different stations. The goodness of fit of MDS was measured by the stress value.

Generally, a stress below 0.05 represents an excellent fit while a figure below 0.1 indicates good relationship and below 0.2 is considered useful (Clark, 1993).

Results and Discussion

Characteristics of turret shells: Shell is small to large, up to 100 mm long and attenuate with numerous whorls. Aperture is proportionately small, rounded or angled at the top. Outer lip is thin with a convex outer margin. There is no umbilicus. Sculpture consists of spiral striations or ridges. Operculum is chitinous, circular, thin and rnultispiral with a central nucleus.

Head is large and bears long tentacles having eyes at their outer bases. Foot is short, truncate anteriorly and narrow posteriorly, with a groove on the ventral side. It possesses a pedal gland at the posterior end. Mantle margin is fringed and has a siphonal fold on the right side. Mantle cavity consists of a monopectinate ctenidiunl and a string-like osphradium. Radula is taenioglossate, 3-1-1-1-3. Digestive system contains small salivary glands, long and narrow oesophagus and a large two-chambered stomach. Sexes are separate. Male is without a penis. Some deposit eggs in stalked capsules, while some brood the young within the oviduct. Veliger larva may be of short duration.

Majority of the species prefer muddy sands of tropical waters. These are detritus feeders and occur from intertidal to offshore, mostly beyond the low tide line. It is a large family consisting of five subfamilies embracing 18 genera and an estimated 150 species. In Myanmar four genera, *Turritella*, *Torcula*, *Haustator* and *Neohaustator*, belonging to the subfamily Turritellinae are reported by Soe Thu (1980). These are mainly distributed along the continental shores. The family Turritellidae has two genera, *Turritella* with five species and *Haustator* with two species in Mon coastal areas (Table 1).

Distribution of turret shells: It was observed that, the turret shells of Kawdut, Sitaw and Kyungyi Island were patchy in existence, composed of small clusters or sometime existed as patches of individuals. Turret shells live associated with the marine algae (*Gracilaria* sp., *Catenella* sp., *Padina* sp. and *Acanthophora* sp.) at Kawdut and Sitaw. Most of the turret shell live associated with rock oyster bed and barnacles at the rocky shore area in Kyungyi Island.

Table 1. Classification of family Turritellidae in Mon coastal areas

Phylum	**:**	Mollusca Linnaeus, 1758
Class	**:**	Gastropoda Cuvier, 1795
Order	**:**	Mesogastropoda Thiele 1925
Family	**:**	Turritellidae Lovén, 1847
Genus	**1**	*Turritella* Lamarck, 1799
Species	1	*Turritella attenuata* Reeve, 1849
	2	*Turritella carinifera* Lamarck, 1822
	3	*Turritella duplicata* (Linnaeus, 1758)
	4	*Turritella fastigiata* Adams and Reeve, 1849
	5	*Turritella terebra* (Linnaeus, 1758)
Genus	**2**	*Haustator* Montfort, 1810
Species	1	*Haustator trisulcata* (Lamarck, 1822)
	2	*Haustator variegata* (Linnaeus, 1758)

From the 50 meter line transect it was found that one to three individuals in each quadrat. A total of 135 individuals of turret shells were recorded from three stations. Among them, 78 individuals from Kawdut, 35 individuals from Sitaw and 22 individuals from Kyungyi Island (Table 2), (Fig. 2) and (Fig. 3A). In the present study, the density was low. Density of turret shells recorded in St. 1 was 0.027 ind/m^2 and St. 2 was 0.004 ind/m^2 at Kawdut. Sitaw recorded 0.014 ind/m^2 at St. 3. In addition, Kyungyi Island recorded 0.009 ind/m^2 at St. 4. The higher densities were recorded at St. 1 and the lower densities were recorded at St. 2. Connell and Orias (1964) reported the densities of turret shell in Indian coastal water was up to 14 individuals per m^{-2}. *Turritella attenuata* were presents all the stations. *T. carinifera* only

found at Kawdut and Kyungyi Island. But *T. terebra* was present at Kawdut and Sitaw. The abundance of *T. duplicata* and *T. fastigiata* were at Kawdut and Kyungyi Island. *Haustator variegata* was found at Kawdut and Sitaw but *H. trisulcata* were present at Kawdut. Most of the turret shells were found in the range of 10 cm to 30 cm long. The growth of turret shells is relatively slow (3-4 cm/year), based on annular growth rings in specimens and may live up to 18 years Garrard (1972).

Table 2. Occurrence of turret shells species in the study areas

Species	Kawdut		Sitaw	Kyungyi Island
	St. 1	St. 2	St. 3	St. 4
Turritella attenuata	25	3	20	5
Turritella carinifera	4	1	0	3
Turritella duplicata	8	3	2	6
Turritella fastigiata	18	2	9	8
Turritella terebra	2	0	1	0
Haustator trisulcata	3	0	0	0
Haustator variegata	8	1	3	0
Total	68	10	35	22

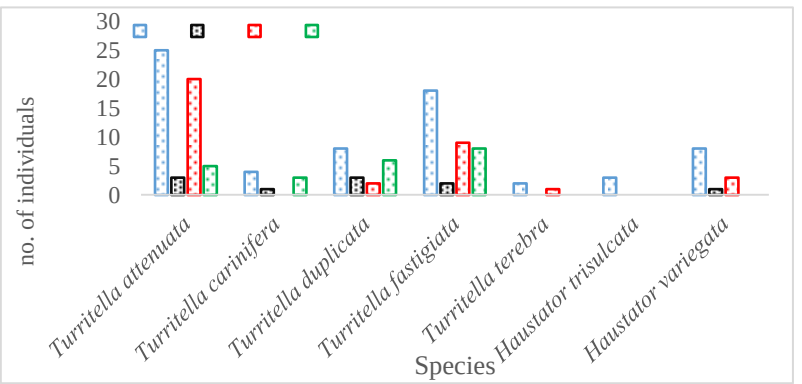

Figure 2. Number of individual present for every study sites

According to Connell and Orias (1964), there was no obvious strong relationship between density of *Turritella attenuata* and various physical and

biological variables in Indian coastal water. However, in Kawdut, Sitaw and Kyungyi Island, turret shells are patchy in their distribution and it is proved by the quadrate line transect result. Only one to three individuals found in each meter square of quadrate. Turret shells from these areas were constantly been sought after by the villagers who frequently visited to the sandy mudflat during low tide period. The low abundance might be largely attributed to this activity.

Table 3. Univariate analysis of the turret shells at study areas

Stations		No. of individuals recorded (N)	Densities (N/m^2)	Taxa (S)	Diversity index (*H'*)	Richness index (*R'*)	Evenness index (*J'*)
Kawdut	St. 1	68	0.027	7	1.631	1.422	0.838
	St. 2	10	0.004	5	1.505	1.737	0.935
Sitaw	St. 3	35	0.014	5	1.145	1.125	0.711
Kyungyi Island	St. 4	22	0.009	4	1.331	0.959	0.959

Species Diversity: From the univariate analysis of turret shells (Table 3), the diversity index recorded higher value at Kawdut St. 1 (1.631) and the lowest value at Sitaw St. 3 (1.145) (Fig. 3B). Highest richness indices values was recorded at Kawdut St. 2 (1.737) and the lowest value at Kyungyi Island St. 4 (0.959) (Fig. 3C). Similarly, the highest evenness indices was recorded at Kyungyi Island St. 4 (0.959) and the lowest value recorded at Sitaw St. 3 (0.711) (Fig. 3D). From the results it is showed that the relatively higher abundance of turret shells at Kawdut as compared to Sitaw and Kyungyi Island. This is further supported by the univariate diversity analysis done for the result gathered in the study area. This study can also confirm that the turret shells are patchy in their distribution.

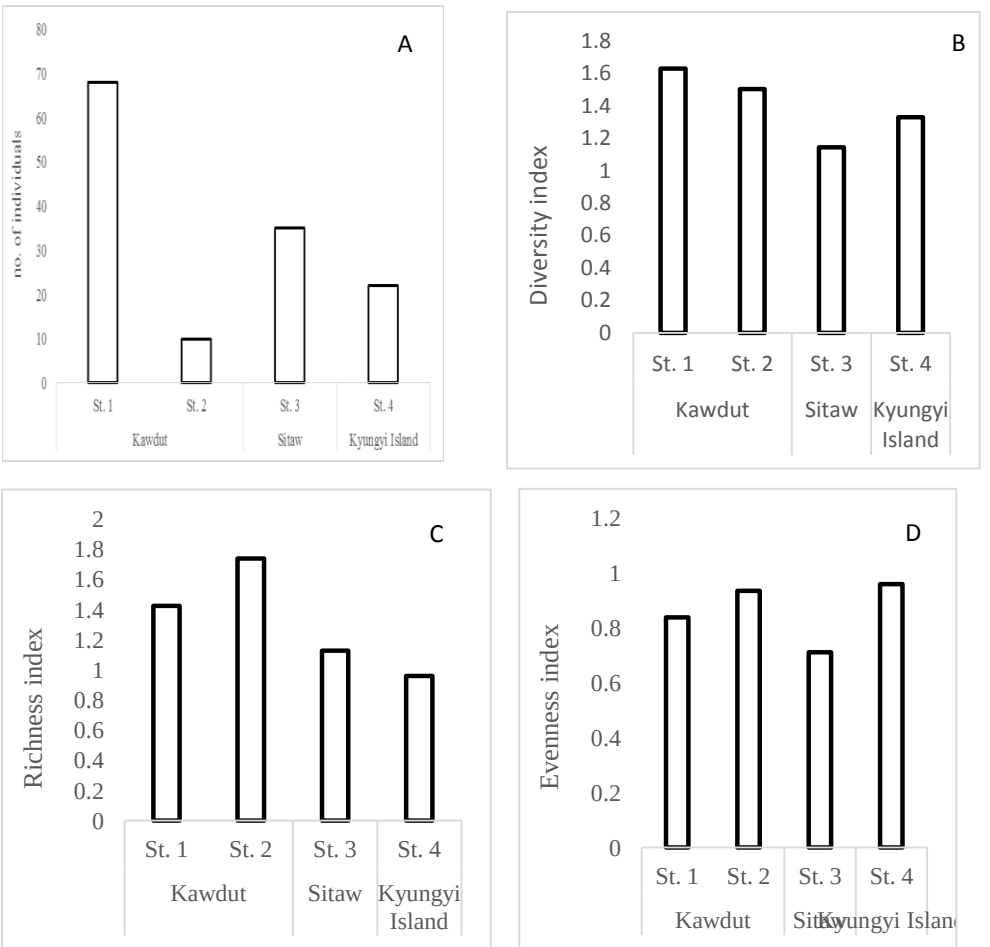

Figure 3. (A-D): Graph of univariate analysis three indices for every study areas. Total number of individuals (A), Diversity index (B), Richness index (C) and Evenness index (D).

As earlier been mentioned, turret shells are distributed as a metapopulations species, where the populations are patchy, composed of small groups or patches of individual (Carpenter and De Angelis, 2016). Boettger (1987) also mentioned that the spatial patchiness of *Turritella*

attenuata must be considered before interpreting the situation. In many areas, *Turritella attenuata* can occur in patches only a few meters to a few tens of meters in diameter separated by gaps of similar size.

Multidimensional Scaling (MDS): Figure 4 and 6 show the MDS ordinations of the clustering communities of turret shell respectively at Kawdut (KD), Sitaw (ST) and Kyungyi Island (KGI). MDS analysis showed a clear separation between the four sites. The stress value quoted together with the ordination showed in zero values and it is indicated that an excellent fit and this showed each sites were clearly different with one other (Fig. 4).

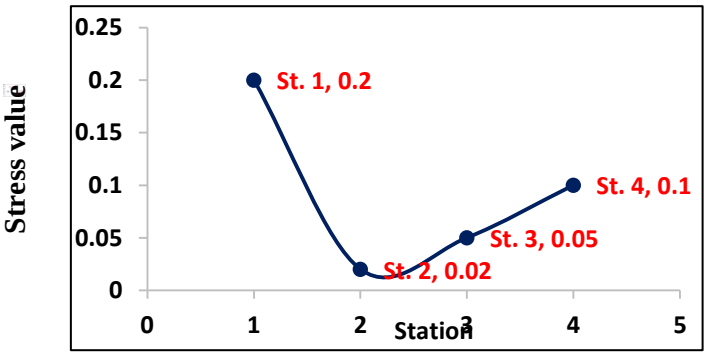

Figure 4. MDS ordinations plot for turret shells at Kawdut, Sitaw and Kyungyi Island

Species Identification: A total of seven species of turret shells were recorded from the sandy mudflat and rocky shore at Kawdut, Sitaw and Kyungyi Island (Table 2). The identification of the species was based on the six characteristics of internal and external of the shells (Fig. 5). Among these seven species, five species belongs to the genus *Turritella* was recorded and two species belongs to genus *Haustator*. *Turritella attenuata* and *Turritella fastigiata* were the dominant species in these study areas.

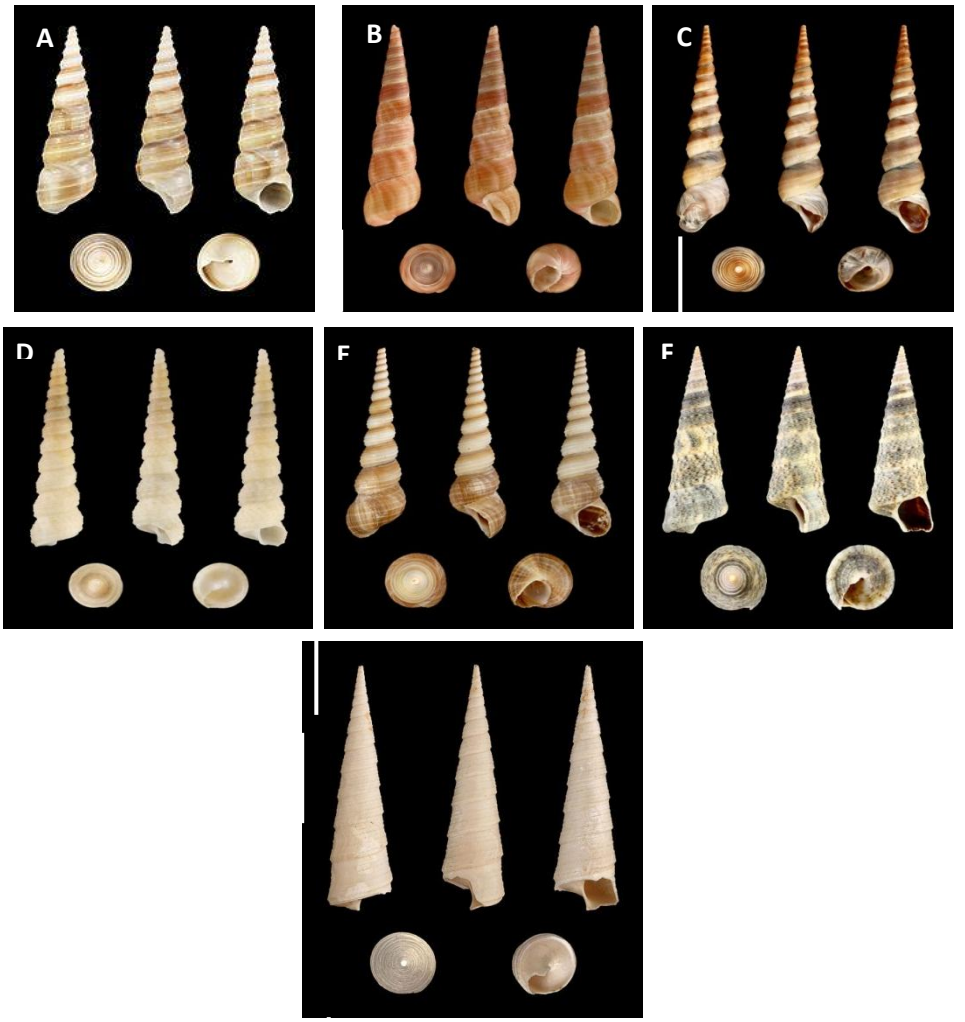

Figure 5. (A-G): Turret shells of Kawdut, Sitaw and Kyungyi Island at Mon coastal areas. A) *Turritella attenuata* Reeve, 1849; B) *Turritella carinifera* Lamarck, 1822; C) *Turritella duplicata* (Linnaeus, 1758); D) *Turritella fastigiata* Adams and Reeve, 1849; E) *Turritella terebra* (Linnaeus, 1758); F) *Haustator trisulcata* (Lamarck, 1822); G) *Haustator variegata* (Linnaeus, 1758). Scale bars = 10 cm.

In term of similarity amongst populations, dendrogram plots show from the study areas there are two areas group together (KD St. 1 and ST St. 3) and (KD St. 2 and KGI St. 4). Result showed KD St. 1 is correlated to ST St. 3 and they can be grouped together at 71% similarity (Fig. 6) and KD St. 2 is correlated to KGI St. 4 which, can be grouped together at 68% similarity. These two groups are correlated by the total number of turret shells existing in each area. KD St. 1 and ST St. 3 recorded higher number of individuals compared to KD St. 2 and KGI St. 4.

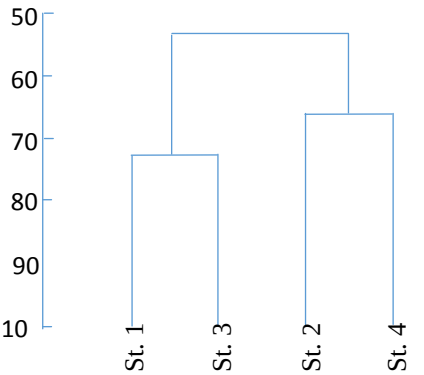

Figure 6. Dendrogram plot for turret shells at Kawdut (KD), Sitaw (ST) and Kyungyi Island (KGI)

In the sandy mudflat and rocky shore areas of Mon coastal areas, *T. duplicata* (Linnaeus, 1758) was recorded at Kawdut, Sitaw and Kyungyi Island and *T. terebra* (Linnaeus, 1758) was recorded at Kawdut and Sitaw. These two species of turret shells was a new distribution recorded in the sandy mudflat and rocky shore areas of southern part of Mon coastal areas. The previous study by Soe Thu (1980) only mention and recorded the existing of turret shell in Myanmar. The statements given are not specific to an exact area. Also Thaw Zin Naing Tun *et.al* (2012), reported the existing of turret shells only at Kampani coastal area, northern part of Taninthayi Coastal Region.

The local fishing communities collect these molluscs for their livelihood and especially gastropods are used for human consumption. They are good source of proteins, mineral and glycogen, and easily digestible compared to other animal foods. The non-edible molluscs were deposited into heaps on the platforms for sun drying and then utilized for domestic and commercial purposes. In the present study 7 species of turret shells were recorded on mud flats, sandy areas, near swamps and mangroves of Ye River Estuary in southern Mon coastal areas. Gastropods were observed to be predominant in this area. The study provides the base line information on malacofauna and it would assist the researchers for further studies on molluscs and manage the resources for sustainability.

Conclusion

From this study it could be concluded that the sandy mudflat and rocky substrate of Kawdut and Sitaw provided rich habitat for turret shells where, *Gracilaria* sp., *Catenella* sp., *Padina* sp. and *Acanthophora* sp. were among the dominant species. However, turret shells also can be found at the rocky shore area in Kyungyi Island. A total of 135 individuals of turret shell were found at four studies areas and they were patchy in distribution. Higher density of turret shells were recorded at St. 1 with 0.027 ind/m^2 and St. 2 recorded lower density with 0.004 ind/m^2 at Kawdut. Seven species of turret shells were recorded from the sandy mudflat and rocky shore at Kawdut, Sitaw and Kyungyi Island. *Turritella attenuata* and *Turritella fastigiata* were found to be dominant in these three study areas. Univariate analysis at Kawdut population recorded higher values for diversity and richness indices compared to the Sitaw and Kyungyi Island but the value of evenness index was quite similar between Kawdut, Sitaw and Kyungyi Island. Multidimensional scaling (MDS) and dendrogram plots found the stress value were zero and it indicated that excellent fit. Two groups were found from MDS and dendrogram plots which Kawdut (St. 1) is correlated to Sitaw (St. 3) and Kawdut (St. 2)

correlated to Kyungyi Island (St. 4). In addition, *Turritella duplicata* (Linnaeus, 1758) and *Turritella terebra* (Linnaeus, 1758) was new distribution records from southern part of Mon coastal areas.

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SPECIES COMPOSITION, ABUNDANCE AND DISTRIBUTION OF PHYTOPLANKTON IN THE ELPHINSTONE ISLAND, MYEIK COASTAL WATERS

Zarni Ko Ko[1]

Abstract

Phytoplankton composition, abundance and distribution were evaluated on June 2013 to January 2014 from Elphinstone Island of Myeik Coastal Waters. Altogether 81 species of phytoplankton; 60 species in 37 genera which belong to 16 families of diatoms and 21 species in 9 genera which include to families of dinoflagellates were identified. The highest species composition (49 species) was found at Grants Island and Zalat Aw Gyi in November and the lowest number (22 species) was also found at Grants Island in August. The abundance of diatom species was found more than that of dinoflagellate species. The highest diversity index (*H'*) and the lowest species richness value (*R'*) were found at Espace Bay. The value of evenness index (*J'*) was not significantly differed by stations.

Keywords: Phytoplankton, Elphinstone Island, Species Composition, Abundance, Distribution.

Introduction

In the phytoplankton, Diatoms (class: Bacillariophyceae) and Dinoflagellates (phylum : dinoglagellata) commonly predominate. Individuals of these two orders are unicellular plants with a size range of about 15 - 400 µm in maximum dimension, but dinoflagellates contain a larger proportion among very small forms.

Diatoms are remarkedly distinguishable into two orders, the Centrales and the Pennales. The Centrales, or centric diatoms, have a radial symmetry and are successful as plankton in marine waters. Their frustules, or shells, can also be triangular or quadrate. The centric diatoms are mostly planktonic and non-motile.

The Pennales, pennate diatoms, occupy and dominate the freshwater, soil, and epiphytic environments. Although they also thrive in marine habitats,

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their typical environmental niche is in fresh water. The Pennales have bilateral symmetry. Most marine diatoms tolerate a wide range of temperatures typically ranging from 19º C to 30.5º C.

Marine dinoflagellates are unicellular, eukaryotic algae. Dinoflagellates are the second most abundant form of autotrophic life in the marine ecosystem. Majority of dinoflagellates are autotrophic and a few are holozoic, saprophytic orphagotrophic. In the autotrophic dinoflagellates, the products of the photosynthesis are starch and lipids (Hunter 2007).

Myeik Archipelago extends from Mali Island to Similand Island and comprises about 800 islands covering on area of about 34,340 squares kilometer in is lying up to 30 km offshore. Elphinstone Island (Thayawthadangyi kyun) is one of the largest outer islands of Myeik Coastal Waters. Around this island, there are some populated fishing villages and culture farms (pearl oyster farm and old seaweed farms).

The study areas; Escape Bay and Grants Island are developed with pearl oyster (*Pinctada maxima*) farms and Mway Kyun station was also developed with seaweed (*Kappaphcus alverazii*) farms. The objectives of the present study are: (1) to identified what kinds of phytoplankton species and (2) to analyze the community structures of phytoplankton species.

Materials and Methods

Phytoplankton samples were collected from five sampling stations; Station (1) Escape Bay (Lat 12° 16' N and Long 98° 00' E), Station (2) Myaw Island (Lat 12° 22' N and Long 98° 06' E), Station (3) Grants Island (Lat 12° 23' N and Long 98° 06' E), Station (4) Zalat Aw Nge (Lat 12° 18' N and Long 98° 01' E) and Station (5) Zalat Aw Gyi (Lat 12° 18' N and Long 98° 02' E),in the waters off Elphinstone Island, MyeikArchipelago, Taninthayi Region (Figure 1).

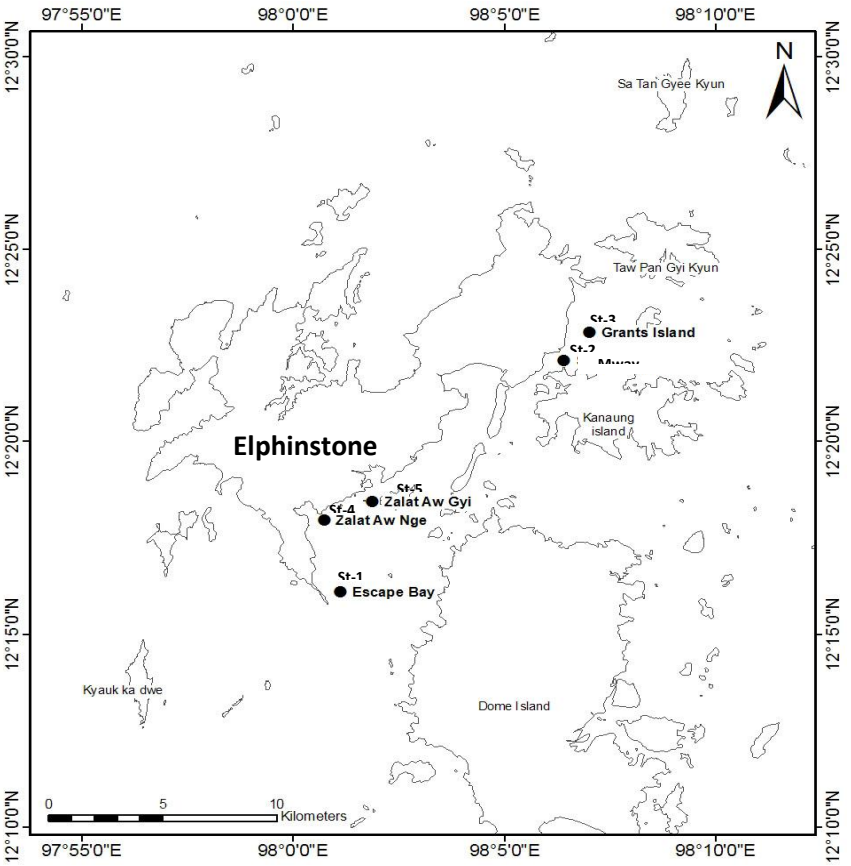

Figure 1. Map showing the study areas of Elphinstone Island

At all study stations, monthly collection of phytoplankton was carried out during June 2013 to February 2014. Phytoplankton net (60cm in length, 25cm in width (diameter) and 25 µm mesh size) was towed horizontally at every station. The collected samples were kept in clean small size plastic bottles and preserved in 2% formaldehyde immediately. The specimens were identified up to species level with the following references; Newell and Newell (1963), Allen and Cupp (1930), Hendey (1964), Yamaji (1971), Tomas (1997), Wood (1968) and Al-Kandari, *et al*. (2009).

Species diversity indices for each sample were calculated by using the formula of Shannon and Weaver (1963), Pielou (1966) and Margalef (1958). $H'=-\sum$ Pi*lnPi, $J'=H'/$lnS, $R'=S-1/$ln N; where, H' is the index of species diversity, Pi is the population abundance of i^{th} species calculated by ni/N, ni is the number of individual of the i^{th} species, N is the total number of individuals in a station, J' is the index of species eveness, S is the total number of species and R' is the index of species richness.

The dominancy index (D) was calculated by the Simpson's index formula; D= n (n-1)/N (N-1), N is the total number of species of phytoplankton and n is the total number of organisms of a particular phytoplankton species. Similarity index was determined by using Sorenson's index as follows: S=C/A+B, where C is the number of species the two communities have in common, A is the total number of species found in community A and B is the total number of species found in community B (as cited in Huliselan, Tuapattinaja and Pattimura, 2017)

Results

A total of 81 species of phytoplankton were identified during the study period from Elpninstone Island. In 81species of phytoplankton of which, 60 species of diatoms, 21species of dinoflagellates were recorded respectively (Table 6). The highest number of species (49) was found in November at both stations 3 and 5 followed by number of species (47) in also November at stations 2 and 4 and the lowest number was occurred in October at stations 1, 4 and 5 (Table 1 and Figure 2). During the study period, diatoms species were more dominant than dinofagellates at all stations in every month.

Table 1. Distribution and occurrence of phytoplankton in Elphinstone Island in Myeik Coastal Waters

Stations	Months								
	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Total
Escape Bay	37	39	31	44	24	44	41	30	290
Myaw Island	39	41	36	26	43	47	33	35	300
Grants Island	30	41	22	36	39	49	38	43	298
Zalat Aw Nge	47	35	26	41	24	47	43	27	290
Zalat Aw Gyi	41	41	27	41	24	49	33	28	284
Total	194	197	142	188	154	236	188	163	**1462**

Table 2. Dominancy index (D) of phytoplankton communities at all stations during study period.

Stations	Months							
	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan
Escape Bay	0.292	0.325	0.204	0.415	0.121	0.415	0.360	0.191
Myaw Island	0.282	0.312	0.240	0.124	0.344	0.411	0.201	0.226
Grants Island	0.170	0.321	0.090	0.246	0.290	0.460	0.275	0.353
Zalat Aw Nge	0.400	0.220	0.120	0.304	0.102	0.400	0.334	0.130
Zalat Aw Gyi	0.340	0.340	0.145	0.340	0.114	0.487	0.219	0.157

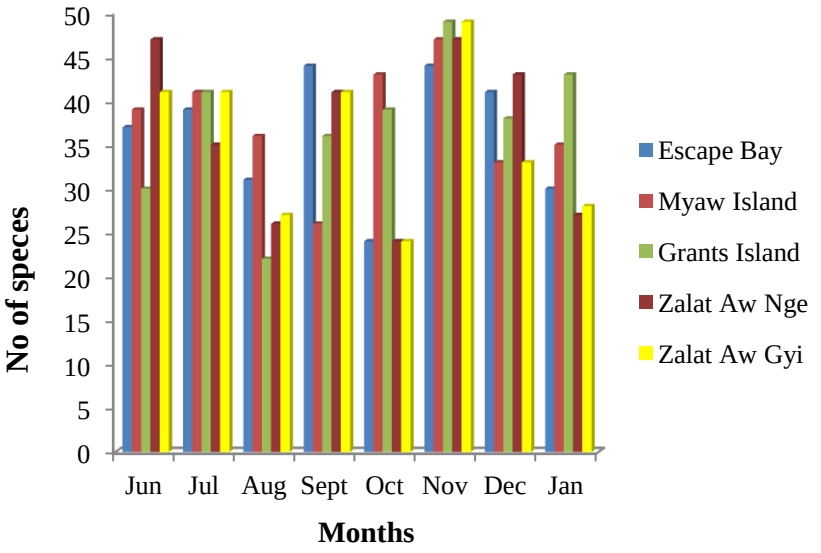

Figure 2. Distribution and occurrence of phytoplankton in Elphinstone Island in Myeik Coastal Waters

During study period, the dominance index varied from 0.090 to 0.487. The maximum (0.487) was recorded at Zalat Aw Gyi in November and the minimum (0.090) was at Grants Island in Auguest (Table 2). The similarities index of phytoplankton at all stations showed that the percentage of similarity ranged from 90% to 95.24%. The value revealed that phytoplankton communities between stations from Elphinstone Island in space were similar. This condition was supported by the value of Dominancy index which was low (Table 3).

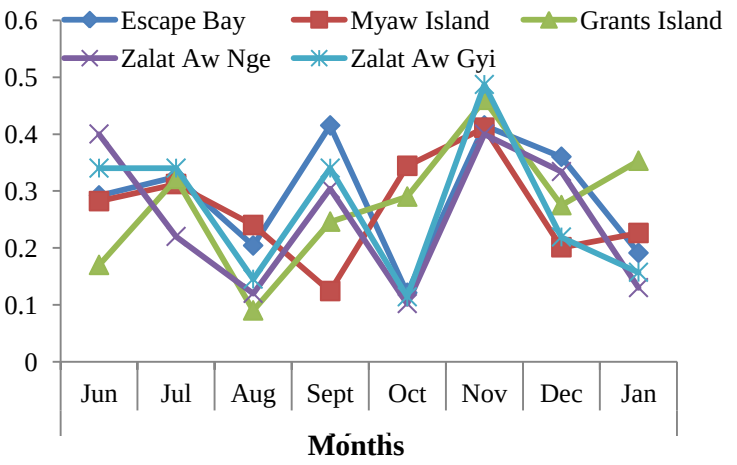

Figure 3. Dominancy index (D) of phytoplankton communities at all stations during study period

Table 3. Similarity (S) of phytoplankton communities at all stations during study period.

Dimension	Object	Similarity (S)	Percentage
Spatial	Stations 1 and 2	0.9503	95.03%
	Stations 1 and 3	0.9000	90.00%
	Stations 1 and 4	0.9296	92.96%
	Stations 1 and 5	0.9275	92.75%
	Stations 2 and 3	0.9517	95.17%
	Stations 2 and 4	0.9524	95.24%
	Stations 2 and 5	0.9371	93.71%
	Stations 3 and 4	0.9315	93.15%
	Stations 3 and 5	0.9014	90.14%
	Stations 4 and 5	0.9028	90.28%

Table 4. The abundance (cells L^{-1}) of phytoplankton communities at all stations from Elphinstone Island.

Table 4.1. The abundance (cells L^{-1}) of phytoplankton communities at Escape Bay station during study period.

Family	Months							
	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan
Biddulphiales	34586	257790	125831	23334	178256	24414	176441	120031
Bacillarineae	1635	96820	449	277103	144	1205	5577	589
Prorocentrales	0	0	0	0	0	0	0	0
Dinophysiales	160	154	64	128	577	128	385	52
Gonyaulacales	577	256	64	2225	0	386	371	103
Dictyochales	0	0	0	0	0	0	38	0

Table 4.2. The abundance (cells L^{-1}) of phytoplankton communities at Myaw Island station during study period.

Family	Months							
	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan
Biddulphiales	64135	229318	82790	3749	565153	23885	7242	857932
Bacillarineae	5077	232825	3298	4135	124474	5240	7659	3076
Prorocentrales	0	0	0	0	0	0	0	0
Dinophysiales	177	67	135	352	180	400	1026	240
Gonyaulacales	317	3904	539	689	1705	1344	849	913
Dictyochales	0	0	0	0	90	0	0	0

Table 4.3. The abundance (cells L^{-1}) of phytoplankton communities at Grants Island station during study period.

Family	Months							
	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan
Biddulphiales	10174	83158	59487	12435	338143	65790	14873	102820
Bacillarineae	577	6922	7629	15385	15751	5205	5769	3429
Prorocentrales	16	0	0	0	0	0	51	0
Dinophysiales	64	128	128	609	174	872	385	962
Gonyaulacales	112	3012	192	1473	1735	3005	921	2787
Dictyochales	0	0	0	0	87	0	0	64

Table 4.4. The abundance (cells L^{-1}) of phytoplankton communities at Zalat Aw Nge station during study period.

Family	Months							
	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan
Biddulphiales	11246	363801	158095	15460	567036	16598	15084	53443
Bacillarineae	499	25667	1499	16880	11751	820	12307	353
Prorocentrales	0	0	0	0	0	0	16	0
Dinophysiales	192	167	83	160	603	282	288	0
Gonyaulacales	1269	1333	83	384	151	975	272	0
Dictyochales	0	0	0	0	0	0	16	0

Table 4.5. The abundance (cells L^{-1}) of phytoplankton communities at Zalat Aw Gyi stations during study period.

Family	Months							
	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan
Biddulphiales	15209	444203	74310	8637	158011	16133	11366	140923
Bacillarineae	558	48692	2846	18814	6904	4422	4974	1205
Prorocentrales	19	19	0	0	0	0	0	0
Dinophysiales	173	231	308	80	285	1106	359	26
Gonyaulacales	1038	3763	616	240	336	1923	77	0
Dictyochales	0	0	0	0	0	0	0	0

During the study, the abundance of phytoplankton at Escape Bay ranged between 52 cells $L^{-1}$and 257790 cells L^{-1}. The minimum (52 cells L^{-1}) was found during the post-monsoon season in January and the maximum (257790 cells L^{-1}) was during monsoon season in July (Table 4.1). At Myaw Island, the abundance varied from 67 cells L^{-1} to 857932 cells L^{-1}. The smallest value (67 cells L^{-1}) was recorded during monsoon seasonin July and the largest value (857932 cells L^{-1}) was during the post-monsoon season in January (Table 4.2). From Grants Island, the range of phytoplankton abundance was 16 cells L^{-1}-338143 cells L^{-1}. The lowest value (16 cells L^{-1}) was occurred during monsoon season in June and the highest value 338143 cells L^{-1} during the post-monsoon season in October (Table 4.3). In Zalat Aw Nge station, the abundance of phytoplankton ranged between 16 cells L^{-1} and 567036 cells L^{-1}. Minimum value (16 cells L^{-1}) was reported during the post-monsoon season in December and maximum (567036 cells L^{-1}) was during the post-monsoon season in October (Table 4.4). At station Zalat Aw Gyi, the phytoplankton abundance varied from 19 cells L^{-1} to 444203 cells L^{-1}. The smallest value (19

cells L^{-1}) was recorded during monsoon season in June and July and the largest value (444203cells L^{-1}) was during the monsoon season in July (Table 4.5).

Table 5. Species diversity index, richness index, evenness at study areas during study period.

	Stations				
	Escape Bay	Myaw Island	Grants Island	Zalat Aw Nge	Zalat Aw Gyi
Diversity index (*H'*)	2.4	1.8	2.4	2.1	2.2
Richness index (*J'*)	5.7	5.5	5.9	5.7	5.8
Evenness (*R'*)	0.6	0.4	0.6	0.5	0.5

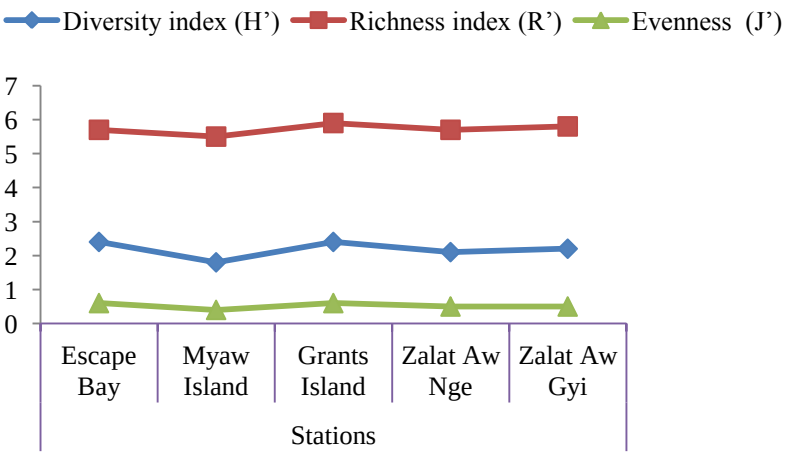

Figure 4. Species diversity index, richness index, evenness at study areas during study period

Table 6. Composition of phytoplankton communities at all stations from Elphinstone Island.

Sir No.	Species Name	Station-1	Satation-2	Station-3	Station-4	Station-5
	Cyclotella striata	+	+	+	+	+
	Lauderia annulata	+	+	+	+	+
	Skeletonema costatum	+	+	+	+	+
	Planktoniella sol	+	+	+	+	_
	Thalassiosira eccentrica	+	+	_	+	+
	Paralia sulcata	+	+	+	+	+
	Corethron criophilum	+	+	+	+	+
	Coscinodiscus occulus-irridis	+	+	+	+	+
	Coscinodiscus centralis	+	+	+	+	+
	Coscinodiscus granii	+	+	+	+	+
	Coscinodiscus radiatus	+	+	+	+	+
	Hemidiscus cuneiformis	+	+	+	+	+
	Rhizosolenia imbricata	+	+	+	+	+
	Rhizosolenia setigera	+	+	+	+	+
	Rhizosoleniarobusta	+	+	+	+	+
	Rhizosoleniacalcar-avis	+	+	+	+	+
	Rhizosolenia bergonii	+	+	+	+	+
	Proboscia alata	+	+	+	+	+
	Guinardia flaccida	+	+	+	+	+
	Guinardia striata	+	+	+	+	_
	Eucampia zodiacus	+	+	+	+	+
	Eucampia cornuta	+	+	+	+	+
	Cerataulina pelagica	+	+	+	+	+

Sir No.	Species Name	Station-1	Satation-2	Station-3	Station-4	Station-5
	Hemiaulus sinensis	+	+	+	+	+
	Climacodium biconcavum	_	+	+	_	+
	Bacteriastrum hyalium	+	+	+	+	+
	Chaetoceros decipiens	+	+	+	+	+
	Chaetoceros curvisetum	+	+	+	+	+
	Chaetoceros diversus	+	+	+	+	+
	Chaetoceros denticulatus	+	+	+	+	_
	Chaetoceros coastatus	+	+	+	+	_
	Chaetoceros pervianum	+	+	+	+	+
	Chaetoceros compressus	+	_	_	_	_
	Bellerochea horologicalis	+	+	+	+	+
	Ditylum sol	+	+	+	+	+
	Helicotheca tamensis	+	+	+	+	+
	Odontella sinensis	+	+	+	+	+
	Odontella mobiliensis	+	+	+	+	+
	Odontella aurita	+	+	_	+	+
	Odontella obtusa	_	_	_	+	_
	Triceratium favus	+	+	+	+	+
	Lamprisus shadboltianum	+	+	+	+	+
	Astrionellopsis glacialis	+	+	_	_	+
	Isthmia nervosa	_	_	_	_	+
	Thalassionema nitzschioides	+	+	+	+	+
	Thalassionemafrauenfeldii	+	+	+	+	+
	Licmophora flabellata	_	_	+	_	+
	Navicula lyra	_	+	+	+	+

Sir No.	Species Name	Station-1	Satation-2	Station-3	Station-4	Station-5
	Pleurosigma normanii	+	+	+	+	+
	Pleurosigma angulatum	+	+	+	+	+
	Pleurosigma elongatum	+	+	+	+	+
	Amphiprora alata	+	+	+	+	+
	Deplonies crabro	_	_	_	+	_
	Bacillaria paxillifera	+	+	+	+	+
	Nitzschia longissigma	+	+	+	+	+
	Nitzschia lorenzian	+	+	+	+	+
	Nitzschia sp.	+	+	+	+	+
	Pseudo-nitzschia seriata	+	+	+	+	+
	Surirella ovalis	+	+	+	+	+
	Tabellaria fenestratea	+	+	+	+	+
	Prorocentrum micans	_	_	_	+	+
	Triposolenia truncata	_	_	+	_	_
	Dinophysis caudata	+	+	+	+	+
	Ornithocercus magnificus	+	+	+	+	+
	Phalacroma circumsutum	_	+	+	+	_
	Ceratium fusus	+	+	+	+	+
	Ceratium fucar	+	+	+	+	+
	Ceratium macroceros	+	+	+	+	+
	Ceratium massiliense	_	+	+	+	_
	Ceratium tripos	+	+	+	+	+
	Ceratium breve	+	+	+	+	+
	Ceratium flacatum	_	_	+	+	_

Sir No.	Species Name	Station-1	Satation-2	Station-3	Station-4	Station-5
	Pyrophacus horologium	+	+	+	+	+
	Protoperidinium depressum	+	+	+	+	+
	Protoperidinium conicum	+	+	+	+	+
	Protoperidinium pentagonum	+	+	+	+	+
	Protoperidinium oceanicum	+	+	+	+	+
	Protoperidinium oblongum	+	+	+	+	+
	Protoperidinium grande	_	+	+	_	+
	Protoperidinium pellucidum	_	+	+	+	+
	Dictyocha fibula	+	+	_	+	+

+ =present, - =absent

Discussion

From the observation of phytoplankton in the waters off Elphinstone Island, 81species of diatoms and dinoflagellates were recorded. In comparison, the diatoms were more abundant than dinofagellates during the present study period. In Myeik, Si Thu Hein (2010), Khin Yu Nwe (2011) and Lett Wai Nwe (2011) observed that diatoms are more dominant than dinoflagellates. Moreover, Zin Mar Aye (2012) and Tin Tin Kyu (2012) reported that diatoms were higher than dinoflagellates in Palaw Waters. However, Zin Lin Khine and Htay Aung (2009) described dinoflagellates occurred to be more abundant than diatoms in the waters off Ayeyarwaddy and Taninthayi coast.

During the whole study period, the highest number of phytoplankton species was found at all stations in November. In this month, the common diatom genera were *Coscinodiscus, Hemidiscus, Rhizosolenia, Proboscia,*

Guinardia, Eucampia, Ditylum, Odontella, Thalassionema and the common dinoflagellates genera were *Amphiprora, Nitzschia, Pseudo-nitzschia, Dinophysis, Ornithocercus* and *Protoperidineum.* However, minimum species numbers were found at all stations in August that was rainy season. Similarly, this result was reported by Caric *et al*., (2011), Zin Mar Aye (2012) and Tin Tin Kyu (2012).

The genera of *Chaetoceros* and *Thalassionema* were observed as dominant in the present study period. Boonyapiwat (1997a) reported these above genera as dominant genera in the Gulf of Thailand and the East Coast of Peninsular Malaysia. Boonyapitwat (1997b) recorded that *Oscillatoria erythrae, Proboscia alata, Rhizosolenia calcar-avis* and *Thalassionema frauenfeldii* were dominant species in Vietnamese. Zin Lin Khin and Htay Aung (2009) also recorded that *Osciallatoria* was dominant species in lower part of Taninthayi Waters. Moreover, Boonyapitwat, *et al.,* (2008) reported *Oscillatoria erythrae* and *Proboscia alata* were the dominance species in north, west and east of the Bay of Bengal. However, the genus *Oscillatoria (Trichodesmiun)* was not found but *T. frauenfeldii* and *P. alata* were found moderately in the present study.

During the study period, the abundance of phytoplankton at all stations varied from 16 cells L^{-1} to 857932 cells L^{-1} by families. The lowest value (16 cells L^{-1}) was occurred during monsoon (June) and post-monsoon season (December) and the highest value (857932 cells L^{-1}) was found during the post-monsoon season (January). The phytoplankton population density varied from 12000 to 92625 cells L^{-1}. The minimum value (12000 cells L^{-1}) was occurred during monsoon (November) and the maximum value (92625 cells L^{-1}) was found during pre-monsoon season (May) (Ananthan, et.al, 2008). The highest cell density (133790 cells L^{-1}) was observed in the Bay of Bengal (Boonyapiwat, *et al.,* 2008).

In present study, the range of dominance index was 0.090-0.487. In the islands of Burung and Buntal, the dominancy index varied from 0.125 to 0.462 (Huliselan, Tuapattinaja and Pattimura, 2017). However, the dominancy index ranged between 8.99 and 53.7was recorded from Ariyankuppam estuary and Verampattinam coast (Ananthan, et.al, 2008). The similarities index of phytoplankton at all stations showed that the percentage of similarities index of phytoplankton at all stations showed from 90% to 95.24%. Phytoplankton communities were similar significantly as well as species by station. Similarity, in Burung and Buntal, that the percentage of similarities index of phytoplankton (73%-96%) was similar significantly between stationand time (Huliselan, Tuapattinaja and Pattimura, 2017)

During the present study, variation of species diversity index values (*H')* ranged from 1.8 to 2.4; the evenness values (*J'*) from 0.4 to 0.6; Species richness index value (*R'*) ranged between 5.5 and 5.9 by stations. Shannon-Wiener diversity index ranged from 0.44 to 3.47, the evenness ranged from 0.13 to 0.86 and species richness index value ranged between 2.45 and 6.8 was observed in South Adaman (Begum *et al.*, 2012). The diversity value at all station of Burung and Buntal Islands ranged from 1.524 to 2.872 and the degree of evenness index of phytoplankton varived from 0.538 to 0.875 (Huliselan, Tuapattinaja and Pattimura, 2017). From Ariyankuppam estuary and Verampattinam coast, diversity index (3.11-5.38) species evenness (1.13-3.91) and species richness index (0.77-0.99) were reported by Ananthan, et.al, 2008.

Conclusion

In the present study, diatoms were more dominated than dinoflagellates. Then, *Chaetoceros curvisetum, Thalassionema nitzschioides* were common at all stations in October. The maximum species composition of phytoplankton was found at all stations during post-monsoon season (October). The high abundance and composition of phytoplankton species were found in all study areas. The community structure of phytoplankton

reflecting by diversity is showed that the community was in a fair state because the Shannon-Wiener diversity indice is fair value ($2< H'<3$). It can be concluded that the study areas were high productive area for phytoplankton. Therefore, the health of water condition had favorable to growth phytoplankton and to success pearl oysters culture.

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FOOD AND FEEDING HABITS OF CHACUNDA GIZZARD SHAD, *ANODONTOSTOMA CHACUNDA* (HAMILTON-BUCHANAN, 1882) NEAR MYEIK WATER

Si Thu Hein[1], Khin May Chit Maung[2], Nyo Nyo Tun[3]

Abstract

The stomach content of *Anodontostoma chacunda* was studied applying the number and occurrence methods. Eight major food groups were observed in the stomach. The dominant diet in the stomach contents was diatoms. The most dominant items were *Bellerocheta* spp., *Coscinodiscus* spp., *Cyclotella sp*., *Lampricsus sp*., *Navicula* spp., *Nitzschia* spp. *Pleurosigma* spp. *Thalassionema* spp. and *Odontella* spp. Larvae also constituted as the main food items. *Anodontostoma chacunda* was omnivores and pelagic feeders.

Introduction

The study of a fish population's food and feeding habits provides valuable data in fishery biology as it helps to determine species distribution. Stomach content analysis supports important insights into fish feeding habits. The quantitative assessment of food intake is essential for the successful management of a fishery and such studies are important in any fishery research program.

Feeding is the dominant activity of the entire life cycle for fish. Food is the basis of their behaviors such as growth, development, abundance, spawning and migration. The analysis of stomach contents can reveal a wide variation in the food and feeding habits among different groups of fishes found at the bottom or at pelagic layers and among different species within a group (Rao, 1965).

The food items of different fishes vary seasonally and locally. Information about the diet of fish can be obtained by analyzing gut content. The information on feeding strategies reveals size and maturation of fish.

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Food availability also influences the movement of fish stock such as horizontal and vertical movement.

Diet composition of Chacunda gizzard shad was studied by Rahardjo *et al*. in Mayangan coastal waters, west Java in 2006. The present study aimed to identify the food items in the diet of *Anodontostoma chacunda*, to determine the most preferred food items and to obtain knowledge about food and feeding habits. Developing a better understanding of *Anodontostoma chacunda* will help fishery management teams to sustainably select fish for culture and to produce optimum yields by utilizing all potential food sources in local waters.

Materials and Methods

The samples for the present study were collected once a month from the local markets and fish landing sites from February 2015 to September 2015. The food items were identified by following the keys of Tomas (1997) and Conway and White (2003). Unidentified food elements in the contents were assigned as miscellaneous. Total lengths of fishes were measured. The belly of the fish was cut open and the sex, stage of maturity and the condition of the stomach were recorded prior to the removal of the stomach. Fish were divided into two groups as immature and mature according to the maturity stage. Those at Stage I-III were considered as immature and those at stage IV and above were mature. After removing the stomach, it was placed in a Petri dish and cut. Water was added and the stomach content of each fish was made up to a known volume (10 ml). After mixing well, a subsample of 1ml was used for microscopic study.

The intensity of feeding was determined by the degree of fullness of the stomach and expressed as actively fed when the stomach was full or ¾ full, moderate when it was ½ full or ¼ full and poor when the stomach was empty or the contents were very little. The number and occurrence methods (Hyslop, 1980) were employed in the analysis of food elements. The number

of stomachs, in which there were food items were recorded and expressed as a percentage of the total number of stomachs examined as follow:

$$F\% = \frac{J_i}{P} \times 100 \qquad \text{(Hynes; 1950)}$$

where, F = Frequency of occurrence

J_i = number of stomachs containing prey i and

P = total number of stomach with food in the sample.

To estimate the percentage in number of each food item, the number of each individual food type in each stomach was counted and expressed as a percentage of the total number of food items in the samples.

Result

The stomach content of *Anodontostoma chacunda* was studied applying the number and occurrence methods (Fig. 1 and 2). The food items found in the stomach of *A. chacunda* were categorized into eight groups: diatoms, dinoflagellates, blue-green algae, protozoans and other zooplankton, crustaceans, larvae, sponge spicules and seaweeds. The diet predominantly consisted of phytoplankton which accounted for 58.1% of the average stomach contents, followed by zooplankton (41.5%) and relatively small quantities of seaweeds (0.3%) and sponge spicules (0.1%) as seen in Fig.3. Phytoplankton food items could be differentiated into three groups as following: (1) diatoms (56%), (2) dinoflagellates (2%) and (3) blue-green algae (0.2%). Zooplankton consisted of larvae (27.6%), protozoans and other zooplankton (12.1%) and crustaceans (1.8%). Partially digested phytoplankton and digested food, such as fragmented carapaces, legs and antennae of zooplankton, were also present but were not included in the diet analysis.

Figure 1. *Anodontostoma chacunda*

Figure 2. Stomach of *Anodontostoma chacunda*

Percentage in numbers

Percentages in number of different food items observed in the stomachs for different months are shown in Table 1 and monthly percentage compositions of different food items are presented in Fig.4. In general, diatoms were the most common food items of *A. chacunda* during the present study period. The percentage in number of diatoms was highest in March (90.7%) and the lowest in June (16.4%). Among the different taxa, *Bellerocheta* spp., *Coscinodiscus* spp., *Cyclotella* sp., *Lampricsus* sp., and *Odontella* spp. were observed throughout the study period.

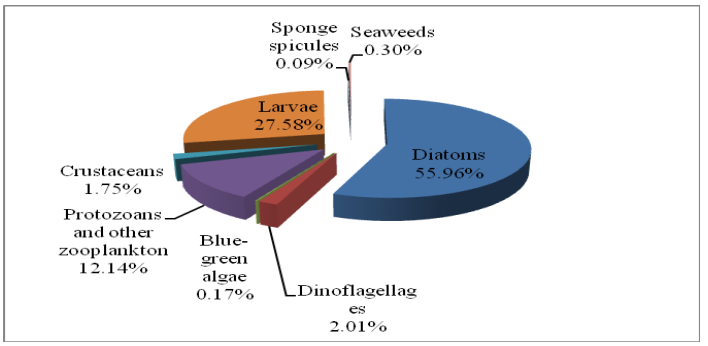

Figure 3. Average percentage compositions of different food items

Dinoflagellates formed a minor portion of the food that occurred during the study period. Their highest percentage (9.8%) in number was in May and the lowest was in March and April. All of the taxa of dinoflagellates found in the stomach were different in the present study. *Ceratium* spp. occurred than more frequently any other dinoflagellate species and *Protoperidinium* spp. occurred second most frequently.

Only one taxon of blue green algae, *Anabaena sp.*, was observed in February, May and June in this study. In these three months, the percentages in number were 0.5%, 0.7% and 0.2% respectively. Overall quantities in the food of *A.chacunda* were very small.

The recorded zooplanktons were categorized in groups such as crustaceans, protozoans and larvae. Four genera of protozoans, *Favella sp.*, *Globigerina* spp., *Leprotintinnus sp.* and *Tintinnopsis* spp. and three taxa of zooplankton *Archnatis sp.*, *Balanus sp.*and *Bolivina sp.* were observed in the present study. They were observed throughout the study period. The highest percentage (56.4%) in number was recorded in February and the lowest (2.6%) in April. Among this group, *Globigerina* spp. were recorded throughout the study except for June and its peak was recorded in February.

The main components of crustaceans were copepods. All of the copepods species, copepod larvae and mysids were found throughout the study period except September. The maximum percentage (5.6%) was recorded in May and the minimum (0.2) was in February. Copepod species were found nearly all months but mysid occurred only in March and May. This item also included the larvae of bivalves, gastropods, worms, crabs and polychaetes. They occurred throughout the study period. On average, it represented the second most abundant food item in the food of *A. chacunda*. Bivalve larvae and worm were observed in almost all months. The highest percentage for this food item was mollusc larvae. Its highest peak (77.1%) was recorded in June and the second highest (67.6%) was in September. Crab larvae were found only in June and gastropod larvae were found only in August.

Sponge spicule were only found in March with a April and its percentage of 0.5% and 0.2% respectively. It was a minor element in the food of *A. chacunda.* Some pieces of seaweed were observed in May and July and their percentage was 0.7% and 1.7 % respectively. It was also a minor component of food items.

Frequency of occurrence

The monthly percentage for frequency of occurrence of different food items of *A. chacunda* is shown in Table 2. In diatoms, the monthly percentage for frequency occurrence of *Coscinodiscus* spp., *Cyclotella sp.*, *Bellerocheta* spp., *Lampricsus sp.* and *Odontella* spp. were in the range of 55.6-100%, 33.3-100%, 10 -100%, 20-100% and 77.8-12.5% respectively. *Melosira sp*. and *Pleurosigma* spp. occurred with a range of 11.1-88% and 12.5-100% but both items were not observed in May. *Navicula* spp., *Nitzschia* spp. and *Surirella sp.* were 11.1-66.7%, 22.2-88.9% and 11.1-70% respectively but these items were not found in September. The percentage of frequency of occurrence of *Thalassionema* spp. varied between 40% and 100% except for August. *Campylodiscus sp*., *Chaetoceros* spp., *Diploneis sp*. and *Teberella sp*.

were only observed during three months with the percentage of frequency of occurrence ranging between 11.1-25%, 22.2-66.7%, 10-33.3% and 11.1-44.4% respectively. The monthly percentage for frequency of occurrence of *Hemiaulus sp*. was 10-33.3% and *Paralia sp*. was 11.1-60% for five months. *Rhizosolenia* spp. ranged between 11.1-77.8% and *Triceratium sp*. was 12.5-80% for four months. *Bacillaria sp*., *Laudaria sp*., *Lucosolenia sp*. *Guinardia sp*., *Helicotheca sp, Proboscia sp*. and *Eucampia sp*. were found in only two months with the frequency of occurrence ranging between 11.1-20%, 11.-66.7%, 20-25%, 11.1-44.4%, 20-22%, 20-22% and 11.1-22.2% respectively. *Gyrosigma sp*., *Planktoniella sp*., *Climacosphenia sp.* and *Staroneis sp*. occurred only in one month and represented 10, 22.2%, 33.3% and 44.4% of percentage of frequency occurrence respectively. The percentages of frequency of occurrence of the remaining diatoms were 11.1%.

The percentages of frequency occurrence of the dinoflagellates were low. Among dinoflagellates, *Ceratium* spp. was more observed than other dinoflagellate species with a percentage between 12.5% and 33.3%. The second most observed dinoflagellates were *Protoperidinium* spp. with the range of 10-88.9. *Diplopsalis sp*. *Gonyaulux sp.* and *Prorocentrum sp*. were only found in two months with their frequency ranging from 20-33.3%, 11.1-12.5% and 22.2-33.3% respectively. The rest of the dinoflagellate species were found in only one month and their percentages were lower, than 22.2%. One species of blue-green algae, *Anabaena sp*., varied from 0.2-0.7% and it was found in February, May and June.

In protozoans and other zooplanktons, the monthly percentage of frequency of occurrence of *Globigerina* spp. varied between 20-60% except July and *Tintinnopsis* spp. were between 11.1-100% but not found in May and September. *Balanus sp. Flavella sp.* and *Bolivina sp.* were observed only in four months with the percentage of frequency of occurrence of 12.5-44.4%, 11.1-20% and 11.1-77.8% respectively. The frequency of occurrence of

Archnatis sp. was 33.3% in February and 90% in August. *Leprotintinnus sp.* occurred only in March and represented 11.1% of frequency of occurrence. The percentage of frequency of occurrence of Crustaceans species varied from 11.1% to 70% except September. Among larvae, frequency of occurrence of 33.3-100% of worms and 20-80% of bivalve larvae were observed more than for other larvae. Gastropod larvae, crab larvae and polychaete larvae were found in only one month and represented 10, 12.5 and 33.3 respectively. In the stomach of *A. chacunda* 11.1% of seaweeds in March and April and sponge spicules 11.1% in May and 20% in July were also observed.

Feeding intensity

The sampled fish stomachs were classified as actively fed, moderately fed and poorly fed based on the fullness of the stomach content. The monthly percentage occurrence of different conditions is shown in Fig.5. The percentage of actively fed ranged from 10 to 50% and was absent in April, July and September. The percentage of fish in the moderately fed group fluctuated in all months and reached its highest (100%) in April and September. Poorly fed fish fluctuated from 0% in, April, June, August and September to 25% in February.

The percentage of stomach contents in various forms of fullness was studied to determine the relationship between feeding intensity and size groups (Fig.6). Most of the size group of 13.1-14cm was observed in the moderately fed category and 16.1-17cm was only in the actively fed category. Only the size group of 14.1-15cm was found in the poorly fed category. The percentage numbers of actively fed, moderately fed and poorly fed in 14.1-15cm were 17.4, 60.9 and 21.7 respectively.

The percentage of occurrence of stomachs in various degrees of fullness was conducted to understand the relationship between feeding intensity and sexual cycle (Fig.7). The percentage among actively, moderately and poorly fed in both immature and mature fish categories did

not vary greatly and more than 71% of both fish categories were classified as moderately fed.

Table 1. Monthly percentage in number of different food items found in the stomach of *Anodontostoma chacund*

	Feb	**March**	**April**	**May**	**June**	**July**	**Aug**	**Sep**
Diatoms								
Actinocyclus sp.		0.2						
Bacillaria sp.			0.2	0.7				
Bacteriastrum sp.				0.5		0.1		
Bellerocheta spp.	6.3	4.2	4.0	0.5	1.8	1.9	0.1	0.8
Campylodiscus sp.		0.1	0.2		0.3			
Certualina sp.		0.1		0.9				
Chaetoceros spp.	0.1			1.6		0.6		
Climacosphenia sp.	0.1							
Coscinodiscus spp.	7.3	2.3	8.1	6.3	3.9	15.6	24.6	18.7
Cyclotella sp.	1.9	4.9	2.0	4.2	0.7	2.3	14.6	0.3
Diploneis sp.		0.3	0.5				0.1	
Ditylum sp.		0.1				0.4		
Eucampia sp.		0.1		1.6				
Fragillaria sp.						0.1		
Guinardia sp.		0.1		10.0				
Gyrosigma sp.							0.5	
Haslea sp.		0.1						

	Feb	**March**	**April**	**May**	**June**	**July**	**Aug**	**Sep**
Helicotheca sp.		0.1	1.8					
Hemiaulus sp.		0.7	0.7	5.8		0.1	0.4	
Lampricsus sp.	6.9	8.6	13.9	19.1	4.5	2.6	0.4	4.3
Laudaria sp.				0.5		4.7		
Lucosolenia sp.					0.3			1.0
Melosira sp.	0.2	0.1	2.5		0.1	5.2	0.9	0.1
Navicula spp.	0.5	23.7	6.0	0.7	1.1	1.1	1.5	
Nitzschia spp.	2.2	21.4	4.0	3.7	0.2	0.8	12.5	
Odontella spp.	0.6	4.3	2.7	4.9	0.1	2.0	0.6	0.7
Paralia sp.		0.1	0.5		0.3		2.4	1.0
Pinnularia sp.		0.1						
Planktoniella sp.						0.6		
Pleurosigma spp.	4.0	10.1	3.8		0.1	8.4	1.8	0.3
Proboscia sp.		0.6	0.5					
Rhizosolenia spp.	0.6	2.1		11.4		0.2		
Staroneis sp.		2.8						
Surirella sp.	0.4	1.5	2.2	0.2	0.2	0.6	4.3	
Teberella sp.		0.1	4.7			1.0		
Thalassionema spp.	8.7	1.3	13.0	4.2	1.8	6.9		1.1
Thalassiosira spp.		0.1		0.7				
Triceratium sp.		0.5			1.0	0.8	3.4	

	Feb	**March**	**April**	**May**	**June**	**July**	**Aug**	**Sep**
Dinoflagellates								
Alexandrium sp.			0.2					
Ceratium spp.	0.1	0.2		7.0	0.2		0.4	
Dinophysis sp.				1.4				
Diplopsalis sp.	0.1							0.3
Gonyaulux sp.					0.1	0.1		
Prorocentrum spp.	0.4			1.4				
Protoperidinium spp.					0.3	2.8	0.1	0.8
Pyrophacus sp.						0.2		
Blue green algae								
Anabaena sp.	0.5			0.7	0.2			
Protozoans and other zooplankton								
Archnatis sp.	0.1						5.0	
Balanus sp.			0.2		0.1	0.4	1.3	2.4
Bolivina sp.		1.4	1.3		2.5	1.9	1.5	
Favella sp.			0.2	0.7	0.1			0.6
Globigerina spp.	55.7	1.3	0.7	4.4		0.2	1.8	0.1
Leprotintinnus sp.		1.6						
Tintinnopsis spp.	0.6	0.1	0.2		0.1	8.4	1.8	
Crustaceans								
Copepod larvae	0.2			1.4				

	Feb	**March**	**April**	**May**	**June**	**July**	**Aug**	**Sep**
Copepods		1	2.0	3.5	0.4	2.6	2.6	
Mysid		0.1		0.7				
Larvae								
Bivalve larvae			17.2	0.7	77.1	17.3	9.9	67.6
Gastropod larvae							0.4	
Crab larvae					0.1			
Polychaete larvae	0.1							
Worms	2.4	3.6	6.5		2.5	8.3	7.1	
Sponge spicules		0.5	0.2					
Seaweeds				0.7		1.7		

Table 2. Monthly percentage of frequency of occurrence of different food items found in the stomach of *Anodontostoma chacunda*

	Feb	**March**	**April**	**May**	**June**	**July**	**Aug**	**Sep**
Diatoms								
Actinocyclus sp.		11.1						
Bacillaria sp.			20	11.1				
Bacteriastrum sp.				11.1		11.1		
Bellerocheta spp.	100	88.9	40	11.1	25	88.9	10	20
Campylodiscus sp.		11.1	20		25			
Certualina sp.		11.1		11.1				
Chaetoceros spp.	66.7			22.2		22.2		

	Feb	**March**	**April**	**May**	**June**	**July**	**Aug**	**Sep**
Climacosphenia sp.	33.3							
Coscinodiscus spp.	100	88.9	80	55.6	75	100	100	60
Cyclotella sp.	33.3	66.7	60	33.3	50	55.6	100	20
Diploneis sp.		33.3	20				10	
Ditylum sp.		11.1				11.1		
Eucampia sp.		11.1		22.2				
Fragillaria sp.						11.1		
Guinardia sp.		11.1		44.4				
Gyrosigma sp.							10	
Haslea sp.		11.1						
Helicotheca sp.		22.2	20					
Hemiaulus sp.		33.3	20	33.3		11.1	10	
Lampricsus sp.	66.7	100	60	66.7	25	77.8	20	20
Laudaria sp.				11.1		66.7		
Lucosolenia sp.					25			20
Melosira sp.	33.3	11.1	20		12.5	88.9	40	20
Navicula spp.	66.7	33.3	60	11.1	50	44.4	40	
Nitzschia spp.	33.3	88.9	60	33.3	25	22.2	70	
Odontella spp.	66.7	55.6	60	22.2	12.5	77.8	30	20
Paralia sp.		11.1	40		12.5		60	20
Pinnularia sp.		11.1						
Planktoniella sp.						22.2		
Pleurosigma spp.	66.7	100	60		12.5	44.4	50	40
Proboscia sp.		22.2	20					
Rhizosolenia sp.	66.7	33.3		77.8		11.1		
Staroneis sp.		44.4						
Surirella sp.	33.3	55.6	40	11.1	12.5	44.4	70	
Teberella sp.		11.1	20			44.4		

	Feb	**March**	**April**	**May**	**June**	**July**	**Aug**	**Sep**
Thalassiosira spp.		11.1		11.1				
Triceratium sp.		44.4			12.5	44.4	80	
Dinoflagellates								
Alexandrium sp.			20					
Ceratium spp.	33.3	22.2		22.2	12.5		20	
Dinophysis sp.				22.2				
Diplopsalis sp.	33.3							20
Gonyaulux sp.					12.5	11.1		
Prorocentrum sp.	33.3			22.2				
Protoperidinium spp.					25	88.9	10	20
Pyrophacus sp.						11.1		
Blue green algae								
Anabaena sp.	66.7			11.1	12.5			
Protozoans and other zooplankton								
Archnatis sp.	33.3						90	
Balanus sp.			20		12.5	44.4	40	20
Bolivina sp.		11.1	40		50	77.8	50	
Favella sp.			20	11.1	12.5			20
Globigerina spp.	33.3	55.6	20	22.2		22.2	60	20

	Feb	**March**	**April**	**May**	**June**	**July**	**Aug**	**Sep**
Leprotintinnus sp.		11.1						
Tintinnopsis spp.	100	11.1	20		12.5	100	30	
Crustaceans								
Copepod larvae	66.7			11.1				
Copepods		33.3	60	66.7	12.5	55.6	70	
Mysid		11.1		11.1				
Larvae								
Bivalve larvae			60	11.1	50	55.6	80	20
Gastropod larvae							10	
Crab larvae					12.5			
Polychaete larvae	33.3							
Worms	100	33.3	80		50	77.8	90	
Sponge spicules		11.1	20					
Seaweeds				11.1		11.1		

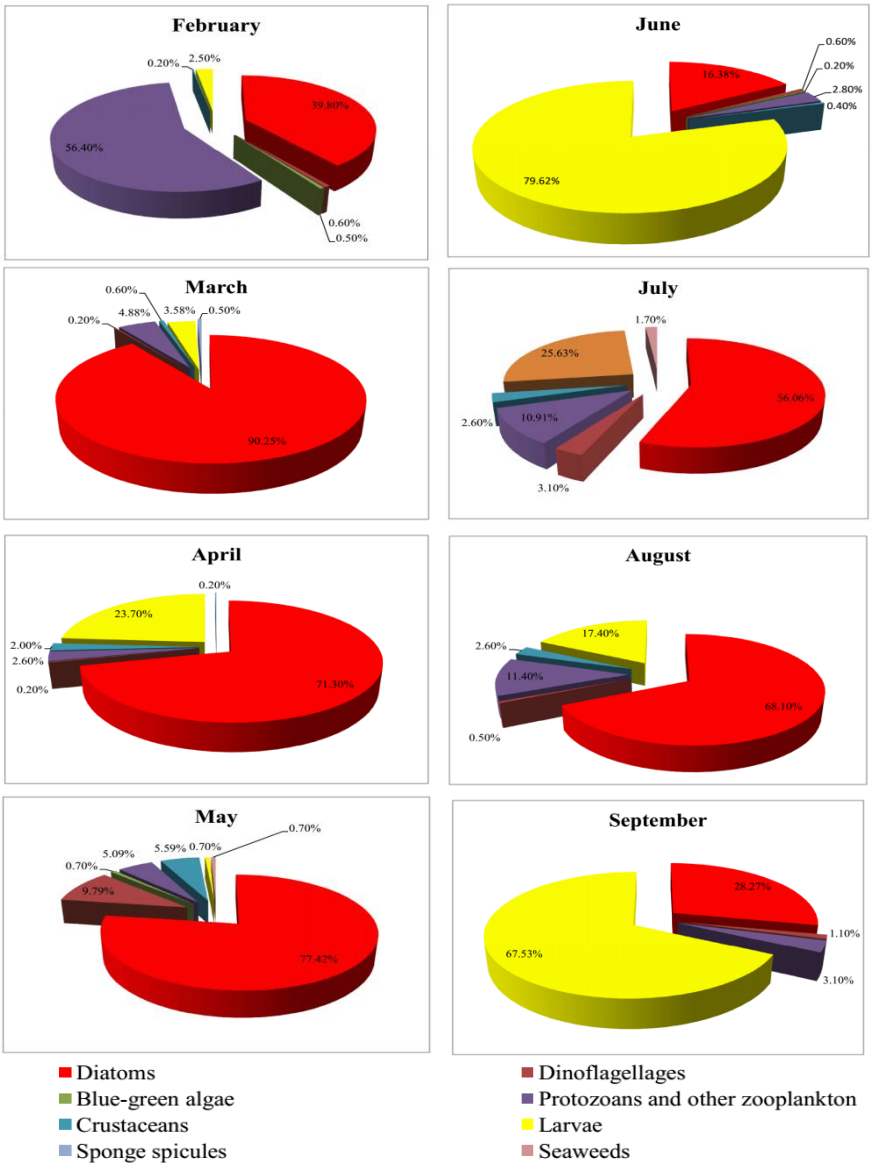

Figure 4. Monthly percentage composition of different food items

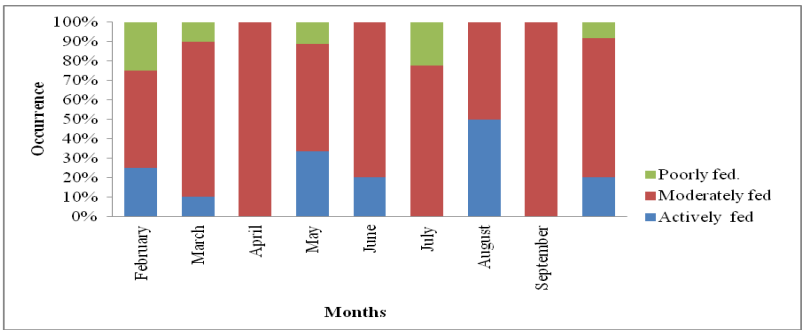

Figure 5. Monthly percentage occurrence of feeding intensity

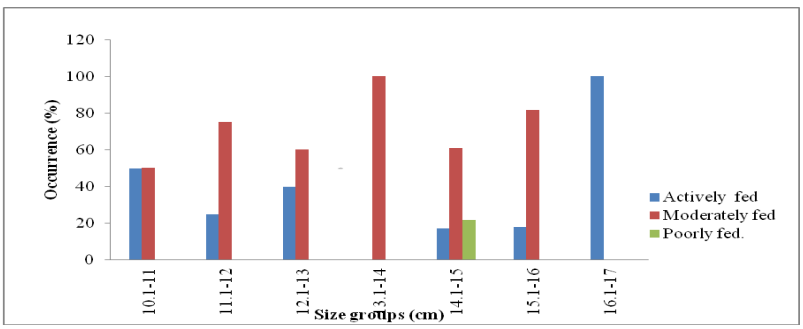

Figure 6. Feeding intensity condition in size groups

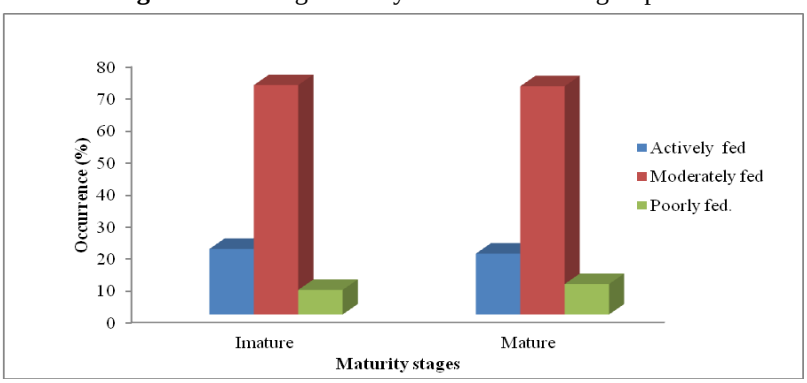

Figure 7. Feeding intensity condition in maturity stages

Discussion

There were eight major food groups found in the stomach contents of *Anodontostoma chacunda*. Diatoms, dinoflagellates, protozoans and larvae were recorded in all months of the study period. Diatoms appeared as the dominant component of phytoplankton in the present study. The percentages of diatoms varied between 16.4% and 90.7% of the total food items. The most dominant diatoms were *Bellerocheta sp.*, *Coscinodiscus* spp., *Cyclotella sp.*, *Lampricsus sp.*, *Navicula* spp., *Nitzschia* spp. *Pleurosigma* spp. *Thalassionema* spp. and *Odontella* spp.

Rahardjo *et al.*, 2006 reported that the dominant diets in the stomach contents of *A. chacunda* were *Coscinodiscus, Pleurosigma,* and *Rhizosolenia* and this supports current data which finds that this fish species prefers *Coscinodiscus, Pleurosigma, Rhizosolenia Gyrosigma, Melosira, Navicula* and copepod. All of these food items were recorded in the sampled stomachs during the study period.

Jambo and Maduako, 2015 showed that the small size group had the highest number of empty stomach and the large size group had no empty stomachs. However, in the present study, empty stomachs were not found in the small size group. The fullness degree insignificantly differed because the feeding behavior was varied from size to size, time to time, and actually it positively correlated to the occurrence and the richness of natural food components (Abdel-Tawwab *et al.* 2004).

Seasonal variation of different food items showed that diatoms formed the most important food items during the study period. Regarding the seasonal variation in the feeding intensity as an index of stomach fullness, Taghavi *et al.*, 2012 reported the maximum number of empty stomachs was recorded during summer season. This is consistent with the findings in the present study. Madkour, 2011 reported that the copepod density was higher during the summer and comparatively low during winter in comparison to other seasons.

In the present observation of total food items, 0.2 % of copepod larvae were found in February and 3.5% of copepod was in May. Small planktonic marine copepods play an important role in pelagic marine food web by serving as prey for icthyoplanktons and other large pelagic carnivores (Nath *et al.*, 2015). Jeyaseelan and Krishnamurthy 1980 stated that *A. chacunda* was omnivore species and it fed on plant and detritus. Therefore, this fish species considered as omnivorous because the gut contents of which contained an appreciable amount of both plant and animals matter in the present study.

Conclusions

The gizzard shad, *A. chacunda*, fed on a variety of food categories. Diatoms and larvae constituted the main food items in the stomach content. According to the results of percentage in number and frequency of occurrence, *A. chacunda* fed on both phytoplankton and zooplankton. Thus they can be considered as omnivores and pelagic feeders. The occurrence of some food items was so low in the stomach contents that it probably was due to accidental consumption. In order to determine if these other food intakes may represent a significant supplementary dietary feature in the food of *A. chacunda*, more research is required. The present observations can be used for further assessment of food and feeding habits of *A. chacunda* and is valuable when then species is selected for culture.

Acknowledgements

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SCREENING OF MARINE ENDOPHYTIC FUNGI ISOLATED FROM SOME SEAGRASSES LEAVES AND THEIR ANTIBACTERIAL ACTIVITIES ON *MICROCOCCUS LUTEUS* NITE83297

Khin Thandar Linn[1]

Abstract

The isolation of endophytic fungi and antibacterial activities of some seagrasses species such as *Halophila ovalis* (R.Brown) Hooker.f, *Thalassia hemprichii* (Ehrenberg) Ascherson and *Syringodium isoetifolium* (Ascherson) Danty were used for the investigation. The samples of seagrasses were collected from (Lat 17˚04'20.36" N and Long 94˚27'08.01"E), Magyi coastal area, Shwe Thaung Yan Sub-township which is located at lower Rakhine Coast. The experimented seagrass samples were stored as herbarium sheets in Marine Science Department, Pathein University. Screening of marine endophytic fungi from seagrass species was carried out by Washing Method . A total of 12 marine endophytic fungi were isolated in this study, 4 different fungi from *Halophila ovalis*, 5 different fungi from *Thalassia hemprichii* and 3 from *Syringodium isoetifolium*. Paper disc diffusion assay method was carried out in the investigation of antibacterial activities. Among the isolation of endophytic fungi, 7 endophytic fungi named KF-01, KF-03, KF-06, KF-07, KF-08, KF-10 and KF-11 showed the antibacterial activities on *Micrococcus luteus* NITE83297.

Key words: antibacterial activities, endophytic fungi, *Halophila ovalis, Micrococcus luteus, Syringodium isoetifolium, Thalassia hemprichii*.

Introduction

Endophytes are microorganisms that can produce the chemical inside the plants (Owen and Hundley 2004). Many of them are capable of synthesizing bioactive compounds that can be used as potential sources of pharmaceuticals leads. Endophytic fungi have been proven useful for novel drug discovery as suggested by the chemical diversity of their secondary

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metabolites. Many endophytic fungi have been reported to produce novel antibacterial, antifungal, antiviral, anti-inflammatory, antitumor, and other compounds (Guo et *al*., 2008, Yu et *al*., 2010). Endophytes are universally present in the world as higher plants, so it was reasoned that plants might support certain endophytic microorganisms that could synthesize important phytochemicals of medicinal plants as well as the plant itself (Strobel,2003).

Plants can be considered as a new isolation source of microorganisms. Among the plants species, seagrasses are also a suitable host for a variety of endophytes. Seagrasses are angiosperms which grow in shallow saline water in tropical marine coastal areas. There are 13 genera and 58 species available all over the world. However, fewer studies have been done on endophytes in seagrasses as difficult to assess and available for seagrasses (Ravikumar et *al*., 2005).

In Myanmar, seagrasses as a relatively small group of flowering plants distributes along the shallow saline coastal regions particular in Rakhine coast and southern Myeik Archipelago. There are 10 species of seagrasses were recorded in Myanmar, according to U Soe Tun, 2016. Seagrasses are also a rich source of secondary metabolites. It is now well documented that endophytic fungi are a good source of bioactive natural products. Most of the studied endophytic fungi have been isolated from terrestrial plants. Bioactive natural products from endophytic fungi from marine plants in particular from seagrasses have been rarely studied (Preuttipon et *al*., 2014).Therefore, the isolation of endophytic fungi from *Halophila ovalis* (R.Brown) Hooker.f, *Thalassia hemprichii* (Ehrenberg) Ascherson and *Syringodium isoetifolium* (Ascherson) Danty from lower Rakhine Coast has been carried out and antibacterial activity of those endophytic fungi were also studied.

Materials and Methods

Sample Collection

Seagrass species such as *Halophila ovalis* (R.Brown) Hooker.f, *Thalassia hemprichii* (Ehrenberg) Ascherson and *Syringodium isoetifolium* (Ascherson) Danty were collected from Magyi coast (Lat 17˚04′ N and Long 94˚27′ E)**,** Shwe-Thaung-Yan Sub-Township, Pathein Township, Ayeyarwady Region. The seagrasses were collected at low tide time of spring tide period as the plants are mostly growing at the subtidal region. At low tide, the plant samples were collected by hand and carried by ice box with natural seawater. In the laboratory, the collected samples were washed thoroughly by using seawater to remove epiphyte, sand, mud and debris for the observation and identification. The samples were preserved with 4% formalin and prepared for herbarium and wet-stacked specimens. These specimens are deposited at the Herbarium of Department of Marine Science, Pathein University and identify based on the external morphologies of vegetative features as compared with other related voucher materials also housed in the same Herbarium. The external morphologies of these specimens were photographed by using a digital camera and the fresh samples were then used to isolate the microorganisms in the laboratory to get superior endophytes.

Figure 1. Map showing the location of sample collecting area

Screening of Marine Endophytic Fungi From Seagrasses

Seagrasses *Halophila ovalis* (R.Brown) Hooker.f, *Thalassia hemprichii* (Ehrenberg) Ascherson and *Syringodium isoetifolium* (Ascherson) Danty were employed for the isolation of endophytic fungi. The isolation was carried out by washing method (Inaba and Ando, 2002). Firstly, the leaves, rhizomes and roots of seagrasses were rinsed with tap water for 15 minutes in the laboratory. The plant parts (leaves and rhizome) were then cut into pieces and then again rinsed by 95% ethanol and 1% sodium hypochloride solution for 5 minutes. It was then washed by 95%ethanol for 15 seconds. Finally, the plants were then stirred and washed with sterile water on vortex mixer. Then, the samples were dried on sterilized filter paper. The dried samples were then cut at the edge on glass plate and placed onto the agar medium. The agar plates were incubated for 3-7 days at 27˚C according to the range of fungi, they start growing on LCA medium. The endophytic fungi were stored in slant culture using potato dextrose agar medium (PDA). After autoclaving chloramphenicol and Penicillin-G were added to the medium.

LCA medium (Ando, 2004)	
Glucose	1.0 g
Sucrose	0.5 g
Yeast Extract	1.0 g
K_2HPO_4	0.2 g
$MgSO_4.7H_2O$	0.05 g
KNO_3	0.1 g
KCl	0.05 g
Agar	1.8 g
DW	100 mL
pH	6.5

PDA medium (Ando, 2004)	
Potato	20 g
Dextrose	1.5g
Agar	1.8 g
DW	100 mL

Antibacterial Activity by Paper Disc Diffusion Assay

Screening (or) Preliminary study for antimicrobial activities was carried out by paper disc diffusion assay (Tomita, 1988). Paper disc having eight millimeter diameter were used for antimicrobial assays. The isolated fungi were grown on GSY medium for 7 days at 25°C for sporulation. Then isolated fungi were inoculated on seed medium and incubated at 25°C for 3 days. Then, 20 mL of seed culture was transferred again into the fermentation medium and fermentation was undertaken at 25°C for 7 days. After that, fermented broth (20µl) was put onto the paper discs (8.0 mm) and allowed to dry. The dry paper discs were placed on assay plates containing test organisms for 24 hours. The assay medium was used for the antimicrobial activity test and one percent of test organism was added to assay medium, then poured into plates. After solidification, paper discs impregnated with samples (fermented broth) were applied on the agar plates and the plates were incubated for 24-36 hours at 28 to 30°C. The clear zones (inhibitory zones) surrounding the test discs can be seen in the next day indicating the presence of bioactive metabolites which inhibit the growth of test organisms.

Seed medium	
BR-BDC-Screening Media (2004)	
Glucose	2.0 g
Polypepton	0.3 g
KNO_3	0.1 g
K_2HPO_4	0.01 g
DW	100 mL
pH	6.5

Fermentation medium	
BR-BDC-Screening Media (2004)	
Glucose	2.0 g
Yeast Extract	0.8 g
K_2HPO_4	0.001 g
$MgSO_4$	0.001 g
$CaCO_3$	0.1 g
DW	100 mL
pH	6.5

Assay medium	
BR-BDC-Screening Media (2004)	
Glucose	1.0 g
Polypepton	0.3 g
KNO_3	0.1 g
Agar	1.8 g
DW	100 mL
pH	6.5

Results

Identification key to the species of seagrasses from Myanmar (U Soe Htun, 2016)

1a. Leaf blade cylindrical----------1. *Syringodium isoetifolium*
1b. Leaf blade flat----------2
2a. Leaves strap-shaped----------3
2b. Leaves paddle-shaped----------8
3a. Leaves with ligula----------4
3b. Leaves without ligula----------7
4a. Leaf tip without serration----------2. *Cymodocea rotundata*
4b. Leaf tip with serration----------5
5a. Leaves greater than 3mm wide----------3. *C.serrulata*
5b. Leaves less than 3mm wide----------6
6a. Leaf tip tridentate without secondary teeth; leaf blade more than 1mm wide----------4. *Halodule uninervis*
6b. Leaf tip with many secondary teeth; leaf blade less than 1mm wide----------5. *H. pinifolia*
7a. Leaves 10-15mm wide----------6. *Enhalus acoroide*
7b. Leaves 4-10 mm wide----------7. *Thalassia hemprichii*
8a. Leaves linear to lanceolate, 1-3 mm wide with 1-3 paralleled veins----------8. *Halophila beccarii*
8b. Leaves lanceolate to oblong, 3-10mm wide with 6-17 cross- veins----------9
9a. Leaf margin serrulate----------9. *H.decipiens*
9b. Leaf margin entire----------10. *H.ovalis*

***Halophila ovalis* (R.Brown) Hooker f.**

Division Anthophyta
Class Monocotyledoneae
Order Helobiae
Family Hydrocharitaceae
Genus *Halophila* Du Petit-Thouars
Species *Halophila ovalis* (R.Brown) Hooker f.
Common name- Paddle weed, spoon grass or dugong grass.

Description - Plants small; rhizomes less than 1mm in diameter, internodes 1.8-2.4cm long; erect shoot at each node, bearing a pair of petiolated leaves; leaf blades lanceolate to obovate or elliptic;1.5-2.2cm long 7-10mm wide, margin entire, apex obtuse, base rounded, petiole 2,2-3.0cm long, midrib prominent with 14-17 cross-veins.

***Thalassia hemprichii* (Ehrenberg) Ascherson**

Family Hydrocharitaceae

Genus *Thalassia* Banks and Solander ex Konig

Species *Thalassia hemprichii* (Ehrenberg) Ascherson

Common name- Turtle grass

Description - Plants moderate in size; intervals of internode 1.9-9.0 cm long; rhizome creeping, leaf blade linear, 1.5-15.0 cm long, 1.5-2.0 mm wide, leaf-tip obtuse, sometime serrulate,2-6 leaves, a thick rhizome prominently marked by several shoot scars between erect shoots.

***Syringodium isoetifolium* (Ascherson) Danty**

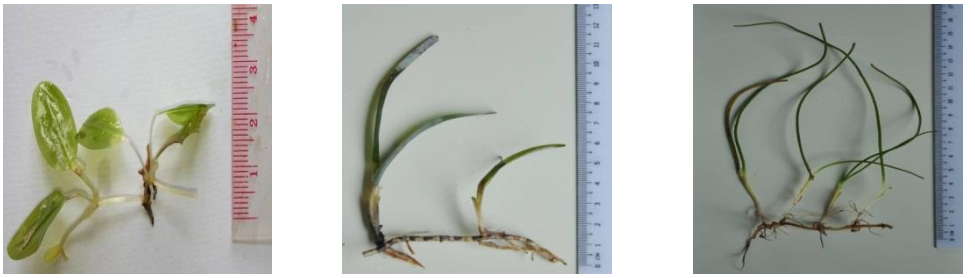
Family Cymodoceaceae

Genus *Syringodium* Kutzing

Species *Syringodium isoetifolium* (Ascherson) Danty

Common name- Noodle grass

Description - Plants erect; rhizome 1mm thick, with internodes, 1.4-2.5 cm long; each node giving a shoot with 1-3 leaves; leaves terete, tapering to the tip, 5.5-12.5 cm in length, 1mm wide, base covered by leaf sheath, 1-3 cm long.



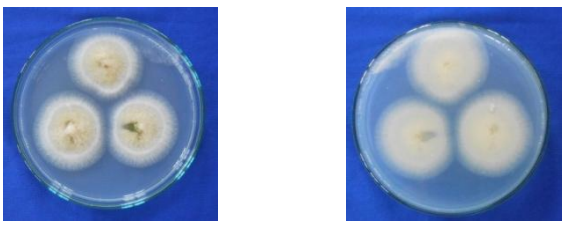
(A) (B) (C)

Figure 2. Morphologies of collected seagrasses ; **(A)** *Halophila ovalis* (R.Brown) Hooker f. **(B)** *Thalassia hemprichii* (Ehrenberg) Ascherson and **(C)** *Syringodium isoetifolium* (Ascherson) Danty

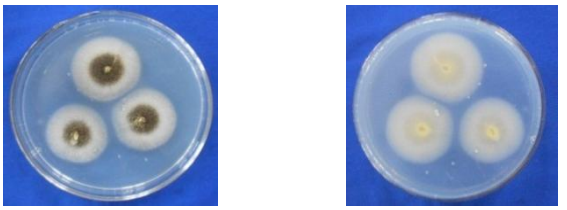
Screening of Marine Endophytic Fungi from Seagrasses

In the study of isolation of endophytic fungi, a total of 12 different fungi were isolated from seagrasses, 4 different fungi namely KF-01, KF-02, KF-03, KF-04 from *H.ovalis*, 5 different fungi from *T.hemprichii* namely KF-05, KF-06, KF-07, KF-08, KF-09 and 3 different fungi namely KF-10, KF-11, KF-12 from *S.isoetifolium*.

KF-01



KF-02



KF-03

KF-04

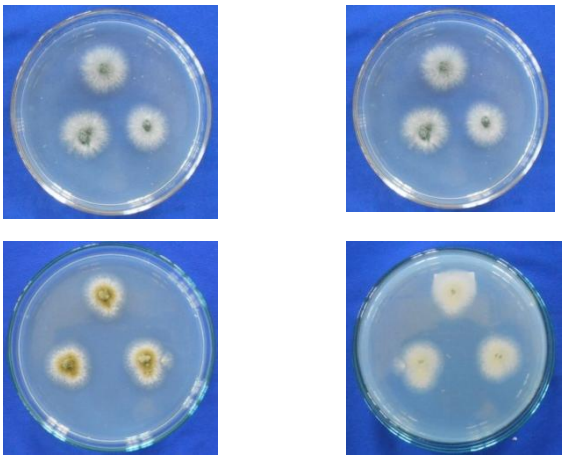
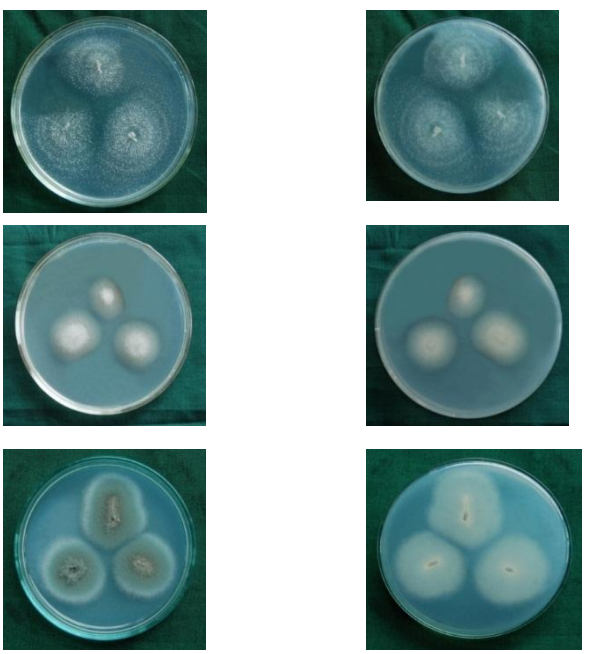


Figure 3. Morphologies of front view and back view of endophytic fungi from *H.ovalis* (KF-01, KF-02, KF-03 and KF-04)

KF-05

KF-06

KF-07

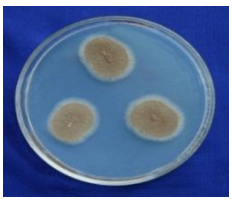


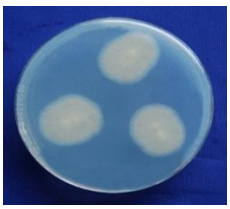
KF-08

KF-09

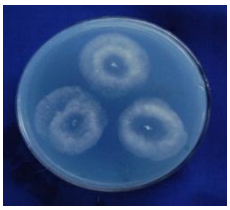
Figure 4. Morphologies of front view and back view of endophytic fungi from *T.hemprichii* (KF-05, KF-06, KF-07, KF-08 and KF-09)

KF-10





KF-11



KF-12

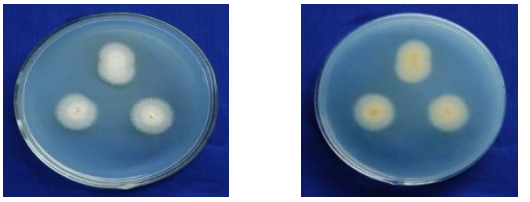


Figure 5. Morphologies of front view and back view of endophytic fungi from *S.isoetifolium* (KF-10, KF-11 and KF-12)

Antibacterial Activities by Paper Disc Diffusion Assay

In the investigation into antimicrobial activities of isolated fungi, KF-01, KF-03, KF-06, KF-07, KF-08, KF-10 and KF-11 showed the activities *Micrococcus luteus* NITE 83297. KF-01 (18.42 mm), KF-03 (21.06 mm), KF-06 (14.57 mm) , KF-07 (21.96 mm), KF-08 (21.72 mm), KF-10 (15.48 mm) and KF-11 (22.53 mm) against respectively.

KF-01

KF-03

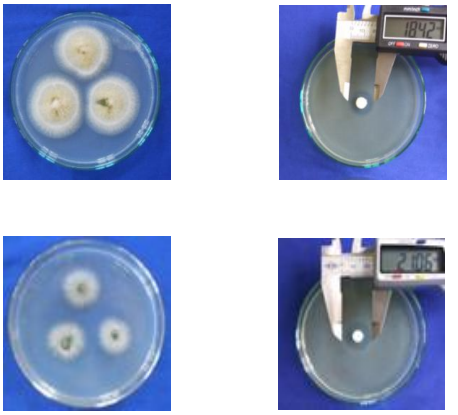


Figure 6. Morphologies and antibacterial activities of isolated endophytic fungi KF-01 and KF-03 from *H.ovalis* against on *M. luteus* NITE 83297

KF-06

KF-07

KF-08

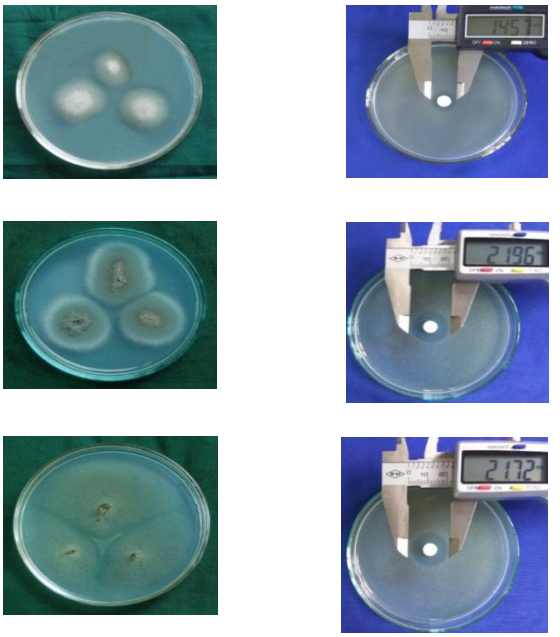


Figure 7. Morphologies and antibacterial activities of isolated endophytic fungi KF-06 ,KF-07 and KF-08 from *T.hemprichii* against on *M. luteus* NITE 83297

KF-10

KF-11

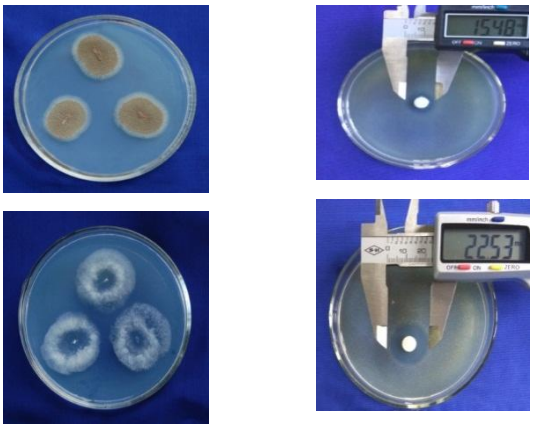


Figure 8. Morphologies and antibacterial activities of isolated endophytic fungi KF-10 and KF-11 from *S.isoetifolium* against on *M.luteus* NITE 83297

Discussion

A variety of medicines and chemical are also prepared from seagrasses and their associates. However, endophytic fungi from seagrasses have been rarely studied. Devarajan et *al*., 2002 stated that some endophytes have been isolated from the leaves of *Halophila ovalis*, *Zostera marina*, *Z. japonica*, *Thalassia testudinum* and *Posidonia oceanica* and from the rhizomes/roots of *H. ovalis* and *Halodule wrightii*. Preuttiporn et *al*.,2014 also reported that seagrasses have been used in traditional medicine in India: roots of *Enhalus acorides* have been applied as a remedy against stings from different kinds of rays and scorpions, *Cymodocea* spp has been used as a tranquillizer for babies, or for soothing help during pregnancy and against coughs and even malaria and some *Halophila* spp produce a strong traditional preparation that can act against malaria, skin diseases and the early stages of leprosy. Ravikumar, S. et *al*., 2010 showed that a new trend in drug discovery from natural sources (like marine seagrasses) emphasize on the investigation of marine ecosystem to explore numerous complex and novel chemical entities. In this study twelve different endophytic fungi remarked were isolated from seagrasses *Halophila ovalis* (R.Brown) Hooker.f, *Thalassia hemprichii* (Ehrenberg) Ascherson and *Syringodium isoetifolium* (Ascherson) Danty and in the investigation of antibacterial activities on Micrococcus luteus NITE83297, seven endophytic fungi showed the activities.

Conclusion

In the screening of endophytic fungi, 12 different fungi namely KF-01, KF-02, KF-03, KF-04, KF-05, KF-06, KF-07, KF-08, KF-09, KF-10, KF-11 and KF-12 were isolated from seagrasses *Halophila ovalis* (R.Brown) Hooker.f, *Thalassia hemprichii* (Ehrenberg) Ascherson and *Syringodium isoetifolium* (Ascherson) Danty, collected from from Magyi Coast, Shwe-Thaung-Yan Sub-Township, Pathein Township, Ayeyarwady Region. In the investigation of antibacterial activities of isolated fungi, fungus KF-01, KF-

03, KF-06, KF-07, KF-08, KF-10 and KF-11 showed antibacterial activity against on *Micrococcus luteus*NITE83297. The endophytic fungi KF-01 and KF-03 were isolated from *H.ovalis*, KF-06, KF-07 and KF-08 were isolated from *T.hemprichii* and KF-10 and KF-11 were isolated from *S.isoetifolium*. The isolated endophytic fungi KF-01showed (18.42 mm), KF-03 showed (21.06 mm), KF-06 showed (14.57 mm) , KF-07 showed (21.96 mm), KF-08 showed (21.72 mm), KF-10 showed (15.48 mm) and KF-11showed (22.53 mm) against respectively.

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ANTICANCER ACTIVITY OF VARIOUS KAN-ZAW EXTRACTS ON HUMAN CERVICAL CANCER CELLS (HELA)*

Moe Moe Lwin[1]

Abstract

Payena paralleloneura Kurz. also called Kan-zaw is a large evergreen tree belonging to the family Sapotaceae which is widely used in the treatment of various cancer and different ailments. In the present work the fatty acid analysis of the Kan-zaw seed oil contains approximately >70% α-eleostearic acid and 3.25 % β-eleostearic acid an unusual conjugated fatty acid that imparts a potent anticancer application and industrially important drying qualities to Kan-zaw oil. The present study also investigated cytotoxic potential of various extracts of Kan-zaw seeds using ethanol, methanol, acetone, chloroform and commercial Kan-zaw oil against human cervical cancer cell line (HeLa) by MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The various extracts of Kan-zaw seeds (ethanol, methanol, acetone, chloroform) and commercial Kan-zaw oil were effective towards tested cell line, with inhibition and they were found that with the highest 87.14%, 81.56%, 87.48%, 87.99% and 88.32% respectively. The *Payena paralleloneura* Kurz. (Kan-zaw) extracts and oil have shown significant anticancer activity on HeLa cells.

Keywords: *Payena paralleloneura* Kurz., Fatty Acid analysis, Anticancer activity

Introduction

Conjugated fatty acids are naturally occurring compounds that have specialized uses in nutraceutical and industrial applications. For example, conjugated linoleic acid (CLA) is a potent anticancer compound present in foods derived from ruminant animals (Belury, 2002). Conjugated fatty acids such as α-eleostearic acid have recently shown promise for anticancer applications (Kohno *et al*., 2002), as well as serum lipid lowering effects in mammals (Koba *et al*., 2002). Oils containing α-eleostearic acid may also be used for industrial drying applications. A third mechanism for generating

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conjugated fatty acids, which is typical of higher plants, involves fatty acid oxidation and bond rearrangement. For example, radiolabeling studies with developing bitter gourd seeds revealed that linoleic acid was modified at the position to produce α-eleostearic acid, Conjugated linolenic acids (CLNs), lack the methylene groups found between the double bonds of linolenic acid. Oils rich in conjugated linolenic acids (CLNs) are important medicinally as a source of nutraceuticals and industrially as drying agents in paints, inks, and varnishes (Biermann *et al*., 2011). α-Eleostearic (α-ESA) acid is the most widespread CLN. Tung *(Aleurites fordii)* and bitter gourd (*Momordica charantia*) seeds are rich source of α-ESA. Other geometrical isomers of α-ESA that are found in nature are punicic acid, catalpic acid, and β-ESA. Punicic acid is found in pomegranate (*Punica granatum*) and snakegourd (*Trichosanthes kirilowii*) seeds, catalpic acid is present in catalpa (*Catalpa bignonoides* and *Catalpa ovata*) seeds, and β-ESA is present in pomegranate, bitter gourd, and catalpa seeds (Joh, *et al.,* 1995; Ozgul, 2005). Additional positional isoforms of α-ESA known are calendic acid and jacaric acid and are present in pot marigold (*Calendula officinalis*) and jacaranda (*Jacaranda mimosifiola*) seeds, respectively (McClean and Clark 1956; Hopkins and Chisholm 1968).

Cervical cancer constitutes the second most common cancer in women. This is due to the infection with Human Papilloma Virus (HPV), notably type 16 and 18 virus (Alvarez - Salas and DiPaolo, 2007).

Natural products are being tested for the treatment but these are yet to prove their efficacy in preclinical and clinical studies. Some of the food plants are believed to be an important source of nutrition as well as chemical substances having potential of therapeutic effects. These plants are effective source of both traditional and modern medicines and are genuinely useful for primary healthcare. Plants have been rich source of medicine because they produce wide range array of bioactive molecule (Agharkar, 1991).

According to local people and traditional practitioners which they used its fixed oil from the seeds as major remedy traditional medicine for the

treatment of breast, cervical, ovarian and various cancers, anti-peptic ulcer, paralysis, bronchitis, rash, chest pain, injury, sores and various other ailments.

These are used for primary healthcare in rural areas in my countries. This tree known under the name of Kan-zaw, produces Kan-zaw oil with high medicinal value. It is highly regarded as a universal panacea in the ayurvedic medicine and large evergreen tree distributed in only Tanintharyi region of Myanmar. The Kan-zaw seeds have not been previously systematic studies on the anticancer activity of Kan-zaw seeds. The main aims of analyzing crude plant extracts are agents for direct use as drugs that can be used as lead substances in the preparation synthetic drugs. There are two main strategies for the selection of plants species in anticancer drug discovery: the first one contain random screening and ethno medical knowledge. The second approach includes plants used in organize traditional medical systems like herbalism and folklore. The search for new anti-cancer drugs is one of the most prominent research areas of natural products. In the present study, the anticancer activity of Kan-zaw seeds were investigated.

Materials and Methods

Fatty acid analysis of Kan-zaw seeds (FAME analysis)

The kernels of the Kan-zaw seeds were dried and crushed into powder in a motor and pestle. Oil was extracted from around 50 mg powder in a glass tube (2cm x 10cm) with a screw cap. To this tube, 3 ml of Hexane was added and vortexed for 10 sec followed by an extraction process under a ultrasonic cleaner (25K Hz) for 1.5 hr. Next, to the sample 400 µl of KOH (5M)/Methanol was added to esterify fatty acid under the ultrasonic for additional 10 min. After cooling to room temperature, 200 µg of methyl heptadecenoate (C 17:1) was added as an internal standard. The mixture was vortexed for 10 sec before centrifugation at 5000 rpm for 5 min. The compounds of fatty acid methyl esters (FAMEs) in the upper organic phase were removed and the residual mixture was extracted for FAMEs with additional 3 ml of Hexane. The pooled FAME extracts were evaporated under nitrogen and then dissolved in 500 µl of Hexane for gas chromatograph

analysis (GC; Agilent 7820A, CA) with a flame ionization detector (FID) on a DB-23 column (30 m by 0.25 mm id., 0.25 µm film; Agilent, CA). The GC conditions were split mode injection (1:20), injector and flame ionization detector temperature, 270°C and 280°C; the oven condition was 80°C for 3 min, with a ramp to 170°C for 10 min with 10°C increments per minute, then increasing at 5°C/min to 220°C. FAME compounds were identified by calibration with a standard (NU-CHEK, USA), a mixture for 37 known FAMEs.

The oil content was then calculated from the famous: Percent oil by dry weight = (100 x total peak area x 0.2 mg/ peak area of internal standard)/mg tissue, where 0.2 mg is the amount of internal standard used per sample. The composition percentage of a single FAME was reflected by a percentage of the peak area of this FAME in total peak area of all FAMEs (Wychen, 2015).

Various extraction of Kan-zaw seeds by using rotary evaporator

The various extraction of Kan-zaw seeds were determined according to (The British Pharmacopoeia, 1965) as follows. Forty gm of Kan-zaw seeds powdered was soaked with 250 ml of ethanol in a closed flask for 72 h and kept over three nights. The mixture was filtered rapidly taking precautions against loss of alcohol and then the filtrate was put into round bottle flask and extracted by using rotary evaporator. The extract was obtained by drying the concentrated pooled extract under vacuum (Suthar and Mulani, 2008).

The above procedure was extracted with methanol, acetone and chloroform instead of ethanol. Commercial Kan-zaw oil for analyses were purchased from the market.

***In vitro* evaluation of anticancer activity by MTT assay**

Cell Culture

The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Sciences (NCCS), Pune and grown in Eagles minimum essential medium (EMEM) containing 10% Fetal Bovine Serum (FBS). All cells were maintained at 37º C, 5% CO2, 95% air and 100% relative humidity.

Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell Treatment

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of

1 x 10^5cells /ml. 100 µl per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37ºC, 5% CO2, 95% air and 100% relative humidity.

After 24 h the cells were treated with serial concentration of the test samples. They were initially dissolved in Dimethyl sulfoxide (DMSO) and using the 250 mg/l concentration various extract four serial dilutions of the extract of 500 µl each was prepared DMSO to get the concentration of the extract from 125-250 mg/l as indicated in Table 2, 3, 4, 5 and 6. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations. Following the treatment with various extract of Kan-zaw seeds (ethanol, methanol, acetone, chloroform) and commercial Kan-zaw oil, the plates were incubated for an additional 48 h at 37ºC 5% CO2, 95% air and 100% relative humidity. The medium without samples were served as control and triplicate was maintained for all concentrations (Slater *et al*, 1963; Alley, *et al*, 1988 and Van de Loosdrecht, 1994).

MTT Assay

After 48 h of incubation, 15 µl of MTT (5 g/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37ºC for 4 hr. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 490 nm using microplate reader. The same procedure was carried out for the

extraction with different solvents. The % cell inhibition was determined using the following formula (Jack, 2005).

Percentage cell inhibition = 100 - Abs (Sample) / Abs (Control) x 100.

Abs (Sample) = Absorbance value of sample

Abs (Control) = Absorbance value of control

Results and Discussion

Fatty acid analysis of Kan-zaw seeds

In this research, the mass spectrum of the FAME corresponding to peak a exhibited a molecular ion at *m/z* = 261, characteristic of a 16:2 methyl ester, where as the spectrum of a prominent molecular ion at *m/z* = 292, indicative of an 18:3 methyl ester. α-Eleostearic acid (α-ESA) is the most widespread CLN Fig. 1. The methyl esters of palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids as shown in Fig. 2. This is the agreement with the findings of John *et al*. (2002) who reported the results. Conjugated fatty acids such as α-eleostearic acid have recently shown promise for anticancer applications and industrially as drying agents in paints, inks and varnishes (Koba *et al*., 2002 and Kohno *et al*., 2002).

The GC chromatogram of FAME derived from the Kan-zaw oil (*Payena parallelonеura* Kurz.) tree contains approximately >70% α-eleostearic acid and β-eleostearic acid methyl esters also presented in Fig. 3. Joh, *et al*., (1995), Ozgul, (2005) and Richa Rawat *et al*. (2012) stated that Tung (*Aleurites fordii*) and bitter gourd (*Momordica charantia*) seeds are rich source of α-ESA and accumulate >80% and >60% of α-ESA, respectively. β-ESA is present in pomegranate, bitter gourd, and catalpa (*Catalpa bignonoides* and *Catalpa ovata*) seeds.

The fatty acid composition of Kan-zaw seeds is presented in (Fig. 3 and Table 1). It comprises of palmitic acid (C16:0) 3.41%, margaric acid (C17:0) 0.23%, stearic acid (C18:0) 2.45%, oleic acid (C18:1) 9.78%, linoleic acid (C18:2) 9.56%, arachidic acid (C20:0) 0.36%, gondoic acid (C20:1)

0.39%, α-ESA 70.57% and β-ESA 3.25%. α-ESA was the principal fatty acid followed by oleic, linoleic, and β-ESA acids. These results are confirmed by the findings of John *et al*. (2002). The presence of high amounts of the essential conjugated fatty acids such as α-eleostearic acid have recently shown promise for anticancer applications and industrially as drying agents in paints, inks and varnishes (Koba *et al*., 2002 & Kohno *et al*., 2002).

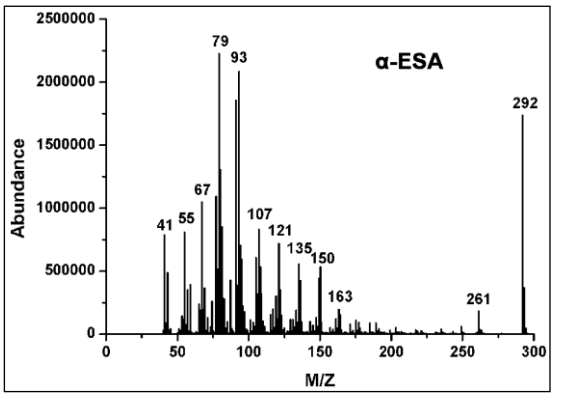

Figure 1. Mass spectra of α-Eleostearic acid methyl ester derived from Kan-zaw seeds

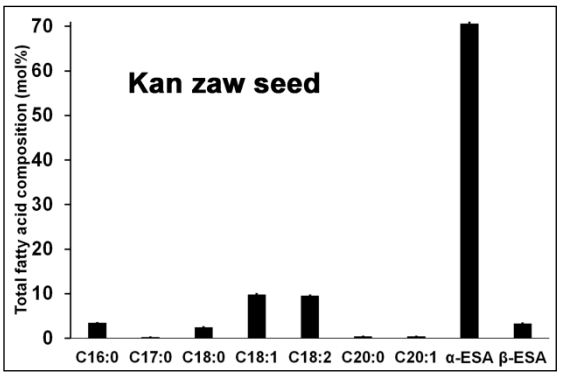

Figure 2. Functional analysis of labeled histogram correspond to the methyl esters of palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids. The GC chromatogram of FAME derived from (Kan-zaw oil) to illustrate the positions of α-Eleostearic and β-Eleostearic acid methyl esters

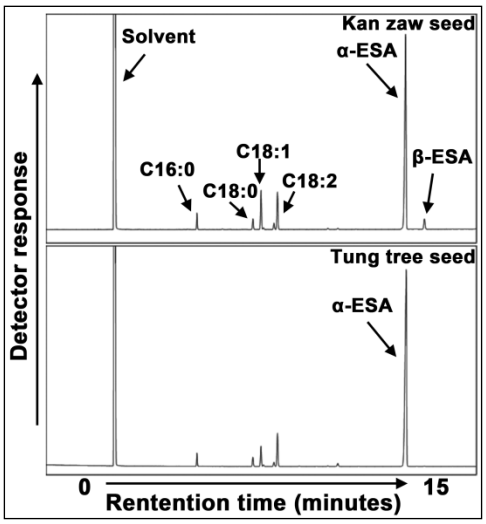

Figure 3. GC analyses of FAMEs extracted from Kan-zaw seeds. Standard includes FAMEs from Tung seeds (*Aleurites fordii* Hemsl.) and fatty acid α-ESA, and β-ESA, respectively from Kan-zaw seeds

Table 1. Fatty acids composition (%) of Kan-zaw seeds.

Fatty acids	**Determined value %**
Palmitic acid (C16:0)	3.41
Margaric acid (C17:0)	0.23
Stearic acid (C18:0)	2.45
Oleic acid (C18:1)	9.78
Linoleic acid (C18:2)	9.56
Arachidic acid (C20:0)	0.36
Gondoic acid (C20:1)	0.39
α - Eleostearic acid	70.57
β - Eleostearic acid	3.25

Anticancer activity by MTT assay

The results for cell growth inhibition by the extract against HeLa cell lines for various concentration are shown in Fig. 4, 5, 6 and Table 2, 3, 4, 5, 6. In the present study the percentage of viable cells remained more than 73% even when cells were treated with 100 µL of concentration for 24 h. But when the doses were increased, the percentages of viable cells were decreased and finally at a dose of 250 mg/l of concentration over 10% cells were viable. The extracts of ethanol, methanol, acetone, chloroform of Kan-zaw seeds and commercial Kan-zaw oil were effective towards tested cell line, among these commercial Kan-zaw oil was found that with the highest 88.32%. These results indicate that concentrations show significant potentiality against the viability and proliferation of cervical carcinoma cell (HeLa cell) line.

The acetone and ethanol extracts of leaves of *Madhuca longifolia* shows the cytotoxic activity against Ehrilich Ascites Carcinoma cell lines using different *in vitro* cytotoxicity assay at 200 g/ml (Yadav *et al*, 2012). The utility of cell lines acquired from tumors allows the investigation of tumor cells in a simplified and controlled environment (Arya *et al*., 2011). In the present study the HeLa cell lines are used as a model for studying cervical cancer. Several mechanisms of action were detected in HeLa cells. Patel *et al.* (2009) stated that the % growth inhibition increasing with increasing concentration steadily up to 0.0196-10 mg/ml *Solanum nigrum* effect on HeLa cell Line.

The IC50 of extract on cell line less than 100 g/ml is categorized as a potential cytotoxic substance (Spavieri *et al*., 2010). In the present study, ethanol, methanol, acetone, chloroform extracts and commercial Kan-zaw oil were found to be moderately cytotoxic towards human HeLa cell in MTT assay.

Table 2. For percentage (%) of cell growth inhibition of ethanol extract of *Payena paralleloneura* Kurz. (Kan-zaw) seeds on HeLa cells by MTT assay.

Concentration of the Extracts (mg/l)	**Absorbance**	**Inhibition of Cell Growth (%)**
250	0.076	87.14
200	0.391	33.84
150	0.415	29.78
125	0.434	26.57
Control	0.591	0

Table 3. For percentage (%) of cell growth inhibition of methanol extract of *Payena paralleloneura* Kurz. (Kan-zaw) seeds on HeLa cells by MTT assay.

Concentration of the Extracts (mg/l)	**Absorbance**	**Inhibition of Cell Growth (%)**
250	0.109	81.56
200	0.219	62.94
150	0.330	44.16
125	0.385	34.86
Control	0.591	0

Table 4. For percentage (%) of cell growth inhibition of acetone extract of *Payena paralleloneura* Kurz. (Kan-zaw) seeds on HeLa cells by MTT assay.

Concentration of the Extracts (mg/l)	**Absorbance**	**Inhibition of Cell Growth (%)**
250	0.074	87.48
200	0.081	86.29
150	0.437	26.06
125	0.447	24.37
Control	0.591	0

Table 5. For percentage (%) of cell growth inhibition of chloroform extract of *Payena paralleloneura* Kurz. (Kan-zaw) seeds on HeLa cells by MTT assay.

Concentration of the Extracts (mg/l)	**Absorbance**	**Inhibition of Cell Growth (%)**
250	0.071	87.99
200	0.170	71.24
150	0.282	52.28
125	0.289	51.1
Control	0.591	0

Table 6. For percentage (%) of cell growth inhibition of commercial oil of *Payena paralleloneura* Kurz. (Kan-zaw) seeds on HeLa cells by MTT assay.

Concentration of the Extracts (mg/l)	Absorbance	Inhibition of Cell Growth (%)
250	0.069	88.32
200	0.322	45.52
150	0.366	38.07
125	0.379	35.87
Control	0.591	0

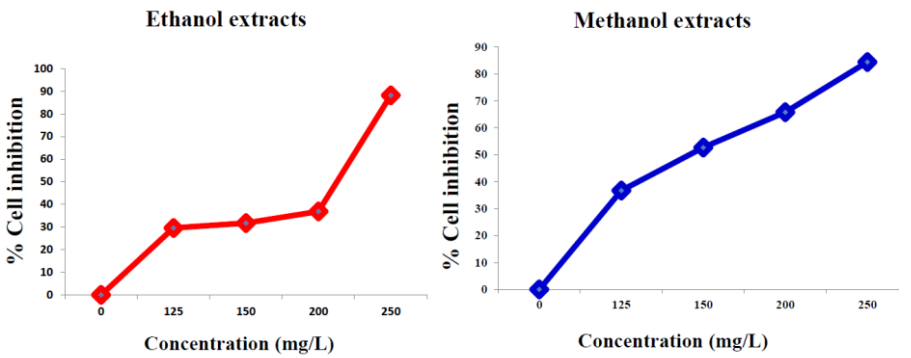

Figure 4. Effect of ethanol and methanol extracts of Kan-zaw seeds on HeLa cells by MTT Assay

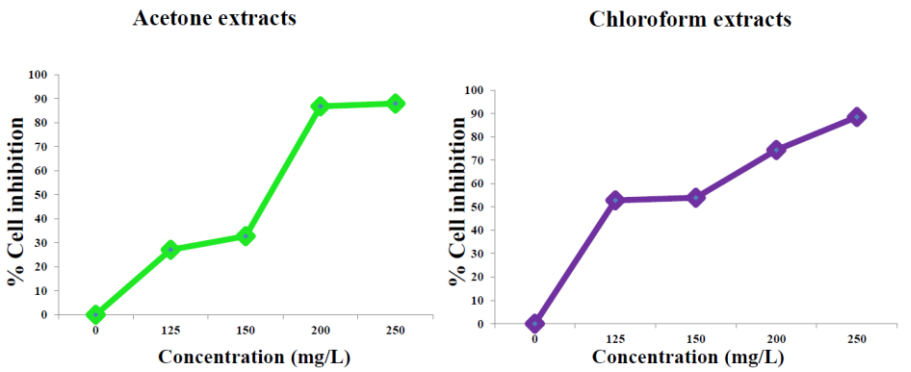

Figure 5. Effect of acetone and chloroform extracts of Kan-zaw seeds on HeLa cells by MTT Assay

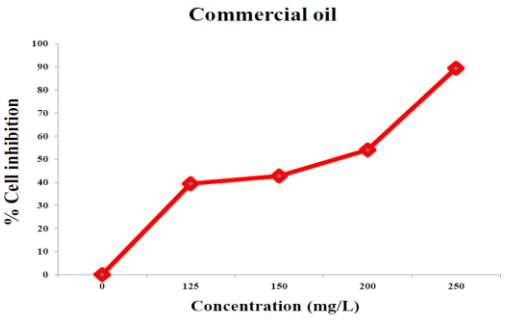

Figure 6. Effect of commercial oil of Kan-zaw seeds on HeLa cells by MTT Assay

Conclusion

The seed oil derived from the Kan-zaw owing to their excellent conjugated fatty acids approximately >70% α-eleostearic acid and β-eleostearic acid methyl esters are naturally occurring compounds that have specialized uses in nutraceutical (anticancer) and industrial applications. The Kan-zaw oil is famous in Myanmar. Although the oil is popularly used by local people and have commercial value, the scientifically research of this plant has not been undertaken previously.

Various extracts of Kan-zaw seeds were prepared for the first time to the best of my knowledge and the synthesized concentration showed a

significant efficacy against cervical carcinoma (HeLa) cell line with different concentrations along with >80% cell killing potentiality. Now overall study evaluate that *Payena paralleloneura* Kurz. (Kan-zaw) various extract and commercial oil exhibit potential effects against (HeLa) cell line in a dose dependent manner. On the basis of review of literature it concluded that research performed on seeds of Kan-zaw to highlight its medicinal properties, but only few experimental research have not been performed for utilizing it as a medicine.

The anticancer property of *Payena paralleloneura* (Kan-zaw) will provide a useful information in the possible application in the prevention and treatment of cancer. So now the time of diversion for commercial utilization of Kan-zaw seeds will also be used in preparation of medicines. This effort may increase the employment and income generation potential of the nation. Further research based on animal models will be conducted *in vivo* efficacy of Kan-zaw seeds.

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EXTRACTION, ISOLATION AND PURIFICATION OF PHYCOCYANIN FROM BLUE GREEN ALGA *SPIRULINA PLATENSIS*

Swe Swe Aye[1]

Abstract

In this study, the blue green alga *Spirulina platensis* was obtained from Aquaculture Section, Research and Development Station, Central Arava in 2016. Phycocyanin, one of the phycobiliproteins, was extracted from blue green algae *Spirulina platensis*, followed by the isolation, and characterization of its purity grade. The phycocyanin was extracted by using SCIENTZ-10N Benchtop Freeze - Drying Lyophilizer (-20 ^{o}C) and characterized by SDS PAGE analysis methods. The results showed that the purity level of crude extract is 0.94, 50% ammonium sulphate resulted in purity of 2.10, whereas dialysis tubing was 2.41. In this condition, the phycocyanin with a purity of 3.24 was obtained based on SDS PAGE analysis. It would be concluded that using low temperature extraction method with PBS buffer (pH-6.5, 0.1M) can help to get the better purity with degraded less phycocyanin molecule, because freezing temperature disrupted completely cell membrane of *Spirulina platensis.*

Keywords: phycobiliprotein, Phycocyanin, dialysis, buffer solution, SDS PAGE, freeze -drying

Introduction

Since previous time, *Spirulina platensis* was useful natural resources mainly served as a food or food supplementary for human beings. Nowadays, it becoming popular trend to use algal products both in functional food items and also raw materials for cosmetic products. An active component of *S platensis*, Phycocyanin, is a colorant, which mainly found in cyanobacteria and red algae. In nature, it exists in the form of monomer, trimers or hexamers; small quantities of oligomers have been found as well (MacColl, 1998). Having special bioactivities, they are increasingly recognized as potential raw materials for making food products that are beneficial to health.

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Many studies have indicated that phycocyanin have bioactivities such as: antitumor, antioxidant, and anti-inflammatory effect (Romay et al., 1998; Eriksen, 2008). Since these plants grow in limiting environment, the pigments are rather expensive, and availability as pure compounds is tend to be quite hard endeavor (Silveria, 2007).

Getting phycocyanin extract from wet biomass have been attempted by various methods. The reported method of freezing and thawing (Soni et al., 2006; Stewart and Farmer, 1984) were done. It would have been suggested that the ratio of absorbance at 620 nm and 280 nm can be employed to indicate the purity of phycocyanin, while the ratio of absorbance at 650 and 280 nm can indicate the purity of allophycocyanin.

The purity of phycocyanin is generally evaluated using the absorbance ratio of A_{620}/A_{280} and a purity of 0.7 is determined as food grade 3.9 as relative grade and greater than 4.0 as analytical grade. Purity is directly related to process costs and in general, the more purified a extracted product is, the more expensive to obtain it. Many obstacles exist in the purification process of Phycocyanin (e.g.- if high purity Phycocyanin is desired, the experimental procedure is rather complicated and need a high production costs). So far, there were some studies have been carried out on extraction and purification of phycocyanin, but there still need to improve in procedure that care about the costs, uses of chemicals and efficiency. In this research work, freeze-drying (-20°C) method has been used to extract phycocyanin to avoid some unnecessary complicated procedures, while it could not be damaging the pigment intensity. Thus, the main focus of the present study was to produce the phycocyanin from *S platensis* using the freeze–drying extraction method, which followed by the dialysis by extracted buffer solution to obtain food grade dye for food industry use. (Soni et al., 2006; Sarada et al.,1999)

Materials and Methods

Materials

Sephaadex G-25, dialysis tubing, markers of known molecular weight were purchased from Sigma (St Lowis,MO, USA). All chemicals were of analytical grade.

Organism and Growth condition

Spirulina platensis was grown in a modified Zarouk medium (1966). Firstly,The algae were grown in a batch culture at 35°C, then illuminated by cool white lamp and finally, stirring was provided by bubbling with a mixture of air with 1.5% CO_2.

Extraction of Phycocyanin from *Spirulina platensis*

The concentrated *Spirulina* cells (50 g) was washed with double distilled water to remove adhered salts. Then, biomass was introduced in Bench - Top Freeze Dryer (at - 20 C for 6 hours) to degrade the cell walls of *Spirulina platensis*. After that, the slurry was put in PBS buffer (pH 6.0, 0.1M). The cell debris was removed by centrifugation (4000 rpm for 20 min) . Finally, the supernatant was obtained as crude phycocyanin.

Isolation and purification of Phycocyanin from *Spirulina platensis*

The isolated crude phycocyanin was precipitated in 50% ammonium sulfate and then recovered by centrifugation (8000rpm, for 20 min). The colourless supernatant was discarded and the blue precipitate was dissolved in the extracted buffer solution to dialyze. Dialyzed was served for thrice against 1000 ml of extraction buffer at room temperature .

The dialyzed solution after centrifugation (13000 rpm , for 10min) was eluted through Sephadex- 25 column (3cm*2cm) pre equilibrated and diluted with same buffer. The column was developed at a flow rate of 0.25ml/min and elution were collected in 2.0 ml fraction tubes .

Spectroscopic Measurements

The isolated phycocyanin pigment was evaluated by the procedures below by the ratio of 280nm for the absorbance of total proteins in phycocyanin to 620 nm of desired protein in the complex .(Liu et al ., 2005)

$$\text{Phycocyanin Purity} = A_{620}/A_{280}$$

SDS – PAGE pattern of phycocyanin

Moreover, the measurement of purity, the molecular weight of isolated phycocyanin was determined by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, Mini-Protean, Bio-red Ltd., Hercules, CA USA). SDS-PAGE was confirmed using a 14% polyacrylamide slab gel and a 4.5 % stacking gel and was confirmed after staining with Coomassie blue R25 (Sigma, St. Louia, MO, USA) and distaining. The molecular weights of the Phycocyanin subunits were confirmed by comparison with a standard ladder (pre-stained SDS-PAGE standard ; Bio-Rad , USA)

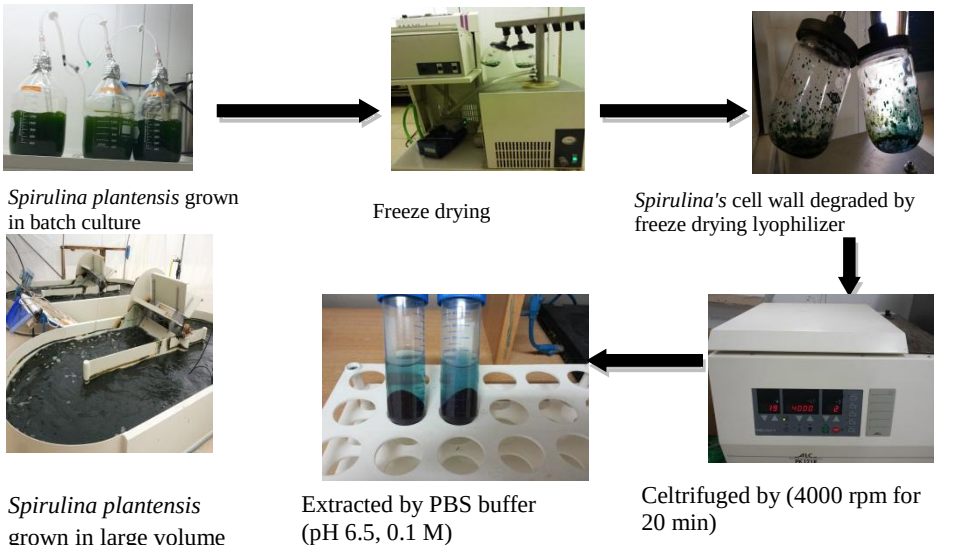

Figure 1. Extraction of crude phycocyanin from *Spirulina plantensis*

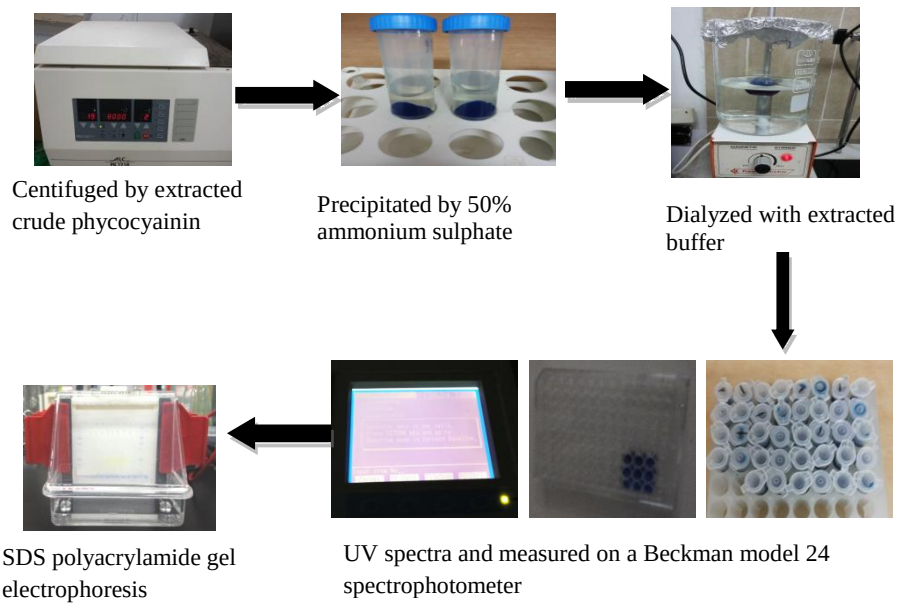

Figure 2. Isolation and purification of phycocyanin from *Spirulina plantensis*

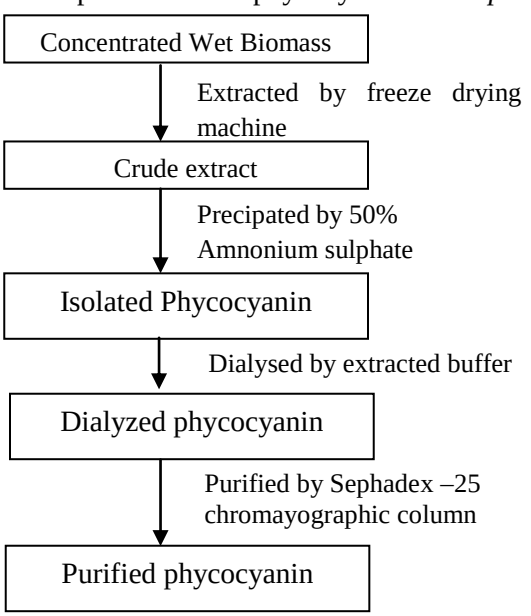

Figure 3. Process flowchart for phycocyanin from *Spirulina plantensis*

Results and Discussion

For obtaining the crude blie pigment, phycocyanin from *Spirulina platensis* is the selection of extraction procedures. In the present study, the Freeze-Drying method (using o.1M PBS buffer, pH 6.0) has been used for extraction of phycocyanin and its purity, which was assessed in Table 1. From this experiment, the purity value for crude extract has been found 0.94, which falls in the food grading. The purity of phycocyanin plays a major role in commercial application and is generally evaluated using Ultra Violet spectrometry. A purity value of isolated pigment up to 0.877 is considered as food grade (Rito – Palmores et al., 2001).

In purification of crude extract, it involves functional precipitation with 50% ammonium sulfate, which is useful in salting out unwanted proteins and at the same time to concentrate Phycocyanin (Boussiba and Richmon, 1979). Experimentally, phycocyanin becomes precipitated in this medium and improves the purity ratio increased to 2.10. After further purification, it was compulsory to be done dialysis for the removal of phycocyanin to improve the purity, which got value up to 2.41. Then, after passing through sephadex G-25 column, this also increased purity ratio to 3.24. Finally, the successive purified fractions from each steps, run on SDS PAGE, the contaminating protein band disappeared and only one band (18 kDa. subunit) was observed, which is consistence with the research found by Patel et al. (2005) Fig (4).

Table1. Purity Ratio of Phycocyanin extracted from *S.platensis* by spectrophotometric method

Steps of purification	280nm	620nm	Purity Ratio (A620/A280)
Crude extract	3.56	3.34	0.94
50%Ammonium sulfate	1.10	2.42	2.10
Dialysis	0.87	2.10	2.41
Sephadex G-25	0.364	1.18	3.24

Isolation & Purification of Phycocyanin from *Spirulina platensis*

The purity of phycocyanin plays a significant role in commercial application and is generally evaluated using the absorbance ratio of A_{620}/A_{280} where A_{620} represents maximum peak height for phycocyanin and A_{280} indicates contamination of aromatic amino acid rich proteins. A purity value up to 0.877 is considered as food grade (Rito – Palmores, 2001) .In this result, the phycocyanin purity value for crude extract was 0.94 . The purification of crude extract involves functional precipitation with 50% ammonium sulfate which is particularly useful in salting out unwanted proteins and at the same time to concentrate phycocyanin (Boussiba and Richmon, 1979). At this step phycocyanin get precipitated and improves the purity ratio is increased to 2.10 . For further purification removal of phycocyanin is compulsory and for this dialysis was carried out and improved the purity value up to 2.41. It was further purified by passing through sephadex G-25 column .This increased purity ratio to 3.24. The purity of phycocyanin was further confirmed by absorption spectral scanning. The successive purified fractions from each step were run on SDS PAGE and thecontaminating protein band disappeared. . Only one band (18 kDa. subunit) was observed. Fig (4).The molecular weight α subunit were being reported from 18 kDa in Spirulina (Patel et al., 2005).

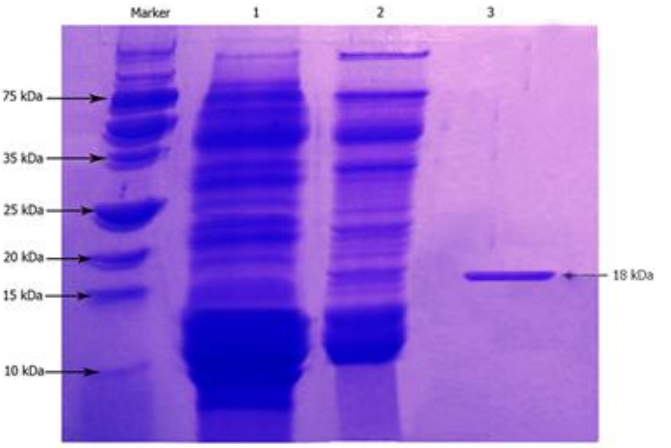

Figure 4. 14% SDS-PAGE at each stage of purification at phycocyanin from *Spirulina plantensis*
1. 50% ammonium sulphate precipitation
2. Dialyzed phycocyanin
3. Sephadex G-25

Conclusion

The present study has been extracted phycocyanin from the natural blue green algae by using freeze - drying method, whereby the resulting compound got better purity with few phycocyanin molecule degradation. It would be described as an effective method of extraction and purification of phycocyanin from *Spirulina* so far. The purity level of phycocyanin at the end of the process achieved at the food grade of 3.24.

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EVALUATION ON WOUND HEALING PROPERTIES OF ROOT EXTRACT OF *FLEMINGIA STROBILIFERA* (L.) R. BR. IN ALBINO RAT MODEL

Swe Zin Soe[1], Wai Wai Thein[2], Aye Win Oo[3]

Abstract

Flemingia strobilifera (L.) R. Br. is medicinally important plant which belongs to the family Fabaceae. In this study, the evaluation on wound healing properties of 70% ethanolic root extract of *Flemingia strobilifera* (L.) R. Br. have been conducted in albino rat model. Male albino rats were divided into 3 groups contains 3 replicates each and anesthesia was administered by an intramuscular injection of xylazine and ketamine. The back side skin of the rats were shaved and an excision of 1 cm diameter on the shaved skin were prepared. In this experiment, group 1 was treated with ethanolic root extract, group 2 by standard treatment (Tetracycline ointment) and group 3 as the control. The rate of epithelialization and complete wound closure was daily recorded. A better healing pattern was observed in rats treated with ethanolic root extract and standard treatment (Tetracycline ointment) compared with the untreated control. The epithelialization period of the group 1 and 2 were much shorter than the group 3. Specimens of healed skin at a thickness of 5µ from each rat were taken at the 20th day of treatment and were fixed in 10% buffered formalin solution for histological studies. These skins were stained with hematoxylin and eosin (H & E) and assessed for histological changes. 70% ethanolic root extract of *Flemingia strobilifera* (L.) R. Br. group was well developed granulation tissues in all layers of skin. According to the results of this experiment, it may be concluded that 70% ethanolic root extract of *Flemingia strobilifera* (L.) R. Br. has the prospective outcome as a new therapeutic agent for wound healing.

Keywords: *Flemingia strobilifera* (L.) R. Br., wound healing, albino rat, a new therapeutic agent

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2. Associate Professor, Department of Botany, Yangon University
3. Deputy Director/Head, Laboratory Animal Services Division, Department of Medical Research

Introduction

Flemingia strobilifera (L.) R. Br. (Fabaceae), which is known as Wild hops in English and Gaung-own-sar or Say-laik-pya or Pa-lan-phyu in Myanmar. *Flemingia strobilifera* (L.) R. Br. is ethano medicinally used in India and Philippines. It is well known for its medicinal effects and is being used for the treatment of various ailments such as to relieve rheumatism, body pain, fever and indigestion.

Plants and their extracts have immense potential for the management and treatment of wound. The phytomedicine for wound healing are not only cheap and affordable but are also purportedly safe as hypersensitive reactions are rarely encountered with the use of these agents (Omale and Isaac, 2012).

Morphological Characters of *Flemingia strobilifera* (L.) R. Br.

Wound healing is a biological process that is initiated by trauma and often terminated by scar formation. Thus, healing is essentially a survival mechanism and represents an attempt to maintain normal anatomical structure and function (Suruse, 2011). The process of wound healing occurs in different phases such as coagulation, epithelialization, granulation, collagen formation and tissue remodeling. Animal wound healing models are important biological tools to understand basic process of tissue repair and to develop and validate strategies for treatment of wounds (Pandey, 2012).

Wound healing potential of *Flemingia strobilifera* (L.) R. Br. root has not been experimentally evaluated so far, hence the present investigation was undertaken to study the wound healing property of 70% ethanolic root extract of *Flemingia strobilifera* (L.) R. Br. on excision wound models(Figure 4).

Materials and Methods

Plant Material

Flemingia strobilifera (L.) R. Br. roots were collected from Bago Region (N- 17^0 16' 19.4" and E- 109^0 28' 15.2") from June 2017 to January 2018. It was identified taxonomically. Herbarium samples have been deposited in the Department of Botany, University of Yangon.

Preparation of Extract

The roots of *Flemingia strobilifera* (L.) R. Br. were collected and dried in shade. The roots were then powdered and extracted with 70% ethanol for a period of 36 hrs in a Soxhlet extractor. The extract was then concentrated and dried.

Experimental Animals

Male albino rats weighing 150-200 g were used. They were housed in standard cages at room temperature (25±2˚C) and provided with food and water. The animals were deprived of food for 24 hrs before experimentation, but had free access to drinking water.

Wound healing studies

Wound healing property of 70% ethanolic root extract was studied on excision wounds using male Albino rats. Animals were divided into 3 groups of 3 each. Group 1 was treated with ethanolic root extract, group 2 by standard treatment (Tetracycline ointment) and group 3 as the control (without root extract). All treatments were made by topical application of ethanolic root extract once a day.

Excision wound model

Hairs were removed from the back side skin of the rats using a blade. Then, an excision of 1 cm diameter on the shaved skin was prepared. The albino rats were divided into three groups and each groups performed three animals. Each rat was anaesthetized with Ketamine hydrochloride and Xylazine and the hair on the back was scrapped off with a pair of curve scissors. This area was disinfected with methylated spirit. An excision of about 1 cm in width area and it was made on the disinfected area of the skin surface (Figure 1 to 3). The wounds Group 1 animals were treated by ethanolic root extract. Group 2 animals were treated on tetracycline ointment. Group 3 animals were kept in their cage without treatment (control). Drugs were topically applied once a day till complete epithelialization, starting from day of excision. Number of days required for falling of scab without any residual raw wound, gave the period of epithelialization (Kodati *et al*., 2011)

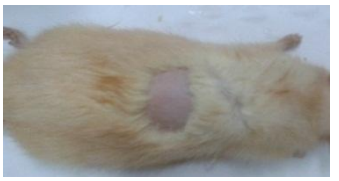

Figure 1. Cleaned and shaved area of albino rats

Figure 2. Excision wound model on albino rat model

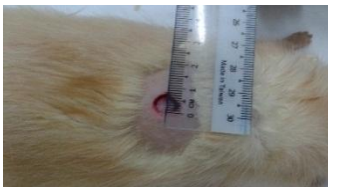

Figure 3. Measure the wound size after wound creating

Results

The morphological characteristics of *Flemingia strobilifera* (L.) R. Br. Showing its habit and root were shown in Figure 4 and 5.

Figure 4. Habit

Figure 5. Root

Appearance of excision wound in day 1

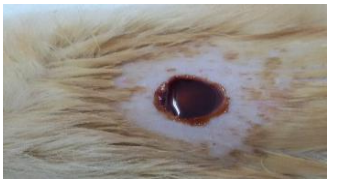

Figure 6. 70% ethanolic root extract

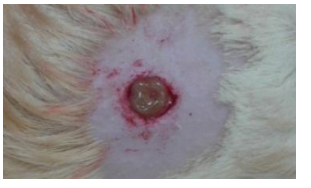

Figure 7. Standard drug (Tetracycline ointment)

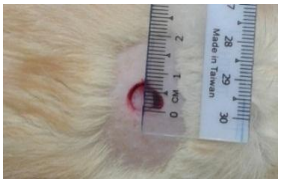

Figure 8. No Treatment

Appearance of excision wound after 20 days

Figure 9. 70% ethanolic root extract

Figure 10. Standard drug (Tetracycline ointment)

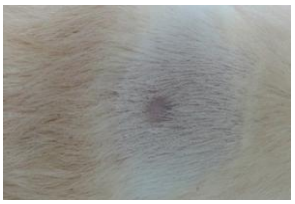

Figure 11. No Treatment

Histological Reports - after 20 days evaluation of wound healing activity of rat skin

Specimens of skin from healed wounds from each rat were taken at the 20 days of treatment and were fixed in 10% buffered formalin solution for histological studies. Specimens of the healed skin were made at a thickness of 5µ and were stained with hematoxyline and eosin (H&E) and assessed for histological changes. The microscopic slides were photographed.

Histological Section of the Skin Tissue from 20 Days Excision Wound Model

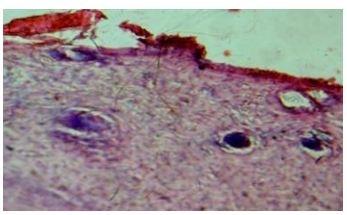

Figure 12. Normal albino rat skin tissue (10X)

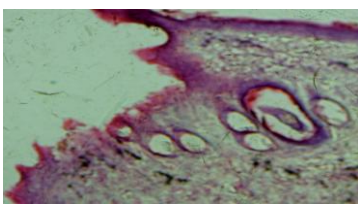

Figure 13. 70% ethanolic root extract treated (10X)

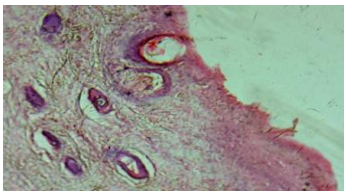

Figure 14. Standard drug (Tetracycline ointment) treated skin (10X)

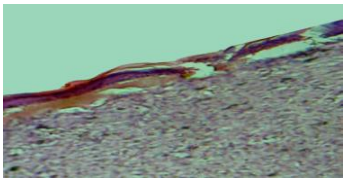

Figure 15. Control (Untreated) skin (10X)

Table 1. Histological report after 20 days of wound healing on Albino rat model

Type of skin	**Epidermis**	**Dermis**	**Subcutaneous tissue**	**Histological Diagnosis**
Normal Skin Figure 12	The papillary pattern of epidermal outline is thick and composed of squamous epithelium cells, normal keratin layer, a lot of sebaceous glands and hair follicles are also noted.	Granulation tissues consist of fibroblast and collagen tissues are noted.	Small blood vessels and part of muscular layer are also noted.	Normal skin
70% ethanolic root extract treated skin Figure 13	The papillary epidermal out line is well developed and composed squamous epithelium cells and thin layer of keratin. Well developed sebaceous glands and hair follicles are also seen.	Granulation tissue is composed of thin layer of fibroblast and collagen tissues.	Normal blood vessels and muscular layers are well developed.	Well developed granulation tissues in all layers of skin

Type of skin	Epidermis	Dermis	Subcutaneous tissue	Histological Diagnosis
Standard drug (Tetra-cycline) Figure 14	The papillary epidermal outline is thin and composed of squamous epithelium. Thin keratin layer is also noted. Loss of some sebaceous and hair follicles are present.	Granulation tissue is composed of thick layer of fibroblast and collagen tissues.	Scanty of small blood vessels and muscular layers are also noted.	Appearance of re-epithelialization was nearly normal but vascularization and granulation tissue formation is reduced.
Untreated (control) Figure 15	The papillary pattern of epidermal outline is thin and composed of squamous epithelium cells. In some areas, keratin layer is thin and scanty in site of disrupted areas. Some sebaceous glands and hair follicles are lost.	Granulation tissues consist of fibroblast and collagen tissues are noted.	Small blood vessels and part of muscular layer are also noted.	Normal degree of re-epitheliali-zation of rat's skin.

Discussion and Conclusion

In this study, ethanolic root extract of *Flemingia strobilifera* (L.) R. Br. showed wound healing activity in albino rats. The results of excision wound model were shown in figure (12 to 15) and table (1). According to this findings, the ethanolic root extract of *Flemingia strobilifera* (L.) R. Br. and standard drug (Tetracycline ointment) exhibited significant wound healing activity as compared to control. It is observed that the wound closure time was faster in both treated groups than in the control. Sebaceous glands and hair follicles were more developed in albino rat treated with ethanolic root extract than standard drug (Tetracycline).

The histological result of wound healing was agreed with Min Htun Min Latt (2014) who stated that sebaceous glands and hair follicle were well developed.

It is concluded that 70% ethanolic root extract of *Flemingia strobilifera* (L.) R. Br. showed better wound healing property, which support the traditional use of *Flemingia strobilifera* (L.) R. Br. for treatment of wounds.

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TAXONOMY AND POLLEN MORPHOLOGY OF THE FAMILY ROSACEAE FOUND IN SOUTHERN AND NORTHERN SHAN STATE

Aye Myint San[1], Swe Swe Linn[2] & Soe Myint Aye[3]

Abstract

The taxonomical and pollen morphological studies of 10 species of the Family Rosaceae were studied. The specimens were collected 1 species such as *Docynia indica* (Wallich) Decaisne. in Pinlaung township and 4 species like *Rubus ellipticus* Smith., *Rubus ellipticus* var. *obcordatus* (Francact) Focke., *R. molucanus* L. & *Pyrus communis* L. in Aungban township and 5 species namely *Chaenomeles japonica* (Thunb.) Lindley, *Prunus cerasoides* D. Don., *P. ceylanica* (Wight) Miq., *P. communis* Huds. & *P. persica* (L.) Batsch., in Lashio township. The genera *Chaenomeles*, *Docynia* and *Pyrus* were belonging to Maloideae, *Rubus* was belonging to Rosoideae and *Prunus* was belonging to Prunoideae. All the specimens were collected from Southern and Nothern Shan State from September 2016 to October 2017. The measurements were based on at least 20 randomly selected, fully developed pollen grains per specimens. All the pollen grains of study species were tricolporate except from *Rubus molucanus* L., which was tri to tetracoporate and *Prunus cerasoides* D. Don. that possessing tricolpate in aperture. All pollen grains were prolate spheroidal in shape. The pollen size was small in 4 specie such as *Docynia indica* (Wallich) Decaisne., *Pyrus communis* L., *Rubus ellipticus* Smith. & *Prunus communis* Huds. and the remaining 6 species were medium. All investigated pollen grains were characterized by striate type of sculpturing. In this research, taxonomic description, the pollen morphological characters and photographs for every species were stated.

Keywords: Taxonomy, Pollen Morphology, Rosaceae

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Introduction

Rosaceae when first established by Jusseu (1789) was a small family with about 40 genera. The family Rosaceae consists of about 1000 species (Hooker 1879), 124 genera and about 3375 species (Dassanayake 1981) and 85 genera and 2000 sexual species (Kubitzki 2004). Watson (2013) described about 85 – 3000 species, cosmopolitan but most diverse in temperate and warm regions of the North Hemesphere. Kress *et al*. (2003) stated that the family Rosaceae consists of about 24 genera with a total of about 219 known species in Myanmar.

The Rosaceae are generally subdivided into four subfamilies: Prunoideae, Pomoideae, Spiraeoideae and Rosoideae. According to the International Code of Botanical Nomenclature, the Pomoideae should be renamed Maloideae, but the original name is still widely used in the literature (Challice 1974).

Taxonomy can be defined as the science dealing with the description, identification, nomenclature and classification of life (Simpson 2006). Since the time of Linnaeus, comparative analysis of reproductive characters has been the principal morphological technique for identifying and classifying angiosperms (Beth 2009).

Palynology is the science of pollen and spore morphology (Erdtman 1952). Palynology is concerned with both the structure and the formation of pollen grains and spores, also with dispersal and their preservation under certain environmental conditions. Palynology is related with cytology, genetics, taxonomy and so on (Moore *et al.* 1991).

Challice (1974) reported the Rosaceae are family of exceptional horticultural significance, with economically important fruit-bearing plants and ornamentals in the Prunoideae, Pomoideae and Rosoideae and a few ornamental shrubs in the Spiraeoideae. As well known examples the following may be mentioned, Prunoideae: plums, cherries, peaches, apricots, almonda, cherry laurels; Pomoideae: apples, pears, quince and numerous ornamental

trees and shrubs; Spiraeoideae: ornamental shrubs; Rosoideae: strawberries, blackberries and raspberries, roses and other garden plants.

In the floristic studies on various region of Myanmar, taxonomical study of a few species belonging to the Family Rosaceae has been studied by various researchers. However, taxonomy and pollen morphology of Rosaceae has not been studied in Myanmar. therefore, a research on the taxonomy and pollen morphology of this family is selected.

The aims and objectives of this research is to record on pollen morphology of the species of the family Rosaceae and to apply the result of palynological characters for taxonomic purposes.

Materials and Methods

The studied plants were collected from Aungban, Pinlaung and Lashio regions during September 2016 to October 2017. All the collected species were recorded by photographs at flowering times. Location of the collected species were described by Global Positioning System (GPS). Description and Classsification of the species were made by fresh specimens. Identification of collected specimens were carried out by using floristic literature of Hooker (1879), Backer (1963), Dassanayake (1981), Lingdi *et al.* (2003), Watson *et al.* (2011). Myanmar names were referred to Hundley and Chit Ko Ko (1987) and Kress *et al*. (2003). Pollen samples were collected from the anther of blooming flowers. Pollen of each species was stored in glass vials with 1cc of glacial acedic acid and the specimen was labelled. Pollen samples were acetolysed by Erdtman method (1960).

Results

Taxonomy and pollen morphology of 10 species belonging to the Family Rosaceae have been studied. The subfamily and general listed according to Lingdi *et al*. (2003) and the species were arranged alphabetically as shown in Table 1.

Table 1. List of collected species

Family	Subfamily	Genus	Species
Rosaceae	Maloideae	*Docynia*	*Docynia indica* (Wallich) Decaisne
		Chaenomeles	*Chaenomeles japonica* (Thunb.) Lindley
		Pyrus	*Pyrus communis* L.
	Rosoideae	*Rubus*	*Rubus ellipticus* Smith.
			R. ellipticus var. *obcordatus* (Franchet) Focke
			R. molucanus L.
	Prunoideae	*Prunus*	*Prunus cerasoides* D.Don.
			P. ceylanica (Wight) Miq.
			P. communis Huds.
			P. persica (L.) Batsch.

1. *Docynia indica* (Wallich) Decaisne, Nouv. Arch. Mus. Hist. Nat. 10: 131. 1874. . (Figure 1. A)

Pyrus indica Wallich, Pl. Asiat. Rer. 2: 56. 1831.

Local Name - Pin SeinThi

English Name -Unknown

Flowering Period - March to April

Perennial trees; stems and branches terete. Leaves simple, alternate; stipules lanceolate, caducous; leaf blade elliptic or oblong lanceolate, broadly cuneate or subroundedat the base, acute or acuminate at the apex, margin shallowly crenate.Flowers bisexual, actinomorphic, pentamerous, perigynous, white; pedicels short or nearly absent, pubescent; bracts lanceolate. Sepals 5,lanceolate or triangular-lanceolate, acute or acuminate at the apex. Petals 5, free, oblobg or oblong-obovate, white, broadly cuneate at the base, ovate at the apex. Stamens numerous, inserted; filaments filiform; anthers dithecous, dorsifixed, dehiscing longitudinally, yellow.Carpels 5, free; ovary superior, ovoid, unilocular with one ovule in each locule on pandulous placentae; styles

slender, as long as stamens; stigma capitate. Fruits pome, subglobose. Seed ellipsoid, glabrous.

Specimens examined: Southern Shan State, Pin Laung Township, 22˚ 56.998' N and 97˚ 44.924' E, Elevation 840 m, Aye Myint San, October 6, 2017; collection no. 29.

Description of pollen morphology (Figure 1. B, C)

Tricolporate, prolate spheroidal, small, 12.6-13.0x12.5µm in length and breadth; amb triangular, colpi longicolpate, 8.8-12.5x5.0-6.3 µm in length and breadth; pori lalongate, 2.5x3.8 µm; exine 1.3-2.5µm thick, sexine thicker than nexine; sculpturing finely striate.

2. *Chaenomeles japonica* (Thunb.) Lindley ex Spach, Hist. Nat. Veg. 2: 159. 1834. (Figure 1. D)

Pyrus japonica Thunb. Nova Acta. Regiae Soc. Sci. Uspal.3: 208. 1780.

Local Name - Chin saw gar thi

English Name -Dwraf Japanese quince

Flowering Period - March to June

Perennial shrubs; stems and branches terete, with slender thorn. Leaves simple, alternate; stipules reniform; leaf blade obovate to broadly ovate, cuneateat the base, obtuse or acute at the apex, margin crenate. Flowers bisexual, actinomorphic, pentamerous, perigynous, dark red; pedicels short or nearly absent, glabrous; Sepals 5,ovate, rarely suborbicular, acuminate at the apex. Petals 5, free, obovate or sub orbicular, dark red, broadly cuneate at the base, ovate at the apex. Stamens numerous, inserted; filaments filiform; anthers dithecous, dorsifixed, dehiscing longitudinally, yellow. Carpels 5, free; ovary superior, ovoid, unilocular with one ovule in each locule on pandulous placentae; styles slender, as long as stamens; stigma capitate. Fruits pome, subglobose. Seed ellipsoid, glabrous.

Specimens examined: Northern Shan State, Lashio Township, Sarsana 2500 Pagoda Hill, 22˚ 56.998' N and 97˚ 44.924' E, Elevation 840 m, Aye Myint San, May 12, 2017; collection no. 18.

Description of pollen morphology (Figure 1. E, F)

Tricolporate, prolate spheroidal, medium, 25.3-25.6x8.8 µm in length and breadth; amb triangular, colpi ¾ way up to the pole, 25.0x10.0-12.5 µm in length and breadth; pori lalongate,7.5x8.8 µm; exine 1.3µm thick, sexine thicker than nexine; sculpturing finely striate.

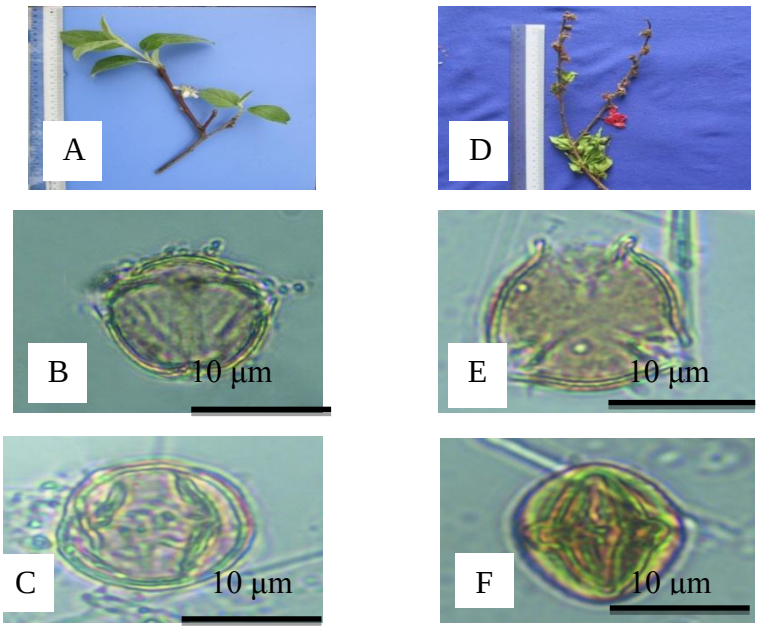


Figure 1. A. Inflorescence of *Docynia indica* (Wallich) Decaisne
B. Polar view of pollen grain of *D. indica* (Wallich) Decaisne
C.Equatorial view of pollen grain of *D. indica* (Wallich) Decaisne
D. Inflorescence of *Chaenomeles japonica* (Thunb.) Lindley
E. Polar view of pollen grain *C. japonica* (Thunb.) Lindley
F. Equatorial view of pollen grain of *C. japonica* (Thunb.) Lindley

3. *Pyrus communis* L., Sp. Pl. 479. 1753. (Figure 2. A)

P. domestica (L.) Ehrn.

Local Name : Thittaw

English Name : Pear

Flowering Period : January to March

Perennial small tree; stems and branches terete, glabrous or slightly pubescent when young. Leaves simple, alternate; stipules linear, caducous; leaf blade broadly ovate or elliptic, rounded at the base, acute at the apex, margin finely serrulate. Inflorescences terminal and axillary, corymbose raceme; peduncles glabrous/. Flowers bisexual, actinomorphic, pentamerous, epigynous, white; bracts lanceolate, caduceus; pedicels terete, glabrous; Calyx campanulate, pubescent, 5-lobed; lobes broadly ovate, acuminate at the apex, pubescent, persistent. Petals 5, free, obovate, white, broadly cuneate at the base, obtuse at the apex. Stamens numerous, inserted; filaments filiform; anthers dithecous, dorsifixed, dehiscing horizontally, yellow. Carpels 5, free; ovary inferior, ovoid, pentalocular with two ovule in each locule on anatrophus placentae; styles 5, free, slender; stigma capitate. Fruits pyriform, rounded. Seed ellipsoid, glabrous.

Specimens examined: Southern Shan State, Pinlong Township, Yeoo village, 20˚ 12.177’ N and 96˚ 45.001’ E, Elevation 1302 m, Aye Myint San, September 13, 2016; collection no. 2.

Description of pollen morphology (Figure 2. B, C)

Tricolporate, Prolate spheroidal, small, 21.3 – 25.0 x 18.8 – 21.3µm in length and breadth; amb convex triangular, colpi ¾ way up to the pole, 16.3 – 20.3 x 6.3µm in length and breadth; pori lolongate, 6.3-7.5 x about 6.3µm; exine 1.3-2.5µm thick, sexine thicker than nexine; sculpturing finely striate, muri 0.5 – 0.9 µm wide, groove 0.5 – 0.9 µm wide.

4. ***Rubus ellipticus*** Smith in Rees, Cyclop. 30: no. 16. 1819. **(Figure 2. D)**

Local Name - Unknown

English Name - Unknown

Flowering Period - January to June

Perennial erect shrubs; stems and branches terete, woody below, flexuous, densely shaggy with spreading rad-brown hair; prickly; princkles stout, hooked, scattered, compress. Leaves pinnately trifoliate compound, alternate; stipules paired, subulate; leaflets elliptic or obovate, unequal; rough, acute at the apex, serreate along the margin, rounded at the base, dark green and glabrous above, pale green beneath, pubescent midrib prominent, minute prickly. Inflorescences axillary or terminal, paniculate cymes; peduncles red-brown shaggy hairy. Flowers, bisexual, actinomorphic, pentamerous, epigynous, white; bracts linear, densely white villous; pedicels subsessile; hypanthium subglobose, pubescent. Sepals 5, free, ovate, persistent, green, densely white bristiles within and without. Petals 5, free, obovate, white, caducous, cuneate at the base, glabrous. Stamens numerous, inserted; filaments filiform; anthers dithecous, dorsifixed, dehiscing longitudinally, small, glabrous, yellow. Carpels many, free; ovary inferior, reniform on distinct receptacle, unilocular, one ovule per locule in basal placentation; styles curved and short; stigma simple, persistant. Fruits aggregate of numerous druplets, globoid, orange, succulent, green at first, orange-yellow when ripe. Seed minute.

Specimens examined: Southern Shan State, Aungban Township, 20˚ 38.443’ N and 96˚ 36.507’ E, Elevation 1297 m, Aye Myint San, September 12, 2016; collection no.4.

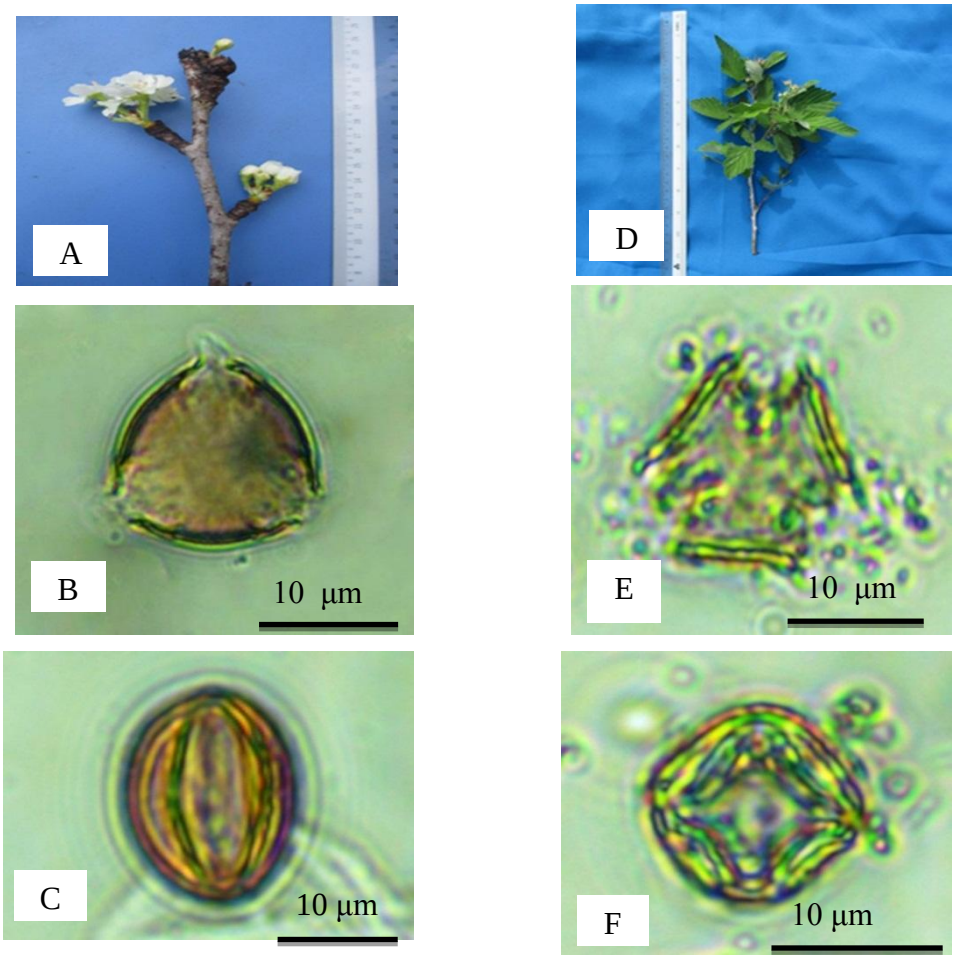


Figure 2. A. Inflorescence of *Pyrus communis* L.
B. Polar view of pollen grain of *P. communis* L.
C. Equatorial view of pollen grain of *P. communis* L.
D. Inflorescence of *Rubus ellipticus* Smith.
E. Polar view of pollen grain of *R. ellipticus* Smith.
F. Equatorial view of pollen grain of *R. ellipticus* Smith.

Description of pollen morphology (Figure 2. E,F)

Tricolporate, Prolate spheroidal, small, 18.8 – 25.0 x 15.0 – 17.5µm in length and breadth; amb convex triangular, colpi ¾ way up to the pole, 15.0

– 18.8 x 5.0 - 0.6µm in length and breadth; pori lolongate, 2.5-5.0 x 2.5-5.0µm; exine 1.3 – 2.5µm thick, sexine thicker than nexine; sculpturing finely striate muri about 0.9µm and groove about 0.5µm wide.

5. ***Rubus ellipticus*** **var.** ***obcordatus*** (Franchet) Focke, Biblioth. Bot. 17 (Heft 72): 199. 1911. **(Figure 3.A)**

Local Name - Unknown

English Name - Unknown

Flowering Period - January to June

Perennial erect shrubs; stems and branches terete, woody below, flexuous, densely shaggy with spreading rad-brown hair; prickly; princkles stout, hooked, scattered, compress. Leaves pinnately trifoliate compound, alternate; stipules paired, subulate; leaflets obovate or obcordate, unequal; rough, truncate or emarginated at the apex, serreate along the margin, obtuse-cuneate at the base, dark green and glabrous above, pale green beneath, pubescent midrib prominent, minute prickly. Inflorescences axillary or terminal, paniculate cymes; peduncles red-brown shaggy hairy. Flowers, bisexual, actinomorphic, pentamerous, epigynous, white; bracts linear, densely white villous; pedicels subsessile; hypanthium subglobose, pubescent. Sepals 5, free, ovate, persistent, green, densely white bristiles within and without. Petals 5, free, obovate, white, caducous, cuneate at the base, glabrous. Stamens numerous, inserted; filaments filiform; anthers dithecous, dorsifixed, dehiscing longitudinally, small, glabrous, yellow. Carpels many, free; ovary inferior, reniform on distinct receptacle, unilocular, one ovule per locule in basal placentation; styles curved and short; stigma simple, persistant. Fruits aggregate of numerous druplets, globoid, orange, succulent, green at first, orange-yellow when ripe. Seed minute.

Specimens examined: Southern Shan State, Aungban Township, 20˚ 38.443’ N and 96˚ 36.507’ E, Elevation 1297 m, Aye Myint San, September 12, 2016; collection

Description of pollen morphology (Figure 3. B,C)

Tricolporate, Prolate spheroidal, medium, 30.0 – 35.0 x 20.0 - 27.5µm in length and breadth; amb convex triangular, colpi ¾ way up to the pole, 18.8 – 28.8 x 5.0µm in length and breadth; pori circular, 2.5-5.0 x 2.5-5.0µm; exine 2.5µm thick, sexine thicker than nexine; sculpturing finely striate muri about 0.9µm and groove abot 0.5µm wide.

6. *Rubus moluccanus* Smith in Rees, Cyclop. 30: no. 16. 1819. **(Figure 3. D)**

Local Name - Unknown

English Name - Unknown

Flowering Period - October to January

Perennial scrambling shrubs; stems and branches solid, terete, woody below, densely white tomentose, prickly; princkles stout, hooked, small, scattered. Leaves simple, alternate; stipules oblong; blades broadly ovate and deeply 5 – lobed, regose, acute at the apex, serrulate along the margin, deeply cordate at the base, dark green and densely woolly beneath, palmately 5 – 7 nerved, prickly on the nerve beneath. Inflorescences axillary or terminal cymes, densely white tomentose, minut prickly; peduncles densely white tomentose. Flowers bisexual, actinomorphic, pentamerous, epigynous, white; bracts oblong, laciniate, densely white villous; pedicels subsessile; hypanthium subglobose, pubescent. Calyx 5 – lobed, campanulate tube, teeth triangular, lanceolate, acute at the apex, persistent, yellowish green,

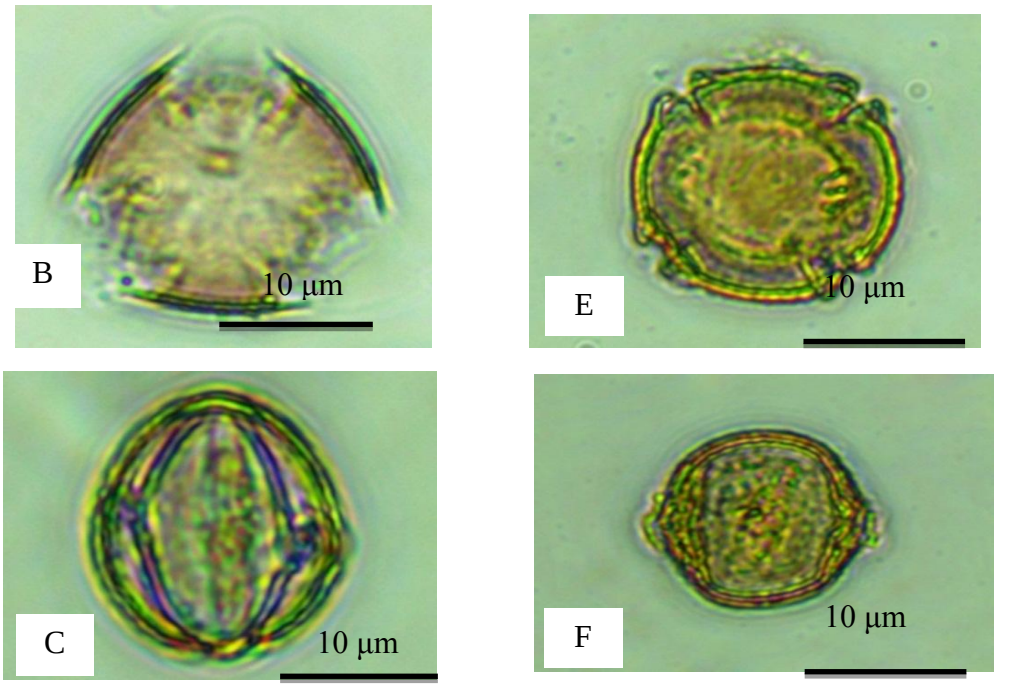


Figure 3. A. Inflorescence of *Rubus ellipticus* var. *obcordatus* (Franchet) Focke,
B. Polar view of pollen grain of *R. ellipticus* var. *obcordatus* (Franchet) Focke,
C. Equatorial view of pollen grain of *R. ellipticus* var. *obcordatus* (Franchet) Focke,
D. Inflorescence of *R. moluccanus* Smith.
E. Polar view of pollen grain of *R. moluccanus* Smith.
F. Equatorial view of pollen grain of *R. moluccanus* Smith.

entire along the margin, enlarged accrescent calyx in fruits, densely villous or white bristiles within and without. Petals 5, free, obovate, white, shorter than the calyx- lobes, rounded at the apex. Stamens numerous, inserted; filaments slender, unequal in length; anthers dithecous, dorsifixed, dehiscing longitudinally. Carpels many, free; ovary inferior, bilocular, two ovule per locule in basal placentae; styles filiform; stigma simple, persistant. Fruits aggregate of small druplets, globoid, succulent, green at first, orange-yellow when ripe. Seed minute.

Specimens examined: Southern Shan State, Aungban Township, 20˚ 54.324' N and 96˚ 36.213' E, Elevation 1474 m, Aye Myint San, September 13, 2016; collection no.6.

Description of pollen morphology (Figure 3. E, F)

Tri to tetracolporate, prolate spheroidal, medium, 37.5 – 46.3 x 26.3 – 33.8µm in length and breadth; amb convex tri to tetraangular, colpi ¾ way up to the pole, 27.5 -35.0 x 6.3 µm in length and breadth; pori circular, 6.3-7.5 x 6.3-7.5 µm; exine 2.5 µm – 3.8 thick, sexine thicker than nexine; sculpturing finely striate muri about 0.9µm and groove 0.9- 1.8µm wide.

7. *Prunus cerasoides* (L.) D. Don, Prod. Fl. Nep: 239. 1825. **(Figure 4. A)**

P. puddum Roxb. ex Wall.Pl. As rar. 2: 37. 1831.

Local Name - Cherry

English Name - Cherry

Flowering Period - December to March

Perennial tree; stems and branches woody, glabrous. Leaves simple, alternate; stipules linear; leaf blade obovate, rounded at the base, acuminate at the apex, margin serrate. Inflorescences terminal and axillary, 2-3 flowered fascicles; peduncles glabrous. Flowers bisexual, actinomorphic, pentamerous, perigynous, pink; scaly bracts; pedicels terete, glabrous; Sepals 5, ovate-oblongate, acute to obtuse at the apex. Petals 5, free, obovate, pink, broadly cuneate at the base, emarginate at the apex. Stamens numerous, inserted; filaments filiform; anthers dithecous, dorsifixed, dehiscing longitudinally, yellow. Carpels 1, free; ovary superior, ovoid, unilocular with one ovule in each locule on pandulous placentae; styles slender; stigma discoid. Fruits a drupe, ovoid. Seed ellipsoid, glabrous.

Specimens examined: Northern Shan State, Lashio Township, Naung Mon Village, 22˚ 46.934' N and 97˚ 39.007' E, Elevation 733 m, Aye Myint San, December 3, 2016; collection no. 20.

Description of pollen morphology (Figure 4. B, C)

Tricolpate, Prolate spheroidal, medium, 26.3 – 35.0 x 18.8 – 27.5µm in length and breadth; amb convex triangular, colpi ¾ way up to the pole, 21.3 – 27.5 x 1.3 – 5.0 µm in length and breadth; pori circular; exine 1.3- 2.5µm thick, sexine thicker than nexine; sculpturing finely striate muri about 0.9 µm and groove 0.5µm wide..

8. *Prunus ceylanica* (Wight) Miq. Fl. Ind. Bat. 1(1): 366. 1855.

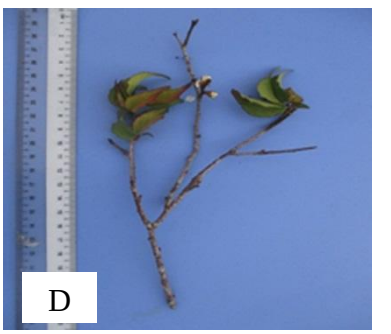
(Figure 4. D)

Local Name - Unknown

English Name - Unknown

Flowering Period - November to February

Perennial small tree; stems and branches woody, glabrous. Leaves simple, alternate; stipules linear; leaf blade ovate or elliptic-lanceolate, cuneate at the base, acute at the apex, margin serrate. Inflorescences terminal and axillary, peduncles glabrous. Flowers bisexual, actinomorphic, pentamerous, perigynous, white; scaly bracts, persistent; pedicels terete, glabrous; Sepals 5, ovate, obtuse at the apex. Petals 5, free, spatulate, white, broadly cuneate



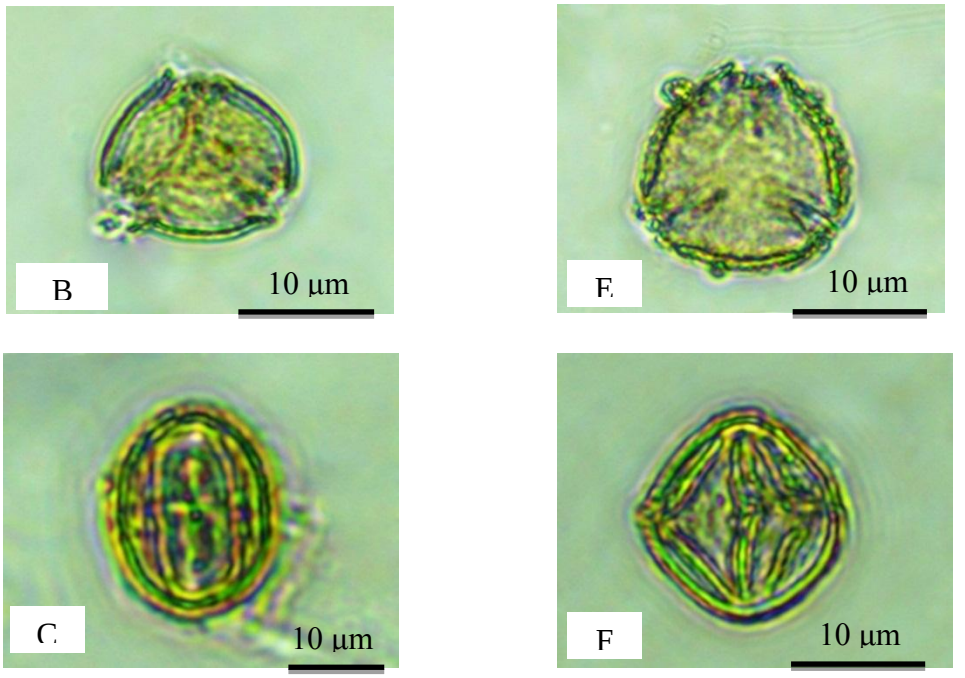


Figure 4. A. Inflorescence of *Prunus cerasoides* D.Don.
B. Polar view of pollen grain of *P. cerasoides* D.Don.
C. Equatorial view of pollen grain of *P. cerasoides* D.Don.
D. Inflorescence of *P. ceylanica* (Wight) Miq.
E. Polar view of pollen grain of *P. ceylanica* (Wight) Miq.
F. Equatorial view of pollen grain of *P. ceylanica* (Wight) Miq.

at the base, acute at the apex. Stamens numerous, inserted; filaments filiform; anthers dithecous, dorsifixed, dehiscing longitudinally, yellow. Carpels 1, free; ovary superior, ovoid, unilocular with two ovule in each locule on pandulous placentae; styles slender; stigma disc-shaped. Fruits a drupe, ovoid to globose. Seed ellipsoid, glabrous.

Specimens examined: Northern Shan State, Lashio Township, Naung Mon Village, 22˚ 46.784’ N and 97˚ 38.944’ E, Elevation 728 m, Aye Myint San, December 3, 2016; collection no. 10.

Description of pollen morphology (Figure 4. E, F)

Tricolporate, prolate spheroidal, medium, 27.5 – 47.5x18.8 - 25.0µm in length and breadth; amb convex triangular, colpi ¾ way up to the pole, 21.3 – 43.8 x 5.0 – 8.8 µm in length and breadth; exine 1.3-2.5µm thick, sexine thicker than nexine; sculpturing finely striate muri about 0.9 µm and groove 0.9-1.8µm wide.

9. ***Prunus communis*** Huds. FL. Angl. (Hudson), ed. 2, 1: 212. 1778.

(Figure 5. A)

Local Name - Metmann

English Name - Unknown

Flowering Period - November to February

Perennial small tree; stems and branches woody, glabrous. Leaves simple, alternate; stipules linear; leaf blade ovate, obtuse at the base, acuminate at the apex, margin serrate. Inflorescences terminal and axillary, 2-3 flowered fascicles; peduncles glabrous. Flowers bisexual, actinomorphic, pentamerous, perigynous, white; scaly bracts; pedicels terete, glabrous; Sepals 5, ovate, acute at the apex. Petals 5, free, obovate, white, broadly cuneate at the base, acute at the apex. Stamens numerous, inserted; filaments filiform; anthers dithecous, dorsifixed, dehiscing longitudinally, yellow. Carpels 1, free; ovary superior, ovoid, unilocular with one ovule in each locule on pandulous placentae; styles slender; stigma discoid. Fruits a drupe, ovoid to globose. Seed ellipsoid, glabrous.

Specimens examined: Southern Shan State, Lashio Township, Naung Mon Village, 22˚ 46.934’ N and 97˚ 39.007’ E, Elevation 733 m, Aye Myint San, December 3, 2016; collection no. 12.

Description of pollen morphology (Figure 5. B, C)

Tricolporate, prolate spheroidal, small, 23.8 – 25.0 x about 18.8µm in length and breadth; amb convex triangular, colpi ¾ way up to the pole, 17.5 – 20.0 x 3.8µm in length and breadth; pori circular; exine 1.3 - 2.5µm thick,

sexine thicker than nexine; sculpturing finely striate muri about 1.8-2.7 µm and groove 0.9-4.5µm wide.

10. *Prunus persica* (L.) Batsch, Beitr. Entw. Pragm. Gesch. 1: 30. 1801 (Figure 5. D)

Amygdalus persica L., Sp. Pl. 472. 1753.

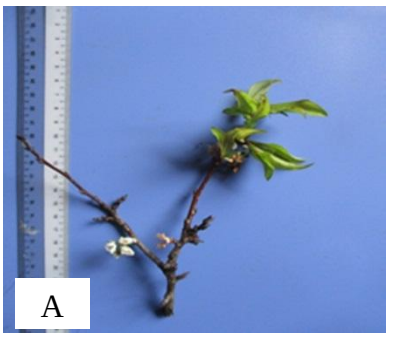
Persica vulgaris Mill., Gard. Dict. ed. 8. 465. 176

Local Name - Met mon

English Name - Peach

Flowering Period - January to March

Perennial small tree; stems and branches terete, glabrous. Leaves simple, alternate; stipules subulate; leaf blade lanceolate, obtuse at the base, acuminate at the apex, margin finely serrate. Inflorescences terminal and axillary, 2-3 flowered fascicles; peduncles glabrous. Flowers bisexual, actinomorphic, pentamerous, perigynous, pink; scaly bracts; pedicels terete, glabrous; Sepals 5, ovate-oblongate, acuminate at the apex. Petals 5, free, obovate, pink, broadly cuneate at the base, ovate at the apex. Stamens numerous, inserted;



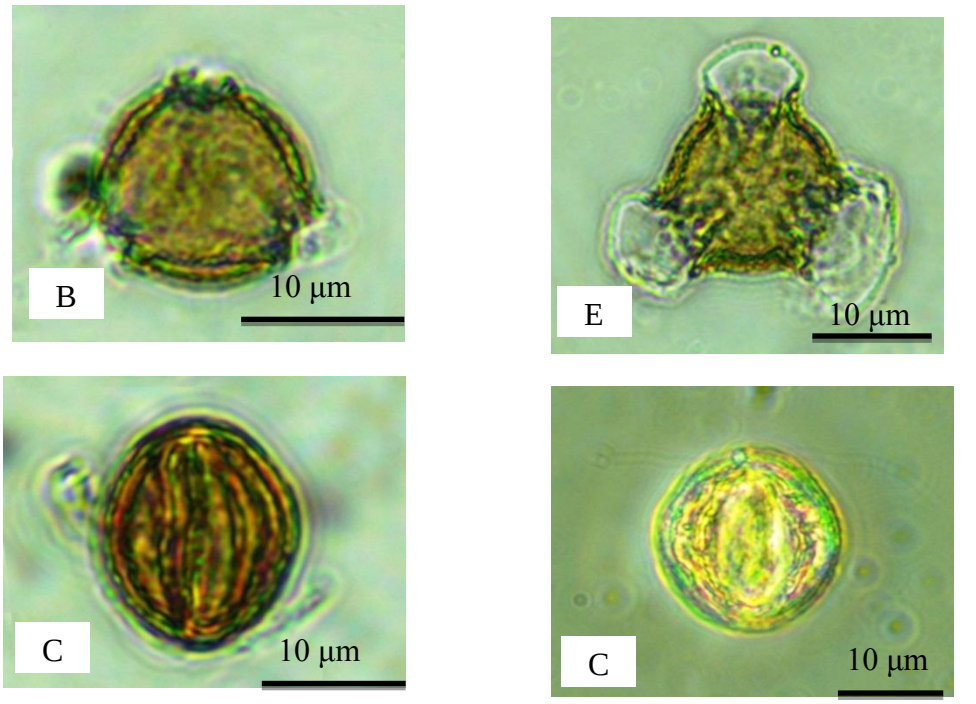


Figure 5. A. Inflorescence of *Prunus communis* Huds.
B. Polar view of pollen grain of *P. communis* Huds.
C. Equatorial view of pollen grain of *P. communis* Huds.
D. Inflorescence of *P. persica* (L.) Batsch.
E. Polar view of pollen grain of *P. persica* (L.) Batsch.
F. Equatorial view of pollen grain of *P. persica* (L.) Batsch.

filament filiform; anthers dithecous, dorsifixed, dehiscing longitudinally, yellow. Carpels 5, free; ovary superior, ovoid, unilocular with one ovule in each locule on pandulous placentae; styles slender; stigma capitate. Fruits a drupe, rounded. Seed ellipsoid, glabrous.

Specimens examined: Southern Shan State, Lashio Township, Sarsana 2500 Pagoda Hill, 22˚ 56.998’ N and 97˚ 44.924’ E, Elevation 840 m, Aye Myint San, December 2, 2016; collection no. 8.

Description of pollen morphology (Figure 5. E, F)

Tricolporate, prolate spheroidal, medium, 26.3 – 33.8 x 20.0 – 22.5µm in length and breadth; amb convex triangular, colpi ¾ way up to the pole, 18.8 – 25.0 x 5.0µm in length and breadth; pori lolongate; exine 2.5µm thick, sexine thicker than nexine; sculpturing finely striate muri about 0.5-0.9 µm and groove 0.5-0.9µm wide.

Discussion and Conclusion

The present study deals with the pollen morphology of the Family Rosaceae found in Southern and Northern Shan State region. Dassanayake (1981) stated that a family of 124 genera and about 3375 species and this family is widely distributed in all parts of the world, mostly in the northern temperate regions. There are about 1000 species, found in all climates and countries, but chiefly in the temperate (Hooker 1879). Worldwide about 85 – 3000 species, cosmopolitan but most diverse in temperate and warm regions of the North Hemesphere (Watson 2013). The family is represented by about 24 genera with a total of about 219 known species in Myanmar (Kress *et al*. 2003). In the present study, taxonomy and pollen morphology of 10 species belonging to the Family Rosaceae have been studied.

Among the collected species 1 species such as *Docynia indica* (Wallich) Decaisne. in Pinlaung township and 4 species like *Rubus ellipticus* Smith., *Rubus ellipticus* var. *obcordatus* (Francact) Focke, *R. molucanus* L. & *Pyrus communis* L. in Aungban township and 5 species namely *Chaenomeles japonica* (Thunb.) Lindley, *Prunus cerasoides* D. Don, *P. ceylanica* (Wight) Miq., *P. communis* Huds. & *P. persica* (L.) Batsch in Lashio township. The genera *Chaenomeles*, *Docynia* and *Pyrus* were belonging to Maloideae, *Rubus* was belonging to Rosoideae and *Prunus* was belonging to Prunoideae. According to the collected data, all studied species were shrubs, unipinnately compound leaves. The flowers were bisexual, actinomorphic, pentamerous and perigynous ovary and the stamens were numerous in all collected species. According to the resulting data, the fruit types were found in achene. These characters were agreed with Hooker

(1879), Backer (1963), Dassanayake (1981), Lu *et al.* (2003), Watson *et al.* (2011).

In the present study, all pollen grains were monad; the aperture types of the pollen grains were mostly tricorporate except from *Rubus moluccanus* Smith; and all species were found in prolate spheroidal in shape. Therefore, these pollen characters were agreed with Erdtman (1971). The pollen size were small in 4 species, *Docynia indica* (Wallich) Decaisne, *Pyrus communis* L., *Rubus ellipticus* Smith, and *Prunus communis* Huds. and the remaining 6 species were medium. Faghir (2015) reported the the Rosaceae pollen grain sizes were small to medium in size however Garaci *et al*. (2012) stated that the pollen grains were medium to large-sized. In the present study, the grains were not found in large size. Therefore the present study were agreed with Faghir (2015). The pori shape of the investigated pollen grains were found to be 2 species of *Docynia indica* (Wallich) Decaisne and *Chaenomeles japonica* (Thunb.) Lindley were found in lalongate; 3 species of *Pyrus communis* L., *Rubus ellipticus* Smith and *Prunus ceylanica* (Wight) Miq. were found in lolongate; and the remaining 4 species were circular in shape. The sexine thicker than nexine. The pollen sculpture of all species were finely striate. These pollen characters were agreed with Erdtman (1971) and Moore *et al*. (1991).

According to the resultting data, the pollen pollen morphological characters were formed to be different within the species. Therefore, these present study was not only for the palynological point of view but also to provided the valuable pollen characters that can be support in classification and identification of the Family Rosaceae.

Acknowledgements

I would like to express my sincerest gratitude to Dr Nu Nu Yee, Professor and Head, Department of Botany, University of Mandalay, for her permission and encouragement to carry out this research work. I am thankful to Dr Aye Pe, Professor and Head, Department of Botany, University of Yangon and Dr Thandar Aye, Professor, Department of Botany, University of Yangon for their grateful encouragement and supportivenss all facilities to accomplish during the study period. Especially, I am also very grateful to my supervisor, Dr

Soe Myint Aye, Professor, Department of Botany, University of Mandalay for his thoughtful suggestions, invaluable suprvision, patient guidance, understanding and encouragements throughout the study period.

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ACUTE TOXICITY AND HYPOGLYCAEMIC ACTIVITY OF 70% ETHANOLIC EXTRACT OF *MIRABILIS JALAPA* L.

Ei Soe Thi Aung[1], Htay Htay Lwin[2]

Abstract

Mirabilis jalapa L. is locally known as Mye-su, Lay-nar-yi-pan, Marvel of Peru or Four o'clock flower and belongs to the family Nyctaginaceae. The plant samples were collected from Kamaryut Township, Yangon University Campus. In acute toxicity study of 70% ethanolic extract of *Mirabilis jalapa* L. were evaluated on albino mice by using method of OECD Guideline 423. There were no sign of toxicity and lethality of mice, even with the maximum dose at 5 g/kg body weight of the extract during the test period of 14 days. Therefore, 70% ethanolic extract of *Mirabilis jalapa* L. had no acute toxic effect up to the dose of 5 g/kg. The hypoglycaemic activity of 70% ethanolic extract was also studied on adrenaline-induced hyperglycaemic rats model by using the method of Gupta *et al*. (1967). In hypoglycaemic activity test on adrenaline-induced hyperglycaemic rats, the extract at the dose of 1 g/kg showed significant blood glucose lowering effect at 4 hrs ($p<0.01$), 2 g/kg at 3 hrs ($p<0.005$) and 4 hrs ($p<0.001$) and 4 g/kg at 2 hrs, 3hrs and 4 hrs ($p<0.001$) when compared with the control. It was found that 4 g/kg of 70% ethanolic extract showed the most effective hypoglycaemic activity among 3 doses of the extract.

Keywords: *Mirabilis jalapa* L., Acute Toxicity, Hypoglycaemic Activity

Introduction

Mirabilis jalapa L. belongs to the family Nyctaginaceae. It consists of about 30 genera and 300 species in tropical and subtropical regions of both the Old and New World (Cronquist, 1981 and Bhattacharyya, 1998). The native of this plant is tropical America (Cooke, 1958).The world is now moving towards the herbal medicine or phytomedicines that repair and strengthening bodily systems and help to destroy offending pathogens without toxic side effects. Today, it has been developed as a separate industry as many people favor herbal medicine over synthetic medicine (Pandey *et al*., 2011).

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Treatment of diseases like cancer, diabetes etc. is not easy for the poor family due to high coast of the treatment. Nowadays, there is widespread interest to promote the traditional health care systems to meet primary health care needs (Pallab *et al*., 2014).

Toxicity is an expression of being poisonous, indicating the state of adverse effects led by the interaction between toxicants and cells. This interaction may vary depending on the chemical properties of the toxicants and the cell membrane, as it may occur on the cell surface, within the cell body, or in the tissues beneath as well as at the extracellular matrix. The toxic effects may take place prior to the binding of the toxicants to the vital organs such as liver and kidneys. Hence, evaluation of toxic properties of a substance is crucial when considering for public health protection because exposure to chemicals can be hazardous and results to adverse effects on human being. (Chen *et al*., 2011).The acute toxicity test in which single dose of the drug is used in each animal on one occasion only for the determination of gross behavior and LD_{50} (the dose which has proved to be lethal causing death to 50% of the tested group of animals) or median lethal dose (Gupta, 2012).

There is hundreds of other bioactive compounds present in plants are helpful for the treatment of diabetic diseases and also used in the lowering of glucose level in the blood (Marles and Farnsworth, 1995). Traditionally plants are also used for the treatment of diabetes throughout the world. Management of diabetes without any side effect is still a challenge for the medical system. This leads to an increasing search for improved antidiabetic drugs (Sarkar *et al*., 2011).

Diabetes is a serious, chronic disease that occurs either when the pancreas does not produce enough insulin (a hormone that regulates blood glucose), or when the body cannot effectively use the insulin it produces. Raised blood glucose, a common effect of uncontrolled diabetes, may, over time, lead to serious damage to the heart, blood vessels, eyes, kidneys and nerves. More than 400 million people live with diabetes. There are two types of diabetes.

Type 1 diabetes is characterized by deficient insulin production in the body. People with type 1 diabetes require daily administration of insulin to regulate the amount of glucose in their blood. If they do not have access to insulin, they cannot survive. The majority of type 1 diabetes occurs in children and adolescents. Symptoms include excessive urination and thirst constant hunger, weight loss, vision changes and fatigue.

Type 2 diabetes results from the body's ineffective use of insulin. Several dietary practices are linked to type 2 diabetes risk, including high intake of saturated fatty acids, high total fat intake and inadequate consumption of dietary fibre, sugar-sweetened beverages, overweight and obesity, unhealthy diet, physical inactivity and also active (as distinct from passive) smoking to increase risk. Diabetes can damage the heart, blood vessels, eyes, kidneys and nerves, leading to disability and premature death (WHO, 2016).

Nowadays, several antidiabetic agents are avaliable but most have certain adverse effects and high cost. The majority of herbal medicines seem to have efficacy, low incidence of serious adverse effects and low cost. The use of herbal medicine is increasing in both developing and developed countries due to growing recognition of natural products and easy availability at affordable prices and sometimes the only source of health care available for rural people (Atmakuri, 2010).

The aim of the present research is to find the medicinal value of *Mirabilis jalapa* L. plant and to promote the intensive application of Myanmar traditional medicine. Screening the acute toxicity and hypoglycaemic activity of 70% ethanolic extracts from *Mirabilis jalapa* L. plant were investigated.

Materials and Methods

Acute toxicity test of 70% ethanolic extract of *Mirabilis jalapa* L. plant on albino mice

The acute toxicity test on mice was carried out according to the method of OECD Guideline 423 (2001). Adult, 18 albino mice, Dutch Denken Yoken strain of female sex, weighting between 25- 30 g, were used for acute

toxicity study. These animals were provided by the Laboratory Animal Services Division, Department of Medical Research, and Yangon.

Materials

Test animals	-	18 female albino mice (ddy strain, body weight 25- 30g)
Test agents	-	Distilled water, 70% ethanol extract from *Mirabilis jalapa* L. plant
Apparatus	-	Mice cages, animal balance, "18" gague intragastric needle, disposal syrings (1ml and 5 ml), rubber gloves and masks
Dose	-	2g/kg and 5g/kg (body weight) of albino mice
Period of observation	-	14 days

Methods

Acute toxicity Test of 70% ethanolic extracts of *Mirabilis jalapa* L. plant was evaluated by the methods of OECD Guidelines 423. The female albino mice were randomly selected and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. According to the test description, total number of 18 female albino mice (ddy strain), weighing between 25- 30 g were selected and divided into three groups. Each group contained six mice and kept in the each mouse cages. At first, the mice were individually marked on the parts of the body and weighed. Required doses based on the body weight of the mice were calculated. The mice were fasted for 18 hours before experiment but were allowed with free access to water. Group (I) mice served as a control group and they were administered 10 ml/kg of distilled water orally. In this study, starting dose 2 g/kg was chosen. The extract was dissolved in distilled water and the required doses were administered orally by using intragastric needle to every mice. A limited test at one dose level of 2 g/kg was carried out with 6 mice (3 mice per step). After administration of the test agent orally, they were allowed to have

food and water. The sign of toxicity such as changes in skin and fur, eyes, salivation, convulsion, cyanosis, tremors and diarrhoea or lethality were observed on test animals. Treated mice were observed individually after dosing at one time during the first 30 minutes hourly up to 4 hours for first 24 hours. After that, all mice were monitored daily up to 14 days.

Thus, another 6 mice (3 mice per step) were administered 5 g/kg. The mice were observed for toxic sign by using the method described above. All the mice were observed to detect the delayed toxicity up to 14 days. The mortality and toxic signs during this period were noted.

Acute toxicity test of 70% ethanolic extract of *Mirabilis jalapa* L. plant on albino mice

Figure 1. Mice cages (each contains 6 mice)

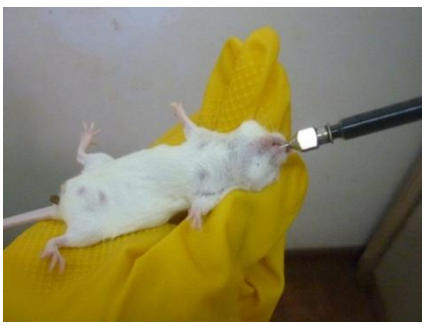

Figure 2. Administration of extract suspension to mice

Hypoglycaemic activity of 70% ethanolic extract of *Mirabilis jalapa* L. plant on adrenaline-induced hyperglycaemic rat model

The hypoglycaemic activity of 70% ethanolic extract was also studied on adrenaline-induced hyperglycaemic rats model by using the method of Gupta *et al*. (1967) at Department of Medical Research (DMR), Yangon.

Materials

Test animals - 8 Wistar strain albino rats of both sexes (body weight 180- 250 g)

Test agent	-	Distilled water, 70% ethanolic extract, Glibenclamide tablets 5 mg (Malaysia), Adrenaline injection (1 mg/ml) (Myanmar Pharmaceutical Factory)
Apparatus	-	Aluminium cages, Animal balance, Spirit cotton wools, disposable syringes with needle (1 ml, 5 ml), Glucometer, Test strips, 18 gauge dosing needle, rubber gloves and masks
Dose Schedule	-	70% ethanolic extract (1 g/kg, 2 g/kg and 4 g/kg) body weight

Methods

Test animal profile

The study of hypoglycaemic effect of 70% ethanolic extract of *Mirabilis jalapa* L. plant was performed by using the method of Gupta *et al*. (1967). Both sexes of eight adult healthy albino rats of Wistar strains weighing (180- 250 g) obtained from Department of Medical Research were used in this experiment. They were kept in clean and dry cages to allow for acclimatization to the laboratory conditions one week before starting the experiment. The rats were fasted overnight for 18 hours before the experiment but water was allowed freely. Firstly, they were served as control group and only distilled water was given orally to them during experiment.

Preparation and administration of drug suspension

Before the experiment, individual rats were marked, weighed and kept without food for 18 hours. The dosage was calculated according to the body weight of rat. Control animals were administered orally with 10 ml/kg of distilled water. The drug suspension (i.e. distilled water) was given orally to each rat by using an intragastric needle connected to a plastic syringe containing the calculated dosage. The syringe was put into the stomach. Then, the piston was pushed to deliver the test agents into the stomach. Immediate sneezing and coughing indicated injecting into the lungs and in such condition, the syringe was withdrawn.

Collection of blood sample and induction of hyperglycaemia in rats

Before the drug administration, the blood sample was collected by cutting about 1 mm at the tip of the tail as the base line blood sample (0hr). The glucometer test strip was inserted into the glucometer and then, one drop of the blood sample was dropped on this strip. Blood glucose concentration was measured by glucometer at 0 hour. The results were expressed in (mg/dl). Then, these rats were orally given with distilled water (10 m/kg) by using "18" gauge intragastric needle. Thirty minutes after administration of distilled water, these rats were subcutaneously injected with (0.4 ml/kg) body weight of adrenaline to the back of the neck. Then, bloods were taken from tail vein and blood glucose levels were determined hourly up to 4 hours with glucometer. After taking the blood sample, the tail of the rat was rubbed with cotton wool soaked in absolute alcohol to protect the puncture against infection.

Determination of hypoglycaemic activity of 70% ethanolic extract (1 g/kg, 2 g/kg and 4 g/kg body weight) of *Mirabilis jalapa* L. plant on adrenaline-induced hyperglycaemic rats

After one week washout period, the same 8 rats were used again and these rats were kept without food for 18 hours before experiment. Only water was allowed orally to them. After that, these rats were orally given ethanolic extract (1 g/kg) body weight by using "18" gauge intragastric needle. After 30 minutes, these rats were subcutaneously injected with (0.4 ml/kg) body weight of adrenaline. Fasting blood was taken from tail vein and blood glucose levels were determined at 0hr, 1hr, 2hr, 3hr and 4 hours with glucometer. Then, all the animals were allowed to rest for one week of drug free period (i.e. washout period). After washout period for one week, the same 8 rats were also tested for determination of blood glucose level with 70% ethanolic extract, (2 g/kg) body weight. Determinations of blood glucose levels were performed as above procedures.

After washout period of one week, the same 8 rats were tested with 70% ethanolic extract, (4 g/kg) body weight for determination of blood glucose level as above procedures.

Determination of hypoglycaemic activity of standard drug, (glibenclamide) on adrenaline-induced hyperglycaemic rats

After drug free interval of one week, the same 8 rats were used again and these rats were kept without food for 18 hours before experiment. Fasting blood glucose levels (0hr) were taken from venous blood obtained by cutting about 1 mm at the tip of the tail and measured by glucometer. After that, these rats were orally given with standard drug glibenclamide (4 mg/kg) body weight by using "18" guage intragastric needle. After 30 minutes, these rats were subcutaneously injected with (0.4 ml/kg) body weight of adrenaline. Then, bloods were taken from tail vein hourly at 1hr, 2hr, 3hr and up to 4 hours and determination of blood glucose levels were done with glucometer.

The study design used in this study was cross over study design in albino rats.

Data management and analysis

Standard statistical methods were used in the calculation of arithmetic mean (X) standard deviation (SD) and standard error (SE). Paired student "t" test were used to analyze the significant differences between means of control and experimental groups (Gupta *et al*., 1967).

Determination of blood glucose concentration

Percent reduction was calculated by the following formula;

$$\text{Percent reduction} = \frac{\text{Difference between rises in blood glucose level of control and test}}{\text{Blood glucose level rise in control}} \times 100$$

$$= \frac{C-T}{C} \times 100$$

C = rise in blood glucose level of control

T = rise in blood glucose level of test

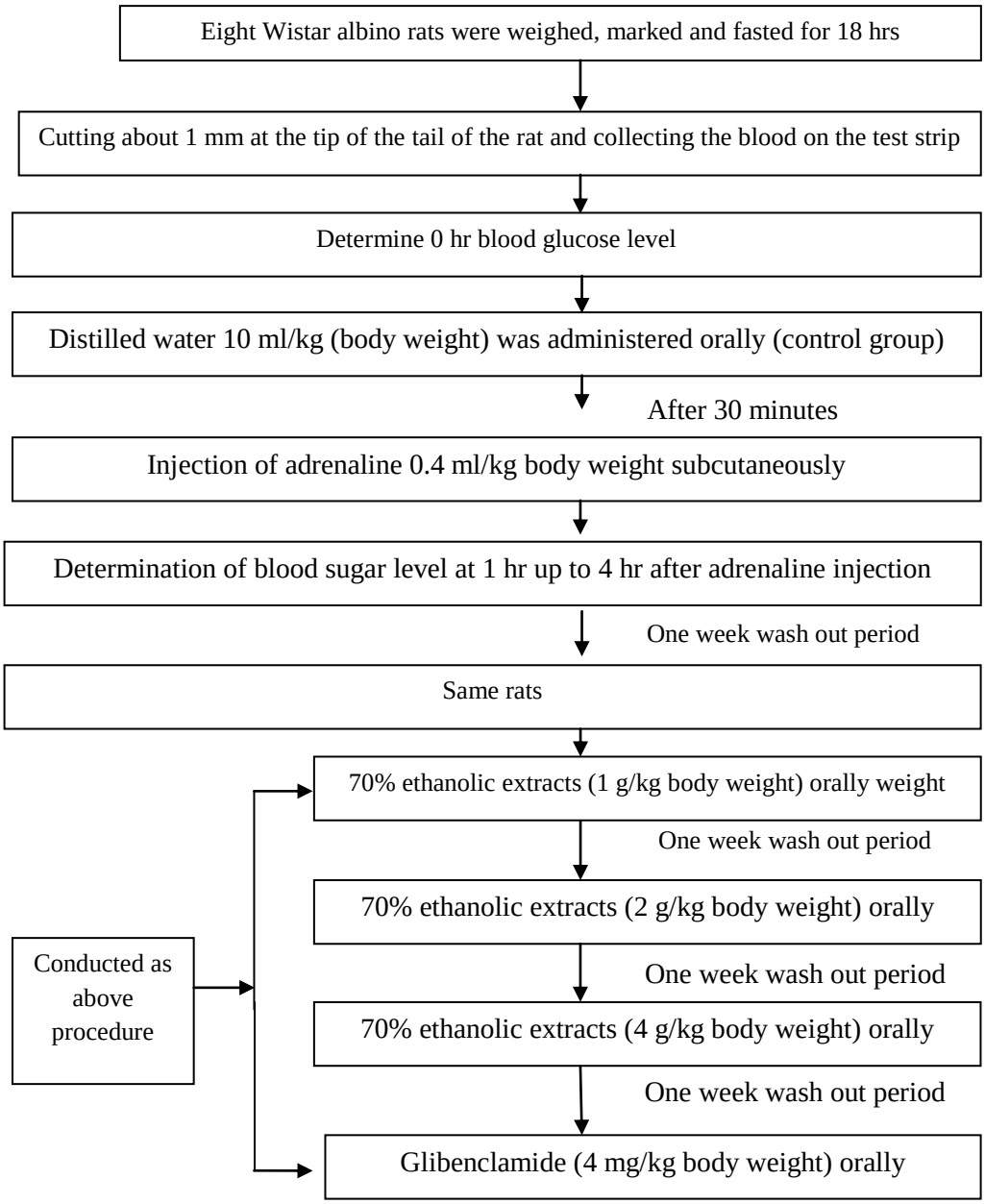

Figure 3. Flow chart for hypoglycaemic activity testing in adrenaline-induced hyperglycaemic rats

Hypoglycaemic activity of 70% ethanolic extract of *Mirabilis jalapa* L. plant on adrenaline-induced hyperglycaemic rat model

Figure 4. Albino rats in cages and each contains 2 rats

Figure 5. Cutting the tip of tail from the rats

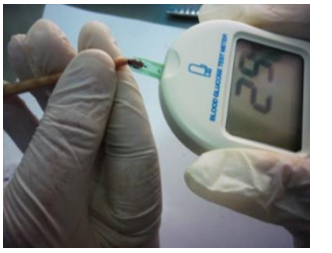

Figure 6. Determination of blood glucose level by using glucometer

Figure 7. Administration of distilled water to rat

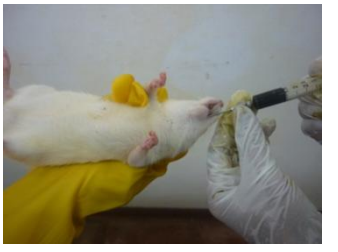

Figure 8. Administration of extracts suspension to rat

Figure 9. Adrenaline injection into nape of neck of albino rat

Results

Acute toxicity test of 70% ethanolic extract of *Mirabilis jalapa* L. plant on albino mice

In this study, the mice were administered with the dose of 2 g/kg (body weight) and 5 g/kg (body weight) of 70% ethanolic extract of *Mirabilis jalapa* L. Each group of mice was still alive and did not show any signs of toxicity in skin, fur and eyes. Salivation, convulsion, cyanosis, tremors and diarrhoea were not detected. Even with the maximum dose of 5 g/kg body weight of 70% ethanolic extracts administration, there was no lethality and toxic effect

up to 14 days of observation period. Therefore, it was observed that median lethal dose (LD_{50}) of the extract was more than 5 g/kg and the extract was not toxic up to the dose of 5 g/kg. The results were shown in Table (1).

Table 1. Acute toxicity test of 70% ethanolic extract of *Mirabilis jalapa* L. plant on albino mice

No. of Group	Type of drug administration	No. of mice tested	Dosage	Observed period	No. of death
I	Control (distilled water)	6	10 ml/kg	14 days	0/6
II	70% ethanolic extracts	6	2 g/kg	14 days	0/6
III	70% ethanolic extracts	6	5 g/kg	14 days	0/6

The mice were found to be alive and healthy during two weeks. No lethality and toxic effect of the mice were observed up to 14 days. These extracts were free from acute toxic effect at the doses 2 g/kg and 5 g/kg.

Hypoglycaemic activity of 70% ethanolic extract of *Mirabilis jalapa* L. plant on adrenaline-induced hyperglycaemic rat model

The hypoglycaemic activity of 70% ethanolic extracts of *Mirabilis jalapa* L. was tested by using adrenaline induced hyperglycaemic albino rats. Eight adult healthy Wistar strain albino rats of both sexes, weighing between (180-250 g) body weight were used for this study. The results of hypoglycaemic activity were shown in Tables (2 - 3) and Figures (10 -11).

Effect of distilled water on blood glucose levels on adrenaline-induced hyperglycaemic rats model (control group)

The mean blood glucose level of the 8 albino rats given orally with distilled water (10 ml/kg body weight) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline 0.4 ml/kg were 62.00 ± 2.62 mg/dl, 191.13 ± 8.93 mg/dl, 249.25 ± 9.77 mg/dl, 226.88 ± 8.52 mg/dl and 210.38 ± 7.4 mg/dl respectively. It was found that blood glucose level significantly increased at 1 hr, 2 hr, 3 hr and 4 hr after injection of adrenaline (0.4 ml/kg) as shown in Table (2).

Effect of different doses levels of 70% ethanolic extract (1 g/kg, 2 g/kg and 4 g/kg body weight) of *Mirabilis jalapa* L. plant on blood glucose level on adrenaline-induced hyperglycaemic rats model

The mean blood glucose level of the 8 albino rats treated with 70% ethanolic extracts of *Mirabilis jalapa* L. **(**1 g/kg body weight) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 ml/kg) were 77.00 ± 3.96 mg/dl, 199.63 ± 5.55 mg/dl, 250.63 ± 6.11 mg/dl, 252.75 ± 10.69 mg/dl and 222.38 ± 11.75 mg/dl respectively. It was observed that the oral administration at 70% ethanolic extracts of *Mirabilis jalapa* L. (1 g/kg body weight) produced a significant decrease in glucose level at 4 hr ($p<0.01$) when compared with that of control group as shown in Table (2) and Figure (10).

The mean blood glucose level of the 8 albino rats treated with 70% ethanolic extracts of *Mirabilis jalapa* L. **(**2 g/kg body weight) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 ml/kg) were 74.63 ± 3.48 mg/dl, 205.00 ± 4.47 mg/dl, 253.25 ± 9.11 mg/dl, 227.75 ± 10.17 mg/dl and 201.00 ± 8.91 mg/dl respectively. It was observed that the oral administration of 70% ethanolic extracts of *Mirabilis jalapa* L. (2 g/kg body weight) produced a significant decrease in glucose level at 3 hr ($p<0.005$) and 4 hr ($p<0.001$) when compared with that of control group as shown in Table (2) and Figure (10).

The mean blood glucose level of the 8 albino rats treated with 70% ethanolic extracts of *Mirabilis jalapa* L. **(**4 g/kg body weight) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 ml/kg) were 63.75 ± 2.17 mg/dl, 201.63 ± 5.84 mg/dl, 223.50 ± 5.61 mg/dl, 199.13 ± 5.7 mg/dl and 178.25 ± 5.51 mg/dl respectively. It was observed that the oral administration at 70% ethanolic extracts of *Mirabilis jalapa* L. (4 g/kg body weight) produced a significant decrease in glucose level at 2 hr ($p<0.001$), 3 hr ($p<0.001$) and 4 hr ($p<0.001$) when compared with that of control group as shown in Table (2) and Figure (10).

Effect of standard drug, glibenclamide on blood glucose level in adrenaline-induced hyperglycaemic rats model

The results of mean blood glucose level of the 8 albino rats treated with standard drug glibenclamide (4 mg/kg body weight) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline 0.4 ml/kg were 72.00 ± 2.34 mg/dl, 149.25 ± 7.93 mg/dl, 193.13 ± 6.58 mg/dl, 172.63 ± 15.38 mg/dl and 114.38 ± 4.24 mg/dl respectively. The results of the oral administration of standard drug, glibenclamide showed that the blood glucose level of adrenaline-induced rats were significant decreased at 1 hr ($p<0.05$), 2 hr ($p<0.001$), 3 hr ($p<0.001$) and 4 hr ($p<0.001$) when compared with that of control group are shown in Table (2) and Figure (10).

Comparison of percent reductions of blood glucose level with different dose of 70% ethanolic extract from *Mirabilis jalapa* L. plant and standard drug, glibenclamide.

The comparison of mean percent reductions of blood glucose levels with 70% ethanolic extracts from *Mirabilis jalapa* L. and standard drug, glibenclamide are shown in Table (3) and Figures (11).

The mean percent reduction of blood glucose level with 70% ethanolic extracts (1 g/kg body weight) were 2.23 ± 6.47%, 6.35 ± 1.75%, 8.95 ± 2.88% and 14.94 ± 3.57% at 1 hr, 2 hr, 3 hr and 4 hr respectively. The mean percent reduction of blood glucose level with 70% ethanolic extracts (2 g/kg body weight) were -5.39 ± 10.15%, 3.92 ± 4.03%, 21.24 ± 4.23% and 26.18 ± 4.48% at 1 hr, 2 hr, 3 hr and 4 hr respectively. The mean percent reduction of 70% ethanolic extracts (4 g/kg body weight) were -10.43 ± 8.63%, 13.45 ± 2.2%, 28.73 ± 3.94% and 31.64 ± 3.11% at 1 hr, 2 hr, 3 hr and 4 hr respectively. The mean percent reduction of blood glucose level with glibenclamide (4 mg/kg body weight) were 33.91 ± 12.15%, 33.99 ± 4.19%, 47.89 ± 6.61% and 60.59 ± 6.72% at 1 hr, 2 hr, 3 hr and 4 hr respectively.

Table 2. Mean blood glucose concentration (Mean ± SE) of 70% ethanolic extract of *Mirabilis jalapa* L. plant (1 g/kg, 2 g/kg, 4 g/kg) and glibenclamide, (4 mg/kg) on adrenaline- induced hyperglycaemic rats model

Group of rats	Blood glucose concentration (mg/dl)				
	0 HR	1 HR	2 HR	3 HR	4 Hr
Control	62.00 ± 2.62	191.13 ± 8.93	249.25 ± 9.77	226.88 ± 8.52	210.38 ± 7.4
70% ethanolic extract 1 g/kg	77.00 ± 3.96	199.63 ± 5.55	250.63 ± 6.11	252.75 ± 10.69	222.38 ± ** 11.75
70% ethanolic extract 2 g/kg	74.63 ± 3.48	205.00 ± 4.47	253.25 ± 9.11	227.75 ± *** 10.17	201.00 ± **** 8.91
70% ethanolic extract 4 g/kg	63.75 ± 2.17	201.63 ± 5.84	223.50 ± **** 5.61	199.13 ± **** 5.7	178.25 ± **** 5.51
Glibenclamide 4 mg/kg	72.00 ± 2.34	149.25 ± * 7.93	193.13 ± **** 6.58	172.63 ± **** 15.38	114.38 ± **** 4.24

*P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.001

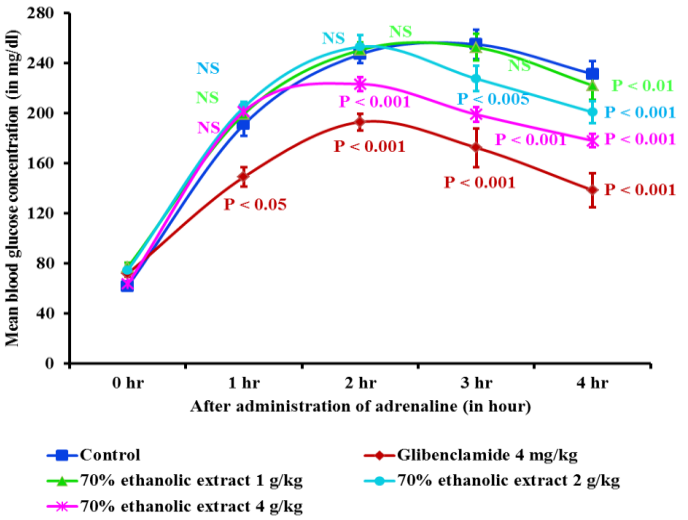

Figure 10. Time course of the effect of 70% ethanolic extracts of *Mirabilis jalapa* L. plant (1 g/kg, 2 g/kg and 4 g/kg) and glibenclamide, (4 mg/kg) on adrenaline- induced hyperglycaemic rats model

Table 3. Percent reduction (Mean ± SE) of hyperglycaemic with 70% ethanolic extract of *Mirabilis jalapa* L. plant and glibenclamide (4 mg/kg) on adrenaline-induced hyperglycaemic rats model

Group of rats	Percent reduction of hyperglycaemic			
	1 HR	2 HR	3 HR	4 HR
Glibenclamide 4 mg/kg	33.91 ± 12.15	33.99 ± 4.19	47.89 ± 6.61	60.59 ± 6.72
70% ethanolic extract 1 g/kg	2.23 ± 6.47	6.35 ± 1.75	8.95 ± 2.88	14.94 ± 3.57
70% ethanolic extract 2 g/kg	-5.39 ± 10.15	3.92 ± 4.03	21.24 ± 4.23	26.18 ± 4.48
70% ethanolic extract 4 g/kg	-10.43 ± 8.63	13.45 ± 2.2	28.73 ± 3.94	31.64 ± 3.11

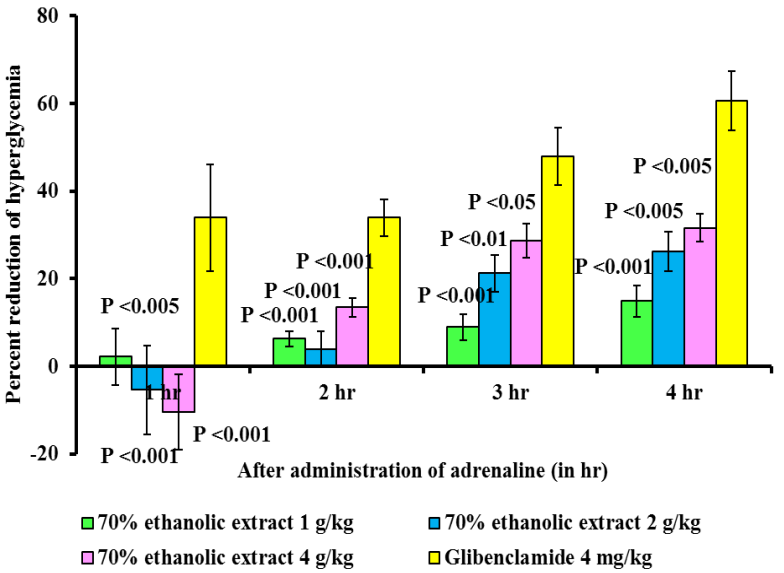

Figure 11. Percent reduction of hyperglycaemic with 70% ethanolic extract of *Mirabilis jalapa* L. plant (1 g/kg, 2 g/kg and 4 g/kg) and glibenclamide, (4 mg/kg) on adrenaline- induced hyperglycaemic rats model. N=8, each point represents the mean of observations and the vertical bars indicate standard errors of the means.

Discussion and Conclusion

In this study, acute toxicity test of 70% ethanolic extract of *Mirabilis jalapa* L. plant was evaluated by using the methods of OECD Guidelines 423. The mice were administered with the dose of 2 g/kg (body weight) and 5 g/kg (body weight) of 70% ethanolic extract of *Mirabilis jalapa* L. All the animals did not show any signs of toxicity and lethality in observation period of 14 days. There were no toxic signs and leathality during the observation period of 14 days with 2 g/kg (body weight) and the maximum dose of 5 g/kg (body weight). Therefore, 70% ethanolic extract of *Mirabilis jalapa* L. plant had no acute toxic effect up to the dose of 5 g/kg.

Prakash *et al*. (2012) reported that the hydro-alcoholic extract of aerial parts of *Mirabilis jalapa* L. possessed antihyperglycaemic activity at dose levels of 200 mg/kg and 400 mg/kg in streptozotocin induced hyperglycaemic animals.

Doss *et al*. (2015) reported that the hydro-ethanolic leaf extract of *Mirabilis jalapa* L. possessed potent antidiabetic activity in streptozotocin induced diabetic rats.

In this study, the hypoglycaemic effect of 70% ethanolic extract of *Mirabilis jalapa* L. plant at the dose of (1 g/kg, 2 g/kg and 4 g/kg) were investigated on adrenaline induced hyperglycaemic rats model by using the method of Gupta *et al*. (1967). 70% ethanolic extract (1 g/kg) significantly decreased the blood glucose concentration of the rats at 4 hour ($p<0.01$) after subcutaneous injection of adrenaline. 70% ethanolic extract (2 g/kg) significantly decreased the blood glucose concentration of the rats at 3 hour ($p<0.005$) and 4 hour ($p<0.001$) after subcutaneous injection of adrenaline. 70% ethanolic extract (4 g/kg) significantly decreased the blood glucose level at 2 hour up to 4 hour ($p<0.001$) after subcutaneous injection of adrenaline. It was found that 4 g/kg of 70% ethanolic extract was more effective than 1 g/kg and 2 g/kg doses. Therefore, hypoglycaemic effects of 70% ethanolic extract from *Mirabilis jalapa* L. plant was found to be in dose dependent manner.

In this study, the hypoglycaemic effects of standard drug, glibenclamide at the dose level of 4 mg/kg showed a significant reduction in

blood glucose level at 1 hour ($p<0.05$) and at 2 hour, 3 hour and 4 hour ($p<0.001$) after the administration of drugs on adrenaline induced hyperglycaemic rats. In this study, 70% ethanolic extract of *Mirabilis jalapa* L. plant at the doses of 1 g/kg, 2 g/kg and 4 g/kg showed significant reduction of blood glucose level on adrenaline induced hyperglycaemic rats.

At 1 hour after subcutaneous injection of adrenaline, mean percent reductions of hyperglycaemia with 70% ethanolic extract of *Mirabilis jalapa* L. plant at the dose of 1 g/kg, 2 g/kg and 4 g/kg were (2.23%, -5.39%, -10.43%) respectively. At 2 hour after subcutaneous injection of adrenaline, mean percent reductions of hyperglycaemia with 70% ethanolic extract of *Mirabilis jalapa* L. plant at the doses of 1 g/kg, 2 g/kg and 4 g/kg were (6.35%, 3.92%, 13.45%) respectively. At 3 hour after subcutaneous injection of adrenaline, mean percent reductions of hyperglycaemia with 70% ethanolic extract of *Mirabilis jalapa* L. plant at the dose of 1 g/kg, 2 g/kg and 4 g/kg were (8.95%, 21.24%, 28.73%) respectively. At 4 hour after subcutaneous injection of adrenaline, mean percent reductions of hyperglycaemia with 70% ethanolic extract of *Mirabilis jalapa* L. plant at the doses of 1 g/kg, 2 g/kg and 4 g/kg were (14.94%, 26.18%, 31.64%) respectively. The mean percent reductions of hyperglycaemia with glibenclamide at 1 hour, 2 hour, 3 hour and 4 hour after subcutaneous injection of adrenaline were (33.91%, 33.99%, 47.89% and 60.59%) respectively.

In the comparison between hypoglycaemic effect 70% ethanolic extract of *Mirabilis jalapa* L. plant and glibenclamide, glibenclamide had more hypoglycaemic effect than 70% ethanolic extract of *Mirabilis jalapa* L. plant.

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ISOLATION, IDENTIFICATION AND ANTIMICROBIAL ACTIVITIESOF ENDOPHYTIC FUNGAL STRAINS FROM DIFFERENT PARTS OF *HESPERETHUSA CRENULATA* (ROXB.) ROEM.

Htet Htet Zaw[1] , Yee Yee Thu[2]

Abstract

In this study, ten endophytic fungal strains were isolated from the plant parts of *Hesperethusa crenulata* (Roxb.) Roem. sample 1 and six fungal strains were also isolated from the plant parts of *Hesperethusa crenulata* (Roxb.) Roem.sample2. *Hesperethusa crenulata* (Roxb.) Roem. sample 1 was collected from Ayartaw Township, Saging Region and *Hesperethusa crenulata* (Roxb.) Roem. Sample 2 was also collected from Yay Sa Kyo Township, Magway Region. The morphological characters of isolated fungal strains were conducted at Microbiology Laboratory, Department of Botany, University of Yangon and microscopic characters were investigatedat Universities' Research Center, YU. In the present study, strains HH 1, 2, 9 and 13 were identified as *Cephalosporium* sp. and strain HH 12 was *Rhizoctonia* sp.. Antimicrobial activities of all isolated strains were conducted by using paper disc diffusion assay with eight test organisms. All isolated strains were exhibited antimicrobial activity on *Agrobacterium tumefaciens, Aspergillus flavus, Bacillus subtilis, Candida albicans, Malassezia furfur, Micrococcus luteus, Salmonella typhus* and *Xanthomonas oryzae.*

Keywords: Antimicrobial activity, Endophytic fungi, *Hesperethusa crenulata* (Roxb.) Roem.

Introduction

Endophytes are organisms, often fungi and bacteria that live between living plant cells. Endophytes, found ubiquitous in all plant species in the world, contribute to their host plants by producing plenty of substances that provide protection and ultimately survival value to the plant. The natural products obtained from endophytic microbes are found to be antimicrobial, antiviral, anticancer, antioxidants, antidiabetic and immunosuppressant.

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The fungal endophytes are known to produce these types of natural products. Nonetheless, poorly explored and underutilized endophytic fungi are known to produce antibiotics in addition to other natural products. The endophytic fungi appear to be a potential source of novel antibiotics. Now the endophytic fungi seem to be a promising alternative potential source of novel antibiotics. Endophytic fungi are as a potential source of novel antibiotics (Walsh, 1992 and Strobel *et al.*, 2002).

Now a day herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Endophyte infected plants often grow faster than non-infected plants. They colonise plant tissue and are remained within the tissue, except that fruiting structures may emerge through the surface of the plant tissue. Indeed, leaves may be fully colonised by a variety of fungi within a few weeks of leaf emergence. The colonies remain asymptomatic and some in perennial plant parts may have a very long life. In amicrobe-plant relationship, endophytes contribute substances that possess various types of bioactivity, suchas antibacterial, antifungal, antibiotic, antitumor, antioxidant, anti-inflammatory etc. The bioactivesubstances in plants are produced as secondary metabolites (Strobel *et al.*, 2002).

The objectives of this study are to isolate endophytic fungal strains from different parts of *Hesperethusa crenulata* (Roxb.) Roem. samples 1 and 2, to investigate morphological and microscopic characters of isolated fungal strains and to evaluate antimicrobial activity of isolated fungal strains.

Materials and Methods

Collection of Plant Samples

Hesperethusa crenulata (Roxb.) Roem. sample 1 was collected from Ayartaw Township, Saging Region and *Hesperethusa crenulata* (Roxb.) Roem. sample 2 was also collected from Yay Sa Kyo Township, Magway Region.

Isolation of Endophytic Fungal Strains

Isolation of endophytic fungal strains can be carried out by the following scheme:

(1) The plants were washed in running water for fifteen minutes. **(**2) The plant parts (leaves, barks and roots) were cut into about 1 cm pieces. **(**3) These parts were sterilized by soaking in 75% ethanol for 2 min. **(**4) They were sterilized by soaking in 5.3% sodium hypochloride for 1 minute. **(**5) After that, these parts were sterilized by soaking in 75% ethanol for thirty seconds. **(**6) These parts were dried on sterilized paper and then they were placed on agar plates containing sucrose/yeast extract medium. **(**7) After 3 to 7 days the microorganisms were picked and purified by subculturing. **(**8) The pure strains are maintained in test tubes as seen in Figure 1 **(**Phay, 1997).

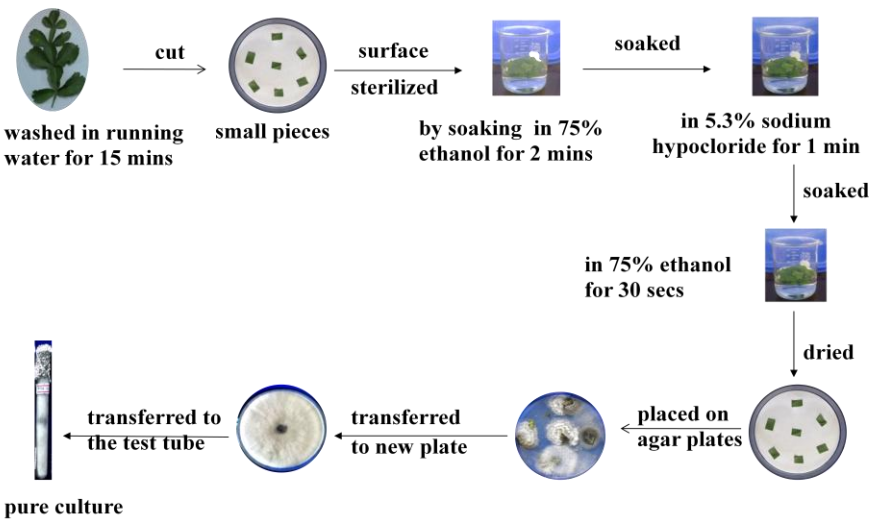


Figure 1. Isolation of endophytic fungal strains

Morphological and Microscopic Characters of Endophytic Fungi

Sixteen isolated endophytic fungi grown on slant culture were transferred into the plates containing SY medium(sucrose 1.0 g, yeast extract 0.3 g, NaCl 0.05 g, $CaCO_3$ 0.01 g, agar 1.8 g, pH7, distilled water 100 ml).

Then, these plates were incubated at 30˚C for 3-7 days.Colony forms, surfaces and reverse pigments of isolated strains were studied for morphology at Microbiology Laboratory, Department of Botany, University of Yangon. Microscopic characters of isolated fungi were investigated by using high magnification of microscope at Universities' of Research Center, YU.

Antimicrobial Activity of Isolated Fungal Strains

Fermentation of isolated strains

Isolated fungal strains grown on 5 days old slant cultures were inoculated into 16 of 50 ml conical flasks containing 25 ml of medium1 or 2 in each.There were 32 conical flasks were utilized for two fermentation media in this study. Next, they were incubated at 30˚C for 3-13 days. Two fermentation media were mentioned in the following.

Test agar plates

Test organisms were *Agrobacterium tumefaciens, Aspergillus flavus, Bacillus subtilis, Candida albicans, Malassezia furfur*, *Micrococcus luteus, Salmonella typhus* and *Xanthomonas oryzae.* Broth culture (50µl) of each test organism was added into 100 ml assay medium and then poured into plates.

Fermentation Medium 1: sucrose 1.0g, yeast extract 0.3g, NaCl 0.05g, $CaCO_3$ 0.01g, pH 7, distilled water 100ml

Fermentation medium 2: glucose 1.0g, yeast extract 0.3g, NaCl 0.05g, $CaCO_3$ 0.01g, pH 7, distilled water 100ml

Paper disc diffusion assay

After solidification, paper discs impregnated with fungal broth sampleswere applied on the test plates.These plates were incubated at room temperature 30˚C for 24 to 48 hrs.After 24 to 48 hrs, clear zones (inhibitory zones) surrounding the test discs were measured.These zones indicate the presence of the bioactive compounds that inhibits the growth of test organism (Phay, 1997).

Results

Outstanding Characters of *Hesperethusa crenulata* (Roxb.) Roem. Sample1

It is imparipinnate leaves with winged rachii, racemes of white-flowered inflorescences and the basifixed anthers. It is aromatic and compact, thin, reddish brown-streaked aromatic bark and the pubescent leavesas shown in Figure 2.

Habit

Leaves

Figure 2. Outstanding characters of *Hesperethusa crenulata* (Roxb.) Roem. sample1

Outstanding Characters of *Hesperethusa crenulata* (Roxb.) Roem. Sample 2

It has the germinate thorns, the imparipinnate leaves with winged rachii, the white-flowered cauliflorous inflorescences and the basifixed anthers. It is zig-zag young stems, the thick, whitish yellow, corky aromatic bark and the leaves which are glabrous except for the nervesas shown in Figure 3.

Habit

Leaves

Figure 3. Outstanding characters of *Hesperethusa crenulata* (Roxb.) Roem. sample 2

Isolation of Endophytic Fungal Strains from *Hesperethusa crenulata* (Roxb.) Roem.

Sixteen fungal strains were isolated from the leaves, barks, stems and roots of *Hesperethusa crenulata* (Roxb.) Roem. sample 1 and sample 2. These strains were given as temporary names HH-1 to HH-16 as shown in Table 1.

Table 1. Isolation of endophytic fungal strains from sample 1 and sample 2

Strains	**Sources**
HH-1 to HH-4	Leaves of *Hesperethusa crenulata* (Roxb.) Roem. sample1
HH-5 to HH-7	Barks of *Hesperethusa crenulata* (Roxb.) Roem. sample1
HH-8 and HH-9	Woods of *Hesperethusa crenulata* (Roxb.) Roem. sample1
HH-10	Roots of *Hesperethusa crenulata* (Roxb.) Roem. sample1
HH-11 to HH-13	Leaves of *Hesperethusa crenulata* (Roxb.) Roem. sample2
HH-14	Barks of *Hesperethusa crenulata* (Roxb.) Roem. sample2
HH-15	Woods of *Hesperethusa crenulata* (Roxb.) Roem. sample2
HH-16	Roots of *Hesperethusa crenulata* (Roxb.) Roem. sample2

Morphological and Microscopic Characters of Isolated Fungal Strains

Morphological and microscopic characters **of** sixteenfungal strains (HH-1 to HH-16) isolated from *Hesperethusa crenulata* (Roxb.) Roem. Samples 1 and 2 were shown in Table 2 and Figures 4 to 19.

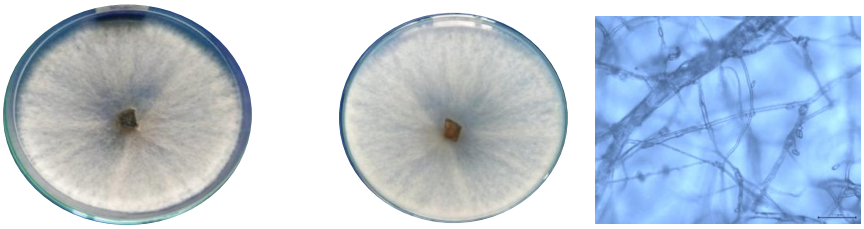
Table 2. Morphological characters of isolated strains

Cultural characters			
Strains	**Surface color**	**Reverse color**	**Hyphae**
HH1	White	White	Septate
HH2	Brownish white	Pale brown	Septate
HH3	White	White	Septate
HH4	Brown	Brown	Septate
HH5	White	White	Aseptate
HH6	White	Pink	Septate
HH7	White	White	Septate
HH8	White	Pale pink	Aseptate
HH9	White	White	Septate
HH10	White	White	Septate
HH11	White	White	Septate
HH12	White	White	Septate
HH13	White	White	Septate
HH14	White	White	Septate
HH15	White	White	Aseptate
HH16	Pink	Pink	Septate

Morphological and microscopic characters of strain HH1

The surface color and reverse color of strain HH1 was the same color white.It shows conidia (phialospores) hyaline, 1 celled, septate hyphae which are typically very fine and narrow. The phialides are separated from hyphae by a septum and taper towards their apices. They usually appear in clusters, in

balls or rarely as fragile chains. It is identified as *Cephalosporium* sp. as shown in Figure 4.

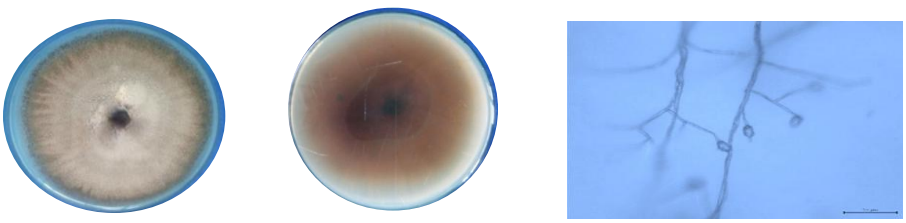


Surface view Reverse view

Figure 4. Morphological and microscopic characters of HH1 (*Cephalosporium* sp.)

Morphological and microscopic characters of isolated strain HH2

The surface color of strain HH2 was brownish white and its reverse color was pale brown. It shows conidia (phialospores) hyaline, 1 celled, septate hyphae which are typically very fine and narrow. The phialides are separated from hyphae by a septum and taper towards their apices. They usually appear in clusters, in balls or rarely as fragile chains. It is identified as *Cephalosporium* sp. as shown in Figure 5.

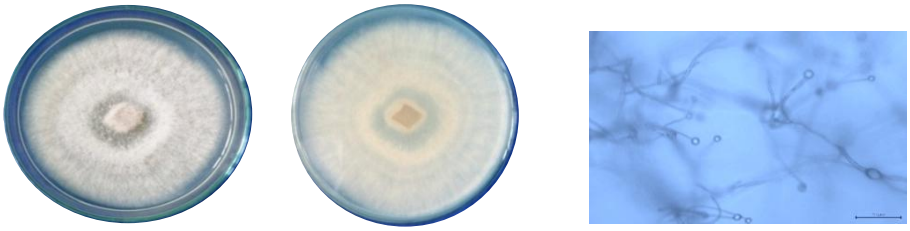


Surface view Reverse view

Figure 5. Morphological and microscopic characters of HH2 (*Cephalosporium* sp.)

Morphological and microscopic characters of isolated strain HH3

The surface color and reverse color of strain HH3 was the same color white.

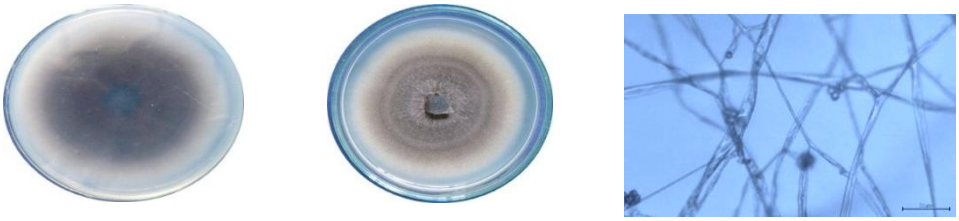


Surface view Reverse view

Figure 6. Morphological and microscopic characters of HH3

Morphological and microscopic characters of isolated strain HH4

The surface color and reverse color of strain HH4 was the same color brown.

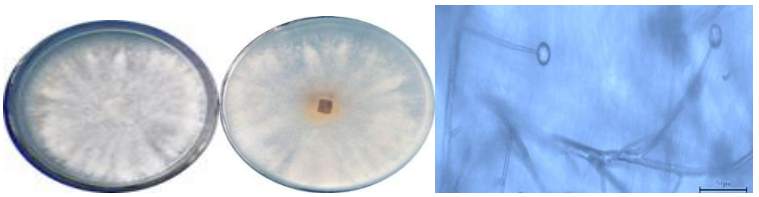


Surface view Reverse view

Figure 7. Morphological and microscopic characters of HH4

Morphological and microscopic characters of isolated strain HH5

The surface color and reverse color of strain HH5 was the same color white.

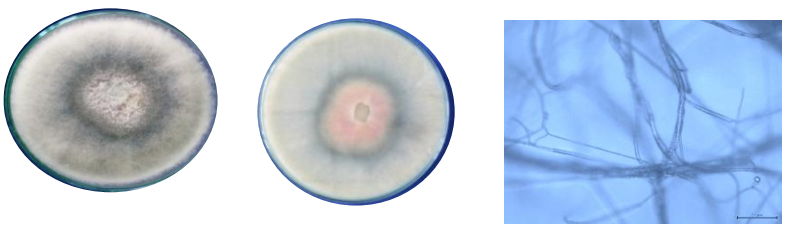


Surface view Reverse view

Figure 8. Morphological and microscopic characters of HH5

Morphological and microscopic characters of isolated strain HH6

The surface color of strain HH6 was white and its reverse color was pink.



Surface view Reverse view

Figure 9. Morphological and microscopic characters of isolated strain HH6

Morphological and microscopic characters of isolated strain HH7

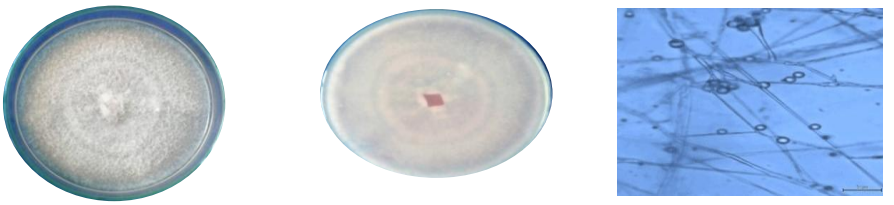
The surface color and reverse color of strain HH7 was the same color white.

Surface view Reverse view

Figure 10. Morphological and microscopic characters of isolated strain HH7

Morphological and microscopic characters of isolated strain HH8

The surface color of strain HH8 was white and its reverse color was pale pink.

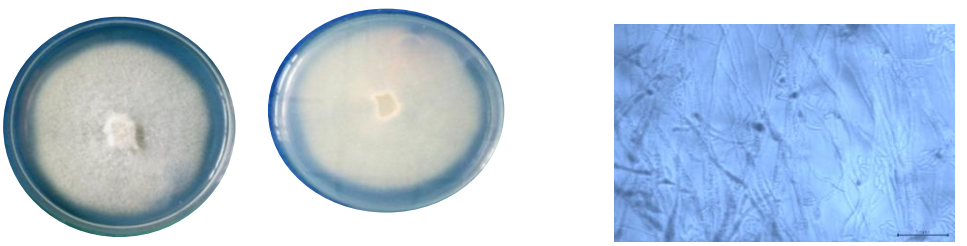


Surface view Reverse view

Figure 11. Morphological and microscopic characters of isolated strain HH8

Morphological and microscopic characters of isolated strain HH9

The surface color and reverse color of strain HH9 was the same color white. It shows conidia (phialospores) hyaline, 1 celled, septate hyphae which are typically very fine and narrow. The phialides are separated from hyphae by a septum and taper towards their apices. They usually appear in clusters, in balls or rarely as fragile chains. It is identified as *Cephalosporium* sp. as shown in Figure 12.



surface view Reverse view

Figure 12. Morphological and microscopic characters of HH9 (*Cephalosporium* sp.)

Morphological and microscopic characters of isolated strain

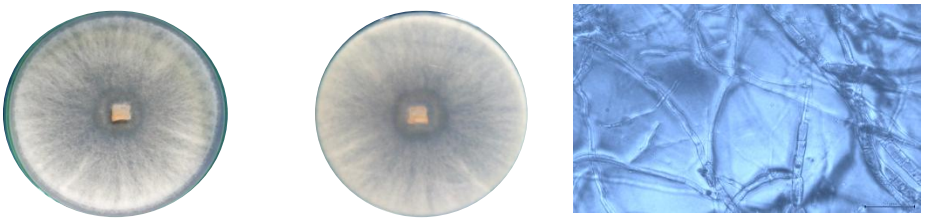
The surface color and reverse color of strain HH10 was the same color white.

surface view Reverse view

Figure 13. Morphological and microscopic characters of HH10

Morphological and microscopic characters of isolated strain HH11

The surface color and reverse color of strain HH11 was the same color white.

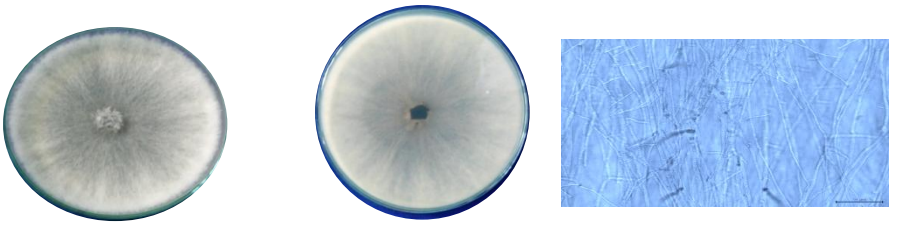


Surface view Reverse view

Figure 14. Morphological and microscopic characters of HH11

Morphological and microscopic characters of isolated strain HH12

The surface and reserve color of strain HH12 was white. Mycelium hyaline was dark. Cells of mycelium are long, septa of branches and set off from the main hyphae. Asexual fruit bodies, conidia absent, sporodocium-like bodies and chlamydospore-like cells were in chains. It is identified as *Rhizoctonia* sp. as shown in Figure 15.

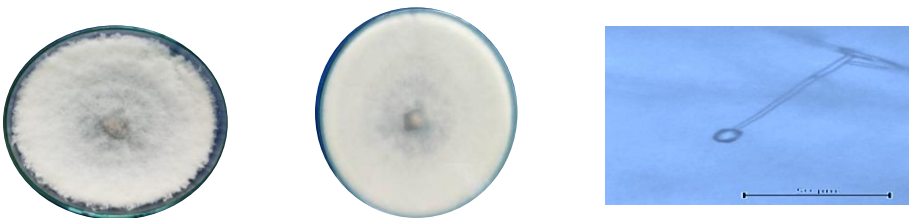


Surface view Reverse view

Figure 15. Morphological and microscopic characters of HH12 (*Rhizoctonia* sp.)

Morphological and microscopic characters of isolated strain HH13

The surface color and reverse color of strain HH13 was the same color white. It shows conidia (phialospores) hyaline, 1 celled, septate hyphae which are typically very fine and narrow. The phialides are separated from hyphae by a septum and taper towards their apices. They usually appear in clusters, in balls or rarely as fragile chains. It is identified as *Cephalosporium* sp. as shown in Figure 16.

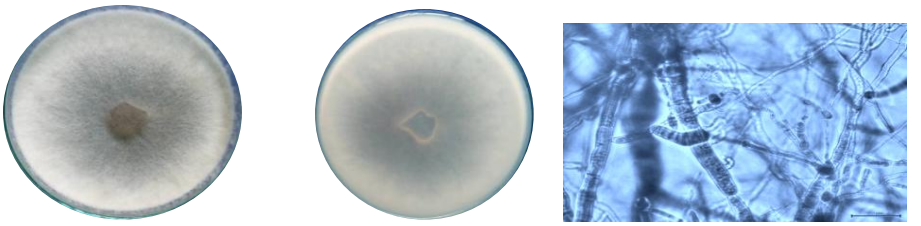


Surface view Reverse view

Figure 16. Morphological and microscopic characters of HH13 (*Cephalosporium* sp.)

Morphological and microscopic characters of isolated strain HH14

The surface color and reverse color of strain HH14 was the same color white.

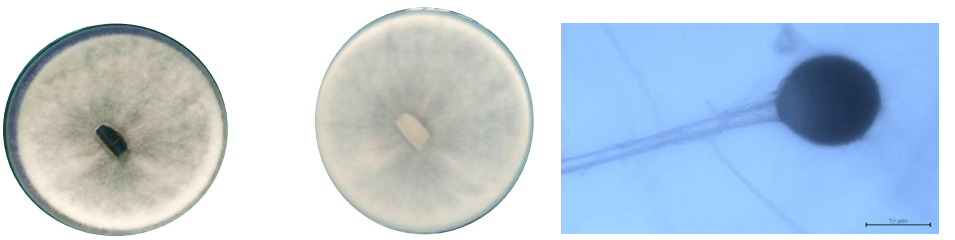


Surface view Reverse view

Figure 17. Morphological and microscopic characters of HH14

Morphological and microscopic characters of strain HH15

The surface color and reverse color of strain HH15 was the same color white.

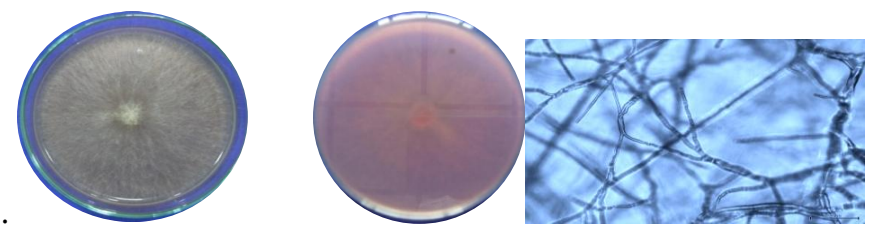


Surface view Reverse view

Figure 18. Morphological and microscopic characters of HH15

Morphological and microscopic characters of isolated strain HH16

The surface color and reverse color of strain HH16 was the same color pink as shown in Figure 19.



Surface view Reverse view

Figure 19. Morphological and microscopic characters of HH16

Antimicrobial Activity of Isolated Fungal Strains

Strain HH 9 showed very high activity against seven test organisms except *C. albicans* on medium 1 while strain HH 8 indicated very high activity against six test organisms except *C. albicans* and *X. oryzae* on medium 2 at 3rd day fermentation. Strains HH1, 9and 14 showed very high activity against eight test organisms on medium 1 whereas strain HH 8 and 15 indicated very high activity against eight test organisms on medium 2 at 4th day fermentation.

Strains HH 2 indicated very high activity against eight test organisms on medium 1 while strain HH 8 also exhibited very high activity against eight test organisms on medium 2 at 5th day fermentation. Strains HH 2, 9 and 13 exhibited very high activity against eight test organisms on medium 1 whereas strains HH 1, 4, 8, 12, 13 and 15 showed very high activity against eight test organisms on medium 2at 6th day fermentation.

Strains HH 1, 2 and 9 exhibited very high activity against six test organisms except *Asp. flavus* and *C. albicans* on medium 1 at 7th day fermentation. Strains HH 8, 11, 12, 13 and 15 showed very high activity against six test organisms except *Asp. flavus* and *C. albicans* on medium 2 at 7th day fermentation. Strains HH 1, 2, 6, 8, 9, 13 and 15 indicated very high activity against eight test organisms on medium 1 and strain HH1, 2, 3, 5, 6, 8, 9, 12, 13 and 15 also exhibited very high activity againsteight test organisms on medium 2 at 8th day fermentation. Strains HH 1, 2, 6, 8, 9 and 13 indicated very high activity against eight test organismson medium 1 while also strain HH1, 2, 3, 5, 6, 8, 9, 12 and 15exhibited very high activity againsteight test organisms on medium 2at 9th day fermentation.

Strains HH1, 2, 3, 5, 6, 8, 9, 10 and 13 indicated very high activity against eight test organismson medium 1 and also strain HH1, 2, 4, 5, 6, 8 and 9exhibited very high activity against eight test organisms on medium 2 at 10th day fermentation. Strains HH 2 indicated very high activity against eight test organismson medium 1 and also strain HH1, 2, 3, 4, 5, 6, 8 and 9 exhibited

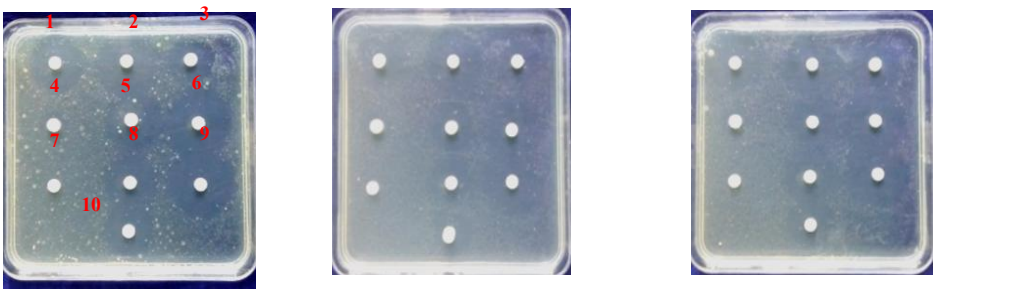
very high activity against eight test organisms on medium 2 at 11 day fermentation.

Strains HH 2 and 13 indicated very high activity against six test organisms except *Asp. flavus* and *C. albicans* on medium 1 and also strain HH2 and 8 exhibited very high activity against eight test organisms on medium 2 at 12 day fermentation.

Strains HH2 indicated very high activity against *Agro. tumefaciens, B. subtilis, S. typhi* and *X. oryzae* on medium 1 and also strain HH8 exhibited very high activity against even test organisms except *C. albicans* on medium 2 at 13 day fermentation as shown in Figures 20-23.

Agrobacterium tumefaciens *Xanthomonas oryzae* *Malassezia furfur*

Figure 20. Inhibitory zones of fermented broth (3 days old) of isolated strains from *Hesperethusa crenulata* sample 1



Salmonella typhi *Xanthomonas oryzae* *Aspergillus flavus*

Figure21. Inhibitory zones of fermented broth (8 days old) of isolated strains from *Hesperethusa crenulata* sample 1

Aspergillus flavus *Xanthanmonas oryzae* *Malassezia furfur*

Figure 22. Inhibitory zones of fermented broth (3 days old) of isolated strains from *Hesperethusa crenulata* sample 2

Salmonella typhi *Micrococcus luteus* *Malassezia furfur*

Figure 23. Inhibitory zones of fermented broth (8 days old) of isolated strains from *Hesperethusa crenulata* sample 2

Discussion and Conclusion

In the present study, sixteen endophytic fungal strains (ten strains from the sample 1 and six strains from the sample 2) were isolated from the leaves, barks, stems and roots of *Hesperethusa crenulata* (Roxb.) Roem. All strains initially showed different antimicrobial activity on *Agrobacterium tumefaciens, Aspergillus flavus, Bacillus subtilis, Candida albicans, Malassezia furfur, Micrococcus luteus, Salmonella typhus* and *Xanthomonas oryzae.* Zhao, *et al.* (2010) isolated endophytic fungi from their host plants for producing the bioactive compounds.

In this study, fourteen strains of all isolated strains indicated their antimicrobial activity from 3 day to 13 days on eight test organisms. However,

3 to 6 days of fermented broths in medium 1 and 2 of strain HH 7 showed their antimicrobial activity. Similarly 3 to 6 days of fermented broths in medium 1 and 2 of strain HH 14 exhibited its antimicrobial activity. Majority of isolated strains were showed their antimicrobial activity until the 13 day fermented broth. Among sixteen isolated fungi, strains HH 1, 2, 6, 8, 9, 11, 12 and 13 strains indicated very high antimicrobial activity on eight test organisms.

Strains HH 1, 2, 9 and 13 were identified as *Cephalosporium* sp. and they showed very high antimicrobial activity on eight test organisms.These findings are agreement with the statements of Crawford *et al* (1952), Selim *et al* (2011) and Prathyusha (2014).Crawford *et al* (1952) has reported that *Cephalosporium* sp. has antibacterial activity against Gram-positive bacteria. Selim *et al*. (2011) stated that endophytic *Cephalosporium* sp. has antimicrobial activity against different pathogenic bacteria and yeasts. Prathyusha (2014) has reported that *Cephalosporium* sp. has antimicrobial (antibacterial and antifungal) activity.

Strain HH12 was identified as *Rhizoctonia* sp. and it exhibited antimicrobial activity on eight test organisms.This finding is agreement with the statements of Prathvi *et al.* (2013) and Andrea *et al.* (2015). Prathvi *et al.* (2013) stated that endophytic *Rhizoctonia* sp. has antimicrobial activity against *Bacillus subtilis* and *Candida albicans.* Andrea *et al.* (2015) has reported that endophytic *Rhizoctonia sp.* has antimicrobial activity especially on *Candida albicans*.

In conclusion, the majority of bioactive fungal strains used in this study were found to inhibit harmful diseases causing agents such as bacteria and fungi. This funding is very beneficial to help humans' health since the bioactive compounds could be produced from the active fungal strains to protect some microbial diseases.

Acknowledgements

We would like to express our sincere thanks to all Chairpersons, Professor & Head Dr. Aye Pe and Professor Dr. Thandar Aye, Department of Botany, University of Yangon for

their valuable advice and kind encouragements to present this paper at the Myanmar Academy of Arts and Sciences.

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ISOLATION AND IDENTIFICATION OF *LACTOBACILLUS* SPP. FROM SAUERKRAUTS AND YOGURTS

Yin Kyay Khin[1] and Htein Htein Lin[2]

Abstract

The purpose of this study was to investigate *Lactobacillus* species from sauerkrauts and yogurts. Microbiological work was conducted from June 2017 to December 2017 in the Fermentation Department, Pharmaceutical Research Department, Ministry of Industry 1, Yangon Region. In the present study, lactic acid bacteria were isolated from the samples that were collected from three different places (Kamayut Township, Mayangone Township and North Dagon Township). Rogosa and tomato juice agar media were used in the isolation of bacteria by the serial dilution method. Six strains (H-1, 2; K-1, 2 and N-1, 2) from the three sauerkrauts and fifteen strains (HD-1 to 5, KB-1 to 5 and ND-1 to 5) from the three yogurts were isolated, purified and identified. In all samples, five isolates were found to ferment galactose, four isolates showed positive results in maltose and eight isolates exhibited positive results in xylose. Therefore, isolates H-1, H-2, K-1 were nearly the same to the characters of *Lactobacillus fermentum*, isolates K-2 and N-2 were mostly alike to the characters of *Lactobacillus bulgaricus*, isolates HD-5, ND-3 and N-1 were possible to the characters of *Lactobacillus hilgardii*, isolates HD-1 to 4, KB-1 to 5 and ND- 1, 2, 4, 5 were similar to the characters of *Lactobacillus heterohiochii* by morphological, staining methods and biochemical tests according to Bergey's Manual.

Introduction

In the food fermentations, microorganisms play an essential role. Lactic acid bacteria have been well known for centuries about their responsible mainly used in food preservation including dairy, meat, vegetables and bakery products due to their fermentative capacities and safety either separately or in combination with other conventional treatment. Keer *et al.*, 1983; Salminen *et al.*, 1996; Tserovska *et al.*, 2002; Gharaei and Eslamifar, 2011 stated that lactic acid bacteria has been isolated from several foods, sewage, manure animals and humans. Yogurt is a dairy product prepared by fermentation of milk with *Lactobacillus* spp. Boor, 2001 said that therapeutic, prophylactic and nutritional properties of yogurt are widely accepted.

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One of the most popular dairy product, yogurt is used as a therapeutic, diet was an old practice in Myanmar. Yogurt is flavored as a meat tenderizer and also used as curing of meat and fish. Sauerkraut is probable one of the oldest forms of lactic acid fermentation preserved food by the bacteria normally present on the plant. Sauerkraut is the clean, sound product of characteristic flavors obtain by fully fermentation chiefly lactic acid of properly prepared and shredded cabbage in the present of not less than 2% or more than 3% salt. It contain valuable quantities of vitamin A, B and C and the undesirable palatability of a good pack if might will be that the home market has distinct potentialities (Wai, 2001).

An important group from the LAB, especially some *Lactobacillus* spp. are commonly used as probiotics in humans and animals. They help maintain the natural balance of organisms (microflora) in the intestines and a healthy digestive system (Dunne *et al*., 1999). In commercial food products, several *Lactobacillus* strains have been isolated from different sources and used as probiotics (Ashraf *et al*., 2009). One of the major members of the lactic acid bacteria, the genus *Lactobacillus* is a group of gram positive bacteria, catalase-negative bacterial species able to produce lactic acid as a main end-product of the fermentation of carbohydrates (Felis and Dellaglio, 2007). In the classification and identification of bacteria, bacterial cell size is still a useful morphological characteristic (Prescot *et al.*, 1993). The aim of this study is to isolate the lactic acid bacteria in yogurt from different areas and to identify the morphological and biochemical characteristics of lactic acid bacteria from yogurt.

Materials and Methods

Microbiological work was conducted in the Fermentation Department, Pharmaceutical Research Department, Ministry of Industry 1, Yangon Region.

Collection of the yogurt samples from different areas

Samples collected from sauerkrauts and yogurts were obtained from Kamayut Township, Mayangone Township and North Dagon Township.

Viable bacteria count in sauerkraut and yogurt samples (Brugger *et al.*, 2012)

The number of bacteria in sauerkraut and yogurt were estimated using colony- forming unit (cfu). The CFU/ml can be calculated using the formula:

$$\text{Colony-forming unit (cfu) / ml} = \frac{\text{(no . of c olo nies x dil utio n fac to r)}}{\text{volume of culture plate}}$$

Isolation of *Lactobacillus* species by serial dilution method and streaking method

(Collin *et al.*, 1995; Dubey and Maheshwari, 2002)

Dilution of sample were plated on Rogosa and tomato juice agar media, and incubated at 37°C for 1-3days. The separate colonies appear and the different types of colonies were cultured in test tubes. The slants of media were repeatedly sub-cultured to obtain pure cultures.

Rogosa Medium (Sharpe, 1960)

Composition per liter	
Tryptone	10.000 g
Yeast extract	5.000 g
Glucose	20.000 g
Sorbitan mono-oleate `Tween 80'	1.000 ml
Potassium dihydrogen phosphate	6.000 g
Ammonium citrate	2.000 g
Sodium acetate, anhydrous	17.000 g
Magnesium sulphate	0.575 g
Manganese sulphate	0.120 g
Ferrous sulphate	0.034 g
Agar	20.000 g
Final pH (at 25℃)	5.4

Tomato Juice Medium (Atlas, 1993)

Composition per liter	
Tomato juice	20.000 g
c10.000 g	
Dextrose	10.000 g
Dipotassium phosphate	0.500 g
Monopotassium phosphate	0.500 g
Magnesium sulphate	0.200 g
Manganese sulphate	0.010 g
Ferrous sulphate	0.010 g
c	0.010 g
Agar	20.000 g
Final P^H (at 25°C)	6.7

Identification of isolated *Lactobacillus* species (Breed *et al.*, 1957; Buchanam and Gibbon, 1974)

(i) Oxygen requirement of the isolated bacteria (aerobic/anaerobic)

5 ml of broth media tubes were inoculated with 1% of *Lactobacilli* cultures. The development of turbidity in culture tubes was recorded as aerobic or anaerobic.

(ii) Staining methods for bacteria

1. Gram staining (Harley and Prescott, 2002)
2. Spore staining (Harley and Prescott, 2002)
3. Acid-fast staining (Bisen and Verma, 1998)

(iii)Motility test (Atlas, 1993)

(iv)Biochemical tests

1. Citrate utilization test (Cruickshank *et al.*, 1968)
2. Indole testone (Hucker, 1948)
3. Nitrate reduction test (Cowan, 1974)
4. Methyl red test (Bisen and Verma, 1998)
5. Voges-Proskauer test (Cruickshank *et al.*, 1968)
6. Hydrogen Sulphide (H_2S) production medium – Triple Sugar Iron (TSI) test (Bisen and Verma, 1998)
7. Gelatin liquefaction test (Hucker, 1948)
8. Catalase test (Salle, 1948)
9. Urease test (Dubey and Maheshwari, 2002)
10. Starch hydrolysis test (Dubey and Masheshwari, 2002)
11. Sugar fermentation test (Cruickshank *et al.*, 1968)

Results

Isolation and identification of *Lactobacillus* species from yogurt samples

The isolation and identification of *Lactobacillus* species from sauerkrauts and yogurts were conducted. Seven bacteria strains (HD-1 to HD-5), (H-1 and H-2) from the Kamayut Township, the other seven bacteria strains (KB-1 to KB-5), (K-1 and K-2) from the Mayangone Township, and next seven bacteria strains (ND-1 to ND-5), (N- 1 and N-2) from the North

Dagon Township were isolated, purified and identified. (HD- 1 to HD-4), (KB-2 to KB-5) and (ND-2 to ND-5) were isolated from Rogosa medium and, (HD-5, KB-1, ND-1) and (H-1, H-2, K-1, K-2, N-1, N-2) were isolated from tomato juice agar medium.

Viable bacteria count in yogurt samples

In sauerkraut samples, total viable counts of bacteria in Kamayut Township was 1.15 x 10^5 cfu/ml, in Mayangone Township was 1.43 x 10^5 cfu/ml and in North Dagon Township was 7.9 x 10^5 cfu/ml. In yogurt samples, total viable counts of bacteria in Kamayut Township was 1.67 x 10^5 cfu/ml, in Mayangone Township was 1.4 x 10^5 cfu/ml and in North Dagon Township was 2.0 x 10^5 cfu/ml.

Figure 1. Twenty-one isolated strains from yogurt and six isolated strains from sauerkraut

Oxygen requirement of the isolated bacteria (aerobic/anaerobic)

In the present study, all isolated bacteria were anaerobes

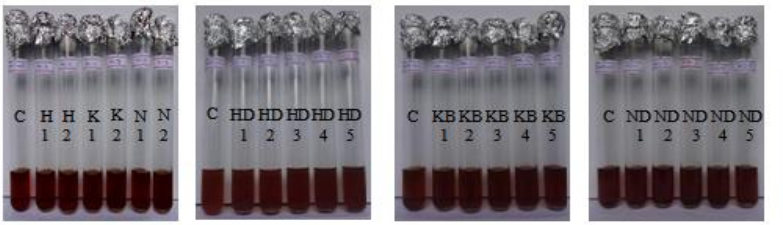


Figure 2. Oxygen requirement of the isolated bacteria (aerobic/anaerobic)

Morphological and microscopic characteristics of *Lactobacillus* species from sauerkraut and yogurt samples

Twenty-one bacterial strains were isolated and purified from the samples. The colony characters of isolates from sauerkraut samples were cream, white, creamish white colours, circular shapes and most isolated strains were entire margin and convex elevation (Table 1 and Figures 3, 4 and 5). The colony characters of isolates from yogurt samples were cream, white, creamish white, grayish white colours, circular and irregular shapes, and most isolated strains were filiform margin and umbonate elevation, HD-5 and ND-3 were entire margin and convex elevation (Table 2).The sizes of isolates were (0.5-1.0) × (1.0-6.0) µm from sauerkraut samples and (0.6-1.0) × (2.0- 5.0) µm from yogurt samples. All of these isolates were gram positive, non-spore forming and unstained in acid-fast staining.

Table 1. Colony morphology of *Lactobacillus* species from sauerkraut samples

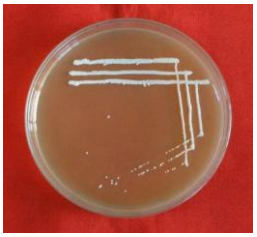
Sample No.		Colony Morphology				Cell Morphology
		Colour	Shape	Margin	Elevation	Cell Size (µm) Width x Length
H	1	Cream	Circular	Entire	Convex	0.5 - 1.0 x 1 - 5
	2	Creamish white	Circular	Entire	Convex	0.5 - 0.8 x 2 - 5
K	1	Creamish white	Circular	Entire	Convex	0.6 - 1.0 x 2 - 6
	2	Cream	Circular	Entire	Convex	0.5 - 1.0 x 2 - 6
N	1	Cream	Circular	Entire	Convex	0.5 - 0.8 x 2 - 5
	2	Creamish white	Circular	Entire	Convex	0.6 - 1.0 x 2 - 5

H - Kamayut Township, K - Mayangone Township, N - Northgon Township

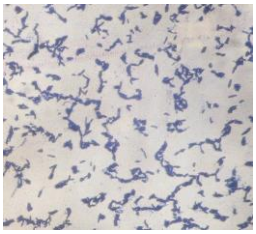
Table 2. Colony morphology of *Lactobacillus* species from yogurt samples

Sample No.		Colony Morphology				Cell Morphology
		Colour	Shape	Margin	Elevation	Cell Size (µm) Width x Length
HD	1	White	Irregular	Filiform	Umbonate	0.7 - 1.0 x 2 - 5
	2	Creamish wh	Irregular	Filiform	Umbonate	0.6 - 1.0 x 2 - 4
	3	Creamish white	Irregular	Filiform	Umbonate	0.7 - 1.0 x 2 - 4
	4	White	Irregular	Filiform	Umbonate	0.7 - 1.0 x 2 - 5
	5	White	Circular	Entire	Convex	0.6 - 1.0 x 2 - 4
KB	1	Creamish white	Irregular	Filiform	Umbonate	0.7 - 1.0 x 2 - 4
	2	Creamish white	Irregular	Filiform	Umbonate	0.6 - 1.0 x 2 - 5
	3	Creamish white	Irregular	Filiform	Umbonate	0.7 - 1.0 x 2 - 5
	4	Creamish white	Irregular	Filiform	Umbonate	0.7 - 1.0 x 2 - 4
	5	White	Irregular	Filiform	Umbonate	0.6 - 1.0 x 2 - 5
ND	1	Grayish white	Irregular	Filiform	Umbonate	0.7 - 1.0 x 2 - 4
	2	Creamish white	Irregular	Filiform	Umbonate	0.7 - 1.0 x 2 - 4
	3	Cream	Circular	Enire	Convex	0.7 - 1.0 x 2 - 4
	4	Creamish white	Irregular	Filiform	Umbonate	0.7 - 1.0 x 2 - 4
	5	Creamish white	Irregular	Filiform	Umbonate	0.7 - 1.0 x 2 - 4

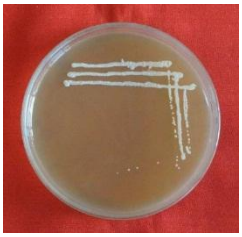
HD - Kamayut Township, KB - Mayangone Township, ND - North Dagon Township



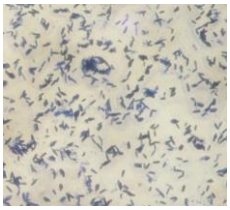
Isolate H-1



Isolate H-1 (1000x)

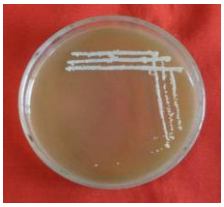


Isolate H-2

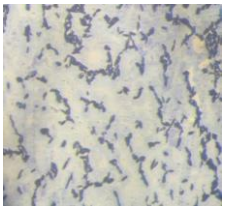


Isolate H-2 (1000x)

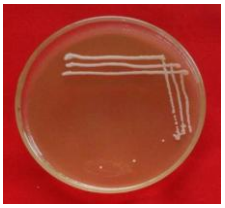
Figure 3. Colony morphology and gram staining of isolates H-1 and H-2 from sauerkraut samples (Kamayut Township)



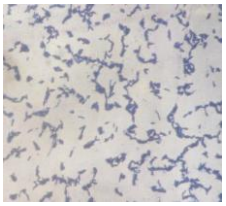
Isolate K-1



Isolate K-1(1000x)



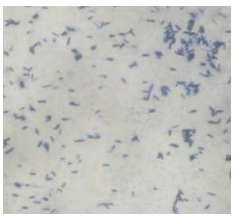
Isolate K-2



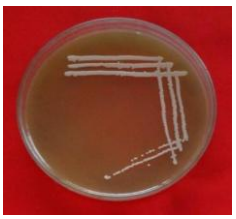
Isolate K-2(1000x)

Figure 4. Colony morphology and gram staining of isolates K-1 and K-2 from sauerkraut samples (Mayangone Township)

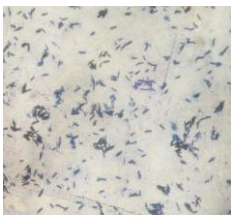
Isolate N-1



Isolate N-1(100x)



Isolate N-2



Isolate N-2(100x)

Figure 5. Colony morphology and gram staining of isolates N-1 and N-2 from sauerkraut samples (North Dagon Township)

Motility test

All isolated *Lactobacillus* species were negative results in motility test shown in Figure 6.

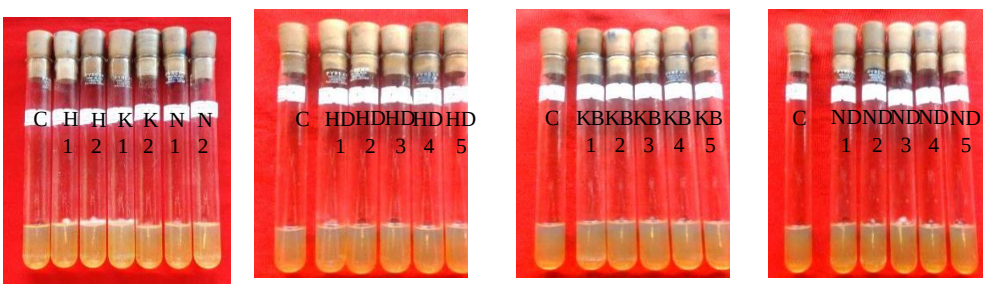


Figure 6. Motility test of isolates

Biochemical characteristics of *Lactobacillus* species from sauerkraut and yogurt samples

The identification of genus level, twenty-one isolated bacteria were carried out by biochemical tests. All isolated *Lactobacillus* species from sauerkrauts and yogurts were negative results in motility test, citrate utilization test, indole test, nitrate reduction test, methyl red test, Voges-Proskauer test, triple sugar iron (TSI) test, H_2S production test, gelatin liquefaction test, catalase teat, urease test and starch hydrolysis test. These results were shown in Table 3 and 4.

Table 3. Biochemical characteristics of *Lactobacillus* species from sauerkraut samples

Code Number	H		K		N	
	1	2	1	2	1	2
Citrate utilization test	-	-	-	-	-	-
Indole test	-	-	-	-	-	-
Nitrate reduction test	-	-	-	-	-	-
Methyl red test	-	-	-	-	-	-
Voges-Proskauer test	-	-	-	-	-	-
TSI	-	-	-	-	-	-
H_2S production test	-	-	-	-	-	-
Gelatin liquefaction test	-	-	-	-	-	-
Catalase test	-	-	-	-	-	-
Urea	-	-	-	-	-	-
Starch hydrolysis test	-	-	-	-	-	-

+ = positive reaction, - = negative reacti

Table 4. Biochemical characteristics of *Lactobacillus* species from yogurt samples

Code Number	HD					KB					ND				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Citrate utilization test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl red test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Voges-Proskauer test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TSI test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H_2S production test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin liquefaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urease test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ = positive reaction, - = negative reaction

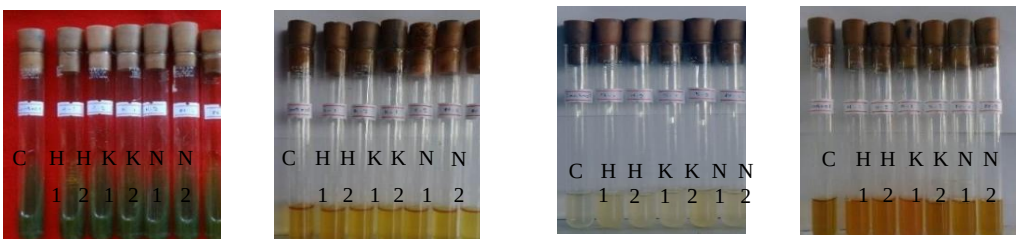


Figure 10. Citrate utilization test, indole test, nitrate reduction test and methyl red test of isolates from sauerkrauts

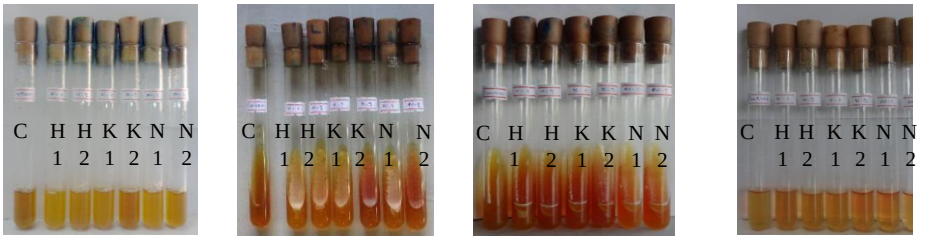


Figure 11. Voges-Proskauer test, Triple sugar iron (TSI) test, Hydrogen Sulphide (H_2S) production test and urease test of isolates from

Figure 12. Gelatin liquefaction test and catalase of isolates from sauerkrauts

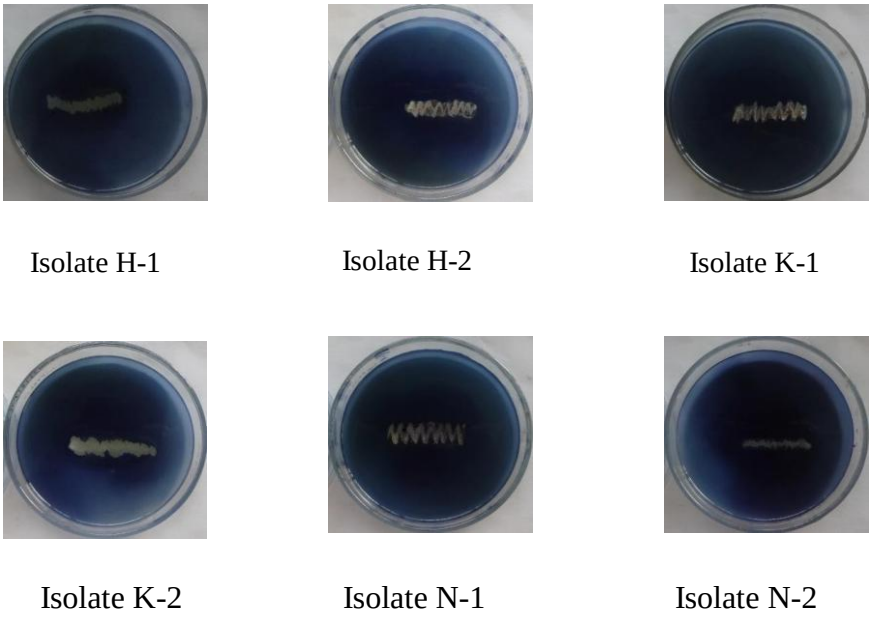


Figure 13. Starch hydrolysis test of isolates from sauerkrauts (H1, H-2, K-1, K-2, N-1 and N-2)

Sugar fermentation tests of Lactobacillus species from sauerkraut and yogurt samples

In the results of sugar fermentation tests in sauerkraut samples, all isolates were found to ferment fructose, glucose, lactose and xylose but could not ferment mannitol. In the sugar fermentation test of cellobiose, galactose, raffinose and sucrose, H-1, H-2, K-1 showed positive results. H-1, H-2, K-1 and N-1 showed positive results in maltose sugar fermentation test. In yogurt samples, all isolates were found to ferment fructose and glucose but could not ferment cellobiose, lactose, sucrose, mannitol and raffinose. In the sugar fermentation test of galactose, maltose and xylose, all isolates showed negative results except HD-5 and ND-3. The results of sugar fermentation test were presented in Tables 5 and 6.

Table 5. Sugar fermentation tests of *Lactobacillus* species from sauerkraut samples

Fermentable sugars	H		K		N	
	1	2	1	2	1	2
Cellobiose	+	+	+	-	-	-
Fructose	+	+	+	+	+	+
Galactose	+	+	+	-	-	-
Glucose	+	+	+	+ (G)	+ (G)	+
Lactose	+	+	+	+	+	+
Maltose	+	+	+	-	+	-
Mannitol	-	-	-	-	-	-
Raffinose	+	+	+	-	-	-
Sucrose	+	+	+	-	-	-
Xylose	+	+	+	+	+	+

+ = able to ferment sugar, - = not able to ferment sugar, (G) = gas produced
H - Kamayut Township, K - Mayangone Township, N - North Dagon Township

Table 6. Sugar fermentation tests of *Lactobacillus* species from yogurt samples

Fermentable sugars	HD					KB					ND			
	1	2	3	4	5	1	2	3	4	5	1	2	3	4
Cellobiose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	-	-	-	-	+	-	-	-	-	-	-	-	+	-
Glucose	+	+	+	+	+ (G)	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	-	-	-	-	+ (G)	-	-	-	-	-	-	-	+	-
Mannitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xylose	-	-	-	-	+	-	-	-	-	-	-	-	+	-

+ = able to ferment sugar, - = not able to ferment sugar, (G) = gas produced
HD - Kamayut Township, KB - Mayangone Township, ND - North Dagon Township

Figure 14. Cellobiose, fructose, galactose, glucose and lactose sugarfermentation tests of isolates from sauerkrauts

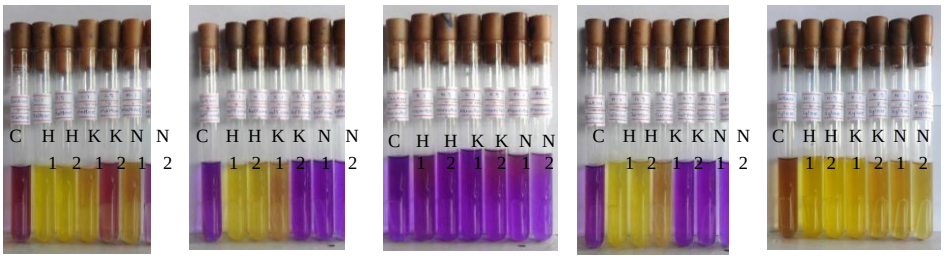


Figure 15. Maltose, raffinose sugar fermentation tests, mannitol fermentation test, sucrose and xylose sugar fermentation tests of isolates from sauerkrauts

Discussion and Conclusion

In the present study, fifteen bacteria strains (HD-1 to 5, KB-1 to 5, ND-1 to 5) were isolated by using Rogosa and tomato juice agar media, six strains (HD-1, 2; K-1, 2 and N-1, 2) were isolated by using tomato juice agar medium from sauerkraut and yogurt samples that were collected from three different areas. These strains were identified by morphological, cultural and biochemical characteristics using Bergey's Manual of Determinative Bacteriology (Breed *et al.*, 1957; Buchanam and Gibbon, 1974).

In sauerkraut samples, total viable counts of bacteria in Kamayut Township was 1.15 x 10^5 cfu/ml, in Mayangone Township was 1.43 x 10^5 cfu/ml and in North Dagon Township was 7.9 x 10^5 cfu/ml. In yogurt samples, total viable counts of bacteria in Kamayut Township was 1.67 x 10^5 cfu/ml, in Mayangone Township was 1.4 x 10^5 cfu/ml and in North Dagon Township was 2.0 x 10^5 cfu/ml. These results are found nearly relevant to the findings of Hoque *et al.*, 2010, Amin *et al.*, 2011. The colony characters of isolates were cream, white, creamish white, grayish white

colours, circular and irregular shapes, and most isolated strains were filiform and entire margins, umbonate and convex elevations. All of these isolates were anaerobes, gram positive, non-motile, non-spore forming and negative in acid-fast staining. Therefore, all isolates might be identified as *Lactobacillus* species according to Schleifer and Ludwig (1995), Kadere and Kutima (2012).

The identification of genus level, the isolated bacteria was carried out biochemical tests. All isolated *Lactobacillus* species provided negative results in motility test, citrate utilization test, indole test, nitrate reduction test, methyl red test, Voges-Proskauer test, triple sugar iron (TSI) test, H_2S production test, gelatin liquefaction test, catalase teat, urease test and starch hydrolysis test. These results were in according with those revealed in Bergey's Manual of Determinative Bacteriology (Breed *et al.*, 1957; Buchanam and Gibbon, 1974). Therefore, all isolates might be identified as *Lactobacillus* species according to Breed *et al.*, (1957), Buchanam and Gibbon (1974), Batt (2000), Holzapfel *et al.*, (2001), Axelsson (2004), Kadere and Kutima (2012), Arimah and Ogunlowo (2014). The *Lactobacillus* isolate exhibited negative pattern of H_2S formation, starch hydrolysis, nitrate reduction and urease activity. These are the common characters of *Lactobacillus* species. Similar results were observed by Schleifer and Ludwig (1995), Forouhandeh *et al.*, (2010), Chakraborty and Bhowal (2015).

In sauerkraut samples, all isolates were found to ferment fructose, glucose, lactose and xylose but could not ferment mannitol. In the sugar fermentation test of cellobiose, galactose, raffinose and sucrose, H-1, H-2, K-1 showed positive results. H- 1, H-2, K-1 and N-1 showed positive results in maltose sugar fermentation test. In the results of sugar fermentation tests in yogurt samples, all isolates were found to ferment fructose and glucose but could not ferment cellobiose, lactose, sucrose, mannitol and raffinose. In the sugar fermentation test of galactose, maltose and xylose, all isolates showed

negative results except HD-5 and ND-3. These results were also similar to the result showed in Bergey's Manual of Determinative Bacteriology (Breed *et al.*, 1957; Buchanam and Gibbon, 1974). All these results are found relevant to the findings of Bhardwaj *et al.,* 2012; Chowdhury *et al.*, 2012. They reported that the sugar fermentation test of most *Lactobacillus* species were acid production without gas from glucose.

In Bergey's Manual, the characters of *Lactobacillus* species are anaerobic or facultative, non-motile, gram-positive, non-sporing, catalase negative, gelatin not liquefied, indole and H_2S not produced (Breed *et al*., 1957; Buchanam and Gibbon, 1974). According to the above characters, in yogurt samples, isolates HD-1 to HD-4, KB-1 to KB-5 and ND-1, 2, 4, 5 were very similar to the characters of *Lactobacillus heterohiochii*, isolates HD-5, ND-3 were nearly the same to the characters of *Lactobacillus hilgardii*. In sauerkraut samples, isolates H-1, H-2, K-1 were mostly alike to the characters of *Lactobacillus fermentum,* isolate N-1 were nearly the same to the characters of *Lactobacillus hilgardii,* and K-2, N-2 were indistinguishable to the characters of *Lactobacillus bulgaricus* (Wai, 2001).

It could be concluded that *Lactobacillus heterohiochii, Lactobacillus hilgardii, Lactobacillus fermentum and Lactobacillus bulgaricus* were possible in the samples of present work and their morphological, biochemical characteristics were investigated from yogurt and sauerkraut samples.

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ISOLATION OF ANTIBACTERIAL COMPOUNDS FROM *PHOMOPSIS* SP. ISOLATED FROM LEAVES OF *PSIDIUM GUAJAVA* L.

Hnin Wit Mhon[1], Mon Mon Thu[2] , Yee Yee Thu[3]

Abstract

An endophytic fungal strain *Phomopsis* sp. was isolated from the leaves of *Psidium guajava* L. For the extraction of the bioactive compounds, 12L large fermentation of strain *Phomopsis* sp. and antimicrobial activity of fermented broth with *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur*, *Salmonella typhi* and *Staphylococcus aureus* were carried out at Microbiology Laboratory, Department of Botany, University of Yangon. Then, the filtrate was extracted with methanol on Ambilites XAD 2 resin column and the methanol extracts showed high against six test organisms. Isolation and purification of the bioactive compounds from the methanol extracts were done by utilizing silica gel columns and Sephadex LH 20 gel columns with various solvent systems at Department of Organic Chemistry, Ramkhamhaeng University, Bangkok, Thailand. The isolated compounds were characterized by FT-IR spectra, 1D-NMR (^{1}H-NMR, ^{13}C-NMR) and 2D-NMR (COSY, HSQC, and HMBC) spectra. The three isolated compounds were identified as phomopsolide B, adenosine and emerimidine C. Antimicrobial activity of the bioactive compounds was evaluated on six test organisms and showed highest activity against *Xanthomonas oryzae* and weak activity against *Candida albicans* and *Escherichia coli*.

Key words: *Adenosine, Emerimidine C, Phomopsis sp., Phomopsolide B*

Introduction

Bioactive metabolites produced by endophytic fungi are the major source of drugs, and the plant clearly provides the proper environment for its growth and survival (Kumaran *et al*., 2010). The bioactive compounds have

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been isolated from microorganisms originating from various terrestrial and marine environments (Strobel and Daisy, 2003). Endophytes have the ability to produce a range of secondary metabolites providing researchers with numerous leads for the compounds of pharmaceutical significance and possible developments as new drugs (Tan and Zou, 2001). Numerous antibiotics have been isolated from a variety of microorganisms, however, studies are still being conducted to identify novel antibiotics effective against pathogenic fungi and bacteria.

Endophytes are proved rich sources of the natural compounds, showing a variety of pharmacological and biological activities. The production and quality of the bioactive compounds from endophytic fungi depend on natural conditions of the association and the nature of the synthetic medium used (Strobe and Daisy, 2003). Several hundreds of the compounds with antibiotic activity have been isolated from microorganisms over the years, but only a few of them are clinically useful (Thomashow *et al.*, 2008).

Materials and Methods

Screening of Endophytic Fungus from *Psidium guajava* L.

The *Psiduum guajava* L. plant sample was collected from Nyaung-Hna-Pin area, Hmawbi Township. The isolation of endophytic fungus was carried out with the following scheme: (1) Plant parts were washed in running tap water for 15 min. (2) Plant parts were cut into about 1 cm pieces. (3) The surfaces of cut-plant pieces were sterilized by soaking it in 75% ethanol for 2 min. (4) Sterile surfaces were socked in 5.3% sodium hypocloride for 5 min. (5) Cut-plant pieces were washed out sodium hypocloride by socking in 75% ethanol for 0.5 min. (6) They were dried and cut into smaller pieces, and placed on agar plates and then incubated for 3 days to 3 weeks (Phay, 1997). Thus, the isolated microorganisms were transferred into a 10 ml test tube containing 5 ml of sucrose/yeast extract medium.

Antimicrobial Activity of 12L Fermentation of *Phomopsis* sp.

The small fungal piece (1 cm) from the plate culture of *Phomopsis* sp. was inoculated into 500 ml of conical flask containing 180 ml of sucrose, yeast extract seed medium. The flask was incubated at room temperature for two days for seed culture. Two days old seed culture (180 ml) was transferred into twelve flasks of 2L conical flask containing 1L of sucrose yeast fermentation medium. These flasks were incubated at 100 rpm for 3-7 days at room temperature. The fermentation flasks indicated antimicrobial activity on *Bacillus subtilis, Candida albicans, Escherichia coli, Malassezia furfur, Salmonella typhi and Staphylococcus aureus* (Strobel and Sullivan, 1999; Phay, 1997).

Extraction of Bioactive Compounds from Fermented Broth of *Phomopsis* sp.

After testing antimicrobial activity, 12L fermented broths were filtered with filter paper. The mycelia from fermented broth were filtered on the filter paper and then the filtrate was applied on an Amberlites XAD 2 resin column. The column was washed with water, followed by six liters of methanol. The methanol extract was evaporated on water bath at 55-60°C. The methanol extract was tested for antimicrobial activity against *Bacillus subtilis, Candida albicans, Escherichia coli, Malassezia furfur, Salmonella typhi* and *Staphylococcus aureus* (Strobel and Sullivan, 1999).

Isolation and Purification of Bioactive Compounds from *Phomopsis* sp.

According to TLC results, silica gel column chromatography was carried out. The silica 34 gel (100 g) column was eluted with hexane:ethyl acetate (100%, 10:1, 10:2, 10:3, 10:5, 1:1, 1:2, 1:3) and ethyl acetate : methanol (100%, 10:0.5, 10:1, 10:2, 10:3, 10:5, 1:1, 100% MeOH) and then fifteen fractions were collected. The column size was 4×17 cm and the flow rate was 2 ml per minute (Grabley *et al*., 1999).

Identification of Isolated Compounds from *Phomopsis* sp.

The identification of the isolated compounds was characterized by 1D-NMR (^{1}H-NMR and ^{13}C-NMR), 2D-NMR (^{1}H-^{1}H COSY, HMBC) 400 MHz at Nuclear Magnetic Resonance and FT-IR spectra at the Department of Chemistry, Ramkhamhaeng University, Bangkok, Thailand. The spectral data of the isolated compounds were compared by ACD (Advanced Chemistry Development) Labs (Robert and Francis, 2014).

Antimicrobial Activity of Isolated Compounds from *Phomopsis* sp.

All the isolated compounds were tested their antimicrobial activity with six test organisms. The volume of each compound was 10µg/disc (conc.1mg/ml).

Paper disc diffusion assay

Broth culture (50µl) of test organisms (*Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur*, *Salmonella enteric*, *Salmonella typhi*, *Staphylococcus aureus* and *Xanthomonas oryzae*) was added to 100 ml assay medium sucrose/yeast medium and then poured into the plates. After solidification paper discs infused with broth samples were applied on the test plates and incubated at 30°C for 24-48 hrs. When clear zones (inhibitory zones) showed around the paper discs, they were measured (Phay, 1997).

Results

Scientific Name - *Psidium guajara* L.
English Name - Guava
Myanmar Name - Malaka
Family - Myrtaceae

Figure 1. Habit of *Psidium guajava* L.

Antimicrobial Activity of 12L Fermented Broth of *Phomopsis* sp.

Twelve fermentation flasks of strain *Phomopsis* sp. showed very high activity against six test organisms. Fermentation flasks 2, 3, 4 and 10 of strain *Phomopsis* sp. indicated high activity against six test organisms. Fermentation flasks 5, 6, 7, 8, 9 and 12 of strain *Phomopsis* sp. exhibited weak activity against six test organisms.

Antimicrobial Activity of Methanol Extracts

The methanol extract showed higher activity than acetone extract against *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur*, *Salmonella typhi* and *Staphylococcus aureus* as shown in Table 1.

Table 1. Inhibitory zones (mm) of methanol extracts from *Phomopsis* sp.

Test Or. / Extracts	*Bacillus subtilis*	*Candida albicans*	*Escherichia coli*	*Malassezia furfur*	*Salmonella typhi*	*Staphylococcus aureus*
Methanol	45	40	45	45	40	35

10-12mm = weak activity, 13 - 18 mm = high activity, >18 mm = very high activity disc size = 6mm

Isolation and Purification of Bioactive Compounds from *Phomopsis* sp.

The two hundred and sixteenth small fractions were collected from silica gel 34 (100g) column. According to their R_f value and colors reaction by reagent on TLC plates under UV 254 nm, they were combined into the large fractions: F1 (1-5), F2 (16-25), F3 (26-40), F4 (41-50), F5 (51-65), F6 (66-80), F7 (81-96), F8 (97-120), F9 (121-146), F10 (148-162), F11 (152-162), F12 (163-180), F13 (181-200), F14 (201-216) and F15 (217-223). All of these fractions, the fraction 8 was crystal and after washing with methanol in three times, it was purified according to their spots on TLC plates under UV 254 nm.

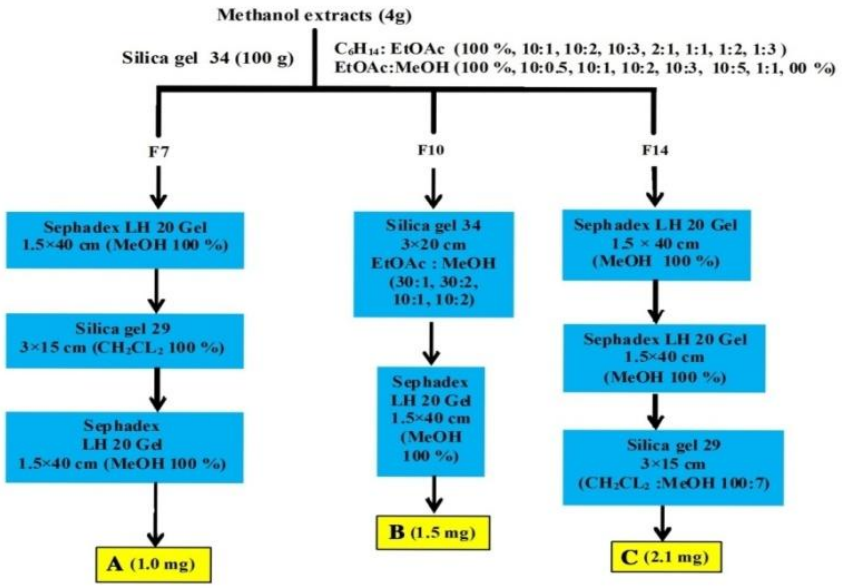
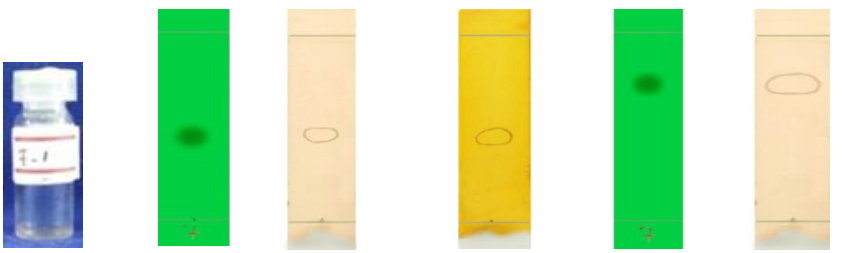


Figure 2. Flow chart for isolation procedure of the active compounds from *Phomopsis* sp.



Compound A | Under UV 254nm | Anisaldehyde | Dragendorff | Under UV 254 nm | Anisaldehyde

Solvent system - CH_2CL_2:MeOH, 5:0.3, 5:0.5,
R_f - 0.35 and 0.69

Figure 3. Identification of the isolated compound A by R_f value

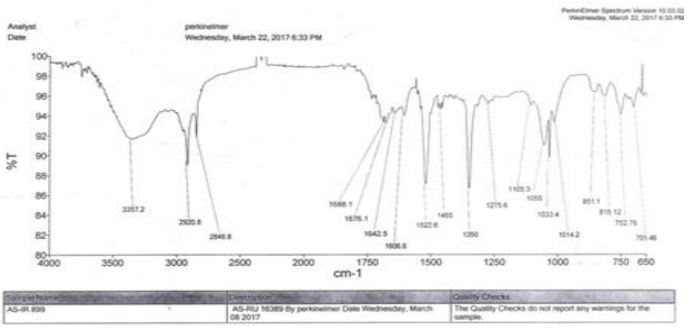


Figure 4. FT-IR spectrum of the isolated compound A

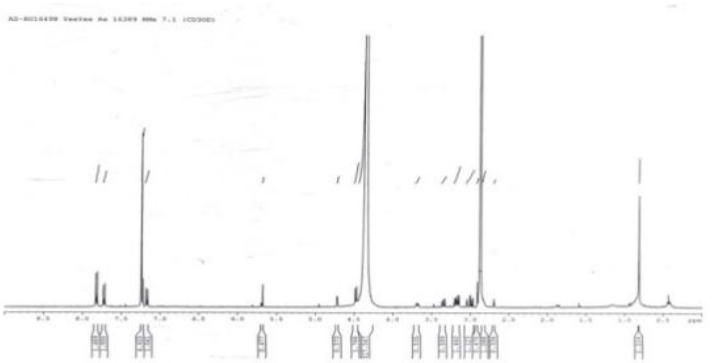


Figure 5. ^{1}H-NMR spectrum (400 MHz, CD_3OD) of the isolated compound A

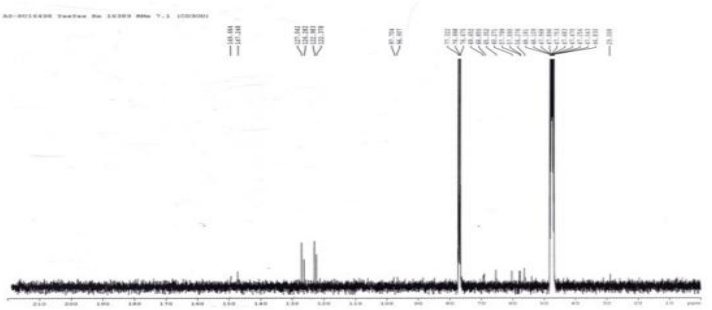


Figure 6. ^{13}C-NMR spectrums (400 MHz, CD_3OD) of the isolated compound A

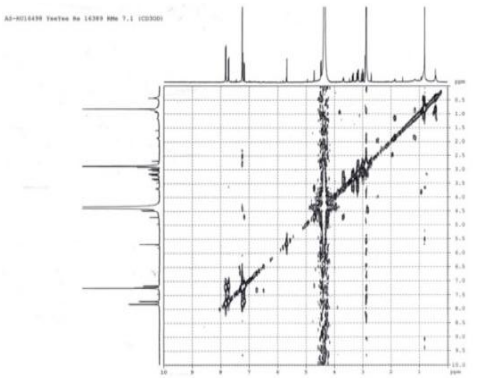


Figure 7. ^{1}H-^{1}H (COSY) spectrum (400 MHz, CD_3OD) of the isolated compound A

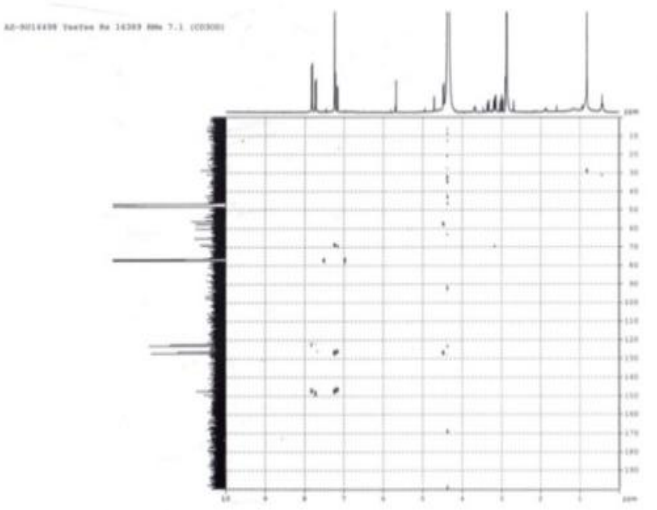


Figure 8. ^{1}H-^{13}C (HMBC) spectrum (400 MHz, CD_3OD) of the isolated compound A

Identification of Isolated Compound A

The isolated compound A was VU active at 254 nm and it does not give any color on TLC plate with anisaldehyde and dragendroff spray reagents. Its R_f value was 0.35 (CH_2CL_2: MeOH, 5:0.3) and 0.69 (CH_2CL_2:MeOH, 5:0.5). This substance is good soluble in chloroform or dichloromethane as shown in Figure 3.

In the FT-IR spectrum, the band for O-H stretching vibration (alcohol and phenol groups) was observed at 3357 cm^{-1}. The bands at 2920 and 2848 cm^{-1}were found for C-H stretching vibration of methyl, methylene and methine groups. C=O stretching vibration (ketone) was showed at 1688 cm^{-1} and C=C stretching vibration group was found at 1676, 1642, 1606, 1522 and 1465 cm^{-1}. The bands for 1350 and 1275 cm^{-1} were found due to the presence of C-H bending vibration of methyl and methylene groups. The bands at 1105, 1055, 1033 and 1014 cm^{-1} showed C-O-C stretching vibration as shown in Figure 4.

According to its ^{1}H-NMR spectrum, aromatic protons were as doublets (*d*) at 7.82 ppm, 7.72 ppm, 7.24 ppm and 7.18 ppm, olefinic protons (C=CH) as singlets (*s*) at 5.67 ppm and as doublets (*d*) at 4.73 ppm, CH_2 protons as doublets (*d*) at 4.47 ppm. CH_2 protons were observed as multiplets (*m*) at 3.69 ppm, as doublet of doublets (*dd*) at 3.35 ppm, as triplet of doublets (*td*) at 3.18 ppm, as doublet of doublets (*dd*) at 3.00 ppm, as singlets (*s*) at 2.93 ppm and as multiplets (*m*) at 2.70 ppm. Alkyl protons were as singlets (*s*) at 1.2 ppm in this compound as shown in Figure 5.

In this ^{13}C- NMR spectrum, aromatic or (C=C) olefinic carbons were found at 149.664 ppm, 147.248 ppm, 127.04 ppm, 126.28 ppm, 122.98 ppm and 122.378 ppm. Acohol (C-OH) groups were at 76.67 ppm, 69.65 ppm, 68.85 ppm, 65.35 ppm, 60.27 ppm, 57.799 ppm, 57.590 ppm and 56.27 ppm as shown in Figure 6.

According to its FT-IR spectral data, ^{1}H-NMR, ^{13}C-NMR and 2D-NMR spectral data, it was identified as phomopsolide B and its molecular formula is $C_{15}H_{20}O_6$ as shown in Fig. 9.

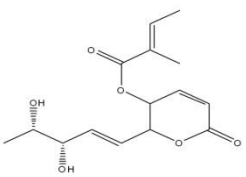
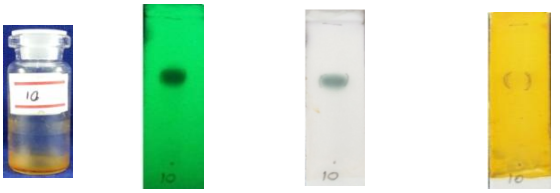


Figure 9. Phomopsolide B



Compound D	Under UV 254 nm	Anisaldehyde	Dragendorff
Solvent system	CH_2CL_2:MeOH, 5:0.3		
R_f	0.60		

Figure 10. Identification of the isolated compound B by R_f value

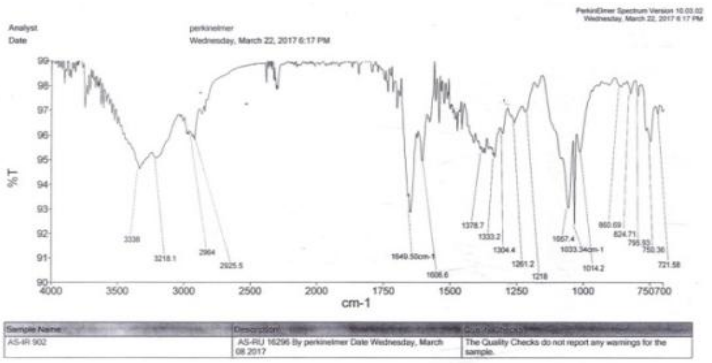


Figure 11. FT-IR spectrum of the isolated compound B

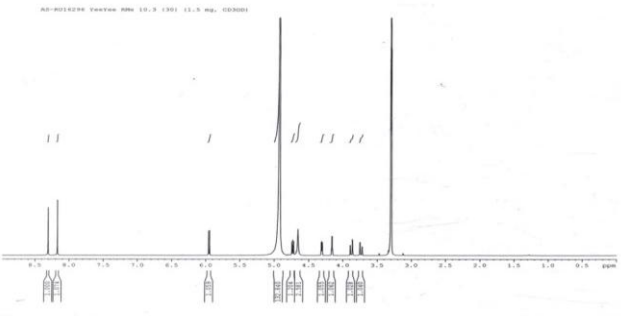


Figure 12. ^{1}H-NMR spectrum (400 MHz, CD_3OD) of the isolated compound B

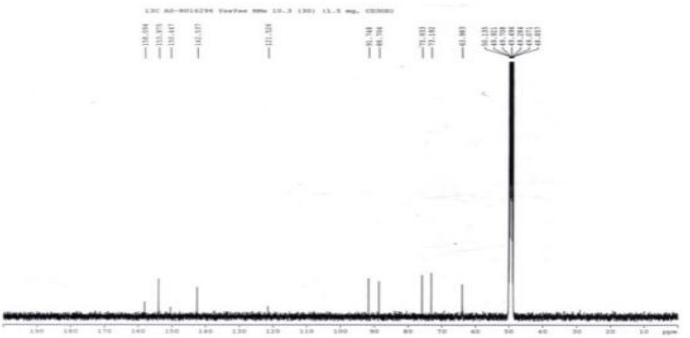


Figure 13. ^{13}C-NMR spectrum (400 MHz, CD_3OD) of the isolated compound B

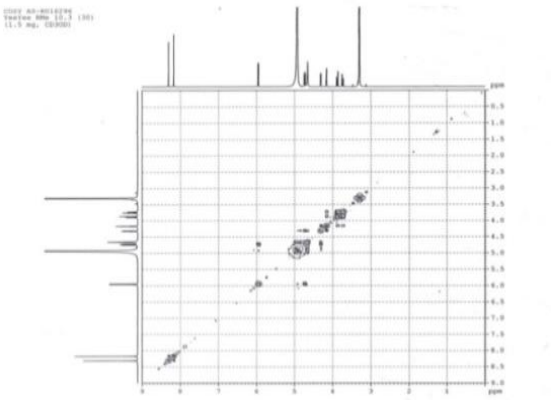


Figure 14. ^{1}H-^{1}H (COSY, 400 MHz, CD_3OD) of the isolated compound B

Identification of the isolated compound B

The isolated compound B was VU active under 254 nm and dark green spot on TLC plate with anisaldehyde spray reagent. Its R_f value was 0.60 (CH_2CL_2:MeOH, 5:0.3). This substance is good soluble in methanol as shown in Figure 10.

Its IR spectrum were showed N-H, O-H stretching vibration (amines, alcohol and phenol groups) at 3338 cm^{-1}. The bands at 2964 and 2925 cm^{-1} were for C-H stretching vibration of methyl and methylene groups. C=C stretching vibration were showed at 1649 and 1606 $cm^{-1}$and C=N stretching vibration was attributed at 1590 and 1690 cm^{-1}. CH_3 and CH_2 bending vibration were found at 1378, 1333, 1304, 1261 and 1218 cm^{-1} and C-N

stretching vibration at 1304 and 1261 cm^{-1}. The bands at 1057, 1033 and 1014 cm^{-1} were showed –C-O-C stretching vibration and the bands at 860, 795 and 750 cm^{1} were found =C-H bending vibration as shown in Figure 11.

According to ^{1}H-NMR spectrum, aromatic protons were found as singlets (*s*) at 8.3 ppm and 8.2 ppm and (CH_2) protons were as doublets (*d*) at 5.9 ppm, as doublet of doublets (*dd*) at 4.73 ppm and as singlets (*s*) 4.65 ppm. Olefinic protons (C=CH) were as quantets (*q*) at 4.31 ppm, as doublet of doublets (*dd*) at 4.16 ppm, at 3.86 ppm, 3.74 ppm in this compound as shown in Figure 12.

By means of ^{13}C NMR, aromatic carbons were at 158 ppm, 153 ppm, 150 ppm and the bands at 142 ppm, 121 ppm, 91 ppm, 88 ppm were found (C=C) olefinic carbons. The (C-OH) alcohol carbons groups were observed at 75 ppm, 73 ppm and 63 ppm as shown in Table 10 and Figure 13.

According to its FT-IR spectral data, ^{1}H-NMR, ^{13}C-NMR and 2D-NMR spectral data, it was identified as adenosine and its molecular formula is $C_{10}H_{13}O_4N_5$ as shown in Figure 15.

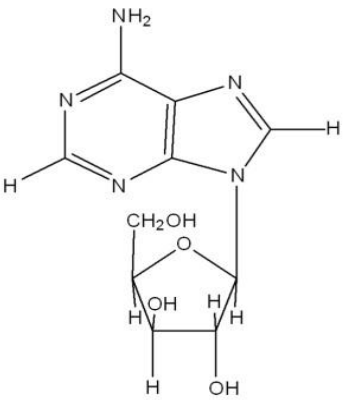
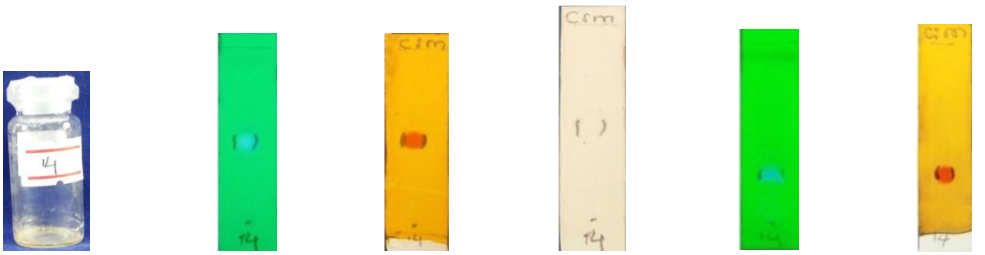


Figure 15. Adenosine



Compound H	Under UV 254 nm	Dragen dorff	Anisalde hyde	Under UV 254 nm	Dragen dorff
Solvent system	- CH_2CL_2:MeOH, 5:0.5, 5:0.3				
R_f	- 0.53, 0.25				

Figure 16. Identification of the isolated compound C by R_f value

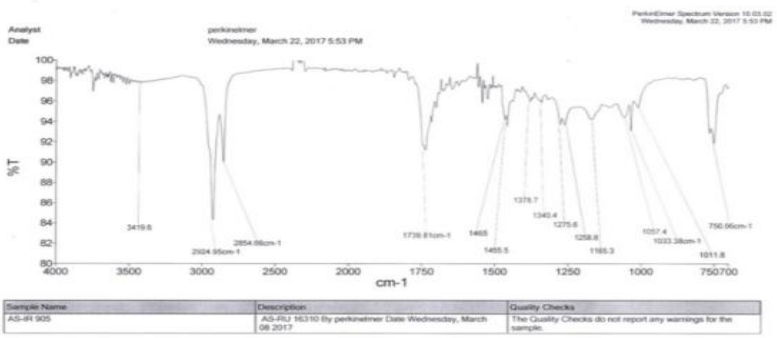


Figure 17. FT-IR spectrum of the isolated compound C

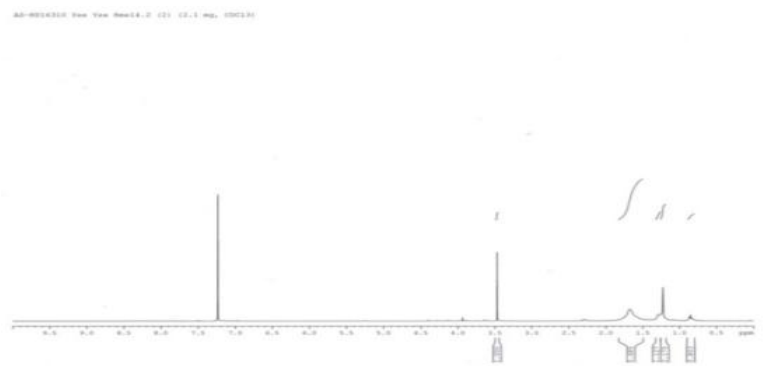


Figure 18. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of the isolated compound C

Identification of the isolated compound C

The isolated compound H was VU active under 254 nm and it gave an orange colour

with dragendoff reagent. Its R_f value was 0.53 (CH_2CL_2:MeOH, 5:0.5) and 0.25 (CH_2CL_2:MeOH, 5:0.3). This substance is good soluble in chloroform or dichloromethane as shown in Figure16.

In the FT-IR spectrum, the band for N-H stretching vibration was observed at 3419 cm^{-1} and the bands at 2924 and 2854 cm^{-1} were found for C-H stretching vibration of methyl and methylene groups. C=O stretching vibration (ketone) was showed at 1739 cm^{-1} and aromatic carbons were found at 1465, 1455, 1378 and 1340 cm^{-1}. The bands at 1165, 1057, 1033 and 1011 cm^{-1} were found alkyl amines. The C-H bending vibration was found at 750 cm^{-1} as shown in Figure 17.

According to its ^{1}H-NMR spectrum, (CH_2) proton was as singlets (*s*) at 3.5 ppm and alkyl protons (CH_3) was as singlets (*s*) at 1.7 ppm, 1.3 ppm and 1.2 ppm in this compound as shown in Figure 18.

According to its FT-IR spectral data and ^{1}H-NMR spectral data, it was identified as emerimidine C and its molecular formula is $C_{10}H_{13}O_3$ as shown in Figure 19.

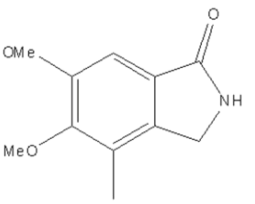


Figure 19. Emerimidine C

Antimicrobial Activity of Isolated Compounds from *Phomopsis* sp.

The compound B and C showed highest activity against *Xanthomonas oryzae* and indicated weak activity against *Candida albicans* and *Escherichia coli* as shown in Table 2.

Table 2. Inhibitory zones (mm) of the isolated compounds

T.O / Compounds	*Bacillus subtilis*	*Candida albicans*	*Escherichia coli*	*Salmonella enterica*	*Salmonella typhi*	*Xanthomonas oryzae*
A	-	-	-	-	-	-
B	-	10	12	-	-	25
C	-	12	10	-	-	32

10 -12 mm = weak activity, 13 - 18 mm = high activity, >18 mm = very high activity, disc size 6 mm

Discussion and Conclusion

In this research work, fungal strain *Phomopsis* sp. was isolated from leaves of *Psidium guajava* L. Twelve liters fermentation was undertaken at room temperature by sucrose/yeast medium and tested its antimicrobial activity with six test organisms. Then the fermentation, the filtrate was extracted with methanol on Ambilites XAD 2 resin column for crude extract. It is in agreement with the statement of Yee Yee Thu (2006). Bicas *et al.*, (2009) stated that the bioactive compounds could be isolated from microbial products through the fermentation.

The methanol extract were tested with six test organisms and it was observed that methanol extract gave good result. Kaczorowski *et al*., (2011) reported that methanol crude extracts of fungi inhibited the growth of all four human pathogens; *B. subtilis*, *S. aureus*, *E. coli* and *S. Typhimurium* by the agar diffusion method. Basha *et al*., (2012) stated that antimicrobial activity of methanol crude extract of fungi showed activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis*, *Escherichia coli*, *Salmonella typhimurium* by agar-well diffusion assay.

The compound A was identified as phomopsolide B. This compound was also isolated from endophytic fungus *Phomosis* sp. by Selim *et al.*, (2012). This compound was also isolated from *Aspergillus terreus* by Rizna *et al.*, (2015) and they also reported that it has antioxidant and antibacterial activities.

The compound B was identified as adenosine according to its spectroscopic methods. Yee Yee Thu (2006) also isolated adenosine from the fermented broth of endophytic fungus *Tricoderma* sp. Xin-guo Zhang *et al.*, (2017) reported that new uncompetitive inhibitor of adenosine deaminase identified from endophyte *Aspergillus niger* sp.

The compound C was identified emerimidine C. This compound was also isolated from endophytic fungi of medicinal plants and showed very high activity against *Xanthomonas oryzae* by Selim *et al.,* (2012).

In conclusion, the active compounds from *Phomopsis* sp. which was isolated from *Psidium guajava* L. exhibited higher activity on *Xanthomonas oryzae* than other test organisms. Therefore, it is essential to do further research concerning with leaf blight *Xanthomonas oryzae* on rice plants as biocontrol. After that, these compounds could be applied for the protection of leaf blight disease caused by bacterium *Xanthomonas oryzae* in the paddy fields. This finding would be helpful to our farmers who make rice, our staple food, as well as all of us in our nation.

Acknowledgements

We wish to mention our sincere thanks to all Professors and Chairpersons from Department of Botany in the seminar of the Myanmar Academy of Arts and Sciences for their invaluable advices and suggestion.

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ISOLATION OF BIOACTIVE COMPOUNDS FROM *ASPERGILLUS* SP.PRODUCED FROM RHIZOME OF *ZINGIBERCASSUMUNAR* ROXB.

Soe Soe Yu Hnin[1], Mon Mon Thu[2], Yee Yee Thu[3] and Aye Pe[4]

Abstract

An Endophytic fungal strain*Aspergillus*sp. was isolated from the rhizome of *Zingibercassumunar*Roxb. For the extraction and isolation of the bioactive compounds, 10L fermentation of strain *Aspergillus*sp. and antimicrobial activity of fermented broth with six test organisms were carried out. After fermentation, the filtrate was extracted with methanol on Ambilite XAD 16 resin column at Microbiology Laboratory, Department of Botany, Universityof Yangon. The methanol extract showed high antimicrobial activity against *Bacillus subtilis*, *Candida albicans*,*Escherichia coli*,*Malassezia furfur*, *Salmonella typhi* and *Staphylococcus aureus.* Isolation of the bioactive compounds from the methanol extract was carried out by using silica gel and Sephadex LH20 gel columns with various solvent systems at Department of Organic Chemistry, Ramkhamhaeng University, Bangkok, Thailand. The isolated compounds were characterized by FT-IR spectra, 1D-NMR (^{1}H-NMR, ^{13}C-NMR) and 2D-NMR spectra.The compounds 1, 2 and 3 were identified as 4-amino-1-(1,3-dihydroxy-1- (4-nitrophenyl) propan-2-yl)-1H-1,2,3-triazol-5(4H)-one; 3,6-dibenzyl-3,6-dimethyl piperazine-2,5-dione and aspergillitine. Antimicrobial activity of all isolated compounds was evaluated on six test organisms and showed antibacterial activity on*Bacillus subtilis*,*Escherichia coli* and *Xanthomonasoryzae.*

Keywords: Antimicrobial activity, Aspergillitine,*Aspergillus*sp. And *Zingibercassumunar*Roxb.

Introduction

In the recent years, numerous metabolites possessing uncommon structures and potent bioactivity have been isolated from strains of bacteria and fungi collected from diverse environments, such as soils, animals, plants

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and sediments (Faulkner *et al*., 2006 and Laatsch, 2010). Endophytic fungi are one of the most unexplored and diverse groups of organisms that make symbiotic associations with higher life forms and may produce beneficial substances for host (Weber, 1981 and Shiomi*et al.,* 2006). Fungi have been widely investigated as a source of the bioactive compounds (Zhang *et al*.,2011). Therefore, several research groups and pharmaceutical companies were motivated to start sampling and screening large collections of fungal strains for antibiotics, antimycotics, antivirals, anticancers and pharmacologically active agents (Song *et al*., 2004).

The objectives of this research work are extraction, isolation and structure elucidation of the bioactive compounds from fermented broth of *Aspergillus* sp., and evaluation of their antimicrobial activity.

Materials and Methods

Isolation of Endophytic Fungus from*Zingibercassumunar***Roxb.**

The *Zingibercassumunar*Roxb.plant sample was collected from Nyaung-Hna-Pin area, HmawbiTownship. The isolation of endophytic fungus was carried out with the following scheme: (1) Plant parts were washed in running tap water for 15 min. **(**2) Plant parts were cut into about 1 cm pieces. **(**3) The surfaces of cut-plant pieces were sterilized by soaking it in 75% ethanol for 2 min. **(**4) Sterile surfaces were socked in 5.3% sodium hypocloride for 5 min. **(**5) Cut-plant pieces were washed out sodium hypocloride by socking in 75% ethanol for 0.5min. **(**6) They were dried and cut into smaller pieces, and placed on agar plates and then incubated for 3 days to 3 weeks (Phay, 1997). Thus, the isolated microorganisms were transferred into a 10ml test tube containing 5ml of sucrose/yeast extract medium.

Antimicrobial Activity of 10L Fermentation from *Aspergillus***sp.**

The small piece (1 cm) of fungus from the plate culture of *Aspergillus* sp.was inoculated into 500 ml of conical flask containing 180 ml of sucrose, yeast extract seed medium. The flask was incubated at room temperature for

two days for seed culture. Two days old seed culture (180 ml) was transferred into ten flasks of 2L conical flask containing 1L of sucrose yeast fermentation medium. These flasks were incubated at 100 rpm for 3-7 days at room temperature. Ten liters fermentation flasks indicated antimicrobial activity on *Bacillus subtilis, Candida albicans, Escherichia coli, Malassezia furfur, Salmonella typhi* and *Staphylococcus aureus*(Strobel and Sullivan,1999;Phay, 1997).

Extraction of Bioactive Compounds from Fermented Broth of *Aspergillus*sp.

After testing antimicrobial activity, 10L fermented broth was filtered with filter paper. The mycelia from fermented broth were filteredon the filter paper and then the filtrate was applied on an Amberlites XAD 16 resin column. The column was washed with water, followed by five liters of methanol. The methanol extract was evaporated on water bath at 55 -60°C. The methanol extract was tested for antimicrobial activity against *Bacillus subtilis, Candida albicans,Escherichia coli,Malassezia furfur, Salmonella typhi* and *Staphylococcus aureus*(Strobel and Sullivan, 1999).

Isolation and Purification of Bioactive Compounds from *Aspergillus*sp.

According to TLC result, silica gel column chromatography was carried out. The silica 34 gel (100 g) column was eluted with hexane : ethyl acetate (100%, 9:1, 8:2, 5:2, 2:1, 1:1, 1:2, 1:3, 1:5) and ethyl acetate : methanol (100% EA, 10:1, 10:2, 10:3, 10:5, 1:1, 100% MeOH) and then fourteen fractions were collected. The column size was 5 × 10 cm and flow rate was 2 ml per minute(Grableyet *al.*,1999).

Identification of Isolated Compounds from *Aspergillus*sp.

The identification of the isolated compounds 1, 2 and 3 were characterized by 1D-NMR (^{1}H-NMR and ^{13}C-NMR), 2D-NMR (^{1}H-^{1}H COSY) 400 MHz at Nuclear Magnetic Resonance and FT-IR spectra at the Department of Chemistry, Ramkhamhaeng University, Bangkok,

Thailand.The spectral data of the isolated compounds were compared by ACD (Advanced Chemistry Development)Labs(Robert and Francis, 2014).

Antimicrobial Activity of Isolated Compounds from *Aspergillus*sp.

All the isolated compounds were tested their antimicrobial activity with six test organisms. The volume of each compound was 10µg/disc (conc.1mg/ml).

Paper disc diffusion assay

Broth culture (50 µl) of test organisms (*Bacillus subtilis*, *Candida albicans*,*Escherichia coli*,*Malassezia furfur*, *Salmonella enteric*, *Salmonella typhi*, *Staphylococcus aureus*and *Xanthomonasoryzae*) was added to 100 ml assay medium sucrose/yeast medium and then poured into the plates. After solidification paper discs infused with broth samples were applied on the test plates and incubated at 30°C for 24-48 hrs. When clear zones (inhibitory zones) showed around the paper discs, they were measured (Phay, 1997).

Results

Outstanding Characters of Plant Sample

Scientific name	-*Zingibercassumunar*Roxb.
English Name	- Bengal ginger
Myanmar name	- Meik-Tha-Lin
Family	- Zingiberaceae

Figure 1. Habit of *Zingibercassumunar*Roxb.

Outstanding characters

Herbs with aromatic rhizome, rhizomes bright yellow; Leaves opposite and distichous, simple; Inflorescence borne separately from the leaves, peduncle, ovate spike, bracteolate; Flowers pale yellow, complete, bisexual, irregular, zygomorphic, epigynous; Sepals (3), synpetalous; Petals (3),

synpetalous; Stamens 1+(2)st + 2st, epipetalous; Filaments exserted, anthers dithecous, dorsifixed, longitudinal dehiscence; Pistil 1, tricarpellary, syncarpous, axile placentation, style long and slender, stigma capitatesinferior; Fruits and seeds not seen.

Morphological and MicroscopicalCharacters of Isolated Fungus*Aspergillus*sp.

The surface and reverse colour of *Aspergillus* sp.was dark green and yellow. Conidiophores are upright, simple, terminating in a globose swelling bearing phialides at the apex, conidia 1 celled, globose, often variously colored in mass.Therefore, this strain was identified as *Aspergillus*sp.

Antimicrobial Activity of 10L Fermented Broth of*Aspergillus*sp.

In this study, allfermentation flasks showed antimicrobial activities against *Bacillus subtilis,Candida albicans, Escherichia coli, Malassezia furfur, Salmonella typhi* and *Staphylococcus*.

Antimicrobial Activity of Methanol Extract

Methanol extract showed highest activity against *Bacillus subtilis,Candida albicans, Escherichia coli, Malassezia furfur,Salmonella typhi*and *Staphylococcus aureus*in Table 1.

Table 1. Inhibitory zones (mm) of methanol extract of *Aspergillus*sp.

Extracts	*Bacillus subtilis*	*Candida albicans*	*Escherichia coli*	*Malassezia furfur*	*Salmonella typhi*	*Staphylococcus aureus*
Methanol	42	40	45	50	36	35

10 -12 mm = weak activity, 13 - 17 mm = high activity, >18 mm = very high activity, disc size: 6 mm

Isolation and Purification of Bioactive Compounds from *Aspergillus*sp.

The two hundred and eight small fractions were collected from silica gel 34 column. According to theirR_f value and colouron TLC plates under UV 254 nm, they were combined into large fractions such as F1 (1-5), F2 (6-12), F3 (13-17), F4 (18-30), F5 (31-80), F6 (81-95), F7 (96-125), F8 (126-135), F9 (136-163), F10 (164-180), F11 (181-198), F12 (199-202), F13 (203-208).All of these fractions, the fraction 9 (the compound 1) was crystal after washing with dichloromethane as shown in Figure 2.

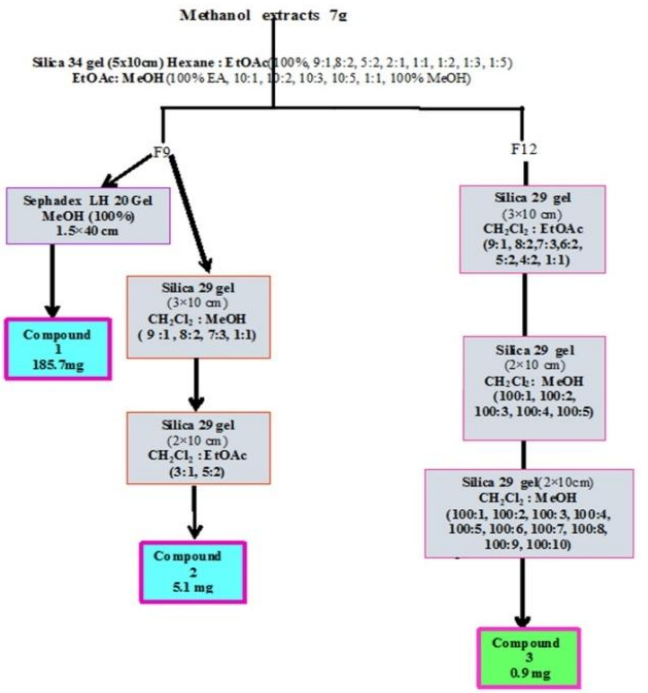
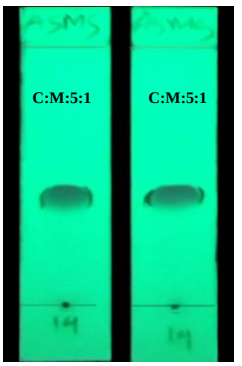


Figure 2. Isolation procedure of the bioactive compounds

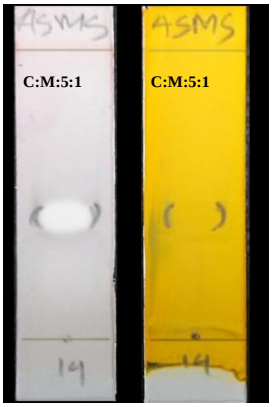
Identification of Isolated Compounds from *Aspergillus*sp.

Identification of the isolated compound 1

SephadexLH20 gel column 1.5×40 cm



UV 254nm



Staining colour (Anisaldehyde and Dragendroff)

Needle shaped crystal

Eluting solvent	= MeOH (100%)
Fraction size	= 1 ml
Flow rate	= 2ml / min
R_f	= 0.4 (CH_2Cl_2:MeOH; 5:1)

Figure 3. Identification of the isolated compound 1 by R_f value

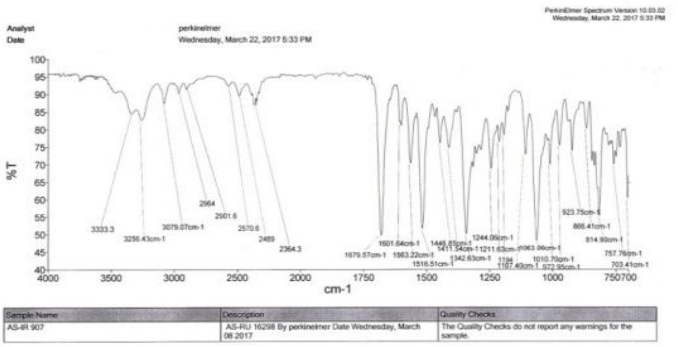


Figure 4. FT-IR spectrum of the isolated compound 1

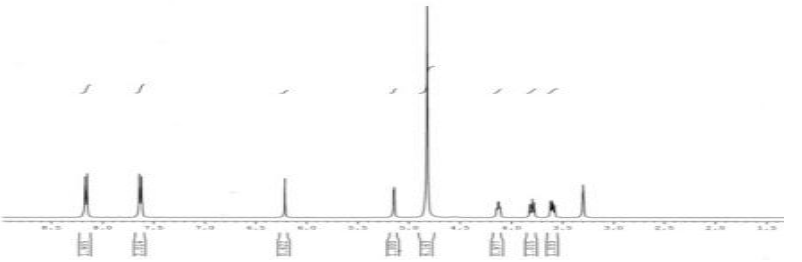


Figure 5. ^{1}H-NMR spectrum (400 MHz, CD_3OD) of the isolated compound 1

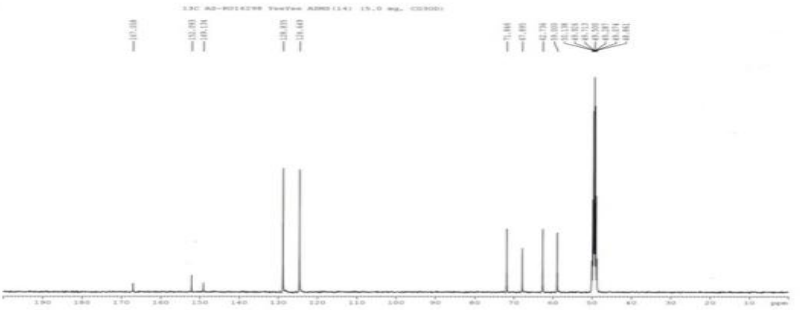


Figure 6. ^{13}C-NMR spectrum (400 MHz, CD_3OD) of the isolated compound 1

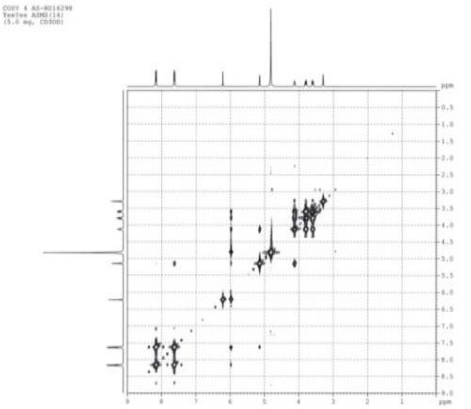


Figure 7. ^{1}H -^{1}H (COSY) spectrum (400 MHz, CD_3OD) of thecompound 1

The compound 1 was found in the fraction 9 as an UV absorbing band at 254 nm and has R_f0.4 (CH_2Cl_2 :MeOH,5:1).It showed white colour spot with anisaldehyde reagent but no active with dragendroff reagent(Figure 3). This substance is needle shaped crystal and good soluble in methanol. In FT-

IR spectrum, N-H and O-H in phenolic group were found at 3333, 3256, 3079 cm^{-1}. C-H stretching vibration of methyl and methylene groups was found at 2964, 2901 cm^{-1}. C=O stretching vibration of ketone was found at 1679 cm^{-1}. C=C aromatic group was observed at 1601, 1516, 1446 cm^{-1}. N-H bending was found at 1563 cm-1. C-H bending of methyl and methylene groups was found at 1411,1342, 1244 cm^{-1}. The bands at 1107 cm^{-1} were attributed C-C-stretching vibration. In addition, C-H out of plane bending vibration was found at 972 cm^{-1}as shown in Figure 4.

According to its ^{1}H-NMR spectrum, aromatic protons (Ar-H) between 8.2-6.22 ppm, as doublet at 8.2 and 7.63 ppm and as singlet at 6.22 ppm. Olefinic protons (C=CH) between 5.15-4.13 ppm, as singlet at 5.15-4.83 ppm,as multiplet at 4.13 ppmand methylene protons(CH_2) between 3.8-3.3 ppm, as quantet at 3.8-3.6 ppm and as singlet at 3.3 ppm are present in this compound as shown in Figure 5.

As a result of ^{13}C-NMR spectral data, C=Oketone carbon was found at 167 ppm, 152 and 149 ppm contained aromatic carbons, olefinic carbons was found at 128, 124 and 71.8 ppm, 67.8 ppm was C-OH carbon, 62.7 ppm contained methylene carbon and methine carbon was observed at 59.0 ppm are present in this compound as shown in Figure 6.

According to 1D-NMR (^{1}H-NMR and ^{13}C-NMR), 2D-NMR (HMBC, HSQC, ^{1}H-^{1}H COSY) and FT-IR spectral data, the compound 1 was identified as 4-amino-1-(1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl)-1H-1,2,3-triazol-5(4H)-one. Its molecular formula is $C_{11}H_{13}O_4N_5$ as shown in Figure 8.

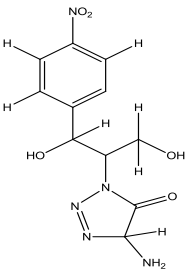
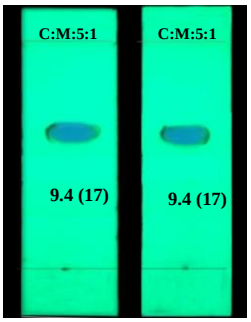


Figure 8. 4-amino-1-(1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl)-1H-1,2,3-triazol-5(4H)-one

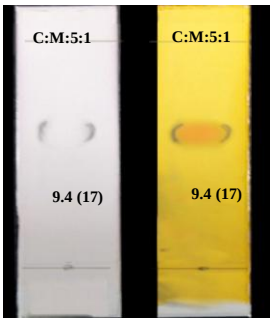
Identification of the isolated compound 2

Silica 29 gelcolumn (3 x10cm)

Silica 29 gelcolumn (2 x10 cm)



UV 254 nm



Staining colour(Anisaldehyde and Dragendroff)

Eluting solvent = CH_2Cl_2 :MeOH (9 :1 , 8 : 2, 7:3, 1:1)
= CH_2Cl_2 :EtOAc (3:1, 5:2)
Fraction size= 1.5 ml(3 x 10cm)
= 1 ml(2 x 10cm)
Flow rate = 2ml / min
R_f = 0.54 (CH_2Cl_2 :MeOH; 5:1)

Figure 9. Identification of the isolated compound 2 by R_f value

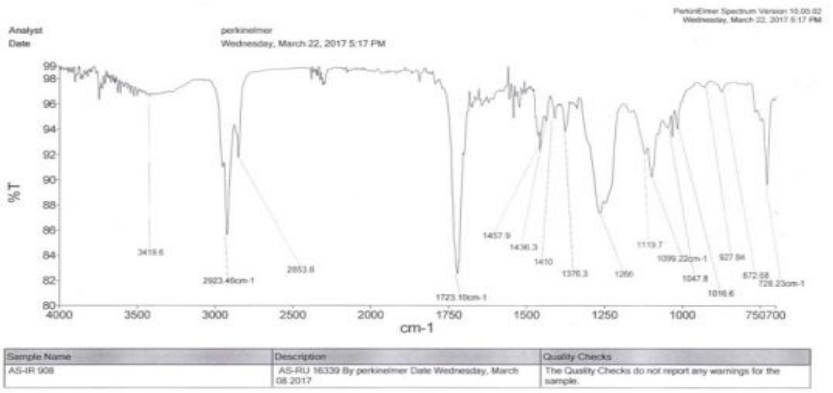


Figure 10. FT-IR spectrum of the isolated compound 2

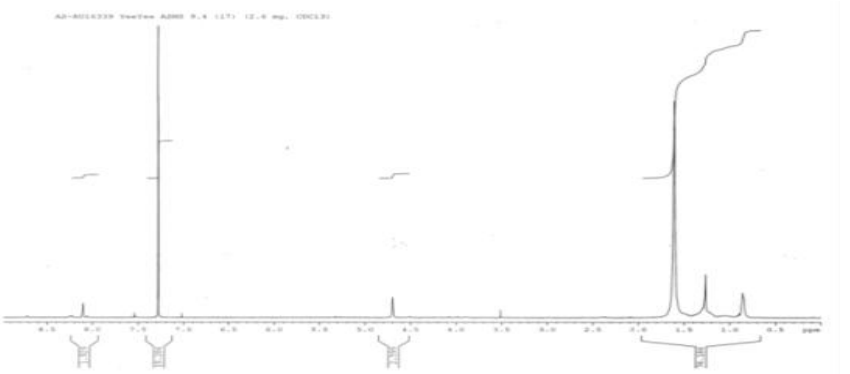


Figure 11. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of the isolated compound 2

During the isolation of *Aspergillus* sp., an additional compound, the compound 2, was also isolated from the fraction 9 and has R_f0.54 (CH_2Cl_2 :MeOH,5:1). It gave an orange colour with dragendroffreagent (Figure 9). This substance is soluble in chloroform or dichloromethane.

According to the FT-IR spectral data, N-H stretching vibration group showed at 3419 cm^{-1}. C- H stretching of methyl and methylene groups was found at 2923 and 2853 cm^{-1}. C=O stretching vibration (Ketone) was observed at 1723 cm^{-1}. The bands at 1457, 1436 and 1410 cm^{-1} were attributed C-H bending of methyl and methylene groups. C-O-C- stretching

vibration of ether was found at 1376, 1266, 1099 and 1047 cm^{-1}. In addition, the bands for C-H out of plane bending vibration were observed at 927,872 and 728 cm^{-1}as shown in Figure 10.

According to its ^{1}H-NMR spectrum, aromatic protons (Ar-H) as singlet at 8.1 ppm, olefinic protons (C=CH) as singlet at 4.7 ppm and alkyl proton (CH_3) as singlet at 1.6-0.85 ppm are present in this compound as shown in Figure 11.

As a result of its ^{1}H-NMR spectrum and FT-IR spectral data, the compound 2 was identified as 3,6-dibenzyl-3,6-dimethylpiperazine-2,5-dione. Its molecular formula is $C_{20}H_{22}N_2O_2$ as shown in Figure 12.

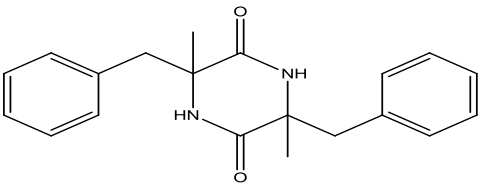
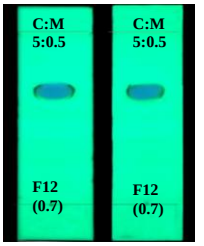


Figure 12. 3,6-dibenzyl-3,6-dimethylpiperazine-2,5-dione

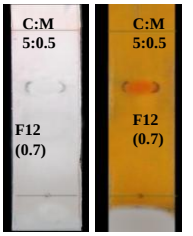
Identification of the isolated compound 3

Silica 29 gel Column(3x10 cm)

Silica 29 gelColumn (2 x10 cm)



UV 254 nm



Staining colour(Anisaldehyde and Dragendroff)

Eluting solvent	= CH_2Cl_2 :EtOAc (9:1, 8:2, 7:3, 6:2, 5:2, 4:2, 1:1) = CH_2Cl_2 :MeOH(100:1, 100:2, 100:3, 100:4, 100:5)
Fraction size	= 1.5 ml(3 x 10cm) = 1 ml(2 x 10cm)
Flow Rate	= 2ml / min
R_f	= 0.68 (CH_2Cl_2 :MeOH; 5:0.5)

Figure 13. Identification of the isolated compound 3 by R_f value

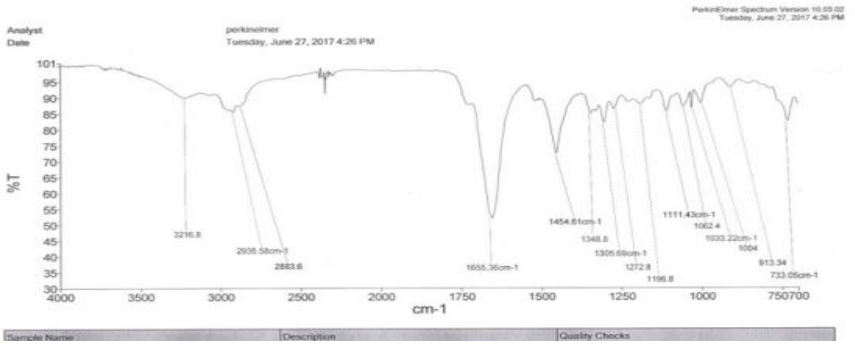


Figure 14. FT-IR spectrum of the isolated compound 3

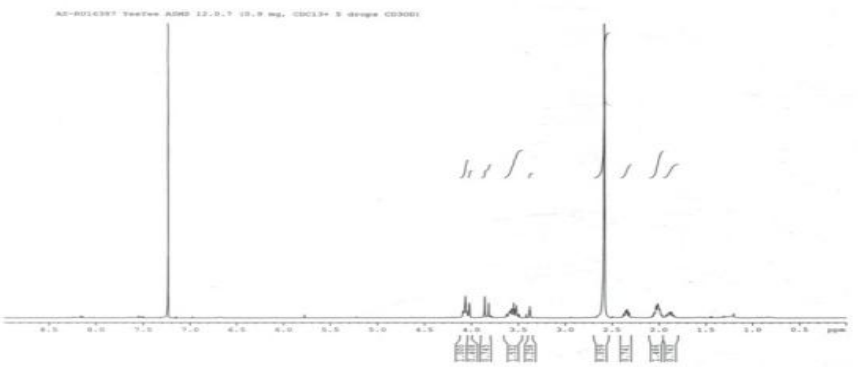


Figure 15. ^{1}H-NMR spectrum (400 MHz, CD_3OD) of the isolated compound 3

During the isolation of this strain, the compound 3 was isolated from the fraction 12 and has R_f0.68 (CH_2Cl_2 :MeOH,5:0.5). It showed orange colour with dragendroffreagent (Figure 13).In FT IR spectrum, N-H stretching vibration showed at 3216 cm^{-1}. C-H stretching of methyl andmethylene groups was found at 2935 and 2883 cm^{-1}. Its FT-IR spectrum showed C=O stretching vibration group at 1700 cm^{-1}. The bands at 1655 cm^{-1}were attributed C=N stretching vibration group. C=C aromatic stretching vibration was observed at 1454 cm^{-1}. Aromatic amines stretchingvibration showed at 1348 and 1305 cm^{-1}. C-O-C-stretching vibrationswere found at 1272 and 1196 cm^{-1}. In addition C-N bending vibration showed at 1111, 1062 and 1033 cm^{-1} as shown in Figure 14.

According to its ^{1}H-NMR spectrum, methine protons between 3.55-3.84 ppm, as multiplet at 3.55 ppm, as doublet at 3.84 ppm; allylic protons (C=C-CH2)as multiplet at 2.35 ppm and alkyl protons (CH3) 2.0-1.8 ppmas multiplet at 2.05 - 1.88 ppm are present in this compound as shown in Figure 15.

As a result of its ^{1}H-NMR spectrum and FT-IR spectral data, the compound 3 was identified as aspergillitine. Its molecular formula is $C_{15}H_{13}NO_2$as shown in Figure 16.

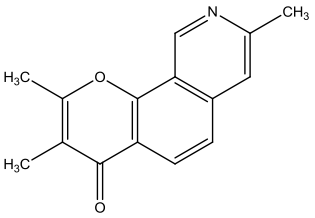


Figure 16. Aspergillitine

Antimicrobial Activity of the Isolated Compounds from*Aspergillus*sp.

Antimicrobial activity of the isolated compounds,the compounds 1;4-amino-1-(1,3-dihy-droxy-1-(4-nitrophenyl)propan-2-yl)-1H-1,2,3-triazol-5(4H)-oneshowed highest activity against *Bacillus subtilis*, *Escherichia coli* and*Xanthomonasoryzae*.The compounds 2;3,6-dibenzyl-3,6-dimethylpiperazine-2,5-dione; showed highest and high activities against *Escherichia coli* and s*Xanthomonasoryzae*. The compound 3 did not show antibacterial activity as shown in Table 2.

Table 2. Antibacterial activity of the isolated compounds

Test organisms	Compound		
	C-1	C-2	C-3
Bacillus subtilis	30	-	-
Escherichia coli	27	21	-
Xanthomonasoryzae	37	16	-

10 -12 mm = weak activity, 13 -17 mm = high activity, >18 mm = very highactivity, disc size = 6 mm

Discussion and Conclusion

Endophytic fungal strain *Aspergillus* sp. was isolated from the rhizome of *Zingibercassumunar*Roxb. in this research work. For the extraction of the bioactive compounds, 10L fermentation of*Aspergillus*sp. and antimicrobial activity of fermented broth with six test organisms were carried out. After testing the antimicrobial test, the active filtrate of fermented broth was applied on XAD 16 resin column followed bymethanol. Shaaban*et al*. (2013) also used XAD-16 resin toextract the filtrate with methanol.

The methanol extract were tested antimicrobial activity by six test organisms in this study.According to the result, methanol extract showed good result on six test organisms. In isolation and purification of the bioactive compounds from *Aspergillus*sp., the methanol extract was utilized on silica gel columns, 34 gel, 29 gel, SephadexLH 20 gel columnswith various solvent systems.

The compound 1 was identified as 4-amino-1-(1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl)-1H-1,2,3-triazol-5(4H)-one and showed highest activity against *Batcillussubtilis*, *Escherichia coli* and *Xanthomonasoryzae*. This compound was also isolated from *Aspergillusterreus*by Riznа*etal*. (2015) and they also reported that it has antioxidant and antibacterial activities. The compound 2 was identified as 3,6-dibenzyl-3,6-dimethylpiperazine-2,5-dione compound. This compound 2 showed antibaterial activity on*Escherichia coli* and *Xanthomonasoryzae*. It was also isolated from *Aspergillusterreus* by Wen Gu and Chao (2012). They also reported that this compound has antibacterial activity on*Escherichia coli*.The compound 3 was identified as aspergillitine. This compoundwas also isolated from the *Aspergillusversicolor*by Vijaya (2017).

Manila*et al*. (2014) reported that alkaloids, phenolic and terpenoid compounds were the main phytochemicals presented in endophytes including *Aspergillus*sp. Furthermore they also stated that strains of various

*Aspergillus*sp. could exhibit the highest antioxidant activity ranging from 50% to 80%.In this study the isolated compound 1; 4-amino-1-(1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl)-1H-1,2,3-triazol-5(4H)-one; and the compound2; 3,6-dibenzyl-3,6-dimethylpiperazine-2,5-dione showed antibacterial activity on *Bacillus subtilis*and*Xanthomonasoryzae.* This finding is in agreement with the statement of Hameed*et al*. (2015) who stated that *Aspergillusniger* produced many important secondary metabolites with high biological activities.

Moreover Hameed*et al*. (2015) also reported that drugs for the treatment of many diseases could produce based on the significance of employing bioactive compounds in pharmacy, the purification of compounds produced by *Aspergillusniger*can be useful. In this study the isolated compounds;4-amino-1-(1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl)-1H-1,2, 3-triazol-5(4H)-one;showed antibacterial activity on *Bacillus subtilis*.This finding is in agreement with the statement of Olivia *et al.* (2015).

It could be concluded that the bioactive compounds were isolated from fermented broth of *Aspergillus* sp. produced from the rhizome of *Zingibercassumunar*Roxb. in this research. These active compounds indicated high activity on *Bacillus subtilis*, *Escherichia coli* and *Xanthomonasoryzae*. Therefore, these compounds could be applied to treat diseases caused by *Bacillus subtilis,Escherichia coli* and *Xanthomonasoryzae*.These findings could help the health of mankind and also help to suppress the *Xanthomonas* caused leaf blight disease in the paddy fields.

Acknowledgements

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THE MORPHOLOGY, SPORES FORMATION AND GERMINATION, LOCAL DISTRIBUTION OF *CLADOPHORA FLEXUOSA* (O.F. MULLER) KUTZING AND *CLADOPHORA PROLIFERA* (ROTH) KUTZING FROM MYANMAR

Moe Moe Khaing[1]

Abstract

Two species of genus *Cladophora* belonging to the Cladophorales, Chlorophyta collected from the three Coastal Regions of Myanmar in 2009 to 2013 were identified as *C. flexuosa* (O.F. Muller) Kutzing and *C. prolifera* (Roth) Kutzing. The descriptions of each taxon with emphasis on the shape, size, colour and branching type of cell and rhizoid. Culture study on spores formation, germination on two species of the genus *Cladophora*. In addition, the distribution ranges of each species along the three coastal regions of Myanmar were presented.

Keywords: *Cladophora*, morphology, laboratory culture, local distribution.

Introduction

Marine algae are the large primary producers of the sea: during the process of photosynthesis inorganic carbon (CO_2) is transformed into organic compounds using radiation energy from the sun and with the simultaneous release of oxygen. Algae were probably the first photosynthetic organisms and untitle appearance of plants on earth, the only photosynthesizers for billions of year. This process is the basis for all plant and animal life. Marine algal beds provide feeding, spawning and nursery grounds for marine living organisms (Chin *et.al*., 2015).

There are 1061 species names in the genus *Cladophora*, of which 188 have been flagged as currently accepted taxonomically (Guiry, 2013). In 2010, Soe-Htun reported the current status of marine algae with a total of 111

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genera and 229 species growing along the three Coastal Regions of Myanmar. Kyaw Soe and Kyi Win (1977) reported the occurrence of twelve species of *Cladophora* along the Coastal Regions of Myanmar. Moreover, Soe-Htun *et.al.* (2009) accounted the five species of *Cladophora*, viz., *C. prolifera* (Roth) Kutzing, *C. rupestris* (Linnaeus) Kutzing, *C. vagabunda* (Linnaeus) Hoek, *C. saracenica* Boergesen and *C. sibogae* Reinbold along the three Coastal Regions of Myanmar.

The purposes of this study are: 1) to identify the two species of *Cladophora* Kutzing, 2) to study the reproductive structures, 3) to record the distributional ranges of two species of *Cladophora* Kutzing both along the three Coastal Regions of Myanmar.

Materials and Methods

The samples were collected from intertidal zone, along the three Coastal Regions of Myanmar from 2009 to 2013. Fresh and living plants were collected from several localities and saved in ice box and brought to the laboratory of Mawlamyine University for observation. Fresh and living plants of specimens were fixed and preserved in 5 % formaldehyde solution which was prepared with seawater. Some of these were prepared for herbarium specimens. All voucher materials in the forms of wet-stack or herbarium specimens were deposited at the Herbarium of Department of Marine Science, Mawlamyine University, Myanmar (MMB). Liquid-preserved specimens were used for detail investigations on external morphology and habit of the plants. Cultures were kept in freezer-incubators illuminated with cool white fluorescent lamps (100-200 fc). Culture Petridishes were incubated under 16 light : 8 dark photoperiod in incubators and in room temperature. Early development of sporelings was examined and diameter (in length) of sporangia was measured under electron microscope with the help of ocular meter of 5 days intervals. Culture medium was changed after each examination.

Results

The two species of *Cladophora* Kutzing were collected from the three Coastal Regions of Myanmar.

A classification system of the genera *Cladophora*

Phylum : Chlorophyta

Class : Chlorophyceae

Order : Cladophorales

Family : Cladophoraceae

Genus : *Cladophora* Kutzing

Species : (i) *Cladophora flexuosa* (O.F. Muller) Kutzing

(ii) *Cladophora prolifera* (Roth) Kutzing

Cladophora flexuosa (O.F. Muller) Kutzing

(Figures. 1 & 2)

Description: Plants are light-green, 0.5-5 cm tall, erect or flexuose, somewhat rigid but delicate primary, rhizoids descending from frond-bases, sometimes septate, 30 μm in diameter, composed of long segments, slightly constricted between each segment; main filaments straight or flexuose, dichotomous or sometimes trichotomous, forming acute axils, 100 - 120 μm in diameter, composed of short segments, 3 - 4 times long provided with thin lateral membrane, 10 - 20 μm thick; branches straight or curved, dichotomous or sometimes opposite, arising from acute axils, 80 - 100 μm in diameter, composed of short segments, 2 - 3 times as long as diameter; branchlets alternate or unilateral, straight or curved, forming acute axils, 60 - 140 μm in diameter, composed of segments 2 - 4 times as long as diameter, with very long top portions, about 5 - 12 segment, ending in obtuse apices; Ultimate branchlets secund or pectinate, arising from every segment or from 2-3 segments, forming somewhat acute axils, straight, long, 20 - 50 μm in diameter, 1-2 segmented, 2 - 4 times longer.

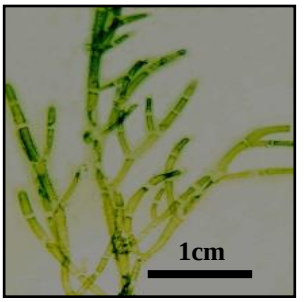

Figure1. Habit of *C. flexuosa* (O.F.Muller) Kutzing

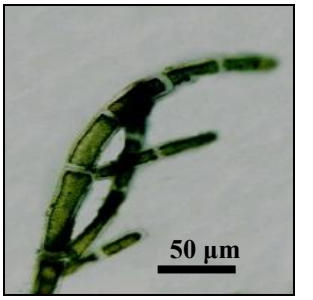

Figure 2. Filament of *C. flexuosa* (O.F.Muller) Kutzing

Spores formation and early germination of the *Cladophora flexuosa* (O. F. Muller) Kutzing (Figs. 3. A-I)

The cell protoplasm of mature thallus changed from yellowish-green to green and later brownish to white due to the abundant spores development (Fig. 3.A). After ten days, the sporangia arise by simultaneous division of the cell. Normally sporangia occurred at the terminal and subterminal of the branch (Fig. 3.B). Each sporangium measured 30-40 µm long and 20-25 µm wide and characterized in brownish colour and spherical and oval shape. After 15 days, cluster of spores were pumped out from the sporangium through a pore. Then individual spores swim from the cluster (Fig. 3.C). Liberated spores were pear shaped and measured 2-3 µm in length. They also swim rapidly with the help of four flagella measuring about 3-4 µm long. The spores settle and round shaped after about 1 minute of free swimming. Some spores and sporangia were embedded in the cell or on the cell wall (Fig. 3.D,E). Germination of released spores was not found until 30 days of watching. On the other hand, embedded spores and spores on the thallus margin gave germination. These embedded spores germinated a new plants. After 20 days, the first germling was measured about 2 µm long (Fig. 3.F). After 25 days, the germling was continuously elongated about 3 µm long. After 30 days, the germling increased to 5 µm long (Fig. 3.G). Some germling were detached from the dying mother plant and grow independently (Fig. 3.H). After 35 days

by cytogenesis two portions were formed, one basal achlorophyllic and the other apical chlorophyllic with a rhizoid (Fig. 3.I). Its cells developed into a mature plant, that is, the chloroplasts became parietal and reticulate and the cell wall became stratified. After 40-50 days, the germling produced the first ramifications.

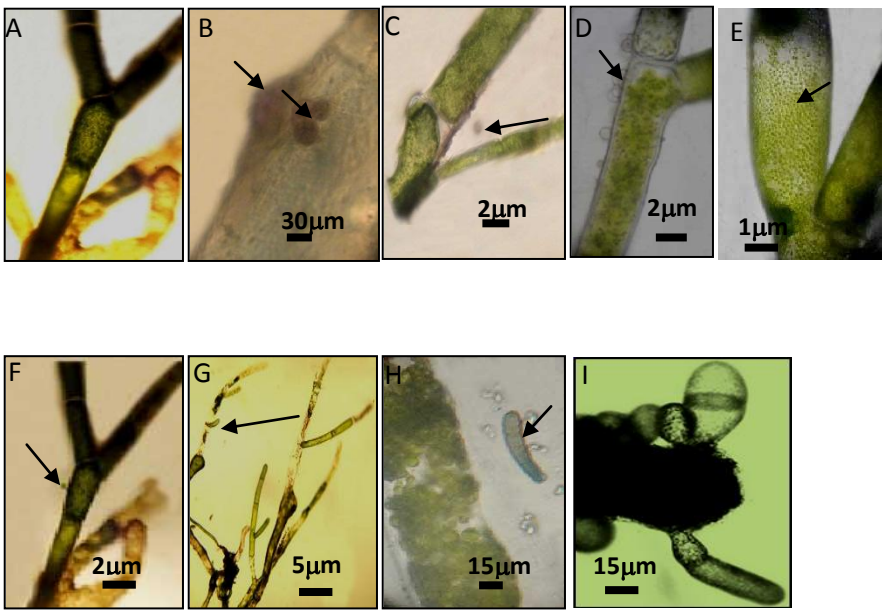

Figure 3. A-I.Sporesformation and early germination of *Cladophora flexuosa* (O. F. Muller) Kutzing

A. Habit of mature plant; **B.** Formation of sporangia; **C.** Released of spores; **D.** Attached spores on the cell wall; **E.** Embedded spores in the cell wall; **F.** Produced the first germling; **G.** Attached the germling with mother plant; **H.** Detached the germling from mother plant; **I.** Formation of two portions germling.

Local Distribution of *Cladophora flexuosa* (O.F. Muller) Kutzing

(Figure. 4. & Table 1)

Local distribution: Tanintharyi Coastal Region- Hmyawyit, Kampani.

Ayeyarwady and Gulf of Martaban Coastal Region- Kayin Thaung, Setse, Yathae Thaung.

Rakhine Coastal Region- Wetthey Gyaing, Magyi Island.

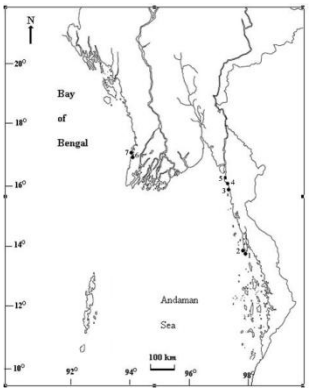

Figure 4. Map showing the collection side of *Cladophora flexuosa* (O. F. Muller) Kutzing along the Coastal Regions of Myanmar. 1. Kampani, 2. Hmyawyit, 3.Yathae Thaung, 4. Setse, 5. Kayin Thaung, 6. Magyi Island and 7. Wetthey Gyaing

Table 1. The distributional range of *Cladophora flexuosa* (O.F. Muller) Kutzing along the Coastal Regions of Myanmar.

Species	TCR		ACR		RCR	
	From	To	From	To	From	To
C. flexuosa (O.F. Muller) Kutzing	Kampani Lat. 14° 02' N Long 98° 04'E	Hmyawyit Lat. 14° 04'N Long. 98° 04'E	Yathae Thaung Lat. 15° 52' N Long. 97° 35' E	Kayin Thaung Lat. 16° 32' N Long. 97° 36' E	Magyi Island Lat. 17° 04' N Long. 94° 27' E	Wetthey Gyaing Lat. 17° 10' N Long. 94° 26' E

Abbreviations:
TCR = The Tanintharyi Coastal Region
ACR = The Ayeyarwady and The Gulf of Martaban Coastal Region
RCR = The Rakhine Coastal Region

***Cladophora prolifera* (Roth) Kutzing**

(Figures. 5 & 6)

Description: Thallus is dark green (blackish when dried) in colour coarse, 1-3 cm high, growing as stiff tufts, composed of densely branched fastigiate filaments. Old cells in the basal and middle parts of the thallus, each giving off one rhizoid with annular constrictions at their basal poles; these rhizoids grow downwards along the cell or cells below, where they entangle and form a conspicuous stipe that attaches to the substratum. Apical cell division is growing into subsequent cell enlargement. Branching originally acropetally organized, becoming irregular in older parts of the thallus because of intercalary growth. Each subapical cell forms a lateral, often immediately after being cut off from the apical cell; lower down a cell may form as a 2nd or sometimes a 3rd lateral. Apical cells cylindrical with rounded tips, 70-120 µm in diameter 2.5 - 4 times as long as breadth ; cells of the terminal branch systems cylindrical, 130 - 170 µm in diameter, 2- 6 times as long as breadth, increasing towards the base of thallus. Cells of the main axes and basal cells elongated and club-shaped, up to 170 µm in diameter, 5 - 7 times as long as breadth, basal parts often with annular constrictions. Rhizoids are 30 - 70 µm in diameter.

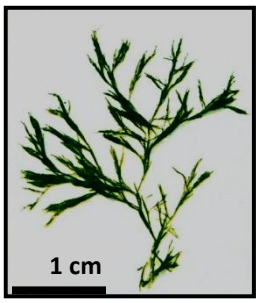

Figure 5. Habit of *C. prolifera* (Roth) Kutzing

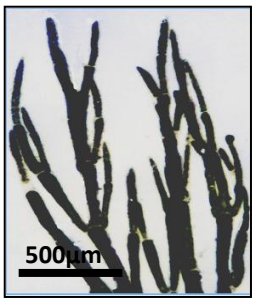

Figure 6. Filament of *C. prolifera* (Roth) Kutzing

Spores formation and early germination of the *Cladophora prolifera* (Roth) Kutzing (Fig. 7. A-I)

The cell protoplasm of mature thallus changed from green to dark-green and later became black due to the spores development (Fig. 7.A). After 5 days, the sporangia arise by simultaneous division of the cell. Normally sporangia occurred at the terminal and subterminal of the branch (Fig. 7.B). Each sporangium measured 50-75 μm long and 20-25 μm wide and characterized in brownish colour and spherical and oval shape. The spores were submerged in sporangia for about 5 days and then released through the pore of the lateral cell wall (Fig. 7.C). Liberated spores from sporangium were pear shaped and measured 1.5-2 μm in length. They also swim rapidly with help of four flagella measuring about 1-1.5 μm long. The spores settle and round shaped after about 2 minutes of free swimming. Some spores and sporangia were embedded in the cell or on the cell wall (Fig. 7.D,E). Germination of released spores was not found until 30 days of watching. On the other hand, embedded spores and spores on the thallus margin gave germination. After 20 days, the first germling was about 2 μm long (Fig. 7.F).

After 25 days, the germling was elongated about 4 μm long. After 30 days and 35 days, the germling was reached to 7 μm and 10 μm long (Fig. 7.G). After 40 days and 45 days the germling was increased up to 20 μm and 30 μm long (Fig. 7.H). After 60 days, the germlings were formed ramification (Fig. 7.I) and after 90 days, it was developed into a mature plant.

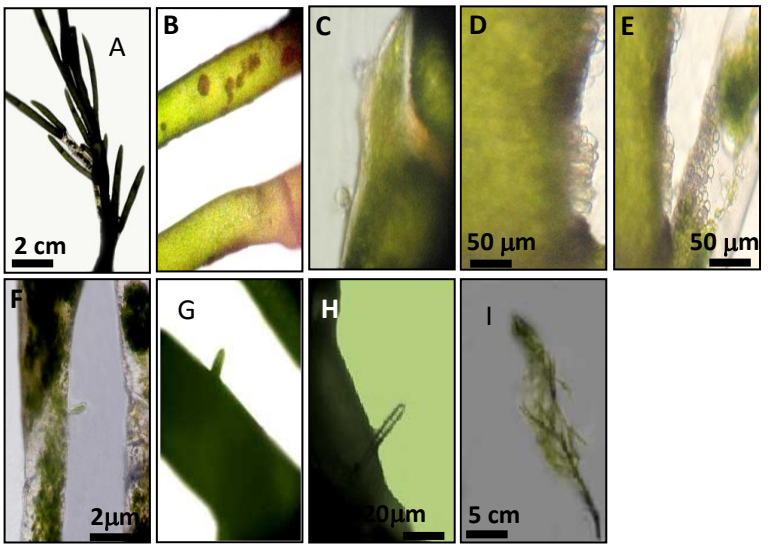

Figure 7. A-I. Spores formation and early germination of *Cladophora prolifera.* (Roth) Kutzing

A .Habit of mature plant**; B.** Formation of sporangia; **C.** Released of spores; **D.** Attached spores on the cell wall; **E.** Embedded spores in the cell wall; **F.** Produced the first germling; **G.** and **H.** Attached the germling from mother plant, **I.** Growing of the new plant.

Local Distribution of *Cladophora prolifera* (Roth) Kutzing

(Fig. 8. & Table 2)

Local distribution: Tanintharyi Coastal Region- Kampani, Hmyawyit.

Ayeyarwady Dalta and Gulf of Martaban Coastal Region- No data.

Rakhine Coastal Region- Maw Shwe Gyaing, Shwe Ya Gyaing, Wetthey Gyaing, Zee Gyaing and Mawtin Point.

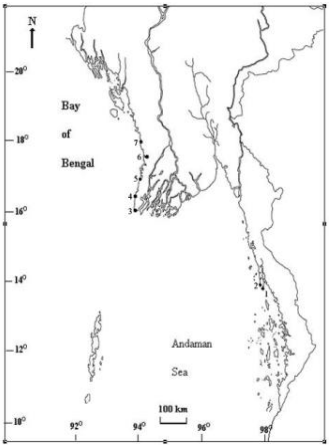

Figure 8. Map showing the collection side of *Cladophora prolifera* (Roth) Kutzing, along the Coastal Regions of Myanmar. 1. Kampani, 2. Hmyawyit, 3. Mawtin Point, 4. Zee Gyaing, 5. Wetthey Gyaing, 6. Shwe Ya Gyaing and 7. Maw Shwe Gyaing.

Table 2. The distributional range of *Cladophora prolifera* (Roth) Kutzing along the Coastal Regions of Myanmar.

Species	TCR		ACR		RCR	
	From	To	From	To	From	To
C. prolifera (Roth) Kutzing	Kampani Lat. 14º 02' N Long. 98º 04' E	Hmyawyit Lat. 14º 04' N Long. 98º 04' E	No data	No data	Mawtin Point Lat. 16º 04' N, Long. 94º 20' E	Maw Shwe Gyaing Lat. 17º 48' N, Long. 94º 29'E

Abbreviations:

TCR = The Tanintharyi Coastal Region
ACR = The Ayeyarwady and The Gulf of Martaban Coastal Region
RCR = The Rakhine Coastal Region

Discussions and Conclusion

The genus *Cladophora* was erected by Kutzing (1845, 1853), who described 85 marine species and varieties from along the German and Austrian sea-coasts. Being either unaware of, or disregarding morphological ranges related to age or environment, Kutzing began the taxonomic chaos by describing numerous species and varieties for every new variant he encountered. Unfortunately, most phycologists after Kutzing continued to apply his lax criteria for describing new *Cladophora* species with predictable results.

Van den Hoek (1963) suggested that combined morphological, like history and cytogenesis studies are necessary to resolve the innumerable taxonomical problems in species of *Cladophora*. Most taxonomists in past years have followed Kutzing (1843) and based their taxonomy principals on structural and morphological features (Collins, 1909; Van den Hoek, 1963; Soderstrom, 1963; Van den Hoek and Womersley, 1984). Cytogenesis information and life histories have been largely neglected since and recognized by Valet (1960), such aspects of these species often difficult to investigate. Only integrated studies can provide reliable results, since the morphology of these species are extremely influenced by the environment (Van den Hoek, 1963; Parodi and Caceres, 1991 and 1995).

The species identification of family Cladophoraceae was carried out base on the shape and size of filament, colour in filaments, composed of cell and branching type, shape and size of cell and shape and size of rhizoid. In the present study, two species of *C. flexuosa* (O.F. Muller) Kutzing and *C. prolifera* (Roth) Kutzing have been identified. The largest size of *C. prolifera* (Roth) Kutzing measured about 1.0 - 3.0 cm in height and the largest size of *C. flexuosa* (O.F. Muller) Kutzing was 0.5 - 5.0 cm in height. Moreover, habit of *C. prolifera* (Roth) Kutzing was stiff and tufts, densely branch. The characters of *C. flexuosa* (O.F. Muller) Kutzing was erect or flexuose and di (or) trichotomous branch possessed. The colour of *C. flexuosa* (O.F. Muller) Kutzing was found light-green and olive-green colour. The wide

cells of *C. prolifera* (Roth) Kutzing had (150-200 μm in wide) and the wide cells of *C. flexuosa* (O.F. Muller) Kutzing had *(*60-100 μm in wide).

In the present study, spores of *Cladophora prolifera* (Roth) Kutzing had ovoid-shaped, equal and quadriflagellate. Spores of *Cladophora flexuosa* (O.F. Muller) Kutzing had pear-shaped, equal and quadriflagellate. The ranges of sporangia were 50-75 μm long, 20-25 μm wide; that of *C. prolifera* (Roth) Kutzing. Sporangia of *C. flexuosa* (O.F. Muller) Kutzing had 30-40 μm long, 20-25 μm wide. Wik-Sjostedt (1970) described *Cladophora flexuosa* (O.F. Muller) Kutzing has a diploid sporophyte with quadriflagellate zoospores and a haploid gametophyte with biflaegllate gametes. *Cladophora flexuosa* (O.F. Muller) Kutzing was very easily cultured and every time that spores from newly collected material were put in culture media, they developed into plants. The ranges of spores were 1.5-2μm of *C. prolifera* (Roth) Kutzing and 2-3 μm of *C. flexuosa* (O.F. Muller) Kutzing. In *C. prolifera* (Roth) Kutzing spores were found abundant in room culture. In *C. flexuosa* (O.F. Muller) Kutzing, spores were found abundant in room culture but not found abundant in incubated culture.

The present study emphasized on the systematic of *Cladophora flexuosa* (O.F Muller) Kutzing and *C. prolifera* (Roth) Kutzing of the genus *Cladophora* Kutzing belonging to the family Cladopharaceae and the order Cladophorales. Seed plants were collected from the three Coastal Regions of Myanmar. In this study, the morphology, spore germination and local distribution of the two species were fully knowledged. *Cladophora flexuosa* (O.F Muller) Kutzing was distribution in Tanintharyi Coastal Region, Ayeyarwady and Gulf of Martaban Coastal Region and Rakhine Coastal Region of Myanmar. *Cladophora prolifera* (Roth) Kutzing was distribution in Tanintharyi Coastal Region and Rakhine Coastal Region of Myanmar. *Cladophora flexuosa* (O.F Muller) Kutzing mainly predominate along the Ayeyarwady and Gulf of Martaban Coastal Region and *Cladophora prolifera* (Roth) Kutzing mainly predominate along the Rakhine Coastal Region of Myanmar.

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I am indebted to Dr Aung Myat Kyaw Sein, Rector of Mawlamyine University, Dr Mie Mie Sein and Dr San San Aye, Pro-Rectors of Mawlamyine University, for their encouragement and supports in preparing this work. I am very grateful to Dr Mar Lar Aung, Professor and Head of Department of Botany, Mawlamyine University for her advice and encouragement in preparing this paper. Specially thanks to U Soe-Htun, Professor and Head (Retd.), Department of Marine Science, Mawlamyine University, for his valuable suggestions and constructive criticisms on this study.

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Pollinial Morphology on Ten Species of Orchidaceae Found in Southern Shan State

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Abstract

Pollinial morphology of 10 species belonging to 10 genera of family Orchidaceae were studied. The specimens of *Coelogyne lactea* Rchb. f., *Cymbidium lowianum* (Rchb. f.) Rchb. f., *Eria stricta* Lindl., *Hemipilia cordifolia* Lindl., *Hygrochilus parishii* (Rchb. f.) Pfitzer, *Papilionanthe teres* (Roxb.) Schltr., *Phaius tankervilleae* var. *pulchra* (King & Pantl.) Karthik., *Robiquetia pachyphylla* (Rchb. f.) Garay, *Thunia alba* (Lindl.) Rchb. f and *Vanda bensoni* Bateman were collected from Southern Shan State from 2016 to 2017. In the present study, the two pollinia were found in *C. lowianum* (Rchb. f.) Rchb. f., *H. cordifolia* Lindl., *P. teres* (Roxb.) Schltr., *R. pachyphylla* (Rchb. f.) Garay and *V. bensoni* Bateman; four pollinia were found in *C. lactea* Rchb. f. and *H. parishii* (Rchb. f.) Pfitzer; eight pollina were observed in *E. stricta* Lindl., *P. tankervilleae* var. *pulchra* (King & Pantl.) Karthik. and *T. alba* (Lindl.) Rchb. f.The smallest pollinarium (4.8 - 5.4 × 4.8 - 5.4 mm) was found in *E. stricta* Lindl. and the largest pollinarium (33.6 - 36.0 × 50.4 - 54.0 mm) was observed in *T. alba* (Lindl.) Rchb. f. The number, shape, size and colour of pollinia were differ from one species to another. The attachment of caudicle or stipe and viscidium to the pollinia were vary from one another. The pollinial morphology provides the knowledge for identification of the species and future systematic research work of family Orchidaceae.

Key words: Orchidaceae, Pollinia, Caudicle, Stipe, Viscidium

Introduction

Palynology (Gr. palynos, dust) is the study of spores and pollen grains. The features of spores and pollen grains can often be used to identify a particular plant taxon (Simpson 2006). The pollen grains are usually bound together by threads of a clear, sticky substance (viscin) in masses called pollinia (Dodson 2015).The pollinarium is defined as pollinia, a pollen mass

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and accessory organs such as a caudicle, a stipe, and a viscidium. In Orchidaceae, this feature is an informative source both in taxonomy and phylogenetics (Freudenstein & Ramussen 1999 as cited in Hidayat *et al.* 2006).

The ancestral number of pollinia per pollinarium is eight and that from there, were independent reductions to six, four or two pollinia (Dressler 1993 as cited in Damon & Nieto 2012). Taxonomic study of family Orchidaceae had been studied on various regions of Myanmar. However, pollinial morphology of Orchidaceae is left to be studied and recorded. Therefore, pollinial morphology of Orchidaceae were selected and studied.

The present study aimed to investigate the morphological differences in pollinia of Orchidaceae, to share knowledge the development and variation in number and structure of pollinia in the family Orchidaceae and to provide the valuable pollinial information in plant classification and identification of Orchidaceae from the palynological point of view.

Materials and Methods

The Orchidaceous plants were collected from Taunggyi Township, Hopong Township and Kalaw Township, Southern Shan State from 2016 to 2017. All the collected species were recorded by digital images. Identification of specimens were carried out by referring to the key and description stated by Hooker (1894), Schweinurth (1960), Backer & Brick (1968), Holttum (1964), Dassanayake (1981) and Seidenfaden (1992). Myanmar names of the collected species were referred to Hundley & Chit Ko Ko (1961), and Kress *et al*. (2003). For pollinarium and pollen preparation, the methods described by Chase (1987) as cited in Hidayat *et al.* (2006) and Erdtman (1960) were used with several modifications.

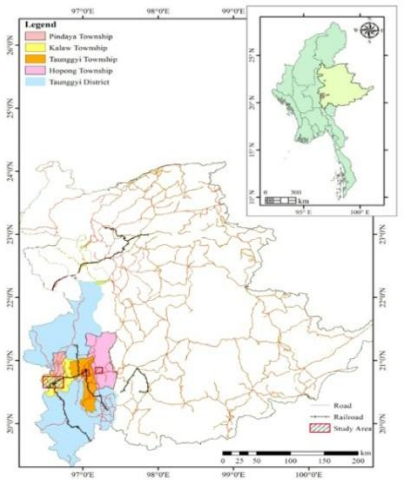

Figure 1. Map of the Collected Species from Southern Shan State

Results

Pollinial morphology of 10 species belonging to 10 genera of Orchidaceae was studied. The lists of collected species are arranged by alphabetically as shown in Table 1 and their pollinial morphology were presented in Table 2 and 3.

Table 1. List of the collected specimens

Family	No.	Scientific Name	Myanmar Name
Orchidaceae	1	*Coelogyne lacteal* Rchb. f.	Ngwe hnin phyu myokywe
	2	*Cymbidium lowianum* (Rchb. f.) Rchb. f.	Pan thet she kya
	3	*Eria stricta* Lindl.	Letset pan
	4	*Hemipilia cordifolia* Lindl.	Unknown
	5	*Hygrochilus parishii* (Rchb. f.) Pfitzer	Taung Karamet
	6	*Papilionanthe teres* (Roxb.) Schltr.	Yo set gyi
	7	*Phaius tankervilleae* var. *pulchra* (King & Pantl.) Karthik.	Zayti thitkhwa
	8	*Robiquetia pachyphylla* (Rchb. f.) Garay	Unknown
	9	*Thunia alba* (Lindl.) Rchb. f.	Kyauk thikhwa phyu
	10	*Vanda bensoni* Bateman	Moe thuzar

1. *Coelogyne lacteal* Rchb. f., Gard. Chron. 1:692.1885. (Figure 2 A-C)

Myanmar name : Ngwe hnin phyu myo kywe

Common name : Unknown

Flowering period : From March to May

Pollinial morphology

Pollinarium 13.8 – 15.6 × 15.6 – 16.8 mm in length and breadth; pollinia number 4; pollinial sac 10.5 – 11.0 × 6.5 – 7.5 mm in length and breadth, gibbous in shape, saffron, attachment of pollinium apical; caudicle not prominent; stipe absent; viscidium 4.6 – 5.2 × 9.5 – 10.8 mm in length and breadth, strap in shape, saffron; pollen tetrad tetragonal in shape, 17.5–30.0 × 21 – 35 μm in length and breadth; individual grain 5 – 11 × 5 – 15 μm in length and breadth; exine 2 – 3 μm thick, sexine as thick as nexine.

2. *Cymbidium lowianum* (Rchb.f.) Rchb.f., Gard.Chron., n.s. 11: 332, f. 56.1879. (Figure 2 D-F)

Cymbidium giganteum var. *lowianum* Rchb. f., Gard. Chron., n.s. 7: 685.1877.

Myanmar name : Pan thet she kya

Common name : Low’s Cymbidium

Flowering period : From February to April

Pollinial morphology

Pollinarium 19.2 – 24.0 × 30 – 36 mm in length and breadth; pollinia number 2; pollinial sac 6.4 – 8.0 × 15 – 18 mm in length and breadth, bell in shape, fulvous, attachment of pollinium ventral; caudicle not prominent; stipe single, 2.4 – 4.8 × 3.6 – 4.8 mm in length and breadth, rectangular in shape, white; viscidium 10.8– 12.0 × 26.3 – 31.5 mm in length and breadth, strap in shape, white; pollen tetrad rhomboidal in shape, 33 – 39 × 35 – 70 μm in

length and breadth; individual grain 10 – 19 × 10 – 23 μm in length and breadth; exine 2.5 – 5.0 μm thick, sexine thicker than nexine.

3. *Eria stricta* Lindl., Coll. Bot. Ad pl. 41B. 1826. (Figure 3A-C)

Myanmar name : Letset pan

Common name : The Rigid Eria

Flowering period : From January to February

Pollinial morphology

Pollinarium 4.8– 5.4 × 4.8 – 5.4 mm in length and breadth; pollinia number 8; pollinial sac 3.0 – 3.3 × 2.0 – 2.3 mm in length and breadth, obovate in shape, beige, attachment of pollinium apical; caudicle not prominent; stipe absent; viscidium 1.0 – 1.3 × 2.4 – 2.7 mm in length and breadth, quadrangular in shape, beige; pollen tetrad rhomboidal in shape, 20.0 – 22.5 × 30 – 40 μm in length and breadth; individual grain 6.5 – 15.0 × 10 – 15 μm in length and breadth; exine 1.5 – 2.0 μm thick, sexine thicker than nexine.

4. *Hemipilia cordifolia* Lindl., Gen. Sp. Orchid. Pl. 296, 1835.

(Figure 3 D-F)

Myanmar name : Unknown

Common name : The heart-shaped leaf Hemipilia

Flowering period : From June to August

Pollinial morphology

Pollinarium 28.8 – 29.4 × 16.0 – 16.3 mm in length and breadth; pollinia number 2; pollinial sac 14.0 – 14.2 × 8.0 – 8.3 mm in length and breadth, obovate in shape, purple, attachment of pollinium apical; caudicle 13.0 – 13.2 × 1.8 – 2.0 mm in length and breadth, strap in shape, tawny; stipe absent; viscidium 1.8 – 2.0 × 2.0 – 2.2 mm in length and breadth, irregular in

shape, mauve; pollen tetrad rhomboidal in shape, 40 – 44 × 55 – 60 μm in length and breadth; individual grain 17.5 – 22.5 × 16.5 – 17.5 μm in length and breadth; exine 1.5 – 2.0μm thick, sexine thicker than nexine.

5. *Hygrochilus parishii* (Rchb.f.) Pfitzer, Nat. Pflanzenfam. 1: 112. 1897.(Figure 4 A-C)

Vanda parishii Rchb. f., Xenia Orchid. 2: 138. 1868.

Myanmar name : Taung karamet

Common name : The Moist Lip Palenopsis

Flowering period : From June to July

Pollinial morphology

Pollinarium 25.2 – 32.4 × 12.6 – 16.2 mm in length and breadth; pollinia number 4; pollinial sac 1 0.2 – 13.0 × 6.3 – 8.1 mm in length and breadth, orbicular in shape, fulvous, attachment of pollinium ventral; caudicle not prominent; stipe single, 18.9 – 24.3 × 6.0 – 7.8 mm in length and breadth, Y like in shape, white; viscidium 8.0 – 10.2 × 7.5 – 8.5 mm in length and breadth, quadrangular in shape, white; pollen tetrad rhomboidal in shape, 17.5 – 20.0 × 25 – 39 μm in length and breadth; individual grain 6 – 9 × 6.5 – 10.0 μm in length and breadth; exine 2.5 – 3.0 μm thick, sexine thicker than nexine.

6. *Papilionanthe teres* (Roxb.) Schltr., Orchis 9:78.1915. (Figure 4 D-F)

Dendrobium teres Roxb., Fl. Ind. (ed.1832) 3: 485. 1832.

Myanmar name : Yo set gyi

Common name : The Terete Leaf Papilionanthe

Flowering period : From March to May

Pollinial morphology

Pollinarium 36 – 42 × 30.0 – 34.8 mm in length and breadth; pollinia number 2; pollinial sac 15 – 20 × 14.5 – 17.4 mm in length and breadth,

orbicular in shape, fulvous, attachment of pollinium ventral; caudicle not prominent; stipe single, 19 – 21 × 7.5 – 8.4 mm in length and breadth, cylindrical in shape, white; viscidium 19.5 – 22.0 × 28.0 – 32.5 mm in length and breadth, obtriangular in shape, white; pollen tetrad rhomboidal in shape, 27.5 – 35.0 × 35 – 45 μm in length and breadth; individual grain 9.0 – 12.5 × 10 – 15 μm in length and breadth; exine 1.0 – 1.5 μm thick, sexine thicker than nexine.

7. *Phaius tankervilleae* var. *pulchra* (King & Pantl.) Karthik., Fl. Ind. Enum:Monocot. 163. 1989. (Figure 5 A-C)

Phaiusblumei var. *pulchra* King & Pantl., Ann. Roy. Bot. Gard. (Calcutta) 8: 109. 1898.

Myanmar name : Zayti thitkhwa

Common name : Unknown

Flowering period : From February to April.

Pollinial morphology

Pollinarium 16.0 – 18.4 × 24.0 – 27.6 mm in length and breadth; pollinia number 8; pollinial sac 5.6 – 6.6 × 10.0 – 12.5 mm in length and breadth, elliptic in shape, fulvous, attachment of pollinium apical; caudicle not prominent; stipe absent; viscidium 7.5 – 8.8 × 7.0 – 8.5 mm in length and breadth, irregular in shape, fulvous; pollen tetrad rhomboidal in shape, 27.5 – 37.5 × 32.5 – 51.5 μm in length and breadth; individual grain 6.5 – 15.0 × 10.0 – 14.5 μm in length and breadth; exine 3 – 5μm thick, sexine as thick as nexine.

8. *Robiquetia pachyphylla* (Rchb.f.) Garay, Bot. Mus. Leafl. 23 (4): 197, 1972.(Figure 5 D-F)

Aerides pachyphylum Rchb.f., Gard. Chron. 14:231. 1880.

Myanmar name : Unknown
Common name : The broad leafed Robiquetia
Flowering period : From April to June

Pollinial morphology

Pollinarium 13.2 – 13.8 × 12.0 – 12.6 mm in length and breadth; pollinia number 2; pollinial sac 5.4 – 5.9 × 5.0 – 5.4 mm in length and breadth, orbicular in shape, cream, attachment of pollinium ventral; caudicle not prominent; stipe single, 8.5 – 9.5 × 2.0 – 2.1 mm in length and breadth, strap in shape, white; viscidium 6.6 – 6.9 × 4.0 – 4.2 mm in length and breadth, rectangular in shape, white; pollen tetrad rhomboidal in shape, 25 – 27 × 32.0 – 37.5μm in length and breadth; individual grain 7.5 – 11.0 × 10.0 – 12.5μm in length and breadth; exine 1.5– 2.0μm thick, sexine thicker than nexine.

9. *Thunia alba* (Lindl.) Rchb.f., Bot. Zeitung (Berlin) 10:764.1852.

(Figure 6 A-C)

Phaius albus Lindl., Pl. Asiat. Rar. 2:, Pl. 198. 1831.

Myanmar name : Kyauk thikhwa phyu
Common name : Unknown
Flowering period : From January to April.

Pollinial morphology

Pollinarium 33.6 – 36.0 × 50.4 – 54.0 mm in length and breadth; pollinia number 8; pollinial sac 29.4 – 31.5 × 13.0 – 13.7 mm in length and breadth, obovate in shape, ocherous, attachment of pollinium apical; caudicle not prominent; stipe absent; viscidium 7.4 – 7.9 × 12.8 – 13.5 mm in length and breadth, irregular in shape, white; pollen tetrad rhomboidal in shape, 27.5 – 35.0 × 35.0 –42.5 μm in length and breadth; individual grain 12.5 – 20.0 × 12.5 – 21.0 μm in length and breadth; exine 1.5 – 2.0μm thick, sexine thicker than nexine.

10. *Vanda bensoni* Bateman, Bot., Mag. 92:, Pl. 5611. 1866.

(Figure 6 D-F)

Myanmar name : Moe thuzar
Common name : Unknown
Flowering period : From March to June

Pollinial morphology

Pollinarium 30 – 36 × 18 – 24 mm in length and breadth; pollinia number 2; pollinial sac 11.4 – 12.5 × 9 – 12 mm in length and breadth, orbicular in shape, tawny, attachment of pollinium ventral; caudicle not prominent; stipe single, 16 – 22 × 11.0 – 12.6 mm in length and breadth, triangular in shape, white; viscidium 13.5 – 18.0 × 17 – 23 mm in length and breadth, quadrangular in shape, white; pollen tetrad rhomboidal in shape, 20 – 33 × 20– 43 μm in length and breadth; individual grain 5.0 – 14.5 × 5 – 17 μm in length and breadth; exine 2.5– 5.0 μm thick, sexine thicker than nexine.

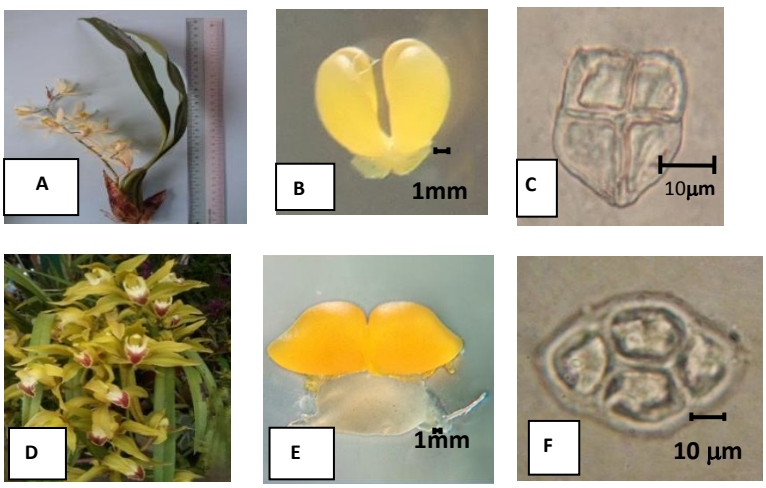

Figure 2. A. Inflorescences of *Coelogyne lacteal* Rchb. f.
B. Pollinarium of *C. lacteal* Rchb. f.
C. Tetragonal tetrad pollen of *C. lacteal* Rchb. f.
D. Inflorescences of *Cymbidium lowianum* (Rchb. f.) Rchb. f.
E. Pollinarium of *C. lowianum* (Rchb. f.) Rchb. f.
F. Rhomboidal tetrad pollen of *C. lowianum* (Rchb. f.) Rchb. f.

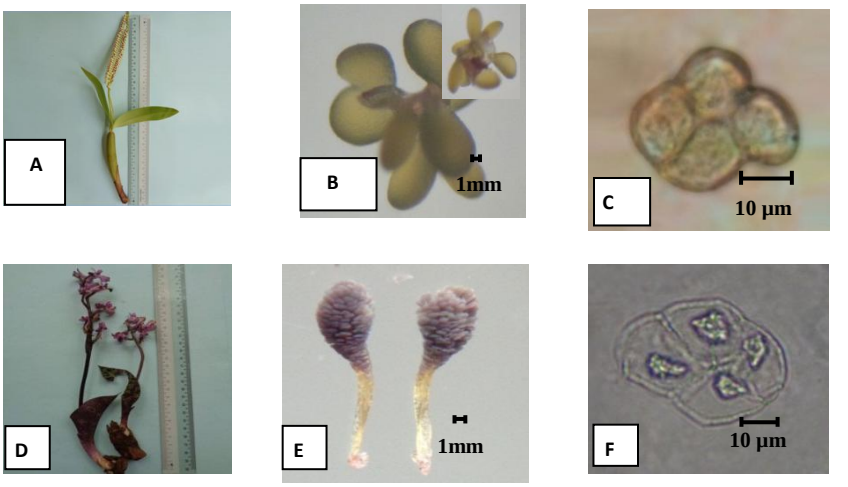

Figure 3. A. Inflorescences of *Eria stricta* Lindl.
B. Pollinarium of *E. stricta* Lindl.
C. Rhomboidal tetrad pollen of *E. stricta* Lindl.
D. Inflorescences of *Hemipilia cordifolia* Lindl.
E. Pollinarium of *H. cordifolia* Lindl.
F. Rhomboidal tetrad pollen of *H. cordifolia* Lindl.

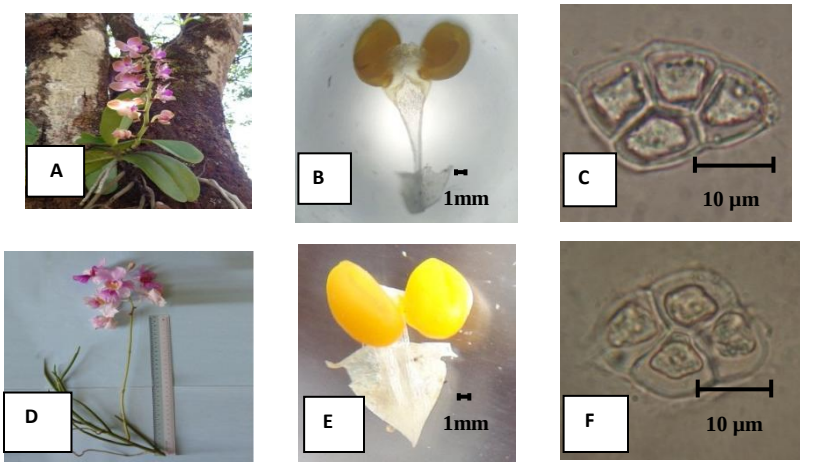

Figure 4. A. Inflorescences of *Hygrochilus parishii* (Rchb. f.) Pfitzer
B. Pollinarium of *H. parishii* (Rchb. f.) Pfitzer
C. Rhomboidal tetrad pollen of *H. parishii* (Rchb. f.) Pfitzer
D. Inflorescences of *Papilionanthe teres* (Roxb.) Schltr.
E. Pollinarium of *P. teres* (Roxb.) Schltr.
F. Rhomboidal tetrad pollen of *P. teres* (Roxb.) Schltr.

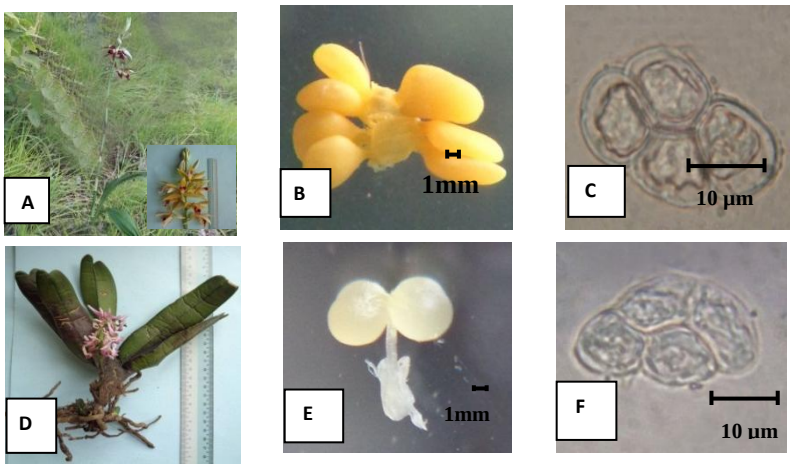

Figure 5. A. Inflorescences of *Phaius tankervilleae* var. *pulchra* (King &Pantl.) Karthik.
B. Pollinarium of *P. tankervilleae* var. *pulchra* (King & Pantl.) Karthik.
C. Rhomboidal tetrad pollen of *P. tankervilleae* var. *pulchra* (King & Pantl.) Karthik.
D. Inflorescences of *Robiquetia pachyphylla* (Rchb. f.) Garay
E. Pollinarium of *R. pachyphylla* (Rchb. f.) Garay
F. Rhomboidal tetrad pollen of *R. pachyphylla* (Rchb. f.) Garay

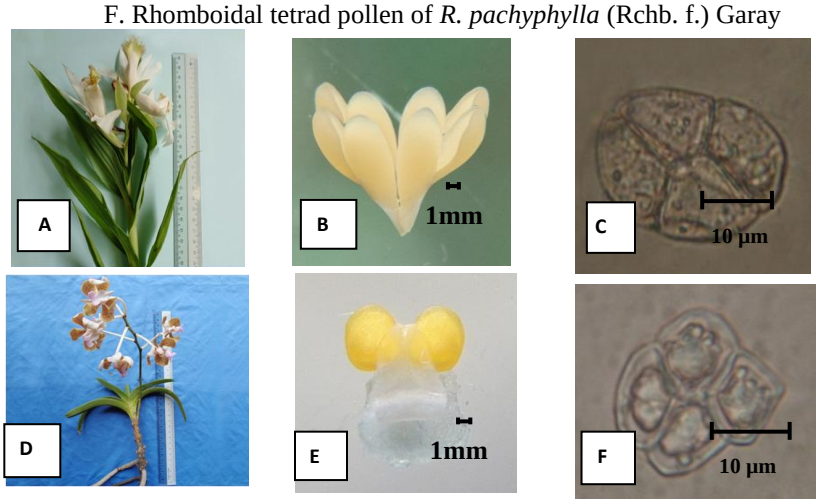

Figure 6. A. Inflorescences of *Thunia alba* (Lindl.) Rchb. f.
B. Pollinarium of *T. alba* (Lindl.) Rchb. f.
C. Rhomboidal tetrad pollen of *T. alba* (Lindl.) Rchb. f.
D. Inflorescences of *Vanda bensoni* Bateman
E. Pollinarium of *V. bensoni* Bateman
F. Rhomboidal tetrad pollen of *V. bensoni* Bateman

Discussion and Conclusion

Pollinial morphology of 10 species belonging to 10 genera of Orchidaceae were studied. The collected species of Orchidaceae were identified and classified according to the number, size, shape, colour, attachment of pollinia, caudicles, stipe and viscidium.

The number of pollinia occurred in Orchidaceae were 2, 4 and 8. Among them, the two pollinia were found in *Cymbidium lowianum* (Rchb. f.) Rchb. f., *Hemipilia cordifolia* Lindl., *Papilionanthe teres* (Roxb.) Schltr., *Robiquetia pachyphylla* (Rchb. f.) Garay and *Vanda bensoni* Bateman; four pollinia were found in *Coelogyne lactea* Rchb. f. and *Hygrochilus parishii* (Rchb. f.) Pfitzer; eight pollina were observed in *Eria stricta* Lindl., *Phaius tankervilleae* var. *pulchra* (King & Pantl.) Karthik. and *Thunia alba* (Lindl.) Rchb. f.

linarium Morphology of 10 Species of Orchidaceae

fic name	Pollinarium		Pollinial sac			Caudicle		Stipe			
	L & B (mm)	Num-ber	L & B (mm)	Shape	Colour	Attach-ment	L & B (mm)	Num-ber	L & B (mm)	Shape	Colour	L & B
lactea	13.8–15.6 × 15.6–16.8	4	10.5 – 11.0 × 6.5 – 7.5	gib	sa	ap	n-pro	ab	Ab	ab	ab	4.6 – 9.5 –
lowianum chb. f.	19.2 – 24.0 × 30 – 36	2	6.4 – 8.0 × 15 – 18	bel	fu	ve	n-pro	si	2.4 – 4.8 × 3.6 – 4.8	rec	wh	10.8 – 26.3 –
Lindl.	4.8 – 5.4 × 4.8 – 5.4	8	3.0 – 3.3 × 2.0 – 2.3	obo	be	ap	n-pro	ab	Ab	ab	ab	1.0 – 2.4 –
ordifolia	28.8 – 29.4 × 16.0 – 16.3	2	14.0 – 14.2× 8.0 – 8.3	obo	p	ap	13.0 – 13.2 × 1.8 – 2.0	ab	Ab	ab	ab	1.8 – 2.0 –
parishii Fitzer.	25.2–32.4 × 12.6 – 16.2	4	10.2 – 13.0× 6.3 – 8.1	orb	fu	ve	n-pro	si	18.9 – 24.3× 6.0 – 7.8	Y	wh	8.0 – 1 7.5 –
ne teres ilt.	36 – 42 × 30.0 – 34.8	2	15 – 20 × 14.5 – 17.4	orb	fu	ve	n-pro	si	19 – 21× 7.5 – 8.4	cy	wh	19.5 – 28.0 –
ervilleae r (Kng & hik.	16.0 – 18.4× 24.0 – 27.6	8	5.6 – 6.6× 10.0 – 12.5	el	fu	ap	n-pro	ab	Ab	ab	ab	7.5 – 7.0 –
pachyphylla iarav.	13.2 – 13.8× 12.0 – 12.6	2	5.4 – 5.9× 5.0–5.4	orb	oc	ve	n-pro	si	8.5 – 9.5× 2.0 – 2.1	st	wh	6.6 – 4.0 –
r (Lindl.)	33.6 – 36.0 × 50.4 – 54.0	8	29.4 – 31.5 × 13.0 – 13.7	obo	oc	ap	n-pro	ab	Ab	ab	ab	7.4 – 12.8 –
oni	30 – 36× 18 – 24	2	11.4 – 12.5× 9 – 12	orb	ta	ve	n-pro	si	16 – 22× 11.0 – 12.6	tr	wh	13.5 – 17 –

h = absent	el = elliptic	obt = obtriangular	sa = saffron	wh = white
p= apical	fu = fulvous	oc = ochreous	si = single	y = yllke
g = beige	gib = gibbous	orb = orbicular	st = strap	mu = mauve
el = bell	ir = iregular	p= purple	ta = tawny	
r = cream	n-pro = not prominent	qu = quadrangular	tr = triangular	
l = cylindrical	obo = obovate	rec = rectangular	ve = ventral	

Table 3. Pollen Morphology of 10 Species of Orchidaceae

No.	Scientific Name	Types of pollen tetrad	Pollen tetrad of length & breadth(μm)	Individual grain of length & breadth(μm)	Exine Thickness (μm)	
1	*Coelogyne lactea* Rchb. f.	tetragonal	17.5–30.0 × 21–35	5–11 × 5–15	2–3	S
2	*Cymbidium lowianum* (Rchb. f.) Rchb. f.	rhomboidal	33–39 × 35–70	10–19 × 10–23	2.5–5.0	S
3	*Eria stricta* Lindl.	rhomboidal	20.0–22.5 × 30–40	6.5–15.0 ×10–15	1.5–2.0	S
4	*Hemipilia cordifolia* Lindl.	rhomboidal	40 – 44 × 55 – 60	17.5 – 22.5 × 16.5 – 17.5	1.5 – 2.0	S
5	*Hygrochilus parishii* (Rchb. f.) Pfitzer	rhomboidal	17.5–20.0 × 25–39	6–9× 6.5–10.0	2.5–3.0	S
6	*Papilionanthe teres* (Roxb.) Schltr.	rhomboidal	27.5–35.0 × 35–45	9.0–12.5 × 10–15	1.0–1.5	S
7	*Phaius tankervilleae* var. *pulchra* (King & Pantl.) Karthik.	rhomboidal	27.5–37.5 × 32.5–51.5	6.5–15.0 × 10.0–14.5	3–5	S
8	*Robiquetia pachyphylla* (Rchb.f.) Garay	rhomboidal	25 – 27 × 32.0 – 37.5	7.5 – 11.0 × 10.0–12.5	1.5 – 2.0	S
9	*Thunia alba* (Lindl.) Rchb. f.	rhomboidal	27.5–35.0 × 35.0–42.5	12.5–20.0 × 12.5–21.0	1.5–2.0	S
10	*Vanda bensoni* Bateman	rhomboidal	20 – 33× 20 – 43	5.0 – 14.5 × 5 – 17	2.5 – 5.0	S

S/N = sexine and Nexine S = N = sexine as thick as nexine S > N = sexine thicker than nexin

The shape of pollinial sac were orbicular in *Hygrochilus parishii* (Rchb. f.) Pfitzer, *Papilionanthe teres* (Roxb.) Schltr., *Robiquetia pachyphylla* (Rchb. f.) Garay and *Vanda bensoni* Bateman; obovate in *Eria stricta* Lindl., *Hemipilia cordifolia* Lindl. and *Thunia alba* (Lindl.) Rchb. f.; gibbous, bell and elliptic in one species each.

The colour of pollinial sac were fulvous, saffron, beige, purple, cream, ochreous and tawny. The fulvous colour was found in *Cymbidium lowianum* (Rchb. f.) Rchb. f., *Hygrochilus parishii* (Rchb. f.) Pfitzer, *Papilionanthe teres* (Roxb.) Schltr. and *Phaius tankervilleae* var. *pulchra* (King & Pantl.) Karthik.; saffron, beige, purple, cream, ochreous and tawny were found in one species each. The caudicles attachment was apical or ventral. The apical attachment was found in *Coelogyne lactea* Rchb. f., *Eria stricta* Lindl., *Hemipilia cordifolia* Lindl., *Phaius tankervilleae* var. *pulchra* (King & Pantl.) Karthik. and *Thunia alba* (Lindl.) Rchb. f.; the ventral attachment of pollinia was observed in *Cymbidium lowianum* (Rchb. f.) Rchb. f., *Hygrochilus parishii* (Rchb. f.) Pfitzer, *Papilionanthe teres* (Roxb.) Schltr., *Robiquetia pachyphylla* (Rchb. f.) Garay and *Vanda bensoni* Bateman.

The morphological characters of pollinarium were different from each other.The size of the pollinaria ranges from 4.8 – 5.4 × 4.8 – 5.4 mm to 33.6 – 36.0 × 50.4 – 54.0 mm. The smallest size was found in *Eria stricta* Lindl. and the largest size was observed in *Thunia alba* (Lindl.) Rchb. f.. The

size of pollinial sac was also different from one species to another. The smallest pollinial sac was *Eria stricta* Lindl. (3.0 – 3.3 × 2.0 – 2.3 mm). The largest pollinial sac was observed in *Cymbidium lowianum* (Rchb. f.) Rchb. f.(6.4 – 8.0 × 15 – 18 mm). The caudicle was prominent in *Hemipilia cordifolia* Lindl. and the not prominent in 9 species.

Orchidaceae have diverse sizes of stipes, the length of stipe was differently observed in various sizes ranging from smallest length 8.5 – 9.5 × 2.0 – 2.1 mm to the largest length 16 – 22 × 11.0 – 12.6 mm. The smallest length of stipe was observed in *Robiquetia pachyphylla* (Rchb. f.) Garay and the largest length of stipe was found in *Vanda bensoni* Bateman. The numbers of stipes were single, double or absent. In this paper, single stipe was found in 5 species. The shape of stipe was strap, cylindrical, triangular, Y like, rectangular in one species each. The colour of stipe was only white in all species.

The viscidium was found in all of the 10 species, the smallest viscidium was found in *Hemipilia cordifolia* Lindl. (1.8 – 2.0 ×2.0 – 2.2 mm) and the largest viscidium was observed in *Papilionanthe teres* (Roxb.) Schltr. (19.5 – 22.0 × 28.0 – 32.5 mm).The studied species were diversed in shape of viscidium. The shape of viscidium was found in strap, quadrangular, irregular, obtriangular and rectangular. The strap shape of viscidium was found in 2 species, quadrangular in 3 species, irregular in 3 species, obtriangular and rectangular shape in one species each. The colour of viscidium was saffron, white, beige, mauve and fulvous. The white colour of viscidium was observed in 6 species, saffron, beige, mauve and fulvous was found in one species each.

Kull *et al.* (2009) described that Orchidaceae have all six possible tetrad types: tetrahedral or tetragonal, decussate, square, rhomboidal, T shape and linear. In the present study, pollen tetrads were rhomboidal in 9 species and tetragonal in 1 species. These findings are comprised Hoen (1999) and Kull *et al.* (2009). The smallest size of tetrad pollen was found in *Coelogyne lacteal* Rchb. f. (17.5 – 30.0 × 21 – 35 µm) and the largest size of tetrad pollen

was observed in *Cymbidium lowianum* (Rchb. f.) Rchb. f.(33 – 39 × 35 – 70 µm).

The numbers of pollinia of *Hygrochilus parishii* (Rchb.f.) Pfitzer was 4, number of stipe was single and caudicle was not prominent. The present results were agreed with the finding of Hidayat *et al.* (2006).

In the present research, on the basis of observation on pollinia, it was stated that the pollinia of different genera vary in morphology. These morphological features of pollinia will be supported for classification and identification of some species in Orchidaceae.

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EFFICACY OF LACTIC ACID BACTERIA FROM EDIBLE FRUITS AGAINST ANTIBIOTICS AND PATHOGENIC BACTERIA

Phoo Pwint Theingi[1], Soe Myint Aye[2] & Htet Htet Win[3]

Abstract

Lactic Acid Bacteria (LAB) were isolated from fresh fruits samples of carambola, pomegranate, guava, papaya, lime, banana and pitahaya by using DeMan Rogos Sharpe (MRS) agar medium in the Microbiology Laboratory, Department of Botany, University of Mandalay during September 2017 to January 2018. The ten bacterial strains, PPTG 1 − 10, were observed and identified based on their colony morphology and biochemical tests. The most number of three LAB strains were observed in papaya with PPTG 4, 5 and 6, followed by two strains in pitayana with PPTG 9 and PPTG 10. The isolated LAB strains PPTG 1, PPTG 5 and PPTG 7 were confirmed as *Lactobacillus* spp.; PPTG 2 and PPTG 8 as *Pediococcus* spp.; and PPTG 3, PPTG 4, PPTG 6, PPTG 9 and PPTG 10 as *Leuconostoc* spp. According to the study on the abilities of antibiotic resistance, *Lactobacillus* sp. (PPTG 1) strain was resistent to ampacillin and amoxicillin; *Leuconostoc* sp. (PPTG 4) strain was resistent to ampacillin, amoxicillin, chloramphenicol and erythromycin; *Lactobacillus* sp. (PPTG 5) strain was resistent to tetracycline, ampacillin, amoxicillin, chloramphenicol and erythromycin; *Leuconostoc* sp. (PPTG 9) strain was resistent to tetracycline, ampacillin and amoxicillin; and *Leuconostoc* sp. (PPTG 10) strain was resistent to ampacillin and amoxicillin. The LABs possessing the antibiotic activities were tested for antimicrobial properties at Biotechnology Research Department, Kyaukse. *Lactobacillus* sp. (PPTG 1) isolated from carambola strain showed maximum zone of inhibition against *Staphylococcus aureus* with 19 mm, a zone of inhibition against *Escherichia coli* with 12 mm, *Bacillus cereus* with 11 mm. The physiological test on five strains showed that they can grow between pH 4.5 and pH 7.2 and salt tolerance was up to 6% NaCl. Therefore, among the study fruits the carambola is the most suitable one to be eaten for the health of human beings. Because of the most LAB strains were observed in papaya, it was also suitable to be consumed in daily life.

Keywords: LABs, edible fruits, antibiotic properties

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Introduction

Microorganisms play an essential role in the food production. The fermentations by Lactic acid bacteria (LAB) were involved for thousands of years in food and are one of the most ancient preservation techniques. The first signs of LAB utilizations date back to 6000BC, describing the fermentation of milk and fermentation of meat 1500BC and vegetable products 300BC. Vegetables and fruits are fundamental sources of water-soluble vitamins (vitamin C and group B vitamins), provitamin A, phytosterols, dietary fibres, minerals and phytochemicals for the human diet. Scientific evidences encouraged the consumption of vegetables and fruits to prevent chronic pathologies such as hypertension, coronary heart diseases and the risk of stroke (Saif 2016).

Lactic acid bacteria (LAB) have been extensively studied for their commercial potential, food preservation and health benefits. Industrial importance of LAB is based on their ability to ferment sugars readily into different metabolites and provide an effective method for preserving fermented food products. These bacteria are genetically diverse group of bacteria encompassing widely recognized genera which include: *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella*. Some authors include the genus Bifidobacterium because of its probiotic role (Emerenini *et al*. 2013).

Abubakr & Al-Adiwish (2017) stated that LABs possess antimicrobial activities and Patel *et al.* (2012) studied that antibiotic resistance or drug resistance can be defined as the ability of bacteria. On account of this, the present study was carried out for the partial fulfillment of isolation, characterization and identification of lactic acid bacteria from selected edible fruits and their efficacy against antibiotics, and antimicrobial properties.

LABs are non-pathogenic bacteria, technologically suitable for industrial processes and their capacity to produce antimicrobial compounds makes them beneficial for health. Obtaining genetically stable strains to be

used in probiotic products has been a concern for researchers in the field. Despite their human origin, recent studies have described novel sources for isolating LAB with potential probiotic benefits, such as wild type fruits and fermented vegetables. However, numerous lactobacilli were found to be abundant in the pollen, suggesting their role in suppressing the growth of molds and other spoilage organisms (Benavides *et al*. 2016).

Most probiotic bacteria are LAB, and among them, *Lactobacillus* is the most common genera. Probiotics are non-pathogenic microorganisms, which exert a positive health benefit on the host when ingested in an adequate amount. The majority of the commercialized and most studied probiotics have been isolated from dairy products and human gastrointestinal tract. Although, dairy foods are recognized to be the best vehicle for the delivery of viable probiotics to the human gut, the increasing number of individuals with lactose intolerance, dyslipidemia, and vegetarianism reinforces the importance of the development of non-dairy probiotic products such as fruit juices (Peres *et al*. 2012).

Raw fruit and their byproducts possess intrinsic physicochemical parameters that resemble those of the human gastrointestinal tract for some traits, such as the acidic environment and presence of anti-nutritional factors (tannins and phenols). The natural adaptation to the intrinsic characteristics of fruit may help fruit-originating bacteria to survive during the processing and storage of fruit-based probiotic formulations as well as in the human stomach (Garcia *et al*. 2016). Martins *et al*. (2016) stated that the variety of fruits ready to eat are containing also the probiotics of high functionality. Mozzi *et al*. (2010) informeded that the probiotic potential of lactic acid bacteria and their role in inhibition of pathogenic organisms have opened new horizons in the fields of medical sciences and food biotechnology.

Hnin Ei Phyu (2012) studied the isolation and characterization of *Lactobacillus* species from yogurt. Khine Zin Mar Thein (2014) studied the isolation and preliminary identification of acetic acid bacteria from honey.

However, the efficacy of lactic acid bacteria from edible fruits against antibiotics and pathogenic bacteria had not been conducted at the Department of Botany, University of Mandalay. Therefore, the present study was carried out for the partial fulfillment of efficacy of lactic acid bacteria from edible fruits against antibiotics and pathogenic bacteria.

The aim and objectives of this research are to isolate the Lactic Acid Bacteria (LAB), to identify by using their morphology and biochemical characteristics, to investigate the antibiotic resistance and the antipathogenic-bacterial activities of the isolated strains.

Materials and Methods

Lactic acid bacteria (LAB) were isolated from 7 samples of fresh fruits (carambola, pomegranate, guava, papaya, lime, banana and pitahaya) obtained from local markets during September 2017 to January 2018.

Preparation of Media and Chemicals Used for Isolation of Bacteria, and physiological and biochemical methods for identification of isolated strains were followed to Breed *et al.* (1957), Cruickshank *et al.* (1968), Speck (1976), Dickey & Kelman (1988), Atlas (1993), Sharma (2007) and Naeem *et al*. (2012). All the experiments were performed at Department of Botany, University of Mandalay. Growth Response of the selected strains at different pH and NaCl concentration were also studied according to Khalil & Anwar (2016).

Antibiotic resistance and antibacterial activity for LAB were carried out at the Biotechnology Research Department, Kyaukse, according to Sukumar & Ghosh (2010).

Results

Morphology of Isolated Lactic Acid Bacteria

The lactic acid bacteria were isolated from edible fruits (carambola, pomegranate, guava, papaya, lime, banana and pitahaya) by using De Man, Rogosa and Sharpe agar (MRS) medium. Ten different kinds of lactic acid

bacterial strains were observed from edible fruits. The lactic acid bacterial strains were named as PPTG 1, PPTG 2, PPTG 3, PPTG 4, PPTG 5, PPTG 6, PPTG 7, PPTG 8, PPTG 9 and PPTG 10. The individual colonies were sub-cultured on the separate media plates to find out the difference in shape, colour and colony formation.

Lactobacillus sp., *Pediococcus* sp. and *Leuconostoc* sp., were isolated from edible fruits. The colonial and microscopic morphology of isolated lactic acid bacteria were presented in Table 1 & 2.

Antibiotic Resistance Test for Lactic Acid Bacteria

Lactobacillus sp. (PPTG 1) strain was resistant to ampacillin and amoxicillin, *Leuconostoc* sp. (PPTG 4) strain was resistant to ampacillin, amoxicillin, chloramphenicol and erythromycin, *Lactobacillus* sp. (PPTG 5) strain was resistant to tetracycline, ampacillin, amoxicillin, chloramphenicol and erythromycin, *Leuconostoc* sp. (PPTG 9) strain was resistant to tetracycline, ampacillin and amoxicillin and *Leuconostoc* sp. (PPTG 10) strain was resistant to ampacillin and amoxicillin. *Pediococcus* sp. (PPTG 2), *Leuconostoc* sp. (PPTG 3), *Leuconostoc* sp. (PPTG 6), *Lactobacillus* sp. (PPTG 7) and *Pediococcus* sp. (PPTG 8) were non-resistant to antibiotics (Table 3).

Table 1. Morphological Characterization of Isolated Bacteria

Cell Morphology	Shape	Size (μm)	colour	Gram staining	Endospore forming	Motility
Lactobacillus sp. (PPTG 1)	Short rod	0.1-0.5 by 1.0-5.0	white	+	–	–
Pediococcus sp. (PPTG 2)	coccus	0.3-0.7	Pale yellow	+	–	–
Leuconostoc sp. (PPTG 3)	spherical	0.3-0.9	yellow	+	–	–

Cell Morphology	Shape	Size (μm)	colour	Gram staining	Endospore forming	Motility
Leuconostoc sp. (PPTG 4)	spherical	0.5-1.0	creamy	+	_	_
Lactobacillus sp. (PPTG 5)	rod	0.7-1.9 by 2.0-6.0	creamy	+	_	_
Leuconostoc sp. (PPTG 6)	spherical	0.2-0.5	yellow	+	_	+
Lactobacillus sp. (PPTG 7)	rod	0.5-1.0 by 2.0-8.0	yellow	+	_	_
Pediococcus sp. (PPTG 8)	coccus	0.1-0.4	white	+	_	_
Leuconostoc sp. (PPTG 9)	coccus	0.3-0.8	white	+	_	_
Leuconostoc sp. (PPTG 10)	coccus	0.2-1.0	Pale yellow	+	_	_

+ = Positive reaction
– = Negative reaction

Table 2. Biochemical Tests for Characterization of Isolated Bacteria

Biochemical Character-istics	Cit. Utiliz. Test	Cata-lase Test	Oxi-dase Test	Fermentation					
				Glu	Suc	Lac	Dex	Man	Mal
Lactobacillus sp. (PPTG 1)	+	+	+	+AG	+AG	_	+AG	+AG	_
Pediococcus sp. (PPTG 2)	_	+	_	+AG	+AG	+AG	+AG	_	+AG
Leuconostoc sp. (PPTG 3)	_	+	_	+AG	+AG	_	+AG	+AG	_
Leuconostoc sp. (PPTG 4)	+	+	+	+AG	+AG	+AG	+AG	+AG	+AG

Biochemical Characteristics	Cit. Utiliz. Test	Catalase Test	Oxidase Test	Fermentation					
				Glu	Suc	Lac	Dex	Man	Mal
Lactobacillus sp. (PPTG 5)	+	+	_	+AG	+AG	+AG	+AG	+AG	+AG
Leuconostoc sp. (PPTG 6)	_	+	_	+AG	+AG	_	+AG	+AG	+AG
Lactobacillus sp. (PPTG 7)	_	+	+	+AG	+AG	_	+AG	+AG	+AG
Pediococcus sp. (PPTG 8)	_	+	_	+AG	+AG	_	_	_	+A
Leuconostoc sp. (PPTG 9)	+	+	_	+AG	+AG	+AG	+AG	+AG	+AG
Leuconostoc sp. (PPTG 10)	+	+	_	+AG	+AG	+AG	+AG	+AG	+AG

+ = Positive reaction, + A = Acid

– = Negative reaction, + AG = Acid with gas

Table 3. Antibiotic Resistance Test for Lactic Acid Bacteria

LABs	Diameter of Inhibition Zone (mm)				
	Tetracycline	Ampacillin	Chloramphenicol	Amoxicillin	Erytho mycin
Lactobacillus sp. (PPTG 1)	–	71.08, 38.48	–	62.97, 41.16	–
Pediococcus sp. (PPTG 2)	–	–	–	–	–
Leuconostoc sp. (PPTG 3)	–	–	–	–	–
Leuconostoc sp. (PPTG 4)	–	28.54, 23.07	14.76, 18.48	23.85, 25.82	17.63
Lactobacillus sp. (PPTG 5)	60.55, 37.45	39.77, 32.24	59.65, 40.73	35.21, 32.49	44.52, 31.38
Leuconostoc sp. (PPTG 6)	–	–	–	–	–

LABs	Diameter of Inhibition Zone (mm)				
	Tetra-cycline	**Ampacillin**	**Chloram-phenicol**	**Amoxicillin**	**Erythomycin**
Lactobacillus sp. (PPTG 7)	–	–	–	–	–
Pediococcus sp. (PPTG 8)	–	–	–	–	–
Leuconostoc sp. (PPTG 9)	16.12	20.11, 21.23	–	24.14, 29.68	–
Leuconostoc sp. (PPTG 10)	–	33.46, 24.74	–	29.68, 26.99	–

– = non resistance to antibiotics

Antibacterial Activity Test for Lactic Acid Bacteria

Lactobacillus sp. (PPTG 1) strain showed maximum zone of inhibition against *Staphylococcus aureus*, average zone of inhibition against *Escherichia coli*, minimum zone of inhibition against *Bacillus cereus* and did not show any zone of inhibition on *Enterococcus faecalis*. *Leuconostoc* sp. (PPTG 4) strain showed zone of inhibition against *Staphylococcus aureus* and did not show any zone of inhibition on *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*. *Lactobacillus* sp. (PPTG 5) strain did not show any zone of inhibition. *Leuconostoc* sp. (PPTG 9) strain showed zone of inhibition against *Staphylococcus aureus* and did not show any zone of inhibition on *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*. *Leuconostoc* sp. (PPTG 10) strain showed zone of inhibition against *Staphylococcus aureus* and did not show any zone of inhibition on *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*. Tetracycline hydrochloride control showed zone of inhibition against *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* (Table 4).

Table 4. Antibacterial Activity Test for Lactic Acid Bacteria

LABs	Diameter of Inhibition Zone (mm)			
	Bacillus cereus	*Escherichia coli*	*Staphylococcus aureus*	*Enterococcus faecalis*
Lactobacillus sp. (PPTG 1)	11	12	19	–
Leuconostoc sp. (PPTG 4)	–	–	11	–
Lactobacillus sp. (PPTG 5)	–	–	–	–
Leuconostoc sp. (PPTG 9)	–	–	9	–
Leuconostoc sp. (PPTG 10)	–	–	9	–
Tetracycline hydrochloride	23	27	13	16

– = non inhibition zone of pathogenic bacteria

Growth Responses of Selected LAB strains

The isolated LAB strains such as *Lactobacillus* sp. (PPTG 1), *Leuconostoc* sp. (PPTG 4), *Lactobacillus* sp. (PPTG 5) and *Leuconostoc* sp. (PPTG 10) possessed the antibiotic properties. Their growth responses were studied at the various pH of 4.5, 6.5 and 7.2. All strains can survive at all study pH level. According to the study on resistance from NaCl 1% to NaCl 6% also all the strains survived in concentration. But *Lactobacillus* sp (PPTG 1) showed the more resistance compared to the remaining 4 strains (Table 5).

Table 5. Physiological Tests for Bacteria possessing antibacterial activity

LABs	Growth at different pH			Growth at different NaCl (%)					
	4.5	6.5	7.2	1	2	3	4	5	6
Lactobacillus sp. (PPTG 1)	+	++	+++	+++	+++	+++	++	++	++
Leuconostoc sp. (PPTG 4)	+	++	+++	+++	+++	+++	++	+	+
Lactobacillus sp. (PPTG 5)	+	++	+++	+++	+++	+++	++	+	+
Leuconostoc sp. (PPTG 9)	+	++	+++	+++	+++	+++	+++	+	+
Leuconostoc sp. (PPTG 10)	+	++	+++	+++	+++	+++	+++	+	+

+ = Positive reaction – = Negative reaction

Discussion and Conclusion

The present study deals with isolation, identification of lactic acid bacteria from selective edible fruits and their efficacy resistance to antibiotics and anti-microbial properties. Seven samples of fresh fruits (carambola, pomegranate, guava, papaya, lime, banana and pitahaya) were obtained from local markets. Ten strains of LABs were isolated from this study and they were relatively named as PPTG 1 to PPTG 10.

Naeem *et al.* (2012) observed that the LABs can be isolated from *Carica papaya* (Papaya), *Musa* spp. (Banana), *Psidium guajava* (Guava) and *Punica granatum* (Pomegranate). In the present study also LABs can be isolated from these species.

Fijan (2014) mentioned that *Lactobacillus* includes the major part of the lactic acid bacteria (LAB) group (including *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Oenococcus*, *Pediococcus*, *Streptococcus* and *Leuconostoc* species) that can convert hexose sugars to lactic acid thus producing an acid environment which inhibits the growth of several species of harmful bacteria. Some *Lactobacilli* are used for the production of yogurt, cheese, sauerkraut, pickles, sourdough, wine and other fermented products. Breed *et al*. (1958) stated that *Pediococcus* are the dominant microbial population on forage crops and silage. Ali (2011) mentioned that the genus *Leuconostoc* have long been known and applied by humans for making different food stuffs. For many centuries, they have been an effective form of natural preservation. Therefore, the resulting of *Lactobacillus* spp., *Pediococcus* spp. and *Leuconostoc* spp. are valuable for the resulting of noble strains for further studies.

According to the present study, *Lactobacillus* sp. (PPTG 1) strain was resistant to ampacillin and amoxicillin; *Pediococcus* sp. (PPTG 2) strain and *Leuconostoc* sp. (PPTG 3) strain were non-resistant to tested antibiotics; *Leuconostoc* sp. (PPTG 4) strain was resistant to ampacillin, amoxicillin, chloramphenicol and erythromycin; *Lactobacillus* sp. (PPTG 5) strain was resistant to tetracycline, ampacillin, amoxicillin, chloramphenicol and erythromycin; *Leuconostoc* sp. (PPTG 6) strain, *Lactobacillus* sp. (PPTG 7)

strain, *Pediococcus* sp. (PPTG 8) strain and *Leuconostoc* sp. (PPTG 9) strain was resistant to tetracycline, ampacillin and amoxicillin, and *Leuconostoc* sp. (PPTG 10) strain was resistant to ampacillin and amoxicillin. These finding are in related with some previous findings of Naeem *et al.* (2012) and Saranya & Hemashenpagam (2011).

Naeem *et al.* (2012) stated that all 15 isolates LAB strains were tested for antibiotic resistance and their susceptibility and resistance against 10 available antibiotics. Saranya & Hemashenpagam (2011) mentioned that Rifampicin, Ketoconazole, Novobiocin, Fluconazole, Gentamycin, Amphotericin, and Chloramphenicol were used to determine antibiotic resistance of lactobacilli strains. The resistances were determined according to the zone formation.

In this present study, *Lactobacillus* sp. (PPTG 1) strain showed maximum zone of inhibition against *Staphylococcus aureus*, average zone of inhibition against *Escherichia coli*, minimum zone of inhibition against *Bacillus cereus* and did not show any zone of inhibition on *Enterococcus faecalis*. *Leuconostoc* sp. (PPTG 4) strain showed zone of inhibition against *Staphylococcus aureus* and did not show any zone of inhibition on *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*. *Lactobacillus* sp. (PPTG 5) strain did not show any zone of inhibition. *Leuconostoc* sp. (PPTG 9) strain showed zone of inhibition against *Staphylococcus aureus* and did not show any zone of inhibition on *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*. *Leuconostoc* sp. (PPTG 10) strain showed zone of inhibition against *Staphylococcus aureus* and did not show any zone of inhibition on *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*. Tetracycline hydrochloride control showed zone of inhibition against *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis.* Therefore, *Lactobacillus* sp. (PPTG 1) is nearly as good as control in antibacterial activity, especially on *Staphylococcus aureus* with up to 19 mm in diameter of inhibition.

Saranya & Hemashenpagam (2011) mentioned that the LAB inhibited all the pathogenic bacteria. The activity of LAB on some gram positive and negative pathogenic bacteria such as *E.coli, Pseudomonas aeroginosa, Klebsiella pneumonia, Staphylococcus aureus* and *Bacillus cereus* and the inhibition zones were in the range of 1.4 to 2.8 cm. The largest zone of inhibition was produced by *L. plantarum* (13mm) against *S. aureus.* In the present study also *Lactobacillus* sp. (PPTG 1) is also possessing antibacterial activity, especially on *Staphylococcus aureus* with up to 19 mm *Staphylococcus aureus* with 19 mm, average zone of inhibition against *Escherichia coli* with 12 mm, minimum inhibition against *Bacillus cereus* with 11 mm. Therefore, the present finding is valuable as previous findings of Saranya & Hemashenpagam (2011).

The isolated LAB produced antimicrobial compounds to varying degree, the increase in the production of lactic acid with time have been attributed to lowered pH which permit the growth of LAB. Garcia *et al.* (2016) stated that the five tested *Lactobacillus* strains displayed the capability to inhibit pathogenic bacteria, including *E. coli*, *L. monocytogenes*, *Salmonella enteritidis*, *Salmonella typhimurium* and *Staphyloccus aureus* in the potato agar or well diffusion assays. Therefore, the present findings agreed with Garcia *et al.* (2016) in possessing the antimicrobial effect on *E. coli* and *S. aureus*.

Owing to the considerable economic importance of LAB, many researchers are now actively working on these bacteria using an array of genetic tools. In the last few decades probiotic potential of LAB and their role in inhibition of pathogenic organisms has opened new horizons in the fields of medicinal sciences and food biotechnology (Naeem *et al*. 2012).

Recent researchers reported that the isolation and identification of the isolate of LAB from different fruits had shown good proteolytic activity and probiotic properties. Martins *et al.* (2016) stated that banana and guava has considerable amounts of prebiotic substrates, which can contribute the viability of persistence of *Lactobacillus rhamnosus* and it has potential to

serve as a probiotic carrier. Previous studies indicated that probiotic culture produces acids that promote the reduction of pH, creating condition unfavourable to microbial growth and also on the growth of pathogens. The presence of such bacteria in raw fruits satisfies the nutritive and microbial profile of fruits to be a healthy food. Garcia *et al*. (2016) stated that the natural adaptation to the intrinsic characteristics of fruit may help fruit-originating bacteria to survive during the processing. The presence of LAB in fruits, according to present study, may promote the health of human beings.

In the present study the most number of LABs were observed in papaya fruit with three isolated strains such as *Leuconostoc* sp. (PPTG4), *Lactobacillus* sp (PPTG 5) and *Leuconostoc* sp (PPTG 6). Because of this finding, papaya is very suitable to be consumed in daily life for the health. The pitahaya fruit also endophytically possess two strains of LAB, *Leuconostoc* spp. (PPTG 9 and PPTG 10). The remaining fruits possess one strain of LAB each. Acording to the study on antipathogenic bacteria properties of LABs, *Lactobacillus* sp. (PPTG 1) strain isolated from carambola showed zone of inhibition against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus*. Therefore, the carambola fruit is also very suitable for the health. Moreover, the selected LAB strains revealed the following desirable probiotic related properties of growth on various pH level and NaCl concentration.

In conclusion, the LAB strains can be isolated from freshly edible fruits and they have noble properties of antibiotic sensitivity, antimicrobial properties, especially on pathogenic bacteria, and also the stains having the ability of growth in different pH and NaCl concentration. The fruits are very important to be consumed not only for their nutritional properties but also as the providers of the beneficial probiotic strains inside the bodies.

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STUDY ON TAXONOMIC CHARACTERS AND PHYLOGENETIC POSITIONS OF SPECIES UNDER DENDROCALAMUS (POACEAE) IN DIFFERENT REGIONS OF MYANMAR

Thida Hlaing[1]

Abstract

In the present research, bamboo plants of the family Poaceae (Gramineae) in Myanmar. Totally 12 genera were described. Most of the bamboos are naturally distributed in higher or lowland of mountain range. The morphological characters of collected species were based on morphology of culm, culm sheath and leaves. According to the phylogenetic study, the phylogenetic positions of genera *Dendrocalamus* have been studied by constructing Dendrogram.

Keywords: Taxonomy, culm, culm sheath and leaves.

Introduction

Bamboo is well known plant all over the world, particularly in Asian and African countries. Most botanists place them in the tribe. Bambuseae within the grass family Poaceae formally called Gramineae. They are worldwide distributed with about 80 genera and about 1000 species. These are about 200 species and 20 genera are found in South-East Asia. In Myanmar, bamboo were found as 18 genera and about 100 species (Hundley & chit koko 1987) Kress *et.al* (2003) recorded 17 genera and 92 species in Myanmar.

Bamboos have evolved to cover a wide range of climates from tropical areas to temperate ones, from plains to high mountains. Some species even occur in the cold temperate zones or on mountains as high as 4,500m above sea level. Geographic distribution of bamboo worldwide may fall into three major regions; namely, the Asian - Pacific Region, the Americas Region and the African Regions. (Yang 2010)

The geographical distribution of bamboo is greatly influenced by human activities. There are about 29 species of *Dandrocalamus* growing in

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South-East Asia, mainly occurring in the lowlands from the Indian subcontinent to Indo-China and Peninsular Malaysia. *Dendrocalamus* Nees comprises 52 species and is distributed from china to India, Myanmar, Thailand, Indo China and Malaysia through Papua New Guinea. A genus containing the largest of all bamboo species, forming clump up to 30m tall. (Stapleton 1994)

Bamboo is an essential part of life for many people around the world. It is used in many ways, including structural support for housing, furniture, musical instruments, toys and innumerable small articles. Bamboo also have ornamental use in landscape gardens and windbreaks (Camus 1913).

Bamboo is used in Chinese medicine for treating infections and healing. It is a low-calorie source of potassium. It is known for its sweet taste and as a good source of nutrients and protein. (Ghosh 2008)

The present work deal with the taxonomic characters and phylogenetic positions of genera *Dendrocalamus* in different regions. The aim and objectives of present work are to identify the taxonomic character of bamboo in phylogenetic relationship among the genera *Dendrocalamus* to disseminate the knowledge of more complete taxonomical characters of bamboo resources plants.

Materials and Methods

1. Morphology

In this study, all the members of the family Poaceae (Tribe-Bambuseae) were collected from different regions of Myanmar. All the collected specimens were recorded and the collected places were determined by using a Global Positioning System (GPS) device. Plant collection and preservation technique were used to make the herbarium specimens. The collected specimens were identified by the culms, culm sheath and leaves referring to the book of Hooker (1897), Stapleton (1994), Wong (1995),

Dransfield (1995) and shouliang *et.al* (2006). The morphological record of all the collected species were stated in figures of photographs.

Phylogenetic Studies

The phylogenetic relationships between these species were to be examined. The classical phylogenetic tree is accomplished by Bor (1960) and Shouliang *et al.* (2006).

The phylogenetic relation was studied basing on morphological characteristics of peculiar plant parts. The individual characters are designated as primitive, derived, or advanced. The selected characters needed in score making for the preparation of phylogenetic scheme are (A) Nature of Rhizome type, (B) Branches type, (C) Culm hollowness, (D) Culm wall thickness, (E) Culm nodes, (F) Culm with white ring, (G) Culm sheath thickness (H) Shape of culm sheath, (I) Ligule, (J) Auricle of culm sheath, (K) Bristles of culm sheath (L) Leaf shape, (M) Ligule of leaf and (N) Leaf auricle. The score of primitive character is 0, intermediate character is 0.5 and advanced character is 1.

Based on the total score of study characters the dendrogram was constructed by using IBM SPSS version 21. The method of data analysis of cluster method was nearest neighbour and Euclidean distance. According from the resulting dendrogram, it can be estimated that which are related among those studied genera or species.

The assessments of primitive and advanced characters are linked with the nature of Rhizome type, culm, culm sheath and leaves as shown in Table 1.

Table 1. Categories of Bamboo Used in Score Making for the Preparation of Phylogenetic Scheme

	Category	Primitive score (0)	Intermediate score (0.5)	Advanced score (1)
A	Rhizome	monopodial	-	sympodial
B	Branches type	simple	moderate	much branches
C	Calm hollowness	solid	nearly solid	hollow
D	Culm wall thickness	thick	-	thin
E	Culm nodes	non swollen	swollen	with thorn
F	White ring	none	present	present in adventitious root
G	Culm sheath thickness	thick	-	thin
H	Shape of culm sheath	cylindrical/ narrow/ linear	triangular/ elongate	oblong/ elliptic / lanceolate/ ovate
I	Ligule	none	short/ present	long
J	Auricle of culm sheath	none	equal/ short/ long	unequal
K	Bristles of culm sheath	none	short	dense
L	Leaf shape	narrow/linear	lanceolate	oblong/ ovate
M	Ligule of leaves	none	short	long
N	Leaf auricle	none	short	long

Results

In the present study a total of twelve genera belonging to the tribe Bambuseae were recorded. The list of the collective genera was arranged according to shouliang *et al*. 2006 and the species of each genus by alphabetical order was shown in Table 2. The resulting species are systematically described and the phylogenies of collected genera based on morphology are also mentioned (Table 3, figure 3).

Table 2. List of Collected Bamboo

No.	Scientific Name	Local Name	Locality	GPS data
1.	*Dendrocalamus asper* (Schultes) Badzer ex Heyne.	Tawwa	Mogok Township	N 22°53′5.46″ E 96°29′45.2″
2.	*Dendrocalamus brandisii* (Munro) Kurz	Lephettaungwa	Pindaya Township	N 20°50′48.08″ E 96°40′57.94″
3.	*Dendrocalumus calostachyus* (Kurz) Kwz	Wagyi	Kalaw Township	N 20°33′25.04″ E 96°36′47.30″
4.	*Dendrocalamus copelandii* Gamble exBrandis	Leikwa	Kalaw Township	N 20°33′25.02″ E 96°36′47.30″
5.	*Dendrocalamus giganteus*Wallich ex Munro	Wabogyi	Nyaungshwe Township	N 20°38′55″ E 96°56′9.02″
6.	*Dendrocalamus hamiltonii* Nees&Arnolt ex Munro	Wabomyetsan gyi	Naypyitaw Township	N 19°57′9.49″ E 95°53′15.8″
7.	*Dendrocalamus latiflorus* Munro	Wani	Nyaungshwe Township	N 20°37′20.28″ E 96°56′56.72″
8.	*Dendrocalamus longispathus* Kurz	Wanet	Magway Township	N 20°10′56.26″ E 94°50′53.43″
9.	*Dendrocalamus membranaceus* Munro	Waya	Kalaw Township	N 21°8′50.6″ E 96°26′33.39″
10.	*Dendrocalamus messri* Blattere&Nees	Waphayaung	Banmauk Township	N 20°33′25.02″ E 96°36′47.30″
11.	*Dendrocalamus pendulus* Ridley	Walaw	Ywangan Township	N 24°24′7″ E 95°49′15″
12.	*Dendrocalamus strictus* (Roxb) Nees	Hmgyinwa	Padan Township	N 19°51′34.22″ E 94°26′37.55″

1. ***Dendrocalamus asper*** (Schultes f.) Backer ex Heyne, Nutt. pl. Ned. Ind, ed. 2, vol. 1:30/ 1927. (Figure. 1)

Bambusa asper Schultes, f. 1830.

Evergreen, loosely tufted, rhizome sympodial, about 20 m height. Culms erect with pendulous tip, 15-20 m long, 10-30 cm in diameter, hollow; walls 3-5 mm thin; nodes not swollen; internodes 25-45 cm long, dark green, glabrous, smooth, white ring absent with white hairs below them, basal nodes with short aerial roots. Culm sheaths caducous, broadly triangular, 20-40 cm long, 20-25 cm wide, thickly leathery, young plant green turning yellow at the age, covered with dark brown hairs on the side, glabrous on the back; blades lanceolate, 20-30 cm long, erect, without auricle and without bristles, acuminate at the apex; ligules short, about 2 mm long, wavy with a ciliate margin. Leaves 3- to 10- grouped at the end of a branched.

2. ***Dendrocalamus brandisii*** (Munro) Kurz, Prelim Rep. Forest Pegu 94. 1875. (Figure. 2)

Bambusa brandisii Munro, Trans. L. Soc. Landon 26: 109. 1868.

Evergreen, loosely tufted, rhizome sympodial, about 25 m height. Culms erect with pendulous tip, 10-30 m long, 10-25 cm in diameter, hallow; walls 2.5-5 mm thin; nodes swollen; internodes 10-40 cm long, arch-grey to dull green, glabrous, white ring present. Culm sheaths deciduous, broadly lanceolate-oblong, 30-50 cm long, 20-35 cm wide, leathery thick, orange to bright yellow covered with shiny black hairs when young. Culm sheaths more longer than culm blade; blades lanceolate to long acuminate, 10-17 cm long, reflexed or nearly erect, with short bristle, acuminate at the apex; ligules short continuous with the sheath top, about 1-2 mm long, margin deeply dentate, auricle short . Leaves 3- to 10- grouped at the tip of the branched.

3. ***Dendrocalamus calostachyus***(Kurz) Kurz, Prelim Rep. Forest Pegu 94. 1875. (Figure. 3)

Bambusa calastachya Kurz, J.Asiat. Soc. Bengal, pl 2, Nat-Hist 42247.1873.

Evergreen, loosely tufted, rhizome sympodial, about 24 m height. Culms erect, tall, about 21-24 m long, 12- 20cm in diameter, hollow; wall 2-4 mm thin; nodes not swollen and annulate; internodes 15-35 cm long, lower most shortest, distally pubescent, white ring present. Culm sheaths broadly ovate, 20-27 cm long, 13-35 cm wide, leathery, thick, pubescent covered with oppressed tawny hairs, truncate at the apex, orange-brown coloured with pale brown hairs on young culm-sheath; blades linear-lanceolate, 6-8 cm long, erect, without bristles, pale brown coloured; ligules short, about 2 mm long and lines of dark hairs, entire with short auricle. Leaves 5-to20- grouped at the end of a branched.

4. ***Dendrocalamus copelandii***(Gamble ex Brandis) N.H. Xia & Stapleton, KewBull52 (2) : 484. 1997 (Figure. 4)

Bambusa copelandii Gamble ex Brandis, Indian Trees: 671. 1906.

Evergreen, loosely tufted, large bamboo, rhizome sympodial, about 30 m height. Culms straight and erect, about 15-30 cm long, 10-35 cm in diameter, hollow; walls 2-3 mm thin; nodes not swollen; internodes 10-40 cm long, lower ones withouthairs covered with copicuous white wax when young, furry, white ring absent. Culm sheaths broadly ovate-oblong, 25 - 40 cm long, 42-55 cm wide, thick, deciduous to persistent, coriaceous, reddish brown coloured when young covered with brown hairs, turning straw-coloured with age, glabrous, apex convexly horizontal; blades lanceolate, 10-25 cm long, erect to spreading, with auricles, continuing from the base of culm-sheath blades as fleshy, crisped lobes without bristles; ligules short, 2 mm long. Leaves 3- to 16- grouped at the tip of the branched.

6. ***Dendrocalamus giganteus***Wallich ex Munro, Trans L. Soc. 26: 150. 1868. (Figure.5)

Bambusa giganteus Hook.f., Fl. Br. Ind. 7: 406. 1897.

Evergreen, densely tufted, rhizome sympodial, the giant bamboo about 30 m height. Culms wide, pale thin wax with arching tip, 30-35 m long, 20 - 30 cm in diameter, hollow; walls 2-4 mm thin; nodes not swollen; internode 20-50 cm long, dark green, furry, covered with a white waxy layer when young, white ring present. Culm sheaths caducous, , broadly ovate-lanceolate, 20-50 cm long, 25-50 cm wide, thickly leathery, whitish to greyish green when young turning pale brown with age, with dark brown hairs on the back; blades reflexed, ovate-lanceolate, 10-30 cm long, with auricles without bristle, acuminate at the apex, pale brown coloured; ligule long, about 12 mm long, serrulate. Leaves 3- to 12- grouped at the tip of the branched.

7. ***Dendrocalamus hamiltonii***Nees&Arnott ex. Munro Trans. L. Soc. Landon 26: 151. 1868. (Figure. 6)

Evergreen, a large densely tufted, rhizome sympodial, about 25 m height. Culms erect and drooping at the top, 20-25 cm long, 15-25 cm diameter, hollow; walls 2-3 mm thick; nodes not swollen; internodes 40-50 cm long, green, glabrous, smooth, covered with a white waxy layer when young, white ring present with hairs below them, basal nodes with short aerial roots. Culm sheaths glabrous, deciduous, broadly ovate, 30-45 cm long, 15-25 cm wide, thick, greywish white coloured with pale brown hairs on young culm sheath, turning green with age, glabrous; blade ovate lanceolate, 20-25 cm long, erect, without auricle acuminate at the apex with long bristle, pale black coloured; ligules long, about 4 mm long with margin waxy and desticulate. Leaves 3- to 5- grouped at the tip of the branched.

8. ***Dendrocalamus latiflorus***Munro, Trans. L. Soc London 26: 152. 1868. (Figure.7)

Bambusa latiflora (Munro) Kurz 1873.

Sinocalamus latiflorus (Munro) McClure 1940.

Evergreen, densely tufted, rhizome sympodial, about 25 m height. Culms erect with pendulous tip, 20-25 m long, 20-45 cm in diameter, furry, green to dark green, hollow; walls 2-5 mm thick; nodes not swollen; internodes 20-60 cm long, green, glabrous, smooth, white ring present. Culm sheaths deciduous, broadly ovate, 15-35 cm long, 25-45 cm wide, hard and brittle, thick, rounded at the apex, orange-yellow when young turning pale brown with age, abaxially dull brown pubescent, margin entire; blades deflexed, ovate to lanceolate, 10-13 cm long, puberulent near the base abaxially, without bristles; ligules long, about 3-5 mm long, margin wavy, finely serrated; auricles short. Leaves 7- to 15- grouped at the tip of the branched.

9. ***Dendrocalamus longispathus***Kurz, For Fl. Brit. Burma 2: 561. 1878. (Figure. 8)

Everygreen, densely tufted, rhizome sympodial, about 20 m height. Culms slender, straight with arching at the bases, 12-20 m long, 8-12 cm in diameter, solid; walls 12 mm thick; nodes slightly swollen; internodes 25-40 cm long, dark green, furry, the lower one bearing aerial root white ring present. Culm sheaths broadly lanceolate, 35-60 cm long, 15.5-18.5 cm wide, yellow green when young turning paleyellow with age, glabrous, fragile with dark brown hairs on the back; blades lanceolate, 30-45 cm long, erect, with long auricle, acuminate at the apex, bearing brown bristles along the edge; ligules long, about 4 mm long, toothed. Leaves 5- to 7- grouped at the tip of the branched.

10. ***Dendrocala musmembranaceus*** Munro, Trans Linn. Soc Landon 26: 149. 1868. (Figure.9)

Evergreen, loosely tufted, rhizome sympodial, about 24 m height. Culms very straight, 10-24 m long, 10-20 cm in diameter, hallow; walls 2-5 mm thin, covered with white powdered deciduous scurf when young, turning green on maturity; nodes swollen; internodes 20-40 cm long, nodes prominent, basal ones with aerial roots, branches arising from all nodes, white ring absent. Culm sheaths deciduous, elliptical to oblong, usually longer than internodes, 17-75 cm long, 9-30 cm wide, thin, papery, smooth, straw-coloured, glabrous, on the smooth back with appressed dark brown hairs; blades narrowly lanceolate, 4.5-30 cm long, reflexed, without bristles brown hairy; ligules long, about 5 mm long, serrulate, auricle short. Leaves 9- to 20- grouped at the tip of the branched.

11. ***Dendrocalamus messeri*** Blattere & Nees, Linnaea 9: 476. 1835. (Figure. 10)

Evergreen, densely tufted, rhizome sympodial, about 20 m height. Culms erect, straight up to 18- 20 m long, 30- 45 cm in diameter, hollow; walls 6- 7 mm thick; nodes swollen, with aerial roots from the nodes; internodes 10-30 cm long, green, glabrous, smooth, white ring present. Culm sheaths broadly ovate- lanceolate, 18-4 cm long, 50-30 cm wide, thick, young plant pale brown to dark purplish green near apex, cover with brown hairs, turning brown with age, glabrous; blades linear lanceolate, 8-10 cm long, erect, without auricle, acuminate at the apex, without bristle; ligules long, 3-4 mm long with dentate. Leaves 7 to 17- grouped at the tip of the branched.

12. ***Dendrocalamus pendulus*** Ridley, Journ Strait Settlem Roy Asiat. Soc. 44. 210. 1905. (Figure.11)

Evergreen, densely tufted, rhizome sympodial, about 30 m height. Culms straight, erect, pendulous at the tip, 20-30 m long, 10-20 cm in diameter hollow; walls 3-6 mm thick; nodes not swollen; internodes 18-42 cm

long dark green, glabrous, smooth, white waxy ring below nodes and pale brown hairs when young. Culm sheaths deciduous, broadly oblong, about 15-25 cm long, 20-30 cm wide, thick,rigid except edges at the top, pale green at their base, yellowish to pinkish near apex, covered with dark brown hairs; blade ovate-lanceolate, 5-10 cm long, spreading (or) deflexed, with short auricle, low rims in lateral extent, bearing long bristles along the edges; ligules short, about 2 mm long, irregularly toothed. Leaves 5- to 11-grouped at the tip of the branched.

12. *Dendrocalamus strictus* (Roxb.) Nees, Linnaea 9: 476. 1835. (Figure. 12)

Bambusa stricta Roxb., Pl. Corom. 1(4): 58. 1798; Hook.f. Fl. Brit. Ind. 7: 404. 1897.

Deciduous, densely tufted with drooping branches, rhizome sympodial, about 20 m height. Culms erect, 8-16 m long, 2.5-8 cm in diameter, hollow; walls 2-3 mm thick; nodes swollen; internodes 30-45 cm long, dull green, glabrous, basal nodes often rooting, branch arising from nearly all nodes, white ring absent. Culm sheaths elongate-triangular, 9-15 cm long, 10-25 cm wide, thick, smooth, pale blue green when young, turning yellowish (or) dull green with age, glabrous; blade erect, persistent, narrowly triangular, 2-4 cm long; blade shorten than sheath, with short auricle, acuminate at the apex, golden brown hairs, on the back, without bristles; ligules short, about 2 mm long , toothed. Leaves 10- to 17- grouped at the tip of the branched.

Figure 1. Showing clump appearance, young shoot, branching type & bud of ***Dendrocalamus asper*** (Schultes f.) Backer ex Heyne.

Figure 2. Showing clump appearance, young shoot, branching type & bud of ***Dendrocalamus brandisii*** (Munro) Kurz.

Figure 3. Showing clump appearance, young shoot, branching type & bud of ***Dendrocalamus calostachyus*** (Kurz) Kurz.

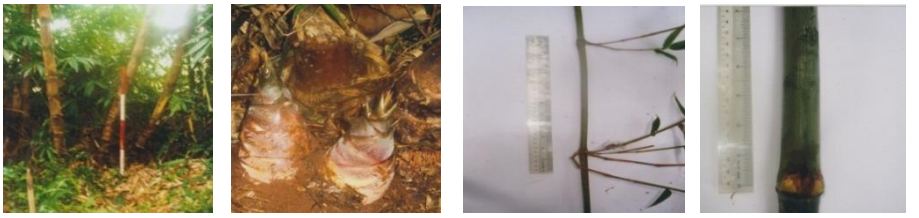

Figure 4. Showing clump appearance, young shoot, branching type & bud of ***Dendrocalamus copelandii*** Gamble ex Brandis.

Figure 5. Showing clump appearance, young shoot, branching type & bud of ***Dendrocalamus giganteus*** Wallich ex Munro

Figure 6. Showing clump appearance, young shoot, branching type & bud of ***Dendrocalamus hamiltonii*** Nees & Arnott ex Munro

Figure 7. Showing clump appearance, young shoot, branching type & bud of ***Dendrocalamus latiflorus*** Munro

Figure 8. Showing clump appearance, young shoot, branching type & bud of ***Dendrocalamus longispathus*** Kurz

Figure 9. Showing clump appearance, young shoot, branching type & bud of ***Dendrocalamus membranaceus*** Munro

Figure 10. Showing clump appearance, young shoot, branching type & bud of ***Dendrocalamus messeri*** Blattere & Nees

Figure 11. Showing clump appearance, young shoot, branching type & bud of ***Dendrocalamus pendulus*** Ridley

Figure 12. Showing clump appearance, young shoot, branching type & bud of ***Dendrocalamus strictus*** (Roxb.) Nees

Phylogenic Study

According to the characters of rhizome, branching, hollowness of culm, culm wall thickness, nodes, white ring, culm-sheath structure, shape of culm-sheath, ligule of culm-sheath, auricle of culm sheath, presence of bristle on culm-sheath, shape of leaves, presence of ligule and presence of leaf-auricle, the phylogeny of the collected genera or species of bamboos were constructed.

The phylogeny among the collected species of genus *Dendrocalamus*

Totally 12 species of the genus *Dendrocalamus* were collected in present study. The value for each characters and total data score are stated in Table 4.7. The total score for *Dendrocalamus asper* (Schultes) Badzer ex Heyne, *D. brandisii* (Munro) Kurz, *D. calostachyus* (Kurz) Kurz, *D. copelandii* Gamble ex Brandis, *D. giganteus* Wallich ex Munro, *D. hamiltonii* Nees & Arnott ex Munro, *D. latiflorus* Munro, *D. longispathus* Kurz, *D. messeri* Nees, *D. membranaceous* Munro, *D. pendulus* Ridley, *D. strictus* (Roxb.) Nees are 7.5, 9.0, 7.5, 7.0, 8.5, 8.0, 8.0, 8.0, 8.5, 9.5, 7.0 and 6.0 respectively. Most primitive and most advanced dendrogram that representing the possible relationship of the genus is stated in Figure 4.60.

According to the resulting dendrogram *D. membranaceous* Munro differs from other species. *D. messeri* Nees, *D. pendulus* Ridley and *D. strictus* (Roxb.)Nees are near to *D. longispathus* Kurz and separated from *D. hamiltonii* Nees & Arnott ex Munro, *D. latiflorus* Munro, *D. copelandii* Gamble ex Brandis, *D. giganteus* Wallich ex Munro, *D. calostachyus* (Kurz) Kurz and *D. brandisii* (Munro) Kurz. Among them *D. copelandii* Gamble ex Brandis, *D. giganteus* Wallich ex Munro and *D.calostachyus* (Kurz) Kurz are in the same level.

Table 3. The Phylogenetic Data Matrix of the Species of the Genus *Dendrocalamus*

No	Species name	Characters														Total
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	
1	*Dendrocalamusasper*(Schultes) Badzer ex Heyne	1	1	1	1	0	0	1	0.5	0.5	0	0	0.5	1	0	7.5
2	*Dendrocalamusbrandisii* (Munro) Kurz	1	1	1	1	0.5	0.5	0	1	0.5	0.5	0.5	0.5	1	0	9
3	*Dendrocalamuscalostachyus*(Kurz) Kurz.	1	0.5	1	1	0	0.5	0	1	0.5	0	0.5	0.5	0.5	0.5	7.5
4	*Dendrocalamuscopelandii* Gamble ex Brandis	1	0.5	1	1	0	0	0	1	0.5	0.5	0	1	0.5	0	7
5	*Dendrocalamusgiganteus* Wallich ex Munro	1	0.5	1	1	0	0.5	0	1	1	0.5	0	1	0.5	0.5	8.5
6	*Dendrocalamushamiltonii* Nees&Arnott ex Munro	1	0.5	1	0	0	1	0	1	1	0	0.5	1	1	0	8
7	*Dendrocalamuslatiflorus*Munro	1	0.5	1	1	0	0.5	0	1	1	0.5	0	1	0.5	0	8.0

Table 3. (Continued)

No	Species name	Characters														Total
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	
8	*Dendrocalamusiongispathus*Kurz	1	0.5	0	0	0.5	1	0	1	1	0.5	0.5	0.5	1	0.5	8
9	*Dendrocalamusmessers*BlattereNe es	1	1	1	0	0.5	0	1	1	1	0.5	0	0.5	0.5	0.5	8.5
10	*Dendrocalamusmembranaceus* Munro	1	1	1	1	0.5	1	0	1	1	0	0	1	1	0	9.5
11	*Dendrocalamuspendulus*Ridley	1	0.5	1	0	0	0.5	0	1	1	0.5	0	1	0.5	0	7.0
12	*Dendrocalamusstrictus*(Roxb) Nees	1	1	1	0	0.5	0	0	0.5	0.5	0.5	0	0	0.5	0.5	6
	Total	12	8.5	11	7	2.5	5.5	2	11	9.5	4	2	8.5	8.5	2.5	94.5
	Average score	1	0.71	0.92	0.58	0.21	0.46	0.17	0.92	0.79	0.33	0.17	0.71	0.71	0.21	7.88

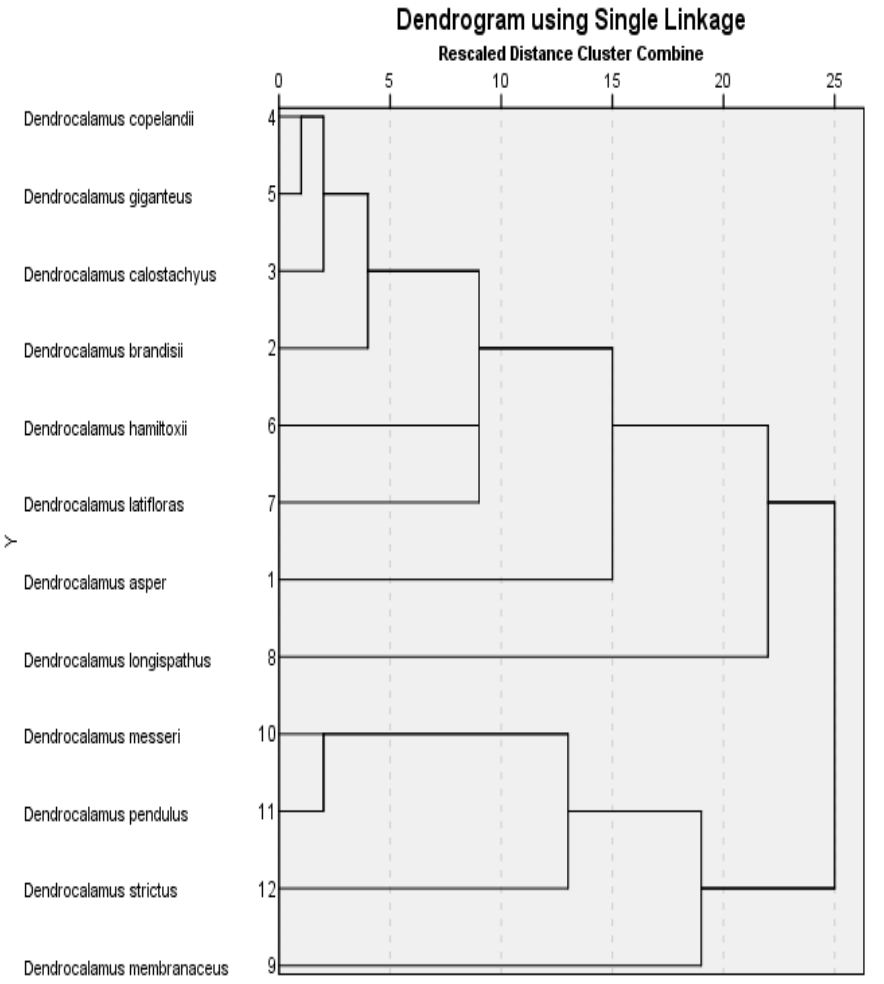

Figure 3. The Cluster Analysis of Collected Species in *Dendrocalamus*

Discussion and Conclusion

The present research deals with taxonomic study on Bamboo growing in Myanmar. It has been observed that totally 12 genera of *Dendrocalamus* were distributed in the study area. The morphology of bamboo can be used for systematic identification for various researchers.

The phylogenetic relationship among the tribe Bambuseae was studied based on the resulting morphological characteristics.

In the plants the presence of rhizome is assumed as advanced characters because it was a modification of stem. Sympodial rhizome is more advanced than the monopodial one. In the branching system, much branching type is advanced. The original stem is solid. Therefore the hollow culms are derived characters and the more thinner the culm is the more advanced in nature. In the stem the cylindrical one without swollen node is primitive and the swollen with thorn form is the advanced characters. Presence of white ring is also assumed at the derived character. In the characters of culm sheath, the thickness is the primitive character and when it is thinner species is more advanced. In addition, presence of ligule, auricle and bristles are also advanced characters and if longer, unequal and denser respectively they are more advanced of characters. In the leaf shape the narrower or linear ones are designated as primitive and the broader large ones are advanced characters. The presence of ligule and auricles in leaves are also advanced morphology of bamboos and the more longer, the more advanced.

The present study deal with 12 genera of Bamboos distributed in Myanmar. The dendrogram for phylogenetic position and relation among the genera were constructed by using the IBM-SPSS version 21 To classify, the data were input in tables of designated characters representing individual score, the hierarchical cluster analysis of genera that needed to analyze was based on nearest neighbor measured by Euclidean distance.

The taxonomy of bamboo or the science of documenting bamboo diversity at species level is the most difficult field for the construction of phylogenetic position of Bambuseae. In lower elevation with dry environment, smaller size of bamboo occurred compared to that in higher elevation of hill and humid place. In some places of the study areas economically important bamboo are cultivated.

Bamboo is one of the most important resources in Myanmar. It is linked with the life and culture of the people of the country that one will hardly find any village homestead without a bamboo grove. The identification, classification of bamboo species are wood product important for future researches. Its high valued utilization not only promotes the economic development in bamboo areas where people are of low income, but also forest resources to protect the ecological environment as a wood substitute. Therefore, the present research work will provide valuable information of bamboo morphology for compilation of the flora of Myanmar.

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ACUTE TOXICITY AND HYPOGLYCAEMIC ACTIVITY OF RHIZOME OF *COSTUS SPECIOSUS* (KOEN.) SM.

Ohn Mar[1]

Abstract

Costus speciosus (Koen.) Sm. is locally known as Phalan-taung-hmwe and belongs to the family Costaceae. The plant was collected from Hpa-an Township, Kayin State. In this study, the acute toxic study of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. was evaluated on albino mice by using method of OECD Guideline 423 (2001). There were no signs of toxicity and lethality of mice, even with the maximum dose of 5g/kg body weight of the extract during the observation period of 14 days. The hypoglycaemic activity of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. (1g/kg, 2g/kg and 4g/kg body weight) was also studied on adrenaline induced hyperglycaemic rats model by using the method of Gupta *et al.,* (1967). The results showed that the extract at dose of 4g/kg showed significant hypoglycaemic effect at 2 hr, 3hr and 4 hr ($p<0.001$) when compare with control. Therefore 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. had significant hypoglycaemic effect in rat model.

Key words: *Costus speciosus* (Koen.) Sm., Acute Toxicity and Hypoglycaemic Activity

Introduction

Costus speciosus (Koen.) Sm. belongs to the family Costaceae. It consists of 4 genera and about 150 species, pantropical in distribution, but best developed in the New World. The plants nearly always grow in wet, shady habitats (Cronquist, 1981).

The native of the plant is Indo-Malayan region, occurring from India to New Guinea (Rodriguez, 2005). *Costus speciosus* (Koen.) Sm. is locally known as Phalan-taung-hmwe in Myanmar and Indian spiral ginger in English (Hundley and Chit Ko Ko, 1987 and Kress *et al.*, 2003).

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Rhizomes are bitter, cooling and useful in aphrodisiac, anthelmintic, febrifuge, expectorant, tonic, constipation, pneumonia, rheumatism, asthma, urinary diseases, jaundice, mental disorders, skin disease and improves digestion (Malabadi *et al.*, 2016).

The most popular species in the genus is *Costus speciosus* (Koen.) Sm. which has emerged as an important antidiabetic plant (Rani *et al*., 2012). The fresh juice from the rhizomes of *Costus speciosus* (Koen.) Sm. are used by the local regions of easten Himalayan belt, Bangladesh in the treatment of diabetes (Rajesh, 2006).

Lijuan (2010) and Revathy *et al*., (2014) reported that ethanolic extract of rhizome of *Costus speciosus* (Koen.) Sm. exhibited hypoglycaemic activities on diabetic rats.

The Government of the Republic of the Union of Myanmar emphasizes on the treatment of six major diseases such as malaria, tuberculosis, diarrhoea, dysentery, hypertension and diabetes mellitus. Diabetes is one of the top priorities among these diseases. So, to fulfill one of the purposes, the present research has been carried out on this plant.

Costus speciosus (Koen.) Sm. is also claimed to possess hypoglycaemic activity. But there is no scientific research data of the acute toxicity and hypoglycaemic activity of this plant in Myanmar. Therefore, this study was aimed to evaluate the acute toxicity and to investigate hypoglycaemic activity from the rhizomes of *Costus speciosus* (Koen.) Sm.

Materials and Methods

Acute toxicity of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm.

The acute toxicity test on mice was carried out according to the method of OECD Guideline 423 (2001). Adult, 18 albino mice, Dutch Denken Yoken strain of female sex, weighting between 25 - 30g, were used for acute toxicity study. These animals were provided by the Laboratory Animal Services Division, Department of Medical Research,Yangon.

Materials

Test animals	-	18 female albino mice (ddy strain, body weight 25-30g)
Test agents	-	70% ethanolic extracts from rhizome of *Costus speciosus* (Koen.) Sm.
Apparatus	-	Mice cages, animal balance, "18" gauge intragastric needle, disposal syrings, rubber glove and mask.
Dose Schedule	-	2g/kg and 5g/kg (body weight) of 70% ethanolic extract of rhizome of *Costus speciosus* (Koen.) Sm.
Period of observation	-	Two weeks

Methods

Acute toxicity Test of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. was evaluated by the methods of OECD Guidelines 423 (2001). The animals were randomly selected and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. According to the test description, total number of 18 female albino mice (ddy strain), weighing between 25 - 30g were selected and divided into three groups. Each group contained six mice and kept in the each mouse cage. At first, the mice were individually marked with picric acid staining on the parts of the body and weighed. Required doses were calculated which based on the body weight of the mice. They were fasted for 18 hours before experiment but were allowed with free access to water. Group (I) mice served as a control group and they were administered 10ml/kg of distilled water orally. In this study, starting dose 2g/kg was chosen. Seventy percent ethanolic extract of *Costus speciosus* (Koen.) Sm. rhizome was dissolved in distilled water and the required doses were administered orally by using intragastric needle to every mouse. There were no lethality and toxic signs at the dose of 2g/kg body weight of the extract.

Thus, another 6 mice were administered 5g/kg body weight. The mice were observed for toxic sign by using the method described above. All the mice were observed to detect the delayed toxicity up to 14 days. The mortality and toxic signs during this period were recorded.

Figure 1. Weighing albino mice in

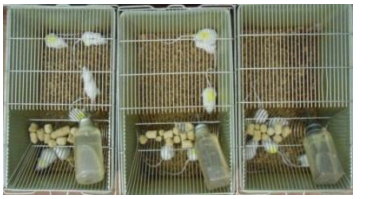

Figure 2. Groups of three mice cages

Figure 3. Administration of extract suspension

Hypoglycaemic activity of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm.

The hypoglycaemic activity of 70% ethanolic extract was also studied on adrenaline induced hyperglycaemic rats model by using the method of Gupta *et al.*, (1967) at Department of Medical Research (DMR), Yangon.

Materials

Test animals - 8 Wistar albino rats of both sexes (body weight 180 - 250g)

Test agent - Distilled water, 70% ethanolic extract, Glibenclamide tablets 5mg, India), Adrenaline injection (1mg/ml) (Myanmar Pharmaceutical Factory)

Apparatus - Aluminium cages, Animal balance, Spirit cotton wools, disposable syringes with needle (1 ml, 5 ml), Glucometer, Test strips, 18 gauge dosing needle, rubber glove and mask

Dose Schedule - 70% ethanolic extracts, 1g/kg, 2g/kg and 4g/kg body weight

Methods

Test animal profile

The study of hypoglycaemic effect of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. was performed by using the method of Gupta *et al*., (1967). Both sexes of eight adult healthy albino rats of Wistar strains weighing (180-250g) obtained from Department of Medical Research were used in this experiment. They were kept in clean and dry cages to allow for acclimatization to the laboratory conditions one week before starting the experiment. The rats were fasted overnight for 18 hours before the experiment but water was allowed freely. Firstly, they were served as control group and only distilled water was given orally to them during experiment. The study design used in this study was cross over study design in albino rats.

Preparation and administration of drug suspension

Before the experiment, individual rats were marked with picric acid, weighed and kept without food for 18 hours. The dosage was calculated according to the body weight of rat. Control animals were administered orally with 10ml/kg of distilled water. The drug suspension (i.e distilled water) was given orally to each rat by using an intragastric needle connected to a plastic syringe containing the calculated dosage. The syringe was put into the mouth about 5 cm until it reached the stomach. Then, the piston was pushed to deliver the test agents into the stomach. Immediate sneezing and coughing indicated injecting into the lungs and in such condition, the syringe was withdrawn.

Collection of blood sample and induction of hyperglycaemic of rats

Before the drug administration, the blood sample was collected by cutting about 1 mm at the tip of the tail as the base line blood sample (0hr). The glucometer test strip was inserted into the glucometer and then, one drop of the blood sample was dropped on this strip. Blood glucose concentration was measured by glucometer at 0 hour. The results were expressed in

(mg/dL). Then, these rats were orally given with distilled water (10 ml/kg) by using "18" gauge intragastric needle. Thirty minutes after administration of distilled water, these rats were subcutaneously injected with 0.4 ml/kg body weight of adrenaline to the back of the neck. Then, blood was taken from tail vein and blood glucose levels were determined hourly up to 4 hours with glucometer. After taking the blood sample, the tail of the rat was rubbed with cotton wool soaked in absolute alcohol to protect the puncture against infection.

Determination of hypoglycaemic activity of 70% ethanolic extracts (1g/kg, 2g/kg and 4g/kg body weight) from the rhizomes of *Costus speciosus* (Koen.) Sm. on adrenaline induced Hyperglycaemic rats

After one week washout period, the same 8 rats were used again and these rats were kept without food for 18 hours before experiment. Only water was allowed orally to them. After that, these rats were orally given ethanolic extract 1g/ kg body weight by using "18" gauge intragastric needle. After 30 minutes, these rats were subcutaneously injected with 0.4ml/kg body weight of adrenaline. Fasting blood was taken from tail vein and blood glucose levels were determined at 0hr, 1hr, 2hr, 3hr and 4 hours with glucometer.

Then, all the animals were allowed to rest for one week of drug free period. After washout period for one week, the same 8 rats were also tested for determination of blood glucose level with 70% ethanolic extracts, 2g/kg body weight. Determinations of blood glucose levels were performed as above procedures. After washout period of one week, the same 8 rats were tested with 70% ethanolic extracts, 4g/kg body weight for determination of blood glucose level as above procedures.

Determination of hypoglycaemic activity of standard drug (glibenclamide) on adrenaline induced hyperglycaemic rats

After drug free interval of one week, the same 8 rats were used again and these rats were kept without food for 18 hours before experiment. Fasting blood glucose levels (0hr) were taken from venous blood obtained by cutting

about 1 mm at the tip of the tail and measured by glucometer. After that, these rats were orally given with standard drug glibenclamide, 4mg /kg body weight by using "18" guage intragastric needle. After 30 minutes, these rats were subcutaneously injected with 0.4ml/kg body weight of adrenaline. Then, blood was taken from tail vein hourly at 1hr, 2hr, 3hr and up to 4 hours and determination of blood glucose levels were done with glucometer.

Data management and analysis

Standard statistical methods were used in the calculation of arithmetic mean ($\overline{X}$) standard deviation (SD) and standard error (SE). Paired student "t" tests were used to analyze the significant differences between means of control and experimental groups (Gupta *et al*., 1967).

Determination of blood glucose concentration

Percent reduction was calculated by the following formula;

$$\text{Percent reduction} = \frac{\text{Difference between rises in blood glucose level of control and test}}{\text{Blood glucose level rise in control}} \times 100$$

$$= \frac{C - T}{C} \times 100$$

C = rise in blood glucose level of control, T = rise in blood glucose level of test

Figure 4. Albino rats in cages

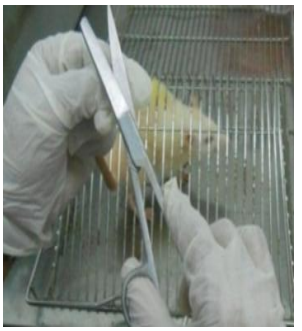

Figure 5. Cutting the tip of tail of the rat

Figure 6. Determining of blood glucose level by using glucometer

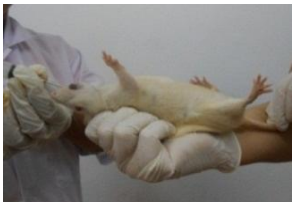

Figure 7. Administration of distilled water to rat

Figure 8. Administration of extracts suspension to rat

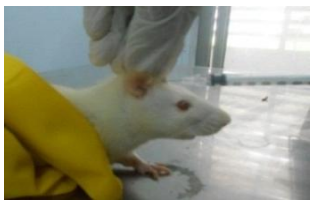

Figure 9. Adrenaline injection into nape of neck of albino rat

Results

Acute toxicity of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. on albino mice

In this study, the mice were administered with the dose of 2g/kg (body weight) and 5g/kg (body weight) of 70% ethanolic extract of rhizome of *Costus speciosus* (Koen.) Sm. Each group of mice was still alive and did not show any signs of toxicity. Even with the maximum dose of 5g/kg body weight of 70% ethanolic extracts, there was no lethality and toxic effect up to two weeks of observation period. Therefore, the extract was observed to be nontoxic. The results were shown in Table 1.

Table 1. Acute toxicity test of 70% ethanolic extracts from the rhizome of *Costus speciosus* (Koen.) Sm. on albino mice

No. of Group	Type of drug administration	No. of mice tested	Dosage	Observed period	No. of death
I	Control (distilled water)	6	10ml/kg	Two weeks	0/6
II	70% ethanolic extracts	6	2g/kg	Two weeks	0/6
III	70% ethanolic extracts	6	5g/kg	Two weeks	0/6

Hypoglycaemic activity of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. on adrenaline induced hyperglycaemic rat model

The hypoglycaemic activity of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. was tested by using adrenaline induced hyperglycaemic albino rats. Eight adult healthy Wistar strain albino rats of both sexes, weighing between 180-250 g body weights were used for this study. The results of hypoglycaemic activity were shown in Tables (2 to 7) and Figure (10).

Effect of distilled water on blood glucose levels on adrenaline induced hyperglycaemic rats model (control group)

The mean blood glucose level of the 8 albino rats given orally with distilled water 10 ml/kg body weight at 0hr, 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline 0.4 ml/kg were 70.88 $\pm$ 2.68 mg/dl, 219.75 $\pm$ 9.58 mg/dl, 249.25 $\pm$ 9.77 mg/dl, 226.88 $\pm$ 8.52 mg/dl and 210.38 $\pm$ 7.4 mg/dl respectively. It was found that blood glucose level significantly increased at 1hr, 2hr, 3hr and 4hr after injection of adrenaline 0.4 ml/kg as shown in Tables (2 and 7) and Figure (10).

Efffect of different dose levels of 70% ethanolic extracts (1g/kg, 2g/kg and 4g/kg body weight) of *Costus speciosus* (Koen.) Sm. rhizome on blood glucose level on adrenaline induced hyperglycaemic rats model

The mean blood glucose level of the 8 albino rats treated with 70% ethanolic extracts of *Costus speciosus* (Koen.) Sm. rhizome 1g/kg body weight at 0hr, 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline 0.4 ml/kg were 69.75 $\pm$ 1.52 mg/dl, 220.13 $\pm$ 7.93 mg/dl, 247.63 $\pm$ 7.24 mg/dl, 224.25 $\pm$ 7.79 mg/dl and 176.13 $\pm$ 5.79 mg/dl respectively. It was observed that the oral administration of 70% ethanolic extracts of *Costus speciosus* (Koen.) Sm. rhizome (1g/kg body weight) produced a significant decrease in glucose level at 4 hr ($p<0.001$) when compared with that of control group as shown in Tables (3 and 7) and Figure (10).

The mean blood glucose level of the 8 albino rats treated with 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. (2g/kg body weight) at 0hr, 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline 0.4 ml/kg were 68.25 $\pm$ 1.69 mg/dl, 218.75 $\pm$ 7.75 mg/dl, 244.25 $\pm$ 8.29 mg/dl, 188.5 $\pm$ 6.69 mg/dl and 161.88 $\pm$ 6.25 mg/dl respectively. It was observed that the oral administration of 70% ethanolic extracts of *Costus speciosus* (Koen.) Sm. rhizome (2g/kg body weight) produced a significant decrease in glucose level at 3hr ($p < 0.001$) and 4 hr ($p < 0.001$) when compared with that of control group as shown in Tables (4 and 7) and Figure (10).

The mean blood glucose level of the 8 albino rats treated with 70% ethanolic extracts of *Costus speciosus* (Koen.) Sm. rhizome (4g/kg body weight) at 0hr, 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline 0.4 ml/kg were 69.63 $\pm$ 2.15 mg/dl, 215.75 $\pm$ 10.66 mg/dl, 224.25 $\pm$ 10.79 mg/dl, 180.38 $\pm$ 6.53 mg/dl and 141.13 $\pm$ 4.04 mg/dl respectively. It was observed that the oral administration at 70% ethanolic extracts of *Costus speciosus* (Koen.) Sm. rhizome (4g/kg body weight) produced a significant decrease in glucose level at 2hr ($p < 0.001$), 3hr ($p < 0.001$) and 4 hr ($p < 0.001$) when compared with that of control group as shown in Tables (5 and 7) and Figure (10).

Efffect of standard drug, glibenclamide on blood glucose level in adrenaline induced hyperglycaemic rats model

The results of mean blood glucose level of the 8 albino rats treated with standard drug, glibenclamide (4mg/kg body weight) at 0hr, 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline 0.4 ml/kg were 70.75 $\pm$ 2.8 mg/dl, 188.75 $\pm$ 5.25 mg/dl, 173.63 $\pm$ 4.56 mg/dl, 148.63 $\pm$ 5.28 mg/dl and 114.38 $\pm$ 4.24 mg/dl respectively. The results of the oral administration of standard drug, glibenclamide showed that the blood glucose level of adrenaline induced rats were significant decreased at 1hr ($p < 0.005$), 2hr ($p < 0.001$), 3hr ($p < 0.001$) and ($p < 0.001$) when compared with that of control group are shown in Tables (6 and 7) and Figure (10).

Comparison of percent reductions of blood glucose level with different dose of 70% ethanolic extracts from rhizome of *Costus speciosus* (Koen.) Sm. and standard drug, glibenclamide

The comparision of mean percent reductions of blood glucose levels with 70% ethanolic extracts from the rhizome of *Costus speciosus* (Koen.) Sm. and standard drug, glibenclamide are shown in Table (8) and Figure (11).

The mean percent reduction of blood glucose level with 70% ethanolic extracts (1g/kg body weight) were -1.44 ± 3.7%, -0.07 ± 1.61%, 0.91 ± 0.9% and 23.54 ± 2.42% at 1hr, 2hr, 3hr and 4hr respectively. The mean percent reduction of blood glucose level with 70% ethanolic extracts (2g/kg body weight) were -1.74 ± 3.05%, 1.11 ± 1.58%, 23.05 ± 1.06% and 33.1 ± 2.33% at 1hr, 2hr, 3hr and 4hr respectively. The mean percent reduction of 70% ethanolic extracts (4g/kg body weight) were 2.07 ± 1.75%, 13.37 ± 2.7%, 28.88 ± 2.0% and 47.98 ± 3.24% at 1hr, 2hr, 3hr and 4hr respectively. The mean percent reduction of blood glucose level with glibenclamide (4mg/kg body weight) were 19.62 ± 3.26%, 41.55 ± 2.5%, 49.67 ± 1.74% and 68.5 ± 1.65% at 1hr, 2hr, 3hr and 4hr respectively.

Table 2. Effect of distilled water on blood glucose concentration of adrenaline induced hyperglycaemic rats (Control group)

Rat Code No.	Blood glucose concentration (mg/dl)				
	0 HR	**1HR**	**2HR**	**3HR**	**4HR**
1	65	251	273	234	212
2	60	185	229	218	195
3	71	230	283	272	255
4	67	185	205	192	183
5	69	205	231	214	209
6	72	211	236	210	203
7	80	249	272	239	216

Rat Code No.	***Blood glucose concentration (mg/dl)***				
	0 HR	***1HR***	***2HR***	***3HR***	***4HR***
8	*83*	*242*	*265*	*236*	*210*
SUM	*567*	*1758*	*1994*	*1815*	*1683*
MEAN	*70.88*	*219.75*	*249.25*	*226.88*	*210.38*
SD	*7.59*	*27.1*	*27.63*	*24.09*	*20.93*
SE	*2.68*	*9.58*	*9.77*	*8.52*	*7.4*

n=8

Table 3. Effect of 70% ethanolic extract of *Costus speciosus* (Koen.) Sm. 1g/kg on blood glucose concentration of adrenaline induced hyperglycaemic rats

Rat Code No.	**Blood glucose concentration (mg/dl)**				
	0 HR	**1HR**	**2HR**	**3HR**	**4HR**
1	67	253	260	235	170
2	62	215	239	214	165
3	72	217	275	265	204
4	69	178	209	189	150
5	71	208	241	215	177
6	77	222	237	216	175
7	71	227	261	235	192
8	69	241	259	225	176
SUM	558	1761	1981	1794	1409
MEAN	69.75	220.13	247.63	224.25	176.13
SD	4.3	22.44	20.47	22.02	16.37
SE	1.52	7.93	7.24	7.79	5.79
P value	0.647	0.948	0.631	0.201	0.000
P value	NS	NS	NS	NS	< 0.001***

n=8, Statistical comparison was made between the test group and control group

Table 4. Effect of 70% ethanolic extract of *Costus speciosus* (Koen.) Sm. 2g/kg on blood glucose concentration of adrenaline induced hyperglycaemic rats

Rat Code No.	**Blood glucose concentration (mg/dl)**				
	0 HR	**1 HR**	**2 HR**	**3 HR**	**4 HR**
1	70	243	274	205	155
2	63	210	229	180	154
3	61	206	264	217	199

Rat Code No.	Blood glucose concentration (mg/dl)				
	0 HR	**1 HR**	**2 HR**	**3 HR**	**4 HR**
4	64	189	213	160	142
5	72	201	224	185	156
6	71	213	228	170	152
7	73	248	269	202	175
8	72	240	253	189	162
SUM	546	1750	1954	1508	1295
MEAN	68.25	218.75	244.25	188.5	161.88
SD	4.77	21.93	23.44	18.92	17.68
SE	1.69	7.75	8.29	6.69	6.25
P value	0.269	0.841	0.135	0.000	0.000
P value	NS	NS	NS	< 0.001***	< 0.001***

n=8, Statistical comparison was made between the test group and control group

Table 5. Effect of 70% ethanolic extract of *Costus speciosus* (Koen.) Sm. 4g/kg on blood glucose concentration of adrenaline induced hyperglycaemic rats

Rat Code No.	Blood glucose concentration (mg/dl)				
	0 HR	**1HR**	**2HR**	**3HR**	**4HR**
1	73	252	261	197	135
2	56	180	188	173	132
3	74	228	235	204	148
4	67	178	185	159	130
5	69	190	201	161	129
6	71	212	220	165	143
7	72	247	259	201	160
8	75	239	245	183	152
SUM	557	1726	1794	1443	1129

Rat Code No.	Blood glucose concentration (mg/dl)				
	0 HR	**1HR**	**2HR**	**3HR**	**4HR**
MEAN	69.63	215.75	224.25	180.38	141.13
SD	6.09	30.15	30.51	18.48	11.44
SE	2.15	10.66	10.79	6.53	4.04
P value	0.535	0.066	0.001	0.000	0.000
P value	NS	NS	< 0.001	< 0.001***	< 0.001***

n=8, Statistical comparison was made between the test group and control group

Table 6. Effect of standard drug, glibenclamide 4mg/kg on blood glucose concentration of adrenaline induced hyperglycaemic rats

Rat Code No.	Blood glucose concentration (mg/dl)				
	0 HR	**1HR**	**2HR**	**3HR**	**4HR**
1	72	190	178	155	121
2	75	185	173	158	120
3	83	205	190	175	137
4	57	162	153	127	101
5	66	174	162	134	105
6	65	192	170	145	109
7	73	198	172	143	104
8	75	204	191	152	118
SUM	566	1510	1389	1189	915
MEAN	70.75	188.75	173.63	148.63	114.38
SD	7.91	14.84	12.91	14.94	11.98
SE	2.8	5.25	4.56	5.28	4.24
P value	0.973	0.003	0.000	0.000	0.000
P value	NS	< 0.005**	< 0.001***	< 0.001***	< 0.001***

n=8, Statistical comparison was made between the test group and control group

Table 7. Mean blood glucose concentration (Mean ± SE) of 70% ethanolic extract of *Costus speciosus* (Koen.) Sm. (1g/kg, 2g/kg, 4g/kg) and glibenclamide 4mg/kg on adrenaline induced hyperglycaemic rats model

Group of rats	Blood glucose concentration (mg/dl)				
	0 HR	1HR	2HR	3HR	4HR
Control	70.88 ± 2.68	219.75 ± 9.58	249.25 ± 9.77	226.88 ± 8.52	210.38 ± 7.4
Ethanolic extract 1g/kg	69.75 ± 1.52	220.13 ± 7.93	247.63 ± 7.24	224.25 ± 7.79	176.13 ± 5.79***
Ethanolic extract 2 g/kg	68.25 ± 1.69	218.75 ± 7.75	244.25 ± 8.29	188.5 ± 6.69***	161.88 ± 6.25***
Ethanolic extract 4 g/kg	69.63 ± 2.15	215.75 ± 10.66	224.25 ± 10.79***	180.38 ± 6.53***	141.13 ± 4.04***
Glibenclamide 4 mg/kg	70.75 ± 2.8	188.75 ± 5.25**	173.63 ± 4.56***	148.63 ± 5.28***	114.38 ± 4.24***

*P < 0.05, **P < 0.01, ***P < 0.001

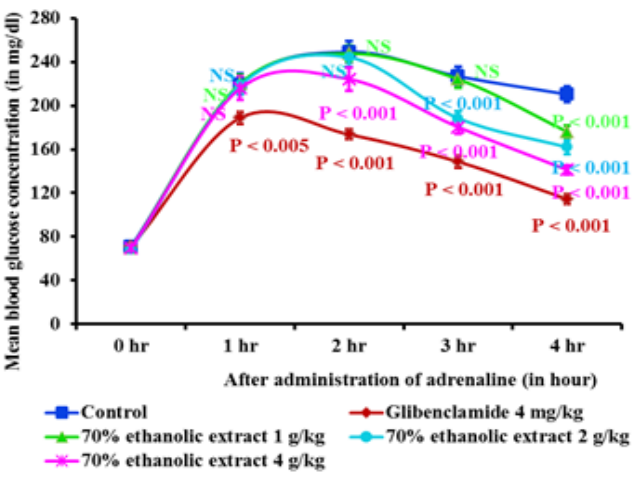

Figure10. Time course of the effect of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. (1 g/kg, 2 g/kg and 4 g/kg) and glibenclamide, 4 mg/kg on adrenaline induced hyperglycaemic rats

Table 8. Percent reduction (Mean ± SE) of hyperglycaemic with 70% extracts of rhizome of *Costus speciosus* (Koen.) Sm. and glibenclamide, 4 mg/kg on adrenaline induced hyperglycaemic rats model

Group of rats	**Percent reduction of hyperglycaemic**			
	1HR	**2HR**	**3HR**	**4HR**
Glibenclamide 4 mg/kg	19.62 ± 3.26	41.55 ± 2.5	49.67 ± 1.74	68.5 ± 1.65
Ethanolic extract 1 g/kg	-1.44 ± 3.7	-0.07 ± 1.61	0.91 ± 0.9	23.54 ± 2.42
Ethanolic extract 2 g/kg	-1.74 ± 3.05	1.11 ± 1.58	23.05 ± 1.06	33.1 ± 2.33
Ethanolic extract 4 g/kg	2.07 ± 1.75	13.37 ± 2.7	28.88 ± 2.0	47.98 ± 3.24

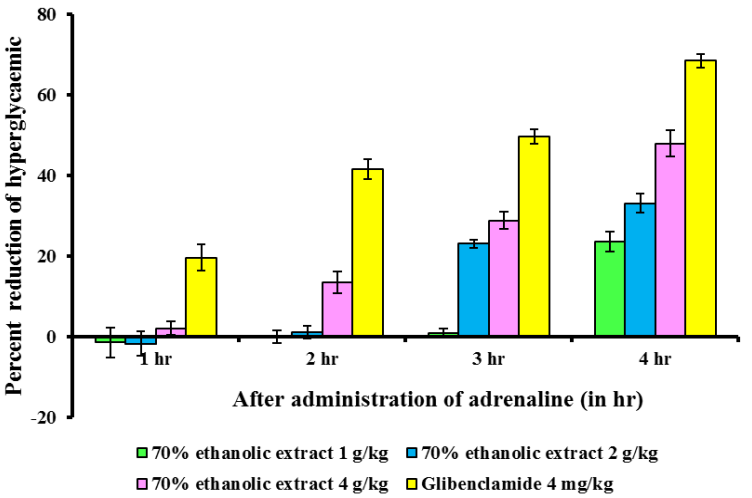

Figure11. Percent reduction of hyperglycaemic with ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. (1g/kg, 2g/kg and 4g/kg) and glibenclamide 4mg/kg on adrenaline induced hyperglycaemic rats model. n=8, Each point represents the mean of observations and the vertical bars indicate standard errors of the means

Discussion and Conclusion

In this research, acute toxicity of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. was evaluated by the methods of OECD

Guidelines 423 (2001). In this study, the mice were administered with the dose of 2g/kg (body weight) and 5g/kg (body weight) of 70% ethanolic extract of rhizome of *Costus speciosus* (Koen.) Sm. All the animals did not show any signs of toxicity and lethality during observation period of 14 days. Therefore, 70 % ethanolic extract of rhizome of *Costus speciosus* (Koen.) Sm. had no acute toxic effect on mice.

In this study, the hypoglycaemic effect of 70% ethanolic extracts from rhizomes of *Costus speciosus* (Koen.) Sm. (1g/kg, 2g/kg and 4g/kg) were also investigated on adrenaline induced hyperglycaemic rats model by using the method of Gupta *et al*., (1967). 70% ethanolic extracts 1g/kg significantly decreased the blood glucose concentration of the rats at 4 hours ($p<0.001$), 2g/kg at 3 hours and 4 hours ($p<0.001$) and 4g/kg at 2 hours up to 4 hours ($p<0.001$) after subcutaneously injection of adrenaline. These results demonstrated that 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. was able to reduce blood glucose levels in adrenaline induced hyperglycaemic rats. The hypoglycaemic effect of standard drug, glibenclamide at the dose level of 4mg/kg showed a significant reduction in blood glucose level at 1hour ($p<0.005$) and at 2 hours, 3 hours and 4 hours ($p<0.001$) after the administration of drugs on adrenaline induced hyperglycaemic rats. Hypoglycaemic effect of glibenclamide 4mg/kg was more than 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm.

The results showed that percent reductions of hyperglycaemic with 70% ethanolic extract of *Costus speciosus* (Koen.) Sm. rhizomes were 23.54% at the doses of 1g/kg, 23.05- 33.1% at 2g/kg and 13.37- 47.98% at 4g/kg. The percent reductions of hyperglycaemic with standard drug glibenclamide 4 mg/kg was 41.55-68.5%. Therefore, hypoglycaemic effect of 70% ethanolic extracts from rhizomes of *Costus speciosus* (Koen.) Sm. 4g/kg had the most effective hypoglycaemic activity among 3 doses of the extracts.

Therefore, 70% ethanolic extract of rhizome of *Costus speciosus* (Koen.) Sm. possessed significant hypoglycaemic activity. These results are agreed with those mentioned by Lijuan (2010) and Revathy *et al*., (2014).

In conclusion, 70% ethanolic extract of rhizome of *Costus speciosus* (Koen.) Sm. possessed significant hypoglycaemic activity although hypoglycaemic effect of the extract is less effective than glibenclamide. Therefore, *Costus speciosus* (Koen.) Sm. rhizome could be beneficial for the treatment of diabetes mellitus.

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ISOLATION OF *RHIZOBIUM* SP. FROM SOIL AND THEIR ANTIMICROBIAL ACTIVITY

Khin May Thi[1] , Zar Zar Yin[2]

Abstract

Soil samples were collected from ten different places of Pyin-Oo-Lwin Township, Mandalay Region, Myanmar. These samples were cultured on Rhizobium Medium (RI) and Yeast Mannitol (YM) agar medium . A total of 50 bacterium colonies were isolated from these soil samples and 31 isolated were obtained from RI Medium and 19 isolated from YM Medium. Isolated bacteria were symbolized as KM. In the colony morphology, the isolated bacteria were small, medium and large in size and color were white, yellow, pale yellow, red and brown. The margins were entire, undulated, circled, lobate and rhizoid and the elevations were raised and flat. Cell morphology of isolated strains were studied by gram staining, colony characters and shape of cell. Thirty nine bacterial strains were short rod and four strains were cocci, seven strains were rod. All isolated bacteria were Gram-negative except ten other strains were positive. All these strains were tested for preliminary study of antimicrobial activity and these strains showed the different levels of antimicrobial activity against ten test organisms. Among them, nine isolates showed the different antimicrobial activity. Especially, KM-40 showed the highest antimicrobial activity (25.99 mm) followed by KM-22 (22.73 mm) on *Bacillus subtilis* respectively.

Key words: antimicrobial activity

Introduction

Microorganisms are present in natural ecosystem such as air, soil and water. They are also present on the himself and living animals and plants. Soil contains many types of microorganisms such as bacterial, actinomycetes, fungi and algae, which are important because they affect the physical, chemical and biological properties of soil. Microorganisms in soil are important because they affect the structure and fertility of different soils (Subba, 1999). Microbes are very small living organism, so small that most of

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them are invisible. Among the soil bacteria, unique group called Rhizobia has a beneficial effect on the growth of plant.

Rhizobium is the most well known species of a group of bacteria that acts as the primary symbiotic fixer of nitrogen. These bacteria can infect the roots of leguminous plants, leading to the formation of lumps or nodules where the nitrogen fixation takes place. The bacterium's enzyme system supplies a constant source of reduced nitrogen to the host plant and the plant furnishes nutrients and energy for the activities of the bacterium. *Rhizibium* bacteria stimulate the growth of leguminous plants and they are able to fix atmospheric nitrogen into soil by interacting symbiotically with leguminous plants, using the nitrogenase enzyme complex (Kiers *et al.*, 2003).

The legume-rhizobium interaction is the result of specific recognition of the host legume by *Rhizobium.* Various signal molecules that are produced by both *Rhizobia* and the legume confer the specificity (Phillips, 1991). Exopolysaccharide (EPS) produced by *Rhizobium* is one such signal for host specificity during the early stage of root hair infection (Olivares *et al.*, 1984).

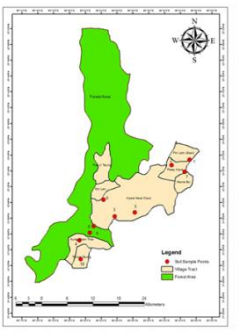
Soil bacteria called rhizobia are gram-negative capable to colonize the soil immediately surrounding roots under the influence of the plant 'rhizosphere' and reduce atmospheric nitrogen into the form available to plants through nitrogen fixation process.

Soil microorganisms specifically bacteria called rhizobia are able to colonise the rhizosphere, infect legume roots and biologically fix nitrogen in the soil through symbiotic process. Rhizobia are very important for crop production because they form symbiotic relationship with legume the process that converts atmospheric elemental Nitrogen (N_2) into accounting for 65% of the nitrogen currently utilized in agriculture. The aim and objectives of present research was to collect the soil samples from Phyin-Oo-Lwin Township, Mandalay Region, to isolate the Rizobium bacteria from these soil samples, to observe the colony morphology and cell shape of isolated bacteria by gram-staining and to study the preliminary study of antimicrobial activity on isolated bacteria with six test organisms.

Materials and Methods

Collection of soil samples

Ten different soil samples were collected from ten different places of Pyin-Oo-Lwin Township, Mandalay Region, Myanmar. This experiment was carried out at the laboratory of Biotechnology and Development Center of Pathein University.



Source-UTM 17.94-8, (Geography Dept. Pathein University)

Figure 1. Location map of Pyin-Oo-Lwin Township in Mandalay Region

Preparation of Glass wares

Pyrex glass wares were used throughout the experiments. The glass wares were treated with the chromosulphuric acid and wasted them with water. After air drying, they were sterilized in an autoclave at 15 psi and 121 Ċ at 15 minutes.

Table 1. Ten different soil samples collected at Pyin-Oo-Lwin Township

Soil samples	Collected places	Soil type	Soil pH	Location
Sample-1	Myaing Gyi	Silty clay	7.63	22' 5.434"N 96' 35.139"E
Sample-2	Pway Kauk	Clay	6.02	22' 4.73"N 96' 32.899"E
Sample-3	Nyaung Ni	Clay	5.94	21'58.221"N 96' 25.685"E
Sample-4	Kyauk	Silty clay	5.58	21' 55.207"E 96' 21.24"E

Soil samples	Collected places	Soil type	Soil pH	Location
Sample-5	Kywe Nwar	Silty clay	6.37	22' 0.355"N 96' 24.26"E
Sample-6	Si Thar	Silty clay	6.58	21' 58.221"N 96' 25.683"E
Sample-7	Htone Bo	Clay	5.97	22' 3.899"N 96' 34.045"E
Sample-8	War Bo Ye	Silty	6.37	21' 57.028"N
Sample-9	Aung Chan	Silty clay	5.46	21' 56.182"N 96' 22.569"E
Sample-10	Pyin Sar	Silty clay	6.58	21' 52.818"N 96' 21.386"E

Serial Dilutions Method of Soil Samples (Collins, 1964)

Serial dilutions of plating and streaking techniques described by Salle (1948), Collins (1964) and Pelezar and Chan (1972) were used for the isolation of bacteria species from soil. An appropriate amount (1 gm) of soil was introduced into a conical flask containing 99 mL of distilled water to make a soil-water dilution ratio of 1:100. The flask was then shaken for about 30 minutes in order to make the soil particles free from each other. This solution was then serially diluted into 10^{-1} to 10^{-5} dilution in separate test tubes and 1 ml each of the above dilutions was separately transferred into sterile petridishes under aseptic condition. A sterile pipette was used for each transfer.

The sterilized medium in conical flask was cooled down to about 45°C and separately poured into each of the petridish containing the respective soil dilution. The inoculated plates were shaken clock-wise and anti clock-wise direction for about 5 minutes so as to make uniform distribution of the bacterial inoculums. When the agar was solidified, the inoculated plates were inverted and incubated at room temperature for 24 hours. Various types of colonies developed on the inoculated plates. They were separately streaked over another set of petridishes containing the same sterile medium. Each of the discrete colonies visible in the second set of inoculated plates was separately transferred to sterile nutrient agar medium. The isolates were maintained in nutrient agar medium for further experimentations.

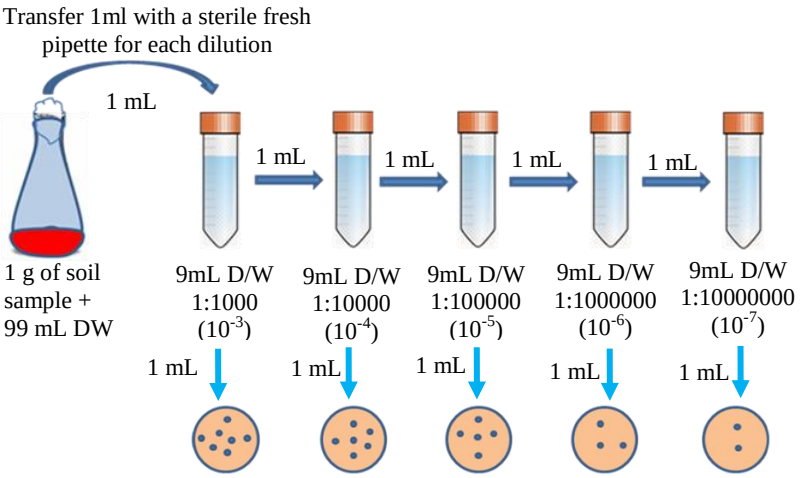


Figure 2. Serial dilution Method

Preparation of Agar Well Method

Isolated strains were subjected with antagonistic activities by agar well method. Cork borer was used to make the wells (8 mm in diameter) in the autoclaved basal antimicrobial test-medium. Wells impregnated with 24, 48 and 72 hours fermented broth (20 µL) were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured. Therefore, the diameter of clear zones has observed as potent activity shown by respective strain. Clear zones surrounding the test wells were indication of the presence of antimicrobial activities which inhibit the growth of the test organisms selectively (Collins 1965). The effectiveness of soil bacteria were rated according to modified Rukhsana Rating Scale (2011) as cited in Kyaut Kay Khaing (2012).

Isolation of pure culture from plate to slant

For pure culture from plate to test tube, about 100 mL of culture media were separately distributed in test tube. These test tubes were plugged with cotton wool and sterilized by autoclaving them at 15 pounds pressure per square inch for 15 minutes at 121ºC. The sterilized media were cooled down.

Each of the separate colonies on petridish was taken out to streak on the slant medium to obtain pure cultures (Atlas, 1993).

Preparation of culture media

Medium I **Rhizobium Medium RI** **(Atlas, 1993)**		**Medium II** **Yeast Mannitol Agar YM** **(Atlas, 1993)**	
$FeCl_3$	0.002 g	Mannitol	10 g
Yeast extract	10 g	Yeast extract	0. 4 g
K_2HPO_4	0. 5 g	K_2HPO_4	0. 5 g
$MgSO_4.7H_2O$	0. 2 g	$MgSO_4.7H_2O$	0. 2 g
NaCl	0. 2 g	NaCl	0.001 g
Agar	18 g	Agar	18 g
D/W	1000 mL	D/W	1000 mL
pH	6.8	pH	6.8

After autoclaving, Nystatin (1.5 mL) was added to the medium

Table 2. Tests organisms and Diseases

No	Tests organisms	Diseases
1	*Agrobacterium tumafaciens* NITE 09678	Plants diseases
2	*Aspergillus paracticus* IFO5123	Fruits diseases
3	*Bacillus subtilis* IFO 90571	Fever
4	*Candida albicans* NITE 09542	Alimentary tract, skin infection
5	*Micrococcus luteus* NITE 83297	Skin diseases
6	*Salmonella typhi* AHU 7943	Skin disease, food poison, wound infection, burns
7	*Staphylococcus aureus* AHU 8465	Food poisoning
8	*Escherichia coli* AHU 5436	Urinary tract infection, cholera, diarrhea and vomiting
9	*Pseudomonas fluorescens* IFO 94307	Rice spoilage
10	*Saccharomyces cerevisae* NITE 52847	Food diseases

Results

Isolation of bacterial from soil samples

Ten different soil samples were collected from Myain Gyi, Pway Kauk, Nyaung Ni, Kyauk Phyar Do, Si Thar, Kywe Nwar Dauk, Htone Bo, War Bo Ye, Aung Chan Thar and Pyin Sar villages in Pyin-Oo-Lwin Township, Mandalay Region. A total of 50 bacterial colonies were isolated from these soil samples. 31 isolated strains were obtained from medium 1 (RI) and 19 strains from medium 2 (YM). These results were shown in Table (1).

Colony Morphology of Isolated *Rhizobium* bacteria

The isolated bacteria were designated as KM 1-50. In the colony morphology, isolated bacteria were small, medium and large in size of colony and the color were white, yellow, pale yellow, red and brown.

The margin were entire, undulated and lobate. The elevation and form were raise and flat. The isolated bacterial strains were rod, short rod and cocci. Among them, 40 strains were gram-negative and 10 strains were gram-positive.

Antimicrobial activity of isolated bacterial strains

Some isolated bacterial strains were tested for antimicrobial activity to ten test organisms with agar well diffusion method and these strains showed different levels of antimicrobial activity. According to results, 2-3 days fermentation culture showed the highest activity (25.99 mm) in KM-40 and other strains were moderate activities (14.43-22.73 mm) respectively. These results were shown in table and figure.

Table 3. Colony morphology of isolated bacteria

Isolated bacteria	Size of Colony	Margin	Color	Elevation and form	Pigment on agar
KM-1	Large	Lobate	White	Flat	white
KM-2	Large	Entire	White	Flat	white

Isolated bacteria	Size of Colony	Margin	Color	Elevation and form	Pigment on agar
KM-3	Large	Undulate	White	Flat	white
KM-4	Large	Undulate	White	Flat	white
KM-5	Large	Entire	White	Flat	white
KM-6	Large	Entire	White	Flat	white
KM-7	Large	Entire	White	Flat	white
KM-8	Large	Entire	White	Flat	white
KM-9	Large	Entire	Pale	Flat	Pale
KM-10	Large	Undulate	White	Flat	white
KM-11	Large	Lobate	White	Flat	white
KM-12	Large	Lobate	White	Flat	white
KM-13	Medium	Entire	White	Flat	white
KM-14	Large	Entire	Yellow	Flat	Yellow
KM-15	Small	Entire	Yellow	Flat	Yellow
KM-16	Medium	Entire	Yellow	Flat	Yellow
KM-17	Small	Entire	Brown	Flat	Brown
KM-18	Medium	Entire	Red	Flat	Red
KM-19	Medium	Lobate	Yellow	Raised	Yellow
KM-20	Medium	Undulate	White	Flat	White
KM-21	Small	Lobate	White	Flat	white
KM-22	Small	Entire	Yellow	Raised	Yellow
KM-23	Small	Undulate	White	Flat	White
KM-24	Large	Lobate	White	Flat	White
KM-25	Large	Lobate	Yellow	Flat	Yellow
KM-26	Large	Entire	White	Flat	White
KM-27	Medium	Entire	Yellow	Flat	Yellow

Isolated bacteria	Size of Colony	Margin	Color	Elevation and form	Pigment on agar
KM-28	Small	Entire	Red	Flat	Red
KM-29	Large	Rhizoid	White	Flat	White
KM-30	Large	Rhizoid	White	Flat	White
KM-31	Medium	Undulate	White	Flat	White
KM-32	Large	Lobate	White	Flat	White
KM-33	Large	Entire	White	Flat	White
KM-34	Large	Undulate	White	Flat	White
KM-35	Large	Undulate	White	Flat	White
KM-36	Large	Undulate	White	Flat	White
KM-37	Medium	Entire	White	Raised	White
KM-38	Small	Entire	White	Raised	White
KM-39	Small	Entire	White	Raised	White
KM-40	Small	Entire	White	Raised	White
KM-41	Large	Entire	White	Flat	White
KM-42	Large	Undulate	White	Flat	White
KM-43	Medium	Curled	Yellow	Flat	Yellow
KM-44	Large	Rhizoid	White	Raised	White
KM-45	Large	Undulate	Yellow	Raised	Yellow
KM-46	Large	Lobate	Yellow	Flat	Yellow
KM-47	Large	Entire	White	Flat	White
KM-48	Large	Undulate	White	Raised	White
KM-49	Medium	Entire	Purple	Raised	Purple
KM-50	Large	Curled	Yellow	Raised	Yellow

Small < 2 mm (diameter)
Medium = between 2 mm and 5 mm (diameter)
Large > 5 mm (diameter)

Table 4. Cell morphology of isolated bacteria

Strain No.	Cell Morphology	Gram Staining	Strain No.	Cell Morphology	Gram Staining
KM-1	Short-rod	-	KM-26	Short-rod	-
KM-2	Cocci	-	KM-27	Short-rod	-
KM-3	Rod	-	KM-28	Short-rod	-
KM-4	Short-rod	-	KM-29	Short-rod	-
KM-5	Short-rod	-	KM-30	Short-rod	-
KM-6	Short-rod	-	KM-31	Short-rod	-
KM-7	Rod	+	KM-32	Short-rod	-
KM-8	Short-rod	+	KM-33	Short-rod	-
KM-9	Cocci	+	KM-34	Short-rod	-
KM-10	Rod	+	KM-35	Short-rod	-
KM-11	Short-rod	+	KM-36	Short-rod	-
KM-12	Short-rod	-	KM-37	Short-rod	-
KM-13	Short-rod	+	KM-38	Short-rod	-
KM-14	Short-rod	-	KM-39	short-rod	-
KM-15	Cocci	-	KM-40	Rod	+
KM-16	Short-rod	-	KM-41	Short-rod	-
KM-17	Short-rod	-	KM-42	Short-rod	-
KM-18	Cocci	-	KM-43	Rod	-
KM-19	Short-rod	-	KM-44	Rod	+
KM-20	Short-rod	-	KM-45	Short-rod	-
KM-21	Short-rod	-	KM-46	Short-rod	-
KM-22	Short-rod	-	KM-47	Rod	+
KM-23	Short-rod	-	KM-48	Short-rod	-
KM-24	Short-rod	-	KM-49	Short-rod	+
KM-25	Short-rod	-	KM-50	Short-rod	-

\+ = Gram-Positive
\- = Gram-Negative

KM-1-10 (Medium I, RI) | KM–11-20 (Medium I, RI) | KM-21-31 (Medium I, RI)

Figure 6. Pure culture of isolated bacteria from soil samples on the Rhizobium I medium

KM-1-10 (Medium II, YM) | KM-11-19 (Medium II, YM)

Figure 7. Pure culture of isolated bacteria from soil samples on the Yeast Mannitol Agar (YM)

Table 5. Antimicrobial activities of isolated bacterial strains on ten test organisms (2 days fermentation period)

Selected Strains	Test organisms and Antimicrobial activity (mm)									
	Test	**Test**	**Test**	**Test**	**Test**	**Test**	**Test**	**Test**	**Test**	**Test**
KM 1	14.19	-	-	9.70	16.00	11.92	15.02	16.00	12.00	13.87
KM 22	13.00	-	**22.73**	11.21	-	18.00	-	14.64	16.00	15.00
KM 28	16.00	-	-	-	15.00	14.28	14.99	14.65	14.69	17.52
KM 37	13.04	-	22.14	18.39	-	14.01	-	-	16.70	16.00
KM 40	-	20.01	**25.99**	19.14	20.42	20.02	-	18.00	19.00	21.80
KM 43	12.09	-	-	18.03	14.00	16.48	14.25	17.41	16.01	15.00
KM 45	-	-	-	17.30	14.52	16.39	14.43	18.16	16.60	17.01
KM 46	-	17.00	-	16.76	14.76	16.89	14.43	17.78	15.73	15.70
KM 50	-	22.00	-	20.00	20.15	16.11	-	17.22	20.52	18.13

Table 6. Antimicrobial activities of isolated bacterial strains on ten test organisms (3 days fermentation period)

Selected Strains	Test organisms and Antimicrobial activity (mm)									
	Test	**Test**	**Test**	**Test**	**Test**	**Test**	**Test**	**Test**	**Test**	**Test**
KM 1	14.1	-	-	-	-	-	15.1	15.4	-	10.93
KM	10.9	-	11.2	9.70	-	14.2	-	14.5	12.6	-
KM	16.0	-	-	-	-	-	12.3	14.0	12.6	15.77
KM	-	-	-	18.0	-	-	-	-	-	-
KM	-	19.26	22.0	-	13.8	14.5	-	16.3	14.8	-
KM	-	-	-	15.0	11.7	14.0	13.3	14.0	15.1	14.24
KM	-	-	-	16.5	11.0	15.0	13.4	15.9	14.5	16.01
KM	-	-	-	15.2	12.9	13.0	13.0	15.4	14.1	15.43
KM	-	-	-	19.6	14.2	18.4	-	15.0	14.8	17.37

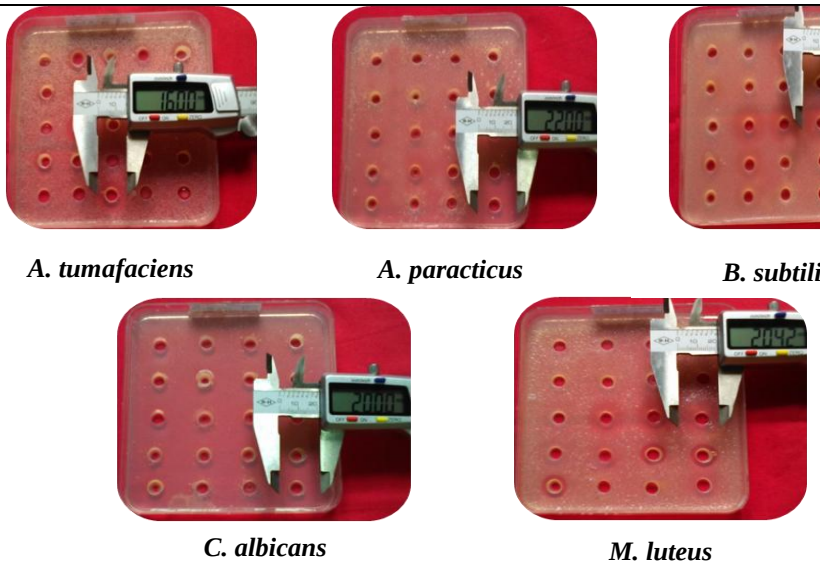


Figure 8. Antimicrobial activity of thirteen isolated bacteria on ten test organisms (2 days period)

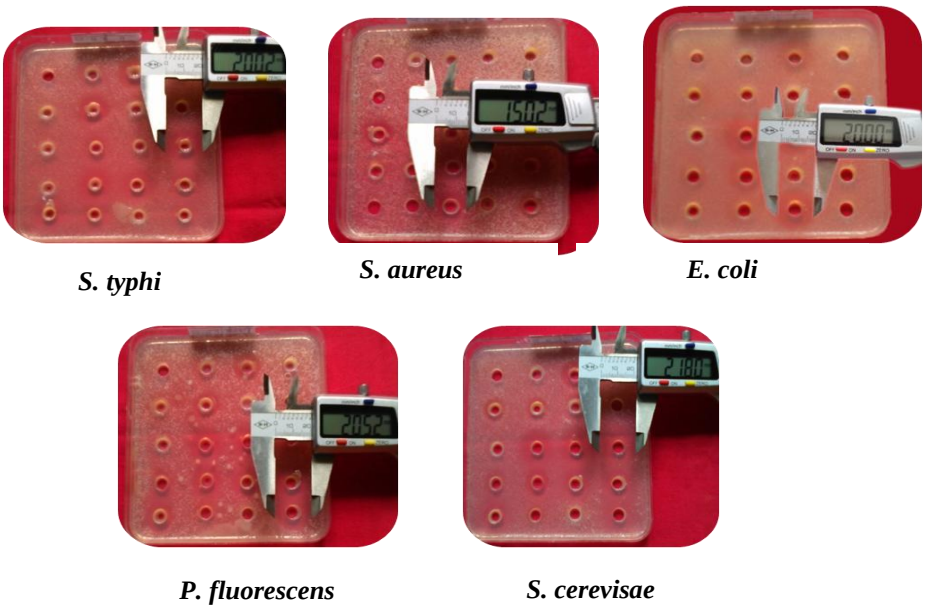


Figure 9. Antimicrobial activity of thirteen isolated bacteria on ten test organisms (3 days period)

Discussion and Conclusion

Ten different soil samples were collected from ten different places of Pyin-Oo-Lwin Township, Mandalay Region, Myanmar Freshly ten different soil samples were isolated by serial dilution method. These samples were cultured on RI medium and YM medium. A total of 50 bacterial strains were obtained. The isolated bacterial strains were designated as KM-1 to KM-50. In the colony morphology, the isolated strains were small, medium, large in size and color were white, pale yellow, yellow, brown, red and purple. The margins of colonies were entire, undulated, lobate, curled, rhizoid and the elevations were flat and raised. Some isolated bacterial strains were tested for antimicrobial activities on ten test organisms with agar well diffusion method

and these strains showed different levels of antimicrobial activities. According to the result, the highest activity was obtained in KM-40 (25.99 mm) and other strains also showed the moderate activities (14.43-22.73 mm) respectively.

Chitra *et al.*, 2013 reported that Rhizobium isolates showed antibacterial activity against *Streptococcus* and *E. coli*. Rhizobium isolates found to be the most potent strain and showed maximum diameter of inhibition zone ranges from 15 mm to 13 mm against *E. coli* and *Streptococcus* respectively. It is necessary Genes confirmation that all isolated strains must be further studied for exploitation such as biochemical characterization and fermentation procedures.

Acknowledgements

Firstly, I wish to express our gratitude to Professor Dr Si Si Hla Bu, Rector, Pathein University for providing me an opportunity to do this work. I also extended my thank to Professor Dr Than Tun and Dr Nilar Myint, Pro-Rectors, Pathein University, for their valuable instruction and guidance. I would like to record my deep thank to Professor Dr Kay Thi Mya, Head of Botany Department, Pathein University and Professor Dr Wah Wah Lwin, Department of Botany, Pathein University for their suggestion and kind understanding during this study. Many thanks are due to my supervisor Dr Zar Zar Yin, Associate Professor, Department of Botany, Pathein University, for her valuable instructions, encouragement and overall supervision for the successful completion of this research paper.

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EFFECT OF WATER HYACINTH COMPOST ON THE VEGETATIVE GROWTH AND YIELD OF *VIGNA RADIATA* L. (PE-DI-SEIN)

Zin Moe Moe[1], Soe Min Min Aye[2]

Abstract

The experiment was conducted in Pyay University Campus, Pyay Township, Bago Region. Water hyacinth, *Eichhornia crassipes* (Mart.) Solms. is a free floating weed and become a valuable soil improver as composting for crop improvement. In this experiment, the compost was made cow dung, water hyacinth, rice straw mixing at 6: 3: 1. The effectiveness of compost were studied by growing of *Vigna radiata* L., Pe-di-sein. There were four treatments and each treatment had five replications with Completely Block Design (CRD). Before experiment, the compositions of soil, cow dung, rice straw, water hyacinth and the water hyacinth compost were analyzed. The germination rate of Pe-di-sein was carried out in the mixture of soil and sand medium. The results of vegetative growth of *Vigna radiata* L. such as the maximum plant height, petiole length, number of leaves per plant, leaf width, leaf length and leaf area were observed from T_4 (10 g plant^{-1}). Moreover, T_4 (10 g plant^{-1}) gave the biggest reproductive growth like the earliest first flowering days, seeds weight per plant, seed yield, pods weight per plant and pod yield. From above the results of this experiment, water hyacinth compost would be suitable organic soil amendment for soil restoration and crop production and also for yield improvement of legume crop to apply of 10 g plant^{-1}. The utilization of water hyacinth compost resulted in several benefits to cultivars and soil fertility.

Keyword: water hyacinth compost, Completely Block Design (CRD), vegetative growth, reproductive growth

Introduction

Legumes, or pulses, are flowering plants in the leguminosae family. This family is also known as Fabaceae and has 690 genera and 18,000 species. Leguminosae family is classified into three sub-families: Papilionoideae,

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Caesalpinioideae, and Mimosoidae. *Vigna radiata* L. is originated from India. Green gram (*Vigna radiata* L.) is an erect, fast-growing, annual, herbaceous legume stems, with fulvous or brown, long, spreading or bristly pubescence. The leaves are trifoliate with ovate leaflets 5-16 long and 3-12 cm wide. The inflorescence is axillary, about 10 cm long, few to numerous flowered. Fruit is spirally dehiscent, straight, turgid with dark brown and seeds oblong-rounded, cylindrical, greenish, brown or blackish (Qi-ming, 2008).

Green gram consists of among major ten crops of Myanmar. Green gram was cultivated 1.12 million hectare at 2011-2012 and 1.14 million hectare at 2015-2016 in Myanmar. Green gram is mainly grown in Magway, Sagaing and Bago Regions. It was produced 1.32 million metric ton and was exported to India, Malaysia and Singapore (JICA, 2013).

Vigna radiata L. is a plant flavoring warm weather but requires a well distributed rainfall of 25 to 35 inches (635-889 mm). This plant is well suited to grow on deep, well drained, loamy soil, as well as on red and black soil. The land is thoroughly ploughed and manured before the seeds are sown broad cost or drilled in rows spaced 9 to 12 inches (22.86-30.48 cm) apart. The crop requires occasional weeding but no irrigation, as it is grown during the monsoon season (Pandey, 2007).

Green gram small oval seeds are highly nutritious, and the green pods are also eaten. It makes a good source of protein. The plants are used as cattle feed and also used for hay. The green gram seed is sweet, oily, laxative, tonic, diuretic and galactactagogue (Kartika and Basu, 1935).

The water hyacinth, *Eichhornia crassipes*, (Liliales, Pontederiaceae), is an invasive plant that is native of the Amazon basin and whose capacity for growth and propagation causes major conservation problems with considerable socioeconomic repercussions. It is a species of great ornamental value used in gardening because of the beauty of its foliage and flowers. Most of the problems associated with *Eichhornia crassipes* are due to its rapid growth rate, its ability to compete with other aquatic plants, and its ease of propagation. These characteristics give rise to enormous amounts of biomass

that cover the water surface of a great variety of habitats often interfering with the use and management of water resources (Tellez *et al.,* 2008). Water hyacinth as a very promising plant with tremendous application in wastewater treatment is already proved. It is used to treat waste water from dairies, tanneries, sugar factories, pulp and paper industries, palm oil mills, distilleries, etc. The water hyacinth have been found to have potential for use as phytoremediation, paper, organic fertilizer, biogas production, human food, fiber, animal fodder (Jha and Singh, 2015). Like seaweed, river grasses, water cresses, etc., the water-hyacinth has very high water content, ranging from 93 to 95 percent. Its composition varies considerably with the media in which it grows. When there is a scarcity of fertilizer elements the plant becomes diminutive, but with plenty of food the growth becomes luxurious, with a deep greenish-blue color. Fresh plant contains 95.5% moisture, 0.04% N, 0.06% P_2O_5, 0.20% K_2O, 3.5% organic matter. On a zero-moisture basis, it is 75.8% organic matter and 1.5% N (Tham, 2012). Composting water hyacinth is a good and feasible way of using harvested plants, especially in developing countries. Composting is a well-known low budget option for improving crop yields and can be carried out by mixing dried water hyacinths with soil, ash and organic municipal waste. Most important nutrients (N, P and K) are retained during the process and tolerable compost can be reached during the relatively short time period of 30 days. Decomposition of water hyacinths resulted in an increased mineralization of nutrients in the soil and as a result enhanced grain yield (Persson *et al*., 2014).

The aims and objectives of this research were to examine the role of water hyacinth compost as an organic fertilizer, to evaluate the growth and yield of *Vigna radiata* L. upon using water hyacinth compost and to distribute the knowledge of water hyacinth compost to the farmers.

Materials and Methods

Experimental Site

The experiment was conducted at the campus of Pyay University, Pyay Township, Bago Region. Soil sample was collected from the growing area of Pyay University campus before the soil preparation.

Analysis of soil sample, water hyacinth, cow dung, rice straw and water hyacinth compost

The collected soil samples, cow dung, water hyacinth, rice straw and the water hyacinth compost were analyzed in the soil laboratory, Land Use Division, Department of Agriculture, Yangon Region.

Soil Preparation

The soil from the growing area was mixed with ash in the ratio of 5:1 and the soil mix was watered and left for a week. Then the soil mixture was put into the bag.

Germination Test

Full check and almost the same size seeds are germinated in the prepared soil mixture (2:1 soil and sand) medium. The medium was put into the tray and this was divided into four plots. After that, the seeds of four plots were germinated in soil and sand (2:1) medium. One plot contained three rows and each row had ten seeds. Therefore, 120 seeds were used in the germination test. The numbers of germinating seeds were recorded. The germination rate was calculated using the method of Soupe (2009).

$$\text{Germination rate (\%)} = \frac{\text{Total No. of Germinated Seedlings}}{\text{Total No. of Cultivated Seeds}} \text{ x } 100$$

Raw materials of compost

Water hyacinth was collected from the lakes located near Pyay University campus and there were chopped into small pieces of about 2-3 cm and air-dried until the remaining of half weight. Cow dung and rice straw

were obtained from fields near by Pyay University campus. The rice straw was also chopped into 2-3 cm small pieces. Water hyacinth, cow dung and rice straw were mixed at 6:3:1 according to Dhal *et al.* (2011).

Composting process

According to Dhal *et al.* (2012), 10 kilograms of the mixture of the water hyacinth, cow dung and rice straw (6:3:1) were placed into the wood bin (length 53 cm, width 38 cm and height 31 cm). Temperature was monitored throughout the composting period. Manual turning up of compost was done in every three days throughout the composting period (Fig. 1).

Planting of *Vigna radiata* L. and Experimental layout

Five seeds of *Vigna radiata* L. were germinated in a bag. One week after sowing seeds, different rates of water hyacinth compost treatment: T_1 - control (without compost), T_2 - 8 g plant^{-1}, T_3 - 9 g plant^{-1} and T_4 - 10 g plant^{-1} were treated to the assigned plantlet. Each treatment had five replicates were laid out in a completely randomized design (CRD) (Fig. 2). The spacing between bags was 30 cm and between rows was 30 cm. Hence the total experimental area was 240 cm x 300 cm. Watering was done every day. Spraying of the organic pesticide such as Tamar pesticide, the extract of *Capsicum annum* and *Alium fruitescens* were carried out when the infestation of plants. Weeding was also carried out whenever it was necessary.

Determination of single Leaf Area

For measuring leaf area, length-width method was used in this experiment (Puttasamy *et al.,* 1976). The leaf sample was collected and measured the length and the maximum width and the area was computed as follow:

$$A = K\ L\ W$$

A = single leaf area, K = adjustment factor (0.6306)

L = leaf length, W = broadest width

Data Collection

Out of three plants in a bag, only two plants were selected for data collection. Germination rates, vegetative growth such as plant height, petiole length, number of leaves per plant, leaf width, leaf length and single leaf area, reproductive growth like first flowering days, pod length, pod width, single pod weight, pods weight per plant, and pod yield were recorded.

Results

Analysis of soil, cow dung, water hyacinth, rice straw and water hyacinth compost

Physico-chemical analysis of the soil revealed that soil was neutral with pH of 7.22. The total nitrogen was 0.46%. It had an exchangeable cation K^+ content of 1.79 meq 100 g^{-1}, an available nutrients P, 66.94 ppm (Olsen), K_2O, 84.19 mg 100 g^{-1}, moisture content of 3.83%, organic carbon, 5.64% and humus content of 10.11% (Table 1). The cow dung had the nitrogen content of 2.41%, phosphorous content of 0.36%, potassium content of 1.14%, organic carbon content of 36.85%, moisture content of 13.65% and C:N of 15.29:1 respectively. Physico-chemical analysis of the water hyacinth revealed that the total nitrogen was 2.019%, total P_2O_5 0.8122%, Total K_2O 5.20%, organic carbon 14.152%, moisture content of 46.701% and C:N of 13.42:1. Physico-chemical analysis of the rice straw stated that the total nitrogen was 0.606%, total P_2O_5 0.116 %, total K_2O 0.369%, organic carbon 9.788%, moisture content of 35.709% and C: N of 34.17:1. Physico-chemical analysis of the water hyacinth compost showed that the total nitrogen was 1.47%, total P_2O_5 1.184%, total K_2O 0.765%, organic carbon 33.527%, moisture content of 39.605%, C: N of 15.63:1 and pH 7.82 (Table 2).

Temperature and reduced weight during water hyacinth composting period

Temperature

The temperature of compost piles were recorded 3 days after composting. The initial temperature was 39°C and which drop up to 28°C

during 30 days period of composting. The mean temperature of compost pile during composting period was 32.8°C (Table 3).

Reduced weight

The reduced weight of water hyacinth compost in 5 replications expressed that out of 10 kg initial weight, the final weight became 6.8 kg in this decomposting period. Therefore, the reduced weight was 32% during 30 days period of decomposting plant materials (Table 4).

Germination test

Among 30 seeds in each plot, plot 1 had 30 germinated plants (100% of germination), plot 2 had numbers of germinated plants 27 (% of germination), plot 3, 28 germinated plants (% of germination) and plot 4, 25 germinated plants (% of germination), respectively. Therefore total germination rate is 91.67 % (Table 5).

Vegetative Growth

Plant height

According to weekly collected data, the results of plant height response to water hyacinth compost treatments showed that the longest height was 17.51 cm T_4 (10 g plant^{-1}) and it was followed by 17.11cm, T_3 (9 g plant^{-1}), then 16.79 cm, T_2 (8 g plant^{-1}) and 13.71 cm, T_1 (control) respectively (Table 6).

Petiole length

The results of petiole length response to water hyacinth compost treatments revealed that T_4 (10 g plant^{-1}) had the longest length 3.93 cm followed by T_2 (8 g plant^{-1}) 3.90 cm, then T_1 (control) 3.69 cm and T_3 (9 g plant^{-1}) had 3.25 cm respectively (Table 7).

Number of leaves per plant

The results of number of leaves per plant response to water hyacinth compost treatments showed that T_4 (10 g plant^{-1}) had much leaves 3.17. The

second highest leaf number was observed 3.12 in T_1 (Control) and 3.07, T_2 (8 g plant^{-1}) and the least number was 2.98, T_3 (9 g plant^{-1}) (Table 8).

Leaf width

The mean value of leaf width among the water hyacinth compost treatments gave that T_4 (10 g plant^{-1}) was highest leaf width 54.77 cm. It was followed by T_2 (8 g plant^{-1}) 50.94 cm, T_1 (control) 48.88 cm and T_3 (9 g plant^{-1}) had least leaf width of 45.75 cm respectively. (Table 9).

Leaf length

The result of the leaf length among the water hyacinth compost treatments showed that T_4 (10 g plant^{-1}) had highest leaf length 40.21 cm. It was followed by T_1 (control) 38.28 cm, T_2 (8 g plant^{-1}) 38.23 cm and T_3 (9 g plant^{-1}) had least leaf length of 38.07 cm respectively (Table 10).

Single leaf area

The biggest single leaf area were 56.89 cm^2 in T_4 (10 g plant^{-1}), followed by T_2 (8 g plant^{-1}) had 52.72 cm^2 and then T_1 (control) had 48.96 cm^2, T_3 (9 g plant^{-1}) had 46.33 cm^2 (Table 11).

All data collection of vegetative growth was not significant. The summarized results of vegetative growth stated the effect of water hyacinth compost on *Vigna radiata* L. that the highest plant height was 17.51 cm, the biggest petiole length 3.93 cm, the maximum number of leaves per plant 3.17, the largest leaf width 54.77 cm, the greatest leaf length 40.21 cm and the broadest single leaf area 56.89 cm^2 in T_4 (10 g plant^{-1}), respectively (Table 12 and Figure 3).

Reproductive Growth

First flowering days

The mean number of the earliest first flowering days is 49 days in T_3 (9 g plant^{-1}) and T_4 (10 g plant^{-1}), followed by 50 days in T_1 (control) and T_2 (8 g plant^{-1}), respectively (Table 13).

Single pod weight

The single pod weight showed that *Vigna radiata* L. was obtained the highest pod weight 0.65 g (T_4, 10 g plant^{-1}), followed by 0.58 g (T_3, 9 g plant^{-1}) and 0.50 g (T_2, 8 g plant^{-1}), 0.36 g (T_1, control) respectively. According to the statistical analysis revealed that all data were highly significant (Table 14).

Pods Weight per plant

The pods weight per plant of green gram had the highest 7.35 g (T_4,10 g plant^{-1}) followed by 4.50 g (T_3, 9 g plant^{-1}), 3.64 g (T_2, 8 g plant^{-1}) and 1.38 g (T_1, control) respectively. According to the statistical analysis showed that all data were highly significant (Table 15).

Pod yield

T_4 (10 g plant^{-1}) had highest pod yield 1422.96 kg ha^{-1} and T3 (9 g plant^{-1}) had second highest yield 871.20 kg ha^{-1}. T_2 (60 g plant^{-1}) produced 704.70 kg ha^{-1} and it had the third yield. Followed by T_1 (control) had 267.17 kg ha^{-1}. According to the statistical analysis showed that all data were highly significant (Table 16).

Seeds weight per Plant

The seeds weight per plant of green gram had the best weight by 2.97 g (T_4, 10 g plant^{-1}) , followed by 1.68 g (T_3, 9 g plant^{-1}), 1.26 g (T_2, 8 g plant^{-1}) and 0.4 g (T_1, control) respectively. According to the statistical analysis showed that all data were highly significant (Table 17).

Seed yield

The seed yield of green gram had the best weight 574.99 kg ha^{-1} (T4,10 g plant^{-1}) , followed by 325.25 kg ha^{-1} (T3, 9 g plant^{-1}), 243.94 kg ha^{-1} (T2, 8 g plant^{-1}) and 196.70 kg ha^{-1} (T1, control), respectively. According to the statistical analysis showed that all data were highly significant (Table 18).

All data collection of reproductive growth was highly significant. The summarized results of reproductive growth stated the effect of water hyacinth compost on *Vigna radiata* L. the maximum single pod weight 0.65 g, pods

weight per plant 7.35 g, pod yield, 1422.96 kg ha^{-1}, seed weight per plant 2.97 g and seed yield 574.99 kg ha^{-1} respectively were observed in T_4, 10 g $plant^{-1}$ (Table 19).

Discussion and Conclusion

In this experiment, the mixture of water hyacinth, cow dung and rice straw in aerobic condition for 30 days was used as compost. The growing of *Vigna radiata* L. using this compost carry out 7 days from germination to harvesting. The reduced weight of decomposed was 32% (Table 4). The initial temperature was 39°C and which drop up to 28°C during 30 days period of composting. The mean temperature of compost pile during composting period was 32.8°C (Table 3). Beesigamukama *et al*. (2018) studied that the highest temperatures were determined in the first week. In all compost treatments, temperature rapidly increased from the initial values of 26°C to peaks ranging between 30 and 43°C before beginning to slope to about 25°C throughout the third week. Thereafter, temperature changes were minimal up to the end. The temperature changes increased from an initial value of 28 to a peak value of 43°C on the second day and decreasing sharply on the eighth day. Sarika *et al.* (2014) reported that he rise in temperature was caused mainly by the metabolic heat generated during degradation of organic matter. The highest peak of temperature (59.7°C) was observed with waste composition of water hyacinth, cattle manure and sawdust (6:3:1) due to optimum proportion of raw materials. Compost accomplished ambient temperature at the end of 20^{th} day indicating the maturity of compost. It also indicated that the microbial activity has reduced due to decrease in the amount of degradable organic matter. Similar results have been obtained by Dhal *et al.* (2012) where maximum temperature was recorded as 57.3°C. Singh and Kalamdhad (2012) reported the maximum temperature of 56°C during water hyacinth composting. Bui *et al.*, (2015) stated that water hyacinth is an excellent organic fertilizer, as it contains 49% moisture, 0.56% total nitrogen, 25.5% phosphorus, 15: 1 C: N and 14.3 organic matters. The chemical properties of water hyacinth were different according to the growing area of water hyacinth which possessed

different climatic conditions. Makind and Ayoola (2012) showed that composition of cow dung was C 36%, N 1.48%, P 0.29%, K 0.75% and C:N 24:1. Cow dung is generated in large quantities from cattle farms. They also contain nitrogen, phosphorus, potassium and essential nutrients. Jusoh *et al.* (2013) revealed that rice straw as an organic waste can be converted to fertilizer throughout the process of composting and contained moisture 55%, N_2 0.49%, Phosphorus 38.2 (mg/kg), C:N 15: 1 and organic matter 12.7%. The physico-chemical analysis of water hyacinth, cow dung and rice straw expressed that the nitrogen content was high in cow dung 2.41% followed by water hyacinth 2.02% and rice straw 0.61%. Among these materials and water hyacinth compost, C: N were the best in water hyacinth, 13.41: 1 followed by cow dung, 15.29: 1, water hyacinth compost, 15.63: 1 and rice straw, 34.17: 1 (Table 2). Sanni and Adesina (2012) showed that water hyacinth compost contains N_2 2.56%, phosphorous 1.9%, potassium 1.35% and organic carbon 33%. There are two methods of decomposition: aerobic and anaerobic decomposition. In this experiment, aerobic decomposition was used by turning up in every three days intervals. Moreover, composting of plant materials such as water hyacinth and rice straw was mixed by 6:1 where three times of cow dung was added as an amendment. Therefore the compost used in this experiment became 6: 3: 1 which was in accordance with the ratio of compost used by Dhal *et al*., (2011). The applied cow dung included *E. coli*, Salmonella, coliform bacteria and fecal coliform (Gong, 2007). These bacteria helped in decomposing of plant materials quickly and efficiently**.** Batham *et al*. (2014) showed that the C:N ratio of a substrate material reflects the organic waste mineralization and stabilization during the process of composting. C:N ratio is considered as a parameter to establish the degree of maturity of compost and its agronomic quality. Decline of C:N ratio to less than 20 indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic wastes. Organic farming in agriculture preserves the ecosystem. It does not involve the use of harmful chemicals and fertilizers. Rather, symbiotic life-forms are cultured, ensuring weed and pest control and optimal soil biological activity, which maintain

fertility. The maximum plant height, the longest petiole length, the largest number of leaves, the best leaf width, the biggest leaf length and maximum single leaf area in T_4 (10 g plant^{-1}) were recorded from this experiment. Padmaja and Paulose (2010) revealed the biggest increase in plant height, the maximum increase in the number of leaves and a significant increase in root volume of green gram using water hyacinth compost, 210 mg plant^{-1}. Sanni and Adesina (2012) stated the biggest number of leaves and the largest plant height applying water hyacinth compost (60 g plant^{-1}) in the growing of spinach. The yield components data experiment showed that the earliest first flowering days, the largest single pod weight, pods weight per plant and pod yield, seed weight per plant and seed yield ^{1} in T_4 (10 g plant^{-1}) were obtained although Padmaja and Paulose (2010) reported maximum yield parameters such as number of flowers per plant, number of pods per plant, single pod weight, number of seeds per pod and hundred seeds weight of green gram were observed by 210 mg plant^{-1} water hyacinth compost. These results can be different according to plant varieties, soil texture, different organic fertilizer, environmental and weather condition. From above results of this experiment, vegetative growth and reproductive growth observed in the water hyacinth compost (10 g plant^{-1}). Ozalkan *et al*. (2010) investigated that the correlation between grain yield was significantly positive correlated with the growth parameters. In this research paper, water hyacinth compost (10 g plant^{-1}) would be suitable organic soil amendment for soil restoration and crop production and also for yield improvement of crop. There is a short point in this experiment was not using the higher value of water hyacinth compost 10 g plant^{-1}. Therefore, it is suggested that level of hyacinth compost higher than (10 g plant^{-1}) was needed to assess for any future experiment of growing green gram. The utilization of water hyacinth compost resulted in several benefits to farmer and enhanced soil fertility. However, there was a weak point in this experiment.

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Table 1. Nutrient contents of analyzed soil

Parameters	Composition
Total N (%)	0.46
Exchangeable cation, K^+ (meq 100 g^{-1})	1.79
Available nutrients, P, ppm (Olsen)	66.94
Available nutrients, K_2O (mg 100 g^{-1})	84.19
pH	7.22
Moisture (%)	3.83
Organic Carbon (%)	5.64
Humus (%)	10.11

Table 2. Nutrient contents of cow dung, water hyacinth, rice straw and water hyacinth compost

Parameters	Nutrient Contents			
	Cow dung	Water hyacinth	Rice straw	Water hyacinth compost
N (%)	2.410	2.019	0.606	1.470
P_2O_5 (%)	0.360	0.812	0.116	1.184
K_2O (%)	1.140	5.200	0.369	0.765
Organic carbon (%)	36.850	14.152	9.788	33.527
Moisture (%)	13.650	46.701	35.709	39.605
C:N	15.29:1	13.42:1	34.17:1	15.63:1
pH (1:2.5)	-	-	-	7.82

Table 3. Temperature of composting period

Days after composting	Temperature (°C)
3	39
6	39
9	38
12	35
15	32
18	31
21	30
24	28
27	28
30	28
Mean	32.8

Table 4. Reduced weight of water hyacinth compost

Compost	Bin I	Bin II	Bin III	Bin IV	Bin IV	Mean
Initial weight (kg)	10	10	10	10	10	10
Final weight (kg)	7	6	8	6	7	6.8
Reduced weight (kg)	3	4	2	4	3	3.2

Table 5. Germination rate of *Vigna radiata* L.

Plot	No. of sown seeds	Germinated plants	Germination %
1	30	30	100.00
2	30	27	90.00
3	30	28	93.33
4	30	25	83.33
Total	120	110	91.67

Table 6. Plant height of *Vigna radiata* L.treated by water hyacinth compost

Treatment	**Plant height (cm)**						
	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS	8 WAS	Mean
T_1 (Control)	11.08	13.45	15.30	16.20	17.30	18.90	13.71
T_2 (8 g plant^{-1})	12.81	14.92	16.40	18.05	18.75	19.80	16.79
T_3 (9 g plant^{-1})	12.83	15.63	16.30	18.40	19.30	20.20	17.11
T_4 (10g plant^{-1})	13.20	15.45	16.90	19.20	19.70	20.60	17.51
F- test	ns	ns	ns	ns	ns	ns	
CV%	13.1	10.9	8.2	7.0	8.1	9.9	
5 % LSD	2.24	2.24	1.83	1.74	1.11	2.72	

WAS = Weeks after sowing CV% = coefficient variation (%),

LSD = least significant difference

Table 7. Petiole length of *Vigna radiata* L.treated by water hyacinth compost

Treatment	Petiole length (cm)						
	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS	8 WAS	Mean
T_1 (Control)	2.28	3.00	3.65	4.14	4.35	4.70	3.69
T_2 (8 g plant^{-1})	2.24	2.91	4.00	4.39	4.55	5.30	3.90
T_3 (9 g plant^{-1})	1.95	2.49	3.20	3.60	3.95	4.30	3.25
T_4 (10 g plant^{-1})	2.28	3.24	4.02	4.38	4.65	5.02	3.93
F- test	ns	ns	ns	ns	ns	ns	
CV%	11.10	13.20	12.70	11.30	10.00	12.10	
5 % LSD	0.33	0.53	0.65	0.64	0.60	0.81	

Table 8. Number of leaves per plant of *Vigna radiata* L. treated by water hyacinth compost

Treatment	Number of leaves per plant						
	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS	8 WAS	Mean
T_1 (Control)	1	2	3	3.9	4.3	4.5	3.12
T_2 (8 g plant^{-1})	1	2	3	3.9	4.1	4.4	3.07
T_3 (9 g plant^{-1})	1	2	2.9	3.8	3.9	4.3	2.98
T_4 (10 g plant^{-1})	1	2	3	3.8	4.4	4.8	3.17
F- test	ns	ns	ns	ns	ns	ns	
CV%	0.00	0.00	7.80	6.80	7.60	11.00	
5 % LSD	0.00	0.00	0.32	0.36	0.44	0.68	

Table 9. Leaf width of *Vigna radiata* L. treated by water hyacinth compost

Treatment	Leaf width (cm)						
	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS	8 WAS	Mean
T_1 (Control)	2.18	7.95	8.80	9.50	9.95	10.50	48.88
T_2 (8g plant^{-1})	2.20	7.88	9.21	9.95	10.70	11.00	50.94
T_3 (9 g plant^{-1})	2.09	7.60	8.30	8.71	9.35	9.70	45.75
T_4 (10 g plant^{-1})	2.10	8.32	10.00	10.60	11.70	12.05	54.77
F-Test	ns	ns	ns	ns	ns	ns	
CV %	6.5	18.4	10.9	8.4	15.1	15.4	
5% LSD	0.19	2.01	1.36	1.13	2.18	2.30	

Table 10. Leaf length of *Vigna radiata* L. treated by water hyacinth compost

Treatment	Leaf length (cm)						
	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS	8 WAS	Mean
T_1 (Control)	4.65	5.08	5.90	6.40	7.04	9.01	38.28
T_2 (8 g plant^{-1})	4.65	5.36	6.03	6.25	6.90	10.37	38.23
T_3 (9 g plant^{-1})	4.49	5.07	5.70	6.22	7.05	9.54	38.07
T_4 (10 g plant^{-1})	4.82	5.67	6.07	6.73	7.19	9.73	40.21
F- Test	ns	ns	ns	ns	ns	ns	
CV %	13.8	10.8	11.6	9.7	13.9	15.1	
5% LSD	0.89	0.79	0.95	0.85	1.35	2.01	

Table 11. Single leaf area of *Vigna radiata* L. treated by water hyacinth compost

Treatment	Single Leaf Area (cm^2)						
	2 WAS	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS	Mean
T_1 (Control)	6.39	25.47	32.74	38.34	44.17	59.66	48.96
T_2 (8g plant^{-1})	6.45	26.63	35.02	39.22	46.56	71.93	52.72
T_3 (9 g plant^{-1})	5.92	24.30	29.83	34.16	41.57	58.35	46.33
T_4 (10 g plant^{-1})	6.38	29.75	38.28	44.99	53.05	73.94	56.89
F- Test	ns	ns	ns	ns	ns	ns	
CV %	16.20	23.60	19.10	14.90	25.80	22.00	
5% LSD	1.56	9.66	9.97	8.99	18.43	22.06	

Table 12. Summarized table of vegetative growth on the *Vigna radiata* L. treated by water hyacinth compost

Treatment	Plant height (cm)	Petiole length (cm)	No. of leaves per plant	Leaf width (cm)	Leaf length (cm)	Single leaf area (cm^2)
T_1 (Control)	13.71	3.69	3.12	48.88	38.28	48.96
T_2 (8 g $plant^{-1}$)	16.79	3.90	3.07	50.94	38.23	52.72
T_3 (9 g $plant^{-1}$)	17.11	3.25	2.98	45.75	38.07	46.33
T_4 (10 g $plant^{-1}$)	17.51	3.93	3.17	54.77	40.21	56.89

Table13. First flowering days of *Vigna radiata* L. treated by water hyacinth compost

Treatments	**First Flowering Days**
T_1 (control)	50
T_2 (8g $plant^{-1}$)	50
T_3 (9 g $plant^{-1}$)	49
T_4 (10 g $plant^{-1}$)	49

Table 14. Single pod weight per plant of *Vigna radiata* L. treated by water hyacinth compost

Treatments	Single pod weight per plant (g)
T_1 (control)	0.36
T_2 (8 g $plant^{-1}$)	0.50
T_3 (9 g $plant^{-1}$)	0.58
T_4 (10 g $plant^{-1}$)	0.65
F. test	**
CV %	38.7
5%LSD	5.28

Table 15. pods weight per plant of *Vigna radiata* L. treated by water hyacinth compost

Treatment	pods weight per plant (g)
T_1 (control)	1.38
T_2 (8 g plant^{-1})	3.64
T_3 (9 g plant^{-1})	4.50
T_4 (10 g plant^{-1})	7.35
F. test	**
CV %	38.7
5%LSD	5.28

Table 16. Pod yield (kg) of *Vigna radiata* L. treated by water hyacinth compost

Treatments	**Pod yield (kg ha^{-1})**
T_1 (control)	267.17
T_2 (8 g plant^{-1})	704.70
T_3 (9 g plant^{-1})	871.20
T_4 (10 g plant^{-1})	1422.96
F. test	**
CV %	37.33
5%LSD	5.05

Table 17. Seeds weight per plant of *Vigna Radiata* L. treated by water hyacinth compost

Treatments	Seeds weight plant^{-1} (g)
T_1 (control)	0.4
T_2 (8 g plant^{-1})	1.26
T_3 (9 g plant^{-1})	1.68
T_4 (10 g plant^{-1})	2.97
F. test	**
CV %	56.2
5%LSD	0.70

Table 18. Seed yield of. *Vigna radiata* L. treated by water hyacinth compost

Treatments	Seed yield (kg ha^{-1})
T1 (control)	196.70
T2 (8 g plant^{-1})	243.94
T3 (9 g plant^{-1})	325.25
T4 (10 g plant^{-1})	574.99
F. test	**
CV %	36.68
5%LSD	5.76

Table 19. Summarized table of reproductive growth on the *Vigna radiata* L. treated by water hyacinth compost

Treatment	First Flowering Days	Single pod weight per plant (g)	pods weight per plant (g)	Pod yield (kg ha^{-1})	Seed weight $plant^{-1}$ (g)	Seed yield (kg ha^{-1})
T_1 (Control)	50	0.36	1.38	267.17	0.4	196.70
T_2 (8 g $plant^{-1}$)	50	0.50	3.64	704.70	1.26	243.94
T_3 (9 g $plant^{-1}$)	49	0.58	4.50	871.20	1.68	325.25
T_4 (10 g $plant^{-1}$)	49	0.65	7.35	1422.96	2.97	574.99

Figure 1. Water hyacinth composting process

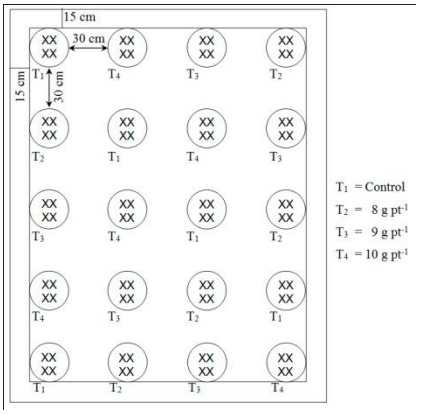

Figure 2. Experimental layout (CRD)

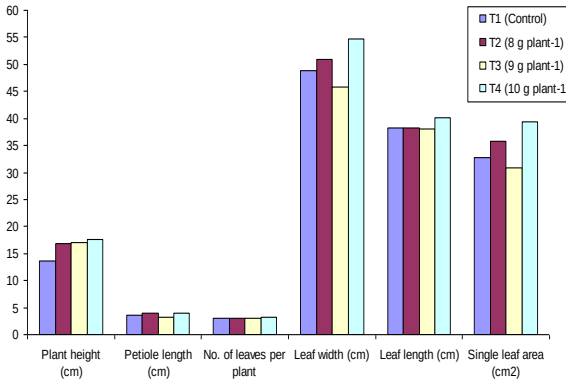

Figure 3. Summarized vegetative growth on the *Vigna radiata* L. treated by water hyacinth compost

TAXONOMIC STUDY ON SOME MEMBERS OF CHLOROPHYTA IN YENWE VILLAGE, TAUNGGYI TOWNSHIP

Ohnmar Ye Win[1], Lay Thinzar Nwe[2]

Abstract

The present study deals with some algae belonging to Chlorophyta in Yenwe village. It is located in Taunggyi Township and is famous for its warm water stream. The specimens were collected from two selected locations. Water temperature of Location I is approximately between 39˚C and 40˚C, and that of Location II is between 25˚C and 29˚C. Algal specimens were collected during June 2009 to February 2010. In this study, 24 species belonging to the 15 genera of Chlorophyta were identified, described and recorded. Keys were constructed according to their morphological characters. Green algal genera such as *Ulothrix*, *Microspora*, *Cladophora*, *Pithophora*, and *Spirogyra* were abundantly seen in Location II. *Microspora crassior* and *Microspora stagnorum* were found in Location I. *Spirogyra rhizobrachialis* was found only in Location I. *Cosmarium pachyderma* and *Cosmarium rectosporum* were found in Location I.

Keywords: Chlorophyta, Yenwe village, morphological characters, hot spring.

Introduction

Earth is often called the "watery planet" because its surface is covered by water. Only 29% of the Earth's surface is made up of land and 71% is covered by water (Manson, 1989). Billions of tiny living creatures drift like a pale mist in the waters of the world. This drifting mass of life is called plankton. Phytoplanktons are the autotrophic component of the plankton community (Thurman, 1997). Phytoplankton prouces 80% of the atmosphere's oxygen through photosynthesis. They vary seasonally in amount, increasing in spring and fall with favourable light, temperature and minerals (Emiliani, 1991). Microscopic algae and dinoflagellates are the smallest plankton organisms. Algae are ubiquitous and multitude of species

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ranging from microscopic unicells to multicells. They occur on shores and coasts, attached to the bottom (benthic species) or live suspended in the water (plankton species) (Hoek, 1998). Aquatic freshwater algae are called pond scums or, when present in great abundance, water blooms (Harold, 1957).

Chlorophyta are commonly known as green algae. This is the most diverse group of algae, with over 7,000 species. Among the oldest of all organisms, the first green algae appeared more than 2 billion years ago in the fossil recording, they are believed to be the most immediate relatives of the green plants (Peter & George, 1990). Green algae may be unicellular, multicellular, colonial or coenocytic (Pandy & Trivedi, 1996). They may occur as free living, attached, epiphytes or parasites. They may be aquatic, amphibious, terrestrial or subaerial. They can adapt to shallow water, and live in both freshwater and marine habitats. Ninety percent of Chlorophyta are freshwater species (Peter & George, 1990).

Today algae are used by humans in many ways: - fertilizers, soil conditioners, livestock feed, food, bakery, cosmetics, pharmaceuticals, leather, textile industries and so on. Algae are nutritious because of their high protein content and high concentrations of minerals, trace elements and vitamins. They have been used for centuries especially in Asian countries. Algae tolerate a wide range of temperature. They can be found growing in hot springs, on snow banks, and also grow on the sea bed, beneath a thick blanket of Arctic or Antarctic sea ice and in the driest deserts (Pandy & Trivedi, 1996).

In Myanmar, the hot springs can be found in some places and Yenwe Village is one of these places. The present study is expected to explore the various algae which are adaptable to not very high temperatures. The research study concerning with this area have not been explored.

Materials and Methods

Study Areas and Sample Collection

Specimens were collected from two selected locations in Yenwe Village during the year 2009 – 2010. It is located in Taunggyi Township, Southern Shan State (N 20˚45′ 22″, E 96˚ 54′ 48″). Its area is 581.559 acres. Water samples were taken from the upper surface and shallow area of the pond and streams. Some were from moist soils. The algal samples were placed under good aeration and favourable light conditions. Water temperatures were measured by thermometer.

Preparation of the Samples for Identification

Fresh and pure specimens were placed on the glass slides and covered with cover slits Measurements were done by using ocular micrometers. The morphological description, classification, and nomenclature were conducted according to the literatures: Tiffany (1937), Presscott (1962), Shirota (1966), Han Maosen Shu Yunfang (1995) & Dillard (1989 – 2000).

Results

In this study, 24 species belonging to 15 genera of Chlorophyta has been identified, described and recorded. Artificial keys: - order keys, genera keys and species keys were made primarily.

Class Chlorophyceae

Key to the Order:

1. The wall is two sections which adjoin at the midregion, constriction or sinus present between the two cell halves (semicells); the walls are often ornamented with spines, teeth, granules, verrucae, sacrobiculations, ---------- Desmidiales

1. The walls not as above ---------- 2

2. Filamentous occurring as coenotypes without cross walls, except where reproductive structures are cut off ---------- Siphonales

2. Plants not as above ---------- 3

3. Filament composed of cells adjoined end to end in definite series, sometimes interrupted ---------- 4

3. Plants filamentous, composed of cells arranged to form filaments; unicellular or colonial, cells varies in shape, spherical, pyramidal, or polygonal, incapable of division in the vegetative state ---------- Chlorococcales

4. Filament unbranched; attached or free – floating ---------- 5

4. Filament with branches, the branches sometimes closely appressed, forming pseudoparenchymatous masses ---------- 7

5. Chloroplast 1 to several, large in the form of spiral band, stellate masses, or plate; pyrenoids conspicuous; reproduce by conjugation ---------- Zygnematales

5. Chloroplast parietal, plate – like, net – like, or small or ovate; reproduce by iso – or heterogametes ---------- 6

6. Cells with a single, parietal, plate – like or broadly discoid chloroplast; cells cylindrical; filaments uniseriate or multiseriate; one or more pyrenoids; isogametes and zoospores are produced in unspecialized cells ---------- Ulotrichales

6. Cells with net – like or sheet – like chloroplast which usually cover both the end and lateral walls; wall composed of two sections which overlap in the midregion, forming H – pieces upon fragmentation; pyrenoids lacking; sexual reproduction unknown ---------- Microsporales

7. Filament composed of cylindrical or rectangular, uninucleate cells; branches which usually are not distinctly bulbous at the base ---------- Chaetophorales

7. Filament composed of cylindrical, multinucleate cells; branches which may become attenuated towards their apices ---------- Cladophorales

Order – Ulotrichales

Family – Ulotrichaceae

Key to the Genera:

1. Filaments often show basal differentiation ---------- 2

1. Filaments not usually show basal differentiation ---------- 3

   2. Chloroplast a parietal band with nearly encircling the cell, protoplast unspecialized cells ---------- *Ulothrix*

   2. Chloroplast a laminate forming a band in the midregion of the cell, protoplast in pairs ---------- *Binuclearia*

3. Cells cylindrical, undifferentiated ---------- *Hormidium*

3. Cells spheroidal broadly ovoid, usually separated from each other ---------- *Geminella*

Ulothrix Kuetzing

Key to the Species:

1. Filaments curved, cells elongate – cylindric, 11.0 – 12.5 µm in diameter 2¼ times longer than wide ---------- *Ulothrix cylindricum*

1. Filaments slender, cells cylindrical, 4.5 – 6.0 µm in diameter & up to 15 µm long ---------- *U. variabilis*

***Ulothrix cylindricum* Prescott.** (Fig. 1) – Filaments long, curved, and lightly entangled. Cells elongate – cylindric, 11.0 – 12.5 µm in diameter, 2¼ times longer than wide; the wall thin and not constricted at the joints. Chloroplast a broad band, nearly equal to the cell in length and folded around ¾ of the circumference; pyrenoids 2.0 – 5.0 µm.

***U. variabilis* Kuetzing** (Fig. 2) – Filaments long, slender, and entangled, forming cottony masses. Cells cylindrical, without constrictions at the cross walls. Chloroplast a folded,parietal plate, ½ to ⅔ the length of the cell, with 1 pyrenoid. Cells 4.5 – 6.0 µm in diameter & up to 15.0 µm long.

Hormidium Kuetzing

***Hormidium subtile* (Kuetz.) Heer.** (Fig. 3) – Long unbranched filaments with no basal – distal differentiation. Cells small, no constricted at the cross walls, chloroplast covering only a small portion of the cell wall. Cells 5.0 – 7.0 µm long 1.5 - 3.0 µm wide.

Geminella Turpin

***Geminella crenulatocollis* Prescott** (Fig. 4) – Uniseriate filaments, oblong cells inclosed by a broad gelatinous sheath and usually separated from each other. Chloroplasts folded parietal plate. Cells 12.0 – 15.0 µm in diameter, 18.0 – 24.0 µm long.

Binuclearia Wittrock

***Binuclearia tatrana* Wittrock** (Fig. 5) – Filaments of long cylindrical cells. Protoplasts oblong with rounded apices; not filling the cells, in pairs. Cells 7.0 – 10.0 µm in diameter, the length sometimes 6 times the width.

Order Microsporales

Family Microsporaceae

Microspora Thuret

Key to the Species:

1. Cell walls thick, the sections evident, 26.0 – 28.0 – (33.0) µm in diameter, 28.0 – 38.0 µm long ---------- *Microspora crassior*

1. Cell walls thin, sections not evident ---------- 2

2.Cells slightly swollen, 14.0 – 17.0 µm in diameter, 22.0 – 29.0 (35.0) µm long; chloroplast usually reticulate ---------- *M. floccose*

2. Cells slightly constricted at the cross walls, 9.0 µm wide, 10.0 – 225.0 (27.0) µm long; chloroplast a granular sheet ---------- *M. stagnorum*

***Microspora crassior* (Hansg.) Hazen** (Fig. 6) – Cell walls thick, the sections evident at the juncture in the mid – region of the cell. Cells cylindrical or

slightly swollen, very slightly constricted at the cross walls, 26.0 – 28.0 – (33.0) µm in diameter, 28.0 – 38.0 µm long. Chloroplast densely granular and covering the entire cell wall.

***M. floccosa* (Vauch.) Thuret** (Fig. 7) – Walls relatively thin, sections not always evident in the mid – region of the cell. Cells cylindrical or slightly swollen; 14.0 – 17.0 µm in diameter, 22.0 – 29.0 (35.0) µm long. Chloroplast usually reticulate.

***M. stagnorum* (Kuetz.) Lagerheim** (Fig. 8) – Walls thin, the two sections not evident. Cells cylindrical or slightly constricted at the cross walls, as much as 3 times their diameter in length; 9.0 µm wide, 10.0 – 225.0 (27.0) µm long. Chloroplast a granular sheet, incompletely covering the wall.

Order Chaetophorales

Family Chaetophoraceae

Key to the Genera:

1. Parenchymatous prostrate system, chloroplast transversely zonate with several pyrenoids ---------- *Stigeoclonium*

1. Pseudoparenchymatous disc of horizontally growing filaments, chloroplast a parietal disc with one pyrenoid ---------- *Protoderma*

Stigeoclonium Kuetzing

***Stigeoclonium longipilum* Kuetzing** (Fig. 9) – Filaments elongate, some branches dichotomous but mostly opposite, arising from node – like zones where a series of 2 or more swollen cells in the main axis develops pairs of branches; branches long and tapering to form slender. Cells short, 1.0 – 3.0 (5.0) µm in diameter, 11.0 – 15.0 (19.0) µm long.

Protoderma Kuetzing

***Protoderma viride* Kuetzing** (Fig. 10) – Thallus an attached disc, irregular in outline, made up of branched filaments which are compact and parenchymatous internally but semi – radiate and spreading at the margin;

terminal cells slightly narrowed. Cells quadrate or cylindrical with thin walls; 3.0 – 6.0 µm in diameter, 10.0 – 15.0 µm in length.

Order Cladophorales

Family Cladophoraceae

Key to the Genera:

1. Filaments repeatedly branched, show distinct basal – distal differentiation, akinetes lacking ---------- *Cladophora*

1. Filaments not repeatedly branched, not clearly show basal – distal differentiation, akinetes cells frequent ---------- *Pithophora*

Cladophora Kuetzing

Key to the Species:

1. Plants free – floating, branches not crowded in the upper limits ---------- 2

1. Plants attached, branches usually crowded in the upper limits ------ *Cladophora glomerata*

   2. Filaments successively branched, cells cylindrical ---------- *C. crispata*

   2. Filaments irregularly branched, cells irregularly swollen ---------- ----- *C. fracta*

***Cladophora crispata* (Roth) Kuetzing** (Fig. 11) – Free – floating except when young, forming rather delicate thalli of successively branched filaments with long, cylindrical cells, gradually attenuated in the branched to slightly narrowed but rounded apices. Main axis 40.0 – 75.0 µm in diameter; branches 20.0 – 35.0 µm in diameter. Cells up to 20 times their diameter in length. Cell walls relatively thin.

***C. fracta* (Dillw.) Kuetzing** (Fig. 12) – Floating, irregularly branched filaments, 60.0 – 120.0 µm in diameter in the main axis, 1 – 3 times their diameter in length; 20.0 – 40.0 µm in diameter in the ultimate branches, 3 – 6 times their diameter in length.

***C. glomerata* (L.) Kuetzing (**Fig. 13) – Thallus successively branched filaments with long, cylindrical cells, gradually attenuated in the branches to slightly narrowed but rounded apices. Main axis 50.0 – 60.0 µm in diameter, branches 20.0 – 25.0 µm in diameter.

Pithophora Wittrock

***Pithophora oedogonia* (Mont.) Wittrock** (Fig. 14) – Filaments slender, 45.0 – 70.0 µm in diameter; branching mostly solitary, rarely opposite. Cells long and cylindrical; as much as 20 times their diameter in length. Akinetes cylindrical, or slightly swollen to cask – shaped, conical, or more often acuminate, when terminal, 57.0 – 144.0 µm in diameter, 95.0 – 380.0 µm long.

Family Hydrodictyaceae

Hydrodictyon Roth

***Hydrodictyon reticulatum* (L.) Lagerheim** (Fig. 15) – Thallus macroscopic with cylindrical cells. Chloroplasts much diffused reticulum, light yellow – green color in the plant mass, meshes pentagonal or hexagonal. Cells up to 200 µm in diameter and up to 1 cm long.

Order Chlorococcales

Family Oocystaceae

Ankistrodesmus Corda

***Ankistrodesmus falcatus* (Corda) Ralfs** (Fig. 16) – Cells needle – like to somewhat spindle – shaped, or narrowly fusiform; solitary or clustered in fascicles, sometimes straight, usually curved, often twisted about one another; without gelatinous envelope. Chloroplast a thin parietal plate covering most of the cell wall; pyrenoid present or absent.

Order Siphonales

Family Vaucheriaceae

Dichotomosiphon Ernst

***Dichotomosiphon tuberosus* (A. Braun) Ernst** (Fig. 17) – Dichotomously branched, chloroplast numerous, without pyrenoids. Filaments dark – green color, 40.0 – 110.0 µm in diameter and up to 10 cm in length.

Order Zygnematales

Family Zygnemataceae

***Spirogyra* Link**

Key to the Species:

1. Filaments less than 90 µm in diameter ---------- 2

1. Filaments more than 90 µm in diameter ---------- *Spirogyra jugalis*

   2. Cells 70.0 – 77.0 µm in diameter, chloroplast 4 ---------- *S. cylindrospora*

   2. Cells 40.0 – 45.0 µm in diameter, chloroplast 3 ---------- *S. rhizobrachialis*

***Spirogyra cylindrospora* W. & G.S. West** (Fig. 18) – Vegetative cells cylindrical, 70.0 – 77.0 µm in diameter, 100.0 – 300.0 µm long, with plane end walls; chloroplasts 4, making 1 – 3 turns, with crenate margins.

***S. rhizobrachialis* Jao** (Fig. 19) – Vegetative cells cylindrical, 40.0 – 45.0 µm in diameter, 115.0 – 240.0 µm long, with plane end walls; chloroplasts 3, making 1.5 – 2.5 turns, with crenate margins.

***S. jugalis* (Fl. Dan.) Kuetzing** (Fig. 20) – Vegetative cells cylindrical, 90.0 – 100.0 µm in diameter, 1 - 1½ times the diameter in length; chloroplasts 3 – 4, making 1 – 2 turns, wall layers smooth.

Order Desmidiales

Family Desmidiaceae

Key to the Genera:

1. Cells without a median constriction, attenuated from the midregion to narrow ---------- *Closterium*

1. Cells with an obvious median constriction, always without extended processes ---------- *Cosmarium*

Closterium Nitzsch

Key to the Species:

1. Cells nearly straight, 80.0 – 100.0 µm wide, 950.0 – 970.0 µm long ---------- *Closterium acerosum*

1. Cells slightly curved, 25.0 – 40.0 µm wide, 280.0 – 320.0 µm long ---------- *C. balliyanum*

***Closterium acerosum* (Schrank) Ehrenberg** (Fig. 21) – The cell is straight, narrowly fusiform, 80.0 – 100.0 µm wide, 950.0 – 970.0 µm long, gradually tapering to the apices; chloroplast with longitudinal ridges and scattered pyrenoids.

***C. balliyanum* Brebisson** (Fig. 22) – The cell nearly straight, slightly curved, 25.0 – 40.0 µm wide, 280.0 – 320.0 µm long, slightly attenuated to the broadly truncate apices; chloroplast with 5 – 8 pyrenoids in series.

Cosmarium Cordaex Ralfs

Key to the Species:

1. Cells truncate pyramidal in shape, 15.0 – 20.0 µm wide, 18.0 – 26.0 µm long ---------- *Cosmarium rectosporum*

1. Cells reniform in shape, 50.0 – 87.0 µm wide, 72.0 – 117.0 µm long ----- *C. pachyderma*

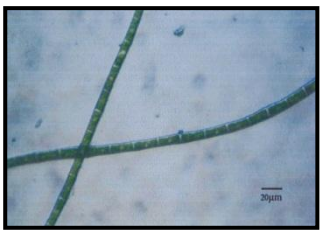
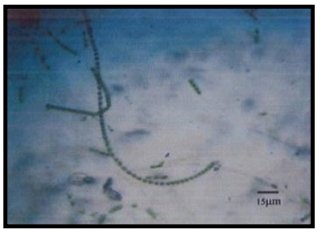
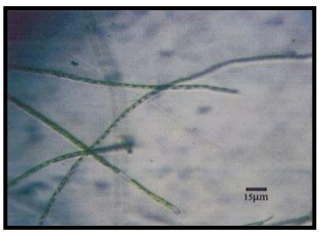
***Cosmarium pachyderma* Lund** (Fig. 23) – Semicells reniform in shape, cell wall smooth, median constriction deep; cells 50.0 – 87.0 µm wide, 72.0 – 117.0 µm long; 1 pyrenoid in each semicell.

***C. rectosporum* Turner** (Fig. 24) – Semicells reniform or truncate pyramidal in shape, lateral margins and apical angles slightly rounded, apex narrow and flat, sinus closed; cells 15.0 – 20.0 µm wide, 18.0 – 26.0 µm long; 1 pyrenoid in each semicell.

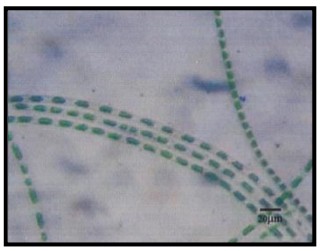
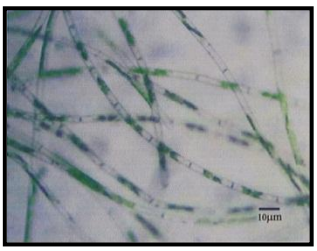
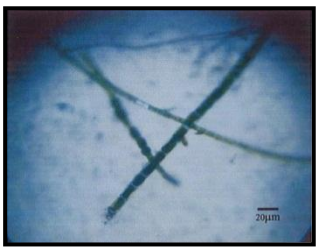
The identified specimens in Chlorophyta were expressed in Table 1.

Table 1. Classification of Chlorophyta found in Yenwe Village

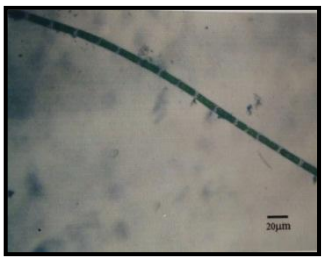
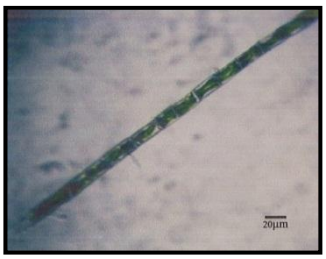
Class	Order	Family	Genus	Species
Chlorophyceae	Ulotricales	Ulotricaceae	*Ulothrix*	*U. cylindricum*
				U. variabilis
			Hormidium	*H. subtile*
			Geminella	*G. crenulatocollis*
			Binuclearia	*B. tatrana*
	Microsporales	Microsporaceae	Microspora	*M. crassior*
				M. floccosa
				M. stagnorum
	Chaetophorales	Chaetophoraceae	*Stigeoclonium*	*S. longipilum*
			Protoderma	*P. varide*
	Cladophorales	Cladophoraceeae	*Cladophora*	*C. crispata*
				C. fracta
				C. glomerata
			Pithophora	*P. oedogonia*
		Hydrodictyaceae	*Hydrodictyon*	*H. reticulatum*
	Chlorococcales	Oocystaceae	*Ankistridesmus*	*A. falcatus*
	Siphonales	Vaucheriaceae	*Dichotomosiphon*	*D. tuberosus*
	Zygnematales	Zygnemataceae	*Spirogyra*	*S. cylindrospora*
				S. rhizobrachiats
				S. jugalis
	Desmidiales	Desmidiaceae	*Closterium*	*C. acerosum*
				C. baillyanum
			Cosmarium	*C. pachyderma*
				C. rectosporum

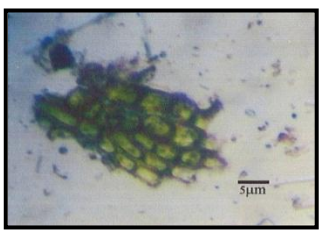
1. *Ulothrix cylindricum* Prescott 2. *Ulothrix variabilis* Kuetzing 3. *Hormidium subtile* Heer

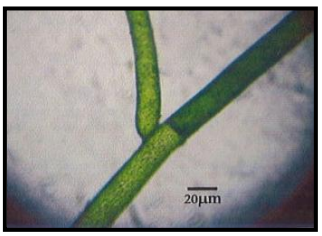




4.*Geminella crenulatocollis* Prescott 5.*Binuclearia tatrana* Wittrock 6. *Microspora rassior*Hazen


7.*Microspora floccose* Thuret 8. *Microspora stagnorum* Lag. 9.*Stigeoclonium longipilum*

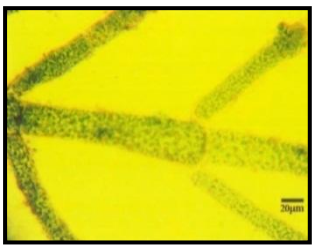
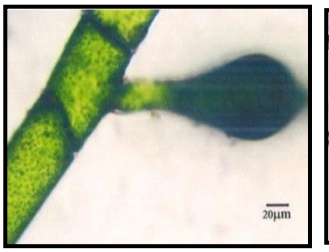
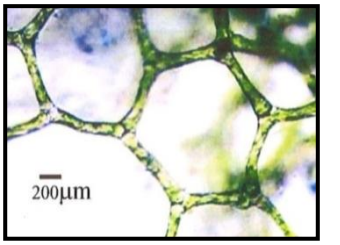




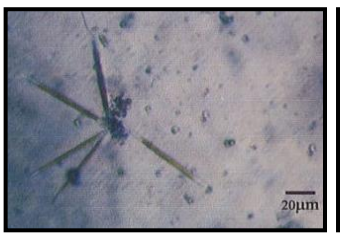
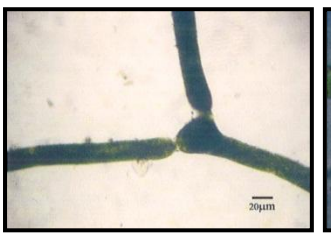
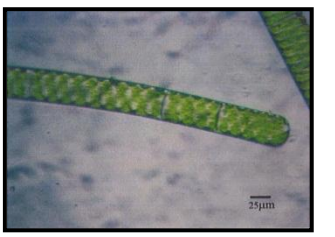
Kuetz.

10. *Protoderma viride* Kuetz. 11.*Cladophora crispate* Kuetz. 12. *Cladophora fracta*

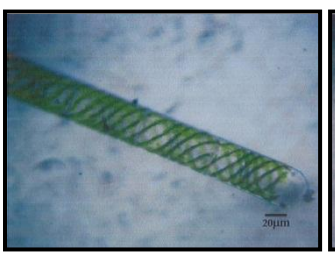
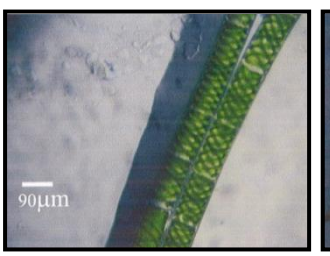
Kuetz.

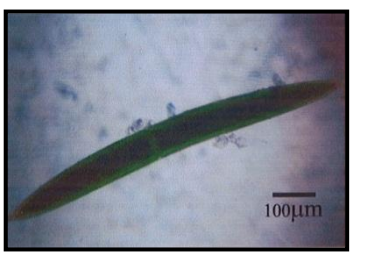




13. *C.glomerata* Kuetz. 14.*Pithophora oedogonia* Witt. 15.*Hydrodictyon eticulatum* La.

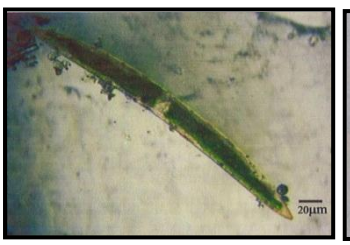
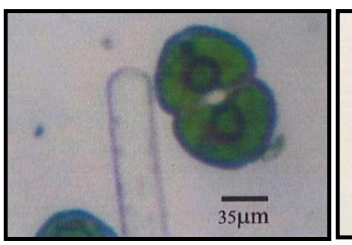




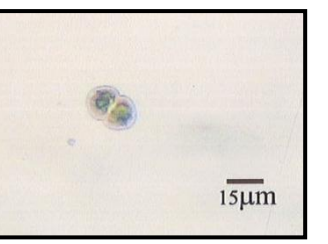
16.*Ankistrodesmus falcatus*Ra. 17.*Dichotomosiphon tuberosus*Er. 18.*Spirogyra cylindrospora*W.



19.*Spirogyra rhizobrachialis* J. 20.*Spirogyra jugalis* Kuetz. 21.*Closterium acerosum* Ehren.



22.*Closterium baillyanum*Bre. 23.*Cosmarium pachyderma* Lu.24.*Cosmarium rectosporum* Tur.

Discussion and Conclusion

Yenwe Village is situated in Taunggyi Township. Many ponds, rice fields and hot springs are found in this village. Because of their presence, different kinds of algae were found abundantly. In the present study, 15 genera and 24 species were collected and identified from two selected locations. The algae represented in Chlorophyta. This study was conducted from June 2009 to February 2010.

German phycologist Skuji (1949) published Flora Burmas in which 83 genera of Chlorophyta were described. Setchell (1903) made observations in Yellowstone Park and reported that the temperature where the algae visible was 75˚C to 77˚C. Khin Cho (2005) made observations in hot springs of Paung Township and reported that the temperature where algae visible was 40˚C to 50˚C. Khin Cho recorded the genera such as *Chrococcus, Gloeocapsa, Synechococcus, Scytonema, Tolypothrix, Calothrix, Radiofilum, Chaetomorpha, Euastrum, Botrydiopsis, Scoliopleura, Tribonema, Cyclotella, Fragilaria, Synedra* and *Cacooneis*. The observations of the present study were not agreed with those mentioned by Khin Cho. This was due to the difference between the temperatures of the study areas.

The present study reported that the temperature where algae visible was 25˚C to 40˚C. Green algae such as *Ulothrix, Microspora, Cladophora, Pithophora,* and *Spirogyra* could abundantly be seen in Location II where the temperature was between 25˚C to 29˚C. In Location I (39˚C to 40˚C), *Microspora, Spirogyra, Closterium* and *Cosmarium* were observed (Table. 2). It can be concluded that the habitats of green algae were strongly dependent on the temperature.

Guiry (2009) stated that green algae were extremely important as a source of food for other aquatic organisms and made a major contribution to

the world's oxygen supply. One of the green algae, *Chlorella* was now grown and sold as a health supplement and *Dunaliella* was as a source of β-carotene. Unfortunately, green algae had negative effects, as when large populations produced an unpleasant taste and odor in drinking water or clog filteration equipment. Algal bloom drastically decreased the oxygen supply available to other life forms in freshwater lakes and ponds.

Johnston, (1996) explained that hot springs were considered to be therapeutic in nature. The hot springs emerged from the earth's crust full of minerals and elements from within. The mineral composition of a hot spring was quite high, and their high temperature facilitated the entry of them into the human body. It can therefore be concluded that green algae found in Yenwe Village had minerals and elements. This can be beneficial and applicable to humans, and their environments.

Table 2. Some Species of Chlorophyta in Two Selected Locations.

No.	Collected Algae in Chlorophyta	Location I	Location II
1.	*Ulothrix cylindricum* Prescott.	-	✓
2.	*Ulothrix variabilis* Kuetzing	-	✓
3.	*Hormidium subtile* (Kuetz.) Heer.	-	✓
4.	*Geminella crenulatocollis* Prescott	-	✓
5.	*Binuclearia tatrana* Wittrock	-	✓
6.	*Microspora crassior* (Hansg.) Hazen	✓	✓
7.	*Microspora floccosa* (Vauch.) Thuret	-	✓
8.	*Microspora stagnorum* (Kuetz.) Lagerheim	✓	✓
9.	*Stigeoclonium longipilum* Kuetzing	-	✓
10	*Protoderma viride* Kuetzing	-	✓
11.	*Cladophora crispata* (Roth) Kuetzing	-	✓
12.	*Cladophora fracta* (Dillw.) Kuetzing	-	✓
13.	*Cladophora glomerata* (L.) Kuetzing	-	✓
14.	*Pithophora oedogonia* (Mont.) Wittrock	-	✓
15.	*Hydrodictyon reticulatum* (L.) Lagerheim	-	✓
16.	*Ankistrodesmus falcatus* (Corda) Ralfs	-	✓
17.	*Dichotomosiphon tuberosus* (A. Braun) Ernst	-	✓

18.	*Spirogyra cylindrospora* W. & G.S. West	-	✓
19.	*Spirogyra rhizobrachialis* Jao	✓	-
20.	*Spirogyra jugalis* (Fl. Dan.) Kuetzing	-	✓
21.	*Closterium acerosum* (Schrank) Ehrenberg	-	✓
No.	**Collected Algae in Chlorophyta**	**Location I**	**Location II**
22.	*Closterium balliyanum* Brebisson	✓	-
23.	*Cosmarium pachyderma* Lund	✓	-
24.	*Cosmarium rectosporum* Turner	✓	-

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I would like to thank Pro-rectors: Dr Myat Nyunt, and Dr Soe Myint Thein, Loikaw University for their permission to submit this article. I am greatly indebted to Dr Sann Sann Oo, Professor and Head, Department of Botany, Loikaw University for her review of this manuscript. I would like to acknowledge to Dr Yee Yee Win, Professor (retired), Department of Botany, for her invaluable suggestions and comments necessary in this work.

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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITIES OF FLOWERS AND SEEDS OF *GYMNANTHEMUM AMYGDALINUM* WALP.

Khin MyoThant[1], Kyawt Kywat Khine[2], San San Myint[3]

Abstract

In this research, the phytochemical analysis and antimicrobial activities of the flowers and seeds of *Gymnanthemum amygdalinum* Walp. were studied. It belongs to the family Asteraceae. The plants were collected from Kyaing Tong Township, Eastern Shan State. English name is bitter leaf plant and Shan name is Sa paung Lone. These investigations had been carried out by (Harbone, 1973 and Trease and Evans, 2002) in the Pharmaceutical Research Department of Ministry of Industry, Yangon Region. Alkaloid, carbohydrate, glycoside, phenolic compound, α-amino acid, saponin, tannin, flavonoids, steroids, terpenoids, reducing sugar and starch were observed in the flowers of studied plant. Tannin and cyanogenic glycoside did not observed in the seeds of this plant. For antimicrobial activities, the different extracts of flowers and seeds of *G. amygdalinum* were applied on some different microorganisms. The antimicrobial activity was done by using agar well diffusion method. Partially active and active were observed on studied microorganisms. The highest inhibition zones (15 mm- 20 mm) were found in ethanol extract of flowers and petroleum ether extracts of seeds. These results can be used for further pharmaceutical studied.

Keywords: *Preliminary phytochemical analysis and antimicrobial activity.*

Introduction

Gymnanthemum amygdalinum Walp. is a tropical plant belonging to the family Asteraceae and is used widely as vegetable and medicinal plant (Ibrahim *et al*., 2000). It is a shrub of about 2 to 5m with a petiolate leaf of about 6 mm in diameter and elliptic shape. The leaves are green with a characteristic odour and bitter taste. It does not produce seeds and has to be distributed or propagated through cutting. It grows under a range of ecological

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zones in Africa and produces a lager mass of forage and it is drought tolerant. It is majorly used for human consumption and has to be washed to remove the bitter taste. Its bitter taste is due to antinutritional factors such as alkaloids, saponins, tannins and glycosides. It stimulates the digestive system as well as reduces fever (Iwu, *et al.*, 1993). This plant contains complex active components that are pharmacologically useful. The roots and the leaves are used in ethno-medicine to treat fever, hiccups, kidney problems and stomach discomfort. The stem and root divested of the bark are used as chew sticks in many West African countries like Cameroon, Ghana and Nigeria (Burkill, 1985). However, extract of bitter leaf had been reported to exert antibiotic action against drug resistant microorganisms and possess antioxidant, anticancer, antiviral, anti-helminthic and anti-inflammatory activities (Akinpelu, 1999; Dahanukar*et al*., 2000). Furthermore, the root provides one of the commonly used chew sticks in Nigeria due to alleged beneficial effect on dental caries (Aregheore*et al*., 1998). The leaves and bark in Ethiopian local medicine are used as purgative, against menstrual pain and wound dressing (Akah and Okafor, 1992; Uhegbu and Ogbuchi, 2004). The present research was aimed to evaluate phytochemical constituents and antimicrobial activities of different solvent extracts of flowers and seeds of these medicinal plants.

Materials and Methods

The flowers and seeds of *Gymnanthemum amygdalinum* were collected from Kyaing Tong Township, Eastern Shan State during the flowering period from January to March in 2017. The identification of species was made by Hooker (1894) and Backer (1963). The samples were dried under shades for three weeks and stored in air tight containers for the phytochemical analysis and antimicrobial activity experiments. For antimicrobial activities, the flowers and seedsof this plantwere extracted by using methanol, ethanol, ethyl acetate, chloroform, pet-ether and water. The solvent extracts were tested six microorganisms (*Bacillus pumalis, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Candida albicans and Escherichia coli*). Since April 2017, these investigations had

been carried out by (Harbone, 1973 and Trease and Evans, 2002) in the Pharmaceutical Research Department of Ministry of Industry, Yangon Region.

Results

1. Outstanding characters

Small tree, much-branched, spreading, bark grey or brown,smooth stems pubescent with asymmetrical T-shaped hairs;leaves petiolate, elliptic, lanceolatebase rounded, margins coarsely serrate, apex shortly acuminate, apiculate;inflorescent capitulate, numerous in terminal compound corymb form cymes,florets 10-24 per capitulum, sweetly scented, bracteolate, green; flowers bisexual, all tubular, actinomorphic, epigynous, outer series 7-8 florets, inner ones 4-6 florets, creamy corolla 5-lobed, tubular; stamens 5, inserted, anther dithecous; ovary inferior, oblong, shortly pubescent, unilocular, solitary basal ovule, stigma bifid, pappus 37-42, creamy; fruits achenes Fig.(A,B and C).

A. Habit B. inflorescence C. Florets

Figure 1. Morphological characters of *Gymnanthemum amygdalinum*

2. Phytochemical Analysis and Antimicrobial Activities of Flowers and Seeds

The phytochemical analysis of *G. amygdalinum* flowers indicated the presence of alkaloids, carbohydrates, glycoside, phenol, amino acid, saponin, tannin, flavonoids, steroids, terpenoids, reducing sugar and starch. Tannin and cyanogenic glycoside were absent in the seeds. These results were shown in Table 1. Petroleum ether, methanol, ethyl acetate and ethanol and watery extract of flowers and seeds showed an effective antimicrobial activity on six

pathogens. Chloroform extract of flowers did not show activity on *Bacillus subtilis.* Chloroform extract of seeds did not show activity on *Candida albicans.* Ethanol extract of flowers and petroleum ether extract of seeds showed the most effective on studied microbes.Among them ethanol extract of flowers and petroleum ether extract of seeds showed the highest activity on *Escherichia coli.* These results were shown in Table 2 and Figure 2 - 7.

Table1. Phytochemical Constituent of Flowers and Seeds

No.	**Sample**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	Flowers	+	+	+	+	+	+	+	+	+	+	+	+	-
2	Seeds	+	+	+	+	+	+	-	+	+	+	+	+	-

(+) = presence (-) = absence

1= Alkaloid, 2= Carbohydrates, 3= Glycoside, 4= Phenol, 5= α-amino acid, 6=Saponin, 7= Tannin, 8= Flavonoid, 9= Steroid, 10=Terpenoids, 11= Reducing sugar, 12=Starch, 13= Cyanogenic glycoside

Table2 . Antimicrobial activities of different extracts of the flowers and seeds

No.	Sample	**Organisms**																	
		***Bacillus pumalis* (mm)**						***Bacillus subtilis* (mm)**						***Candida albicans* (mm)**					
		P	**C**	**M**	**EA**	**E**	**W**	**P**	**C**	**M**	**EA**	**E**	**W**	**P**	**C**	**M**	**EA**	**E**	**W**
1	Flowers	14	16	17	15	15	12	13	-	12	14	13	12	14	15	17	14	16	13
2	Seeds	14	11	13	13	12	11	15	11	12	11	11	12	14	15	-	13	12	11
No.	Sample	**Organisms**																	
		***Escherichia coli* (mm)**						***Pseudomonas aeruginosa* (mm)**						***Staphylcoccus aureus* (mm)**					
		P	**C**	**M**	**EA**	**E**	**W**	**P**	**C**	**M**	**EA**	**E**	**W**	**P**	**C**	**M**	**EA**	**E**	**W**
1	Flowers	12	12	18	14	20	17	12	14	16	13	17	15	12	11	13	12	14	16
2	Seeds	17	13	13	13	12	11	13	12	12	13	12	12	13	11	12	12	12	12

Agar well – <10 mm (inactive) ,10 mm ~ 14 mm (partially active), 15 mm ~ 19 mm (active)

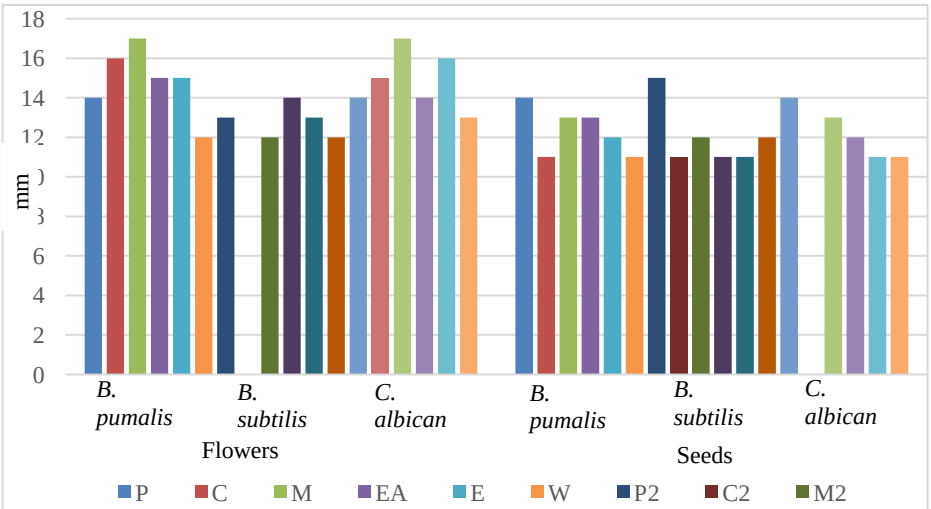


Figure 2. Antimicrobial activities of flowers and seeds of *G.amygdalinum*

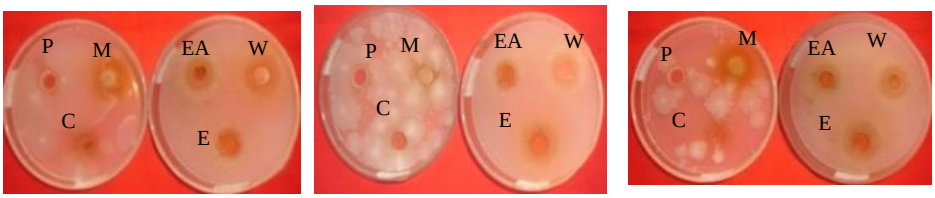


Figure 3. Antimicrobial activities of flowers of *Gymnanthemum amygdalinum*

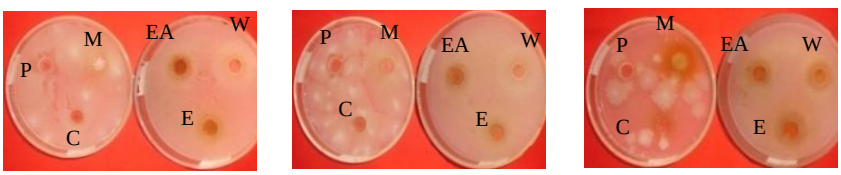


Figure 4. Antimicrobial activities of seeds of *Gymnanthemum amygdalinum*

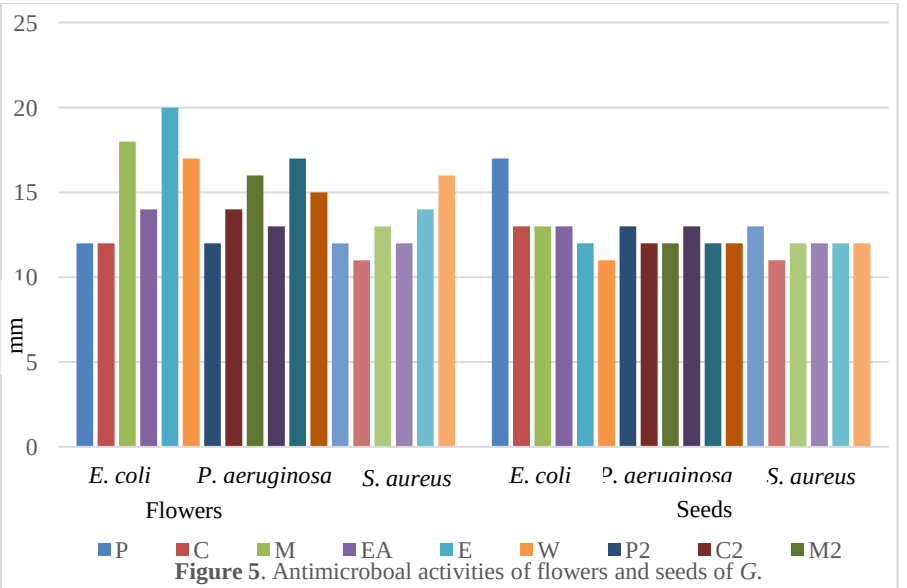
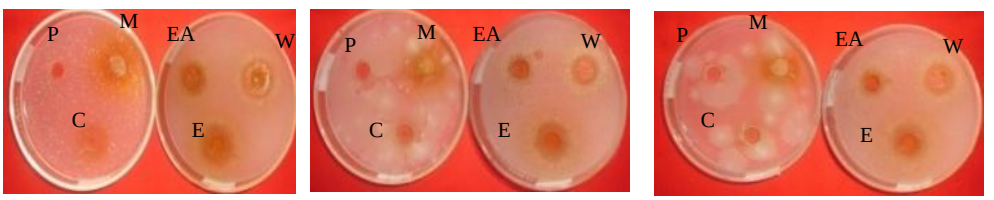
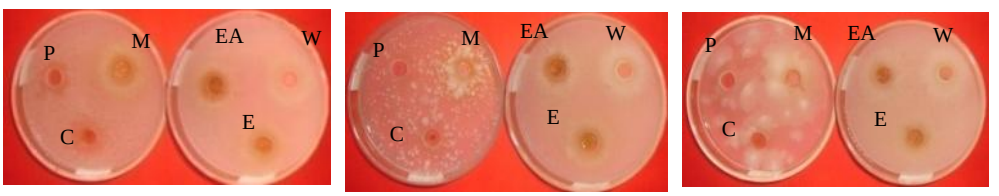


Figure 5. Antimicroboal activities of flowers and seeds of *G. amygdalinum*



Staphylococcus aureus *Pseudomonas aeruginosa* *Staphylococcus aureus*

Figure 6. Antimicrobial activities of flowers of *Gymnanthemum amygdalinum*



Escherichia coli *Pseudomonas aeruginosa* *Staphylococcus aureus*

Figure 7. Antimicrobial activities of seeds of *Gymnanthemum amygdalinum*

P = pet ether, C = chloroform, M= methanol, EA= ethyl acetate, E = ethanol, W= water

Discussion and Conclusion

The phytochemical analysis of the *Gymnanthemum amygdalinum* flowers and seeds showed the presenceof saponin, steroids, terpenoids, and alkaloids. These finding agreed with those mentioned by Adetunji *et al*., 2013. Saponin, flavonoids, alkaloids, and terpenoids were present in the flowers and seeds. These findings agreed with Enyi-Idoh *et al*.,2012. Tannin, alkaloids and saponin were present in flowers and seeds. These findings agreed with Wazis *et al*., 2012. Tannin have accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immunoresponses properties (Chung, 1998). Saponin have a favorable effect on cholesterol, can help boost the immune system, have an antioxidant effect, may even support bone strength and act against cancer cells by Sushant (2018) reported that alkaloids are used as a cure for malaria, as an anesthetic, help in curing diabetes, controlling blood sugar levels and treatment of cancer. Jordan (2017) reported that amino acid supplement with improved muscle growth, increase endurance, greater fat burn, reduce fatigue, increase mental focus muscle sparing, improved recovery and reduced muscle soreness. A carbohydrates intensive diet can cause high blood sugar and unwanted weight grain. Terpenoids have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunodulatory properties (Roslin and Anupam 2011). Flavonoids showed antioxidant activity, anticancer, antitumor activity, hepatoprotective activity, anti-inflammatory activity, anti-diabetes activity, antiviral antibacterial and antifungal activity (Xiao, 2016). Reducing sugar intake and weight loss and can create a stable mood and energy levels (Medibank, 2018). Starch can improve insulin sensitivity, very effective at lowering blood sugar levels after meals and help avoid chronic disease (Kris, 2018). According to the data, these plants can be used as drugs. In antimicrobial activities, pet-ether extract was effect on *Staphylococcus aureus* and *Escherichia coli*. These findings agreed with Gashe *et al*., 2017. Chloroform extract showed active on *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans.* These findings agreed with Zenebe *et al*., 2015, Gashe *et al*., 2017.Methanol extract

was active on *Staphylococcus aureus Escherichia coli, and Candida albicans.* These findings agreed with Zenebe *et al*., 2015, Gashe *et al*., 2017. Ethanol extract showed active on *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. These finding agreed with Adetunji *et al*., 2013, Anibijuwon *et al*., 2012 and Enyi-Idoh *et al*., 2012. *E. coli* infection include diarrhea, abdominal pain and fever. *Pseudomonas aeruginosa* can cause problems ranging from ear infection, bronchitis, respiratory infection and pneumonia (Ann, 2017). *Bacillus* infection are clindamycin and vancomycin and food poisons (Sliman *et al*., 1987). *Candida albicans* can cause skin and vaginal yeast infection (Weng, 2016). Staphylococcus aureus can cause a range of illnesses, from minor skin infections such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome and abscesses to life threating diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic, shock syndrome, bacteremia and sepsis (Larry and Charles 2018).In conclusion, this research showed more effective on studied microbes. Many kinds of secondary metabolites were observed in the flowers and seeds of this plant. So this work can help further pharmaceutical study.

Acknowledgement

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OPTIMUM PARAMETERS IN THE FERMENTATION OF ANTIMICROBIAL COMPOUNDS FROM THE FUNGUS *Stachybotrys* sp. AGAINST *AGROBACTERIUM TUMEFACIENS*

Tin Tin Hla [1]

Abstract

The fungus *Stachybotrys* sp. were studied, glucose as carbon source 2 %, 3 % and 4 % with yeast extract as nitrogen source 0.6 %, 0.7 % and 0.8 % were used to test on *Agrobacterium tumefaciens.* To optimize the fermentation medium, it was found that glucose 4 % with yeast extract 0.8 % yield the best result. There are seven different media in which 4 % glucose is combined with two nitrogen sources. There are also four different media in which 4 % glucose is combined with three different nitrogen sources. In the present study, different types of fermentation media were formulated by using 4 % glucose combined with different types of nitrogen sources and found out maximal antibacterial activity against *Agrobacterium tumefaciens*. In the investigation of ages and sizes of inoculum, 72 hrs ages and 20 % sizes of inoculum were optimized for fermentation. In the fermentation studies, the maximum activity reached at 72 hrs of fermentation for the production of antibacterial compound.

Keywords: fermentation, parameter of carbon and nitrogen ratio

Introduction

Fungi are primarily organisms that cannot synthesize their own food and are dependent on complex organic substances for carbon. Specialized fungi can be pathogenic on the tissues of plants, while others form mutually beneficial relationships with plants and assist in direct nutrient supply to the plants.

Fermentation procedures have to be developed for the cultivation of microorganisms under optimal conditions and for the production of desired metabolites or enzymes by the microorganisms. Several parameters which must be optimized are composition of ingredients, quality, carbon or nitrogen

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relationship, pH value before and after sterilization and changes in the sterilized nutrient solution before inoculation due to increase in temperature and aeration (Malek, 1984).

Media used in the cultivation of microorganisms must contain all elements in a form suitable for the synthesis of cell substances and for the production of metabolic products (Dale and Linden, 1984). Various aspects of microbial media such as carbon and nitrogen sources, minimal salts, trace elements, vitamins and pH have been reviewed (El-Tayeb *et al*., 2004; Rizk *et al*., 2007). Microbial growth kinetics is necessary to understand for the production of primary and secondary metabolites (Omura, 1985 and Crueger, 1989).

Physical factors such as incubation temperature can exert different effects on the growth and production phases of secondary metabolism. The purification of bioactive metabolites from fermented broth of microorganisms largely depends upon the physico-chemical properties of metabolite (Saxena *et al*., 2007).

The main objectives of present study is to investigate to find out pH, size of inoculum, incubation time, effects of carbon and nitrogen sources for the production of antibacterial metabolite.

Materials and Methods

Studies on the microbial growth kinetics of fungus (Omura, 1985, Crueger, 1989)

A loopful of isolated *Stachybotrys* sp. was inoculated into the seed medium (glucose 1.0 %, yeast extract 0.2 %, peptone 0.2 %, DW 100 mL) and incubated for 120 hrs at 100 rpm rotary shaker. The culture samples 5 mL was checked in 12 hr intervals for the growth. The sample 5mL was centrifuged at 2000 rpm for 30 mins and PCV % (Packed cell volume) was calculated.

Sample calculation example

Sample 5mL……..cell volume 0.5 mL

Sample 100mL……..0.5 x 100/5 = 10 %

Packed cell volume……... 10%

(Centrifugation at 2000 rpm/min)

Study on the effects of ages of seed culture on the fermentation (Crueger and Crueger, 1989)

The strain *Stachybotrys* sp. was inoculated into the medium, 48 hrs, 60 hrs, 72 hrs, 84 hr, 96 hrs and 108 hrs were employed with 15 % seed culture in 12 hrs intervals for the growth.

Study on the effects of sizes of seed culture on fermentation (Crueger and Crueger, 1989)

In the investigation of sizes of inoculums, 5 %, 10 %, 15 %, 20 % and 25 % of 72 hrs seed culture were utilized for the fermentation. Fermentation was carried out 5 days and antibacterial activity was tested by paper disc diffusion assay.

The effects of carbon and nitrogen sources on fermentation

In the investigation of carbon and nitrogen sources, the 1.5 % of each carbon source used in these studies glucose, sucrose, fructose, glycerol, glactose, tapioca powder and potato broth.

The 0.5 % 0f each nitrogen sources employed in this investigation were yeast extract, cornsteep liquor, KNO_3, peptone, fishcake and peanut cake. Antibacterial activity was examined at 12hrs intervals by paper disc diffusion assay. The choice of carbon source (glucose- 2 %, 3 % and 4 %) with nitrogen source (yeast extract- 0.6 %, 0.7 % and 0.8 %) were

incorporated into fermentation medium for detection of antibacterial activity against *A. tumefaciens* respectively.

Production of antibacterial compound by *Stachybotrys* sp. against *A. tumefaciens*

The fermentation was carried out for the production of antibacterial compound with 20% size of inoculum and 72 hrs age of culture for 120 hrs. The activity was checked 12 hrs interval using *A. tumefaciens*.

Determination of solvents for the extraction of antibacterial metabolite by bio-assay of paper chromatography (Tomita, 1998)

The filter paper and four solvents such as 20% NH_4Cl, *n-butanol* saturated with water, ethyl acetate - acetic acid - water (3:1:1) and ethyl acetate saturated with water were used for preliminary characterization of the compound. The obtained fermented broth samples (100 µL) were applied on the paper and allowed to dry. The chromatographic papers were tested in each solvent. Then, bioautography was done to check the antibacterial activity of each fermented broth. Each paper was placed on assay agar plate. After one hour the paper was taken out and then the plates were incubated for 24 hours. Then, the inhibitory zone was measured and R_f value for the corresponding metabolite was calculated.

Preliminary extraction of the solvent layer with organic solvents

According to the result of paper chromatography, the fermentation broth was mixed with *n-butanol* in the ratio of 12:4. The activity of organic layers obtained (first supernatant and lower sediment; second supernatant and lower sediment) were tested by paper disc diffusion assay.

Determination of solvents for the extraction of antibacterial metabolite by bio-assay of paper chromatography (Tomita, 1998)

The antibacterial activity of each extract was measured and R_f value for the corresponding metabolite was calculated.

Extraction of antibacterial metabolites adjusted pH with organic solvents (James, 1998)

The *n-butanol* layer adjusting pH 5-10 in the ratio of 12:4 (12- fermented broth and 4 - *n-butanol*). The *n-butanol* layer were tested the antibacterial activity compared with original fermented broth.

Results

Study on the microbial growth kinetics of fungus (Omura, 1985, Crueger, 1989)

In the studies of microbial growth kinetics, it was observed that growth phase of fungus *Stachybotrys* sp. between 48 hrs and 108 hrs. According to Omura, 1985 and Crueger and Crueger, 1989, ages of inoculum 48 hr, 60 hr, 72 hr, 84 hr, 96 hr and 108 hr were utilized for the optimization of fermentation as shown in Figure 1.

Studies on the ages and sizes of fungus against on *Agrobacterium tumefaciens*

It was found that growth phase between 48hrs and 108hrs. According to Crueger and Crueger (1989), ages of inoculums (48, 60, 72, 84, 96 and 108hrs) with 20% sizes of inoculum were utilized for the optimization of fermentation. According to the result of age of inoculum, 20% sizes of inoculums were optimized for the fermentation to produce the antibacterial metabolites.

Study on the effects of carbon and nitrogen sources of fungus *Stachybotrys* sp.

In the formation of fermentation medium, the best carbon source is 4 % glucose and in the case of nitrogen source 0.8 % yeast extract provided the highest antibacterial activity as shown in Table 1-8 and Figure 2-7.

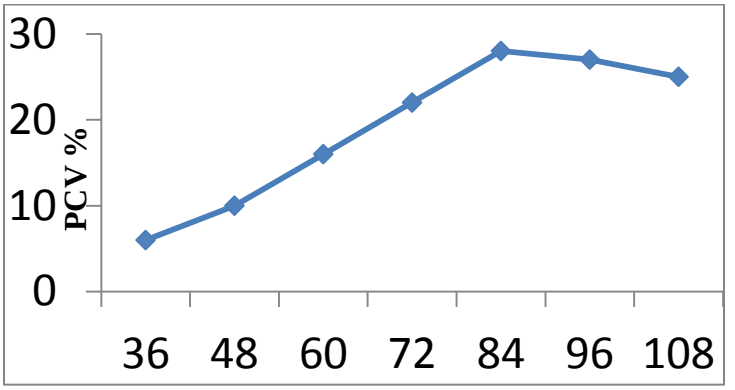


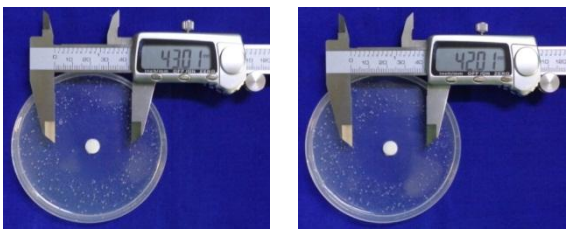
Figure 1. Microbial growth kinetics of fungus

Table 1. The effects of carbon sources on fermentation

No.	Carbon source	Activity (inhibitory zone, mm)
1	**Glucose**	**43.01**
2	Sucrose	42.57
3	Fructose	40.62
4	Glactose	36.83
5	Glycerol	39.01
6	Potato broth	38.00
7	Tapioca powder	39.01

Table 2. The effects of nitrogen sources on fermentation

No.	Nitrogen sources	Activity (inhibitory zone, mm)
1	KNO_3	40.34
2	**Yeast extract**	**42.01**
3	Corn steep	41.62
4	Peptone	39.04
5	Fishcake	37.13
6	Peanut cake	39.70

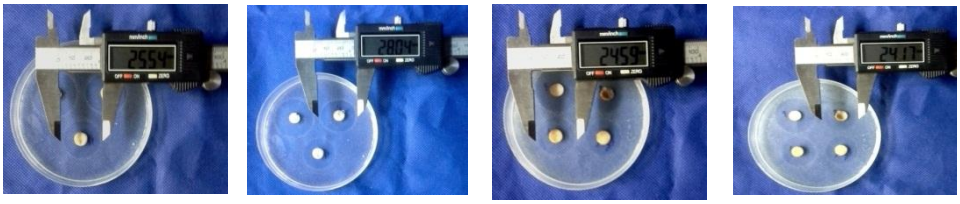


glucose (43.01 mm) Yeast extract (42.01 mm)

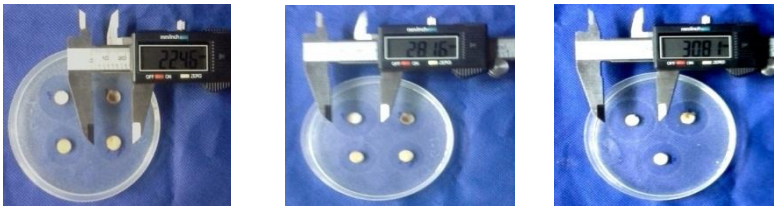
Figure 2. The effects of carbon and nitrogen sources on the antibacterial activity against *A.tumefaciens*

Table 3. The effects of different carbon and nitrogen sources on the antibacterial activity against *A. tumefaciens*

C and N source		Inhibitory zone (mm)
Glucose-2%	Yeast extract-0.6%	25.54
	Yeast extract-0.7%	28.04
	Yeast extract-0.8%	24.59
Glucose-3%	Yeast extract-0.6%	24.17
	Yeast extract-0.8%	22.46
Glucose-4%	Yeast extract-0.6%	28.16
	Yeast extract-0.8%	**30.81**



G-2%,Y-0.6% (25.54mm) | G-2%,Y-0.7% (28.04mm) | G-2%,Y-0.8% (24.59mm) | G-3%,Y-0.6% (24.17mm)



G-3%, Y-0.8% (22.46mm) | G-4%, Y-0.6% (28.16mm) | G-4%, Y-0.8% (30.81mm)

Figure 2. The effect of different carbon and nitrogen sources on the antibacterial activity against *Agrobacterium tumefacien*

Table 4. The effects of 4% glucose with two combination of different nitrogen sources on the antibacterial activity against *A. tumefaciens*

C and N source		Inhibitory zone(mm)
Glucose 4% per 100mL	Peptone, fishcake (Medium 1)	30.14
	Yeast extract, peanut cake (Medium 2)	24.91
	Yeast extract, cornsteep (Medium 3)	**34.73**
	Yeast extract, fishcake (Medium 4)	30.20
	Corn steep, peptone (Medium 5)	34.62
	Peanut cake, peptone (Medium 6)	33.41
	Yeast extract, peptone (Medium 7)	27.15

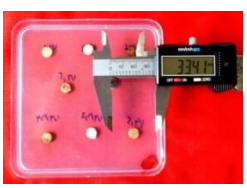
Medium 1(30.14mm)

Medium 2(24.91mm)

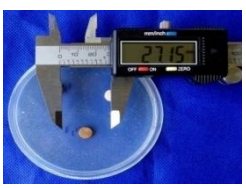
Medium 3(34.73mm)

Medium 4(30.20mm)

Medium 5(34.62mm)



Medium 6(33.41mm)

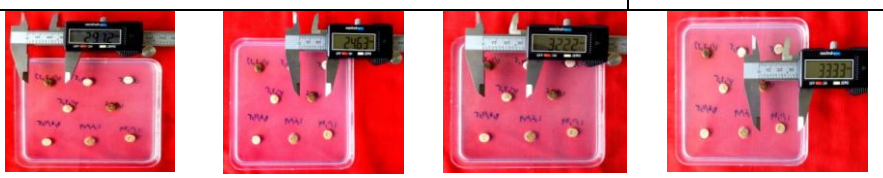


Medium 7(27.15mm)

Figure 3. The effects of different media with 4% glucose various combination of nitrogen sources on the antibacterial activity against *Agrobacterium tumefaciens*

Table 5. The effects of 4% glucose as carbon source and different combination of nitrogen sources on the antibacterial activity against *A. tumefaciens*

C and N source	Inhibitory zone (mm)
Medium 1(glucose with yeast, cornsteep and KNO_3)	29.12
Medium 2(glucose with yeast, cornsteep and fishcake)	24.63
Medium 3(glucose with yeast, cornsteep and peptone)	32.22
Medium 4 (glucose with yeast extract, cornsteep and peanutcake)	**33.33**



Medium 1 Medium 2 Medium 3 **Medium 4**

Figure 4. The effects of 4% glucose as carbon source and different combination of nitrogen sources on the antibacterial activity against *Agrobacterium tumefaciens*

Table 6. The effects of different carbon sources and combination of nitrogen sources on the antibacterial activity against *A. tumefaciens*

C and N ratio		Inhibitory zone (mm)
Glucose and Glycerol	**Yeast** and **cornsteep (Medium 1)**	**34.87**
	Yeast and **peanutcake (Medium 2)**	**34.87**
	Cornsteep and peptone (Medium 3)	30.95
	Peanutcake and peptone (Medium 4)	31.41
	Yeast and peptone (Medium 5)	30.34
	Yeast and fishcake (Medium 6)	31.69
	KNO_3 and peptone (Medium 7)	29.41




Medium1(34.87mm) Medium 2 (34.87mm) Medium 3(30.95mm) Medium 4(31.41mm)



Medium 5(31.69mm) Medium 6 (30.34mm) Medium 7(29.41mm)

Figure 5. The effects of different carbon sources and combination of nitrogen sources on the antibacterial activity against *Agrobacterium tumefaciens*

Table 7. The effects of glucose and potato broth combined with different nitrogen sources on the antibacterial activity against *A.tumefaciens*

C and N ratio		Inhibitory zone (mm)
Glucose and potato broth	KNO_3 and peptone (Medium 1)	31.62
	Yeast extract and fishcake (Medium 2)	29.12
	Yeast and peptone (Medium 3)	34.03
	Yeast extract and peanutcake(Medium 4)	34.88
	Yeast extract and **KNO_3 (Medium 5)**	**34.89**
	Peanutcake and peptone (Medium 6)	32.81

Table 8. The effects of Sucrose and potato broth combined with different nitrogen sources on the antibacterial activity against *A. tumefaciens*

C and N ratio		Inhibitory zone (mm)
Sucrose and potato broth	Yeast extract and fishcake (Medium 1)	25.54
	Peanutcake and peptone (Medium 2)	30.05
	Yeast extract and peanutcake(Medium 3)	29.62
	Yeast extract and **peptone (Medium 4)**	**31.63**

Medium 1 (31.62mm)

Medium 2 (29.12mm)

Medium 3 (34.03mm)

Medium 4 (34.88mm)

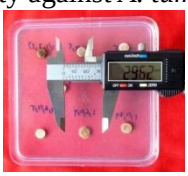
Medium 5 (34.89 mm)

Medium 6 (32.81mm)

Figure 6. The effects of glucose and potato broth combined with different nitrogen sources on the antibacterial activity against *A. tumefaciens*

Medium 1 (25.54mm)

Medium 2 (30.05mm)



Medium 3 (29.62mm)

Medium 4 (31.63mm)

Figure7. The effects of sucrose and potato broth combined with different nitrogen sources on the antibacterial activity against *Agrobacterium tumefaciens*

In the optimization of fermentation, the best carbon source glucose-4% was selected. The fermentation media such as glucose with yeast; glucose with yeast, corn steep liquor (Medium 3); glucose with yeast extract, corn steep liquor, peanut cake (Medium 4); glucose, glycerol with yeast extract, corn steep (Medium 1); glucose, potato broth with yeast extract, KNO_3 (Medium 5) and sucrose, potato broth with yeast extract and peptone (Medium 4) were found on the best antibacterial activities on *Agrobacterium tumefaciens* respectively. Among these media, the maximum result indicated by size of clear zone (34.89 mm) revealed in Medium 5 was selected for the further investigation.

Production of antibacterial compound by *Stachybotrys* sp. against *A. tumefaciens*

In the fermentation studies, the maximum activity reached at 72 hrs as shown in Table 9 and Figure 8.

Screening of solvent for the extraction of antibacterial metabolite by bio-assay of Paper chromatography

According to the R_f values, it was considered that solvent 2, *n-butanol* is suitable for the extraction of antibacterial compound. According to the R_f value, it was considered that antibacterial metabolite is soluble also in solvent 2, *n-butanol* and in solvent 4, ethyl acetate. However, solvent 2, *n-butanol* is more suitable for the extraction of antibacterial metabolite than solvent 4, ethyl acetate because of their apparent R_f value (0.9) solvent 2 than (0.7) solvent 4 as shown in Figure 9.

Preliminary extraction of the solvent layers with *n-butanol*

According to the activity of extraction of antibacterial metabolite, it was considered that the first supernatant with *n-butanol* is suitable for the extraction of antibacterial compound. However, the first sediment and the second supernatant extracted with *n-butanol* very little showed antibacterial

activity but the first supernatant and the second sediment resulted from *n-butanol* extraction showed distinct activity indicated by clear zone as shown in Figure 10. However, the first time upper layer was more suitable for the extraction of antibacterial metabolite than the second time lower layer with *n-butanol* as shown in Figure 10.

Extraction of antibacterial metabolite adjusted pH 7.0 with *n-butanol*

In the present work, the total volume of fermented broth (36 liters) with pH 6.0 was adjusted to pH 7.0 and extracted with *n-butanol* in the ratio of 12:4 (broth : *n-butanol*). They were concentrated *in vacuo* and kept in the chamber for further investigation by chromatography as shown in Table 10 and Figure 11and 12.

Table 9. Time course of fermentation for the production of antibacterial compound

Time course (hrs)	Inhibitory zone (mm)
36	+
48	38.45
60	39.67
72	42.00
84	41.17
96	40.54
108	39.04
120	38.36

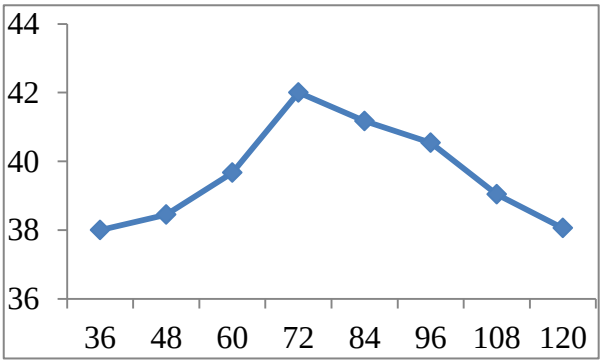
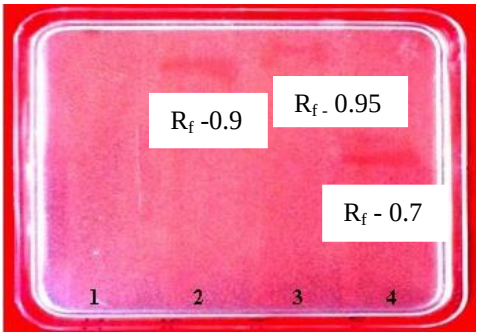


Figure 8. Time course of fermentation for the production of antibacterial compound



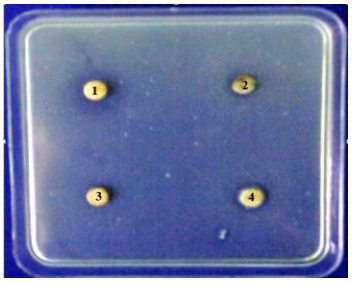
1- 20%NH_4Cl

2 - n- butanol saturated with water

3- ethyl acetate:acetic acid:water(3:1:1)

4- ethyl acetate saturated with

Figure 9. Paper chromatography Bioautographic overlay-assay



1- extract of first supernatant

2- extract of first sediment

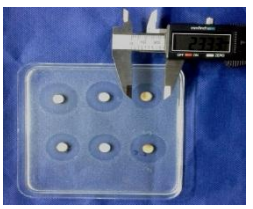
3- extract of second supernatant

4- extract of second sediment

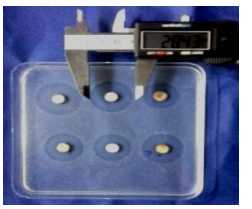
Figure 10. The effect of supernatant and sediment of *n-butanol* extraction on the antibacterial activity

Table 10. The effects of pH on the extraction with *n-butanol* on the antibacterial activity against *Agrobacterium tumefaciens*

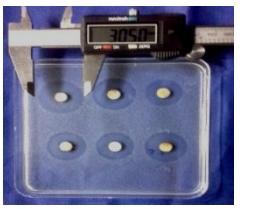
Adjusted pH	Inhibitory zone(mm)
5	23.33
6	28.43
7	**30.50**
8	28.05
9	25.34
10	25.20



pH 5 (23.33 mm)

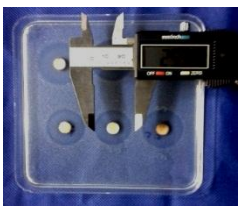


pH 6 (28.43 mm)

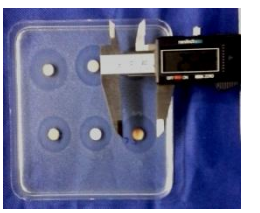


pH 7 (30.50 mm)

pH 8 (28.05 mm)



pH 9 (25.34 mm)



pH 10 (25.20 mm)

Figure 11. The effect of pH on the extraction of compound with *n-butanol* on the antibacterial activity against *Agrobacterium tumefaciens*

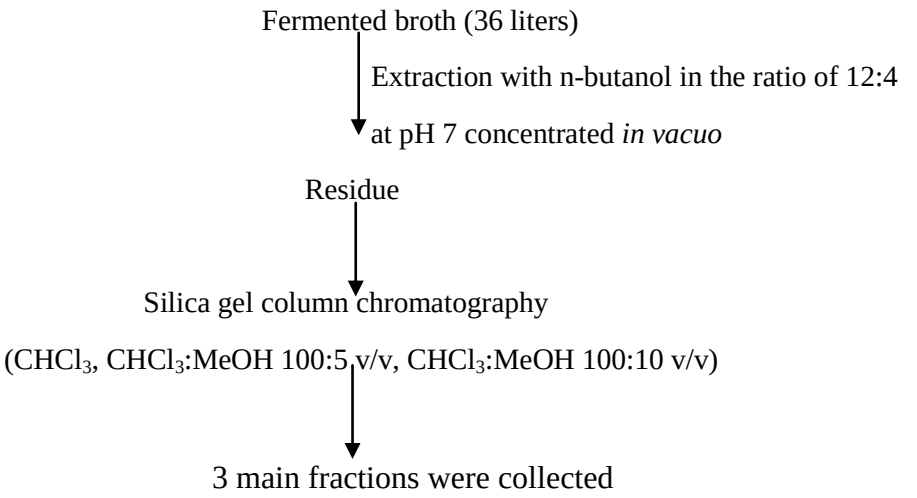


Figure 12. Flow chart of fermentation of medium 5 by using *Stachybotrys* sp.in optimal condition

Discussion and Conclusion

This fungus was observed that growth phase between 48 hrs and 108 hrs. It was observed that 20 % sizes and 72 hrs age were suitable for the production of antibacterial metabolites. In the studies of carbon and nitrogen sources utilization, glucose (43.01 mm, inhibitory zone) and yeast extract (42.01 mm, inhibitory zone) were the best for the production of antibacterial metabolites. In the investigation of carbon and nitrogen sources, carbon source (glucose- 2 %, 3 % and 4 %) with nitrogen source (yeast- 0.6 %, 0.7 % and 0.8 %) were used in the fermentation as antibacterial activity on *A. tumefaciens*. Subsequently, carbon source (No. 1 glucose and No. 4 glycerol) with six kinds of nitrogen sources were used in the fermentation tested on *Agrobacterium tumefaciens*. All fungi need organic carbon compounds as a source of energy and for biosynthesis. Most investigations concerning nitrogen metabolism in fungi, cannot fix atmospheric nitrogen. Most fungi can use nitrate as nitrogen source, but ammonium salts and a number of organic

nitrogen compounds ranging from urea and amino acids to proteins are also good nitrogen sources for many fungi (Anna-Lena Sunesson, 1995).

In the investigation of carbon and nitrogen sources, carbon source (glucose - 2 %, 3 % and 4 %) with nitrogen source (yeast- 0.6 %, 0.7 % and 0.8 %) were used in the fermentation of *Agrobacterium tumefaciens*. It was found that carbon source (glucose 4 %) with nitrogen source (yeast extract 0.8 %) the best inhibitory zone (30.18 mm) on *A. tumefaciens*. Therefore, carbon source, glucose 4 % was selected. Subsequently, carbon source (glucose 4 %) with two kinds of nitrogen sources and three kinds of nitrogen sources were takes placed in the fermentation of *Agrobacterium tumefaciens*. It was observed that carbon source, (glucose 4 %) with two kinds of nitrogen sources (Medium 3**,** yeast extract and cornsteep) the best inhibitory zone (34.73 mm) and carbon source, (glucose 4 %) with three kinds of nitrogen sources (Medium 4, yeast extract, cornsteep and peanutcake) the best inhibitory zone (33.33 mm) were occurred.

However, two kinds of carbon sources such as (No. 1 glucose and No. 4 glycerol), (No. 1 glucose and No. 6 potato broth) and (No. 2 sucrose and No. 6 potato broth) with two kinds of nitrogen sources were used in the fermentation tested *A. tumefaciens*.

In two kinds of carbon sources, (glucose – glycerol) with two kinds of nitrogen sources, **Medium 1** (glucose, glycerol with yeast extract, corn steep, inhibitory zone - 34.87 mm), in glucose – potato broth, **Medium 4** (glucose – potato broth with yeast extract and KNO_3, inhibitory zone -34.89 mm), and in sucrose – potato broth, **Medium 4** (sucrose – potato broth with yeast extract and peptone, inhibitory zone – 31.63 mm) were found on the best antibacterial activities against *A. tumefaciens.* According to the result, the effect of carbon and nitrogen sources utilization for the extraction of antibacterial metabolite was found in 5 days period and the maximum activity reached at 72 hrs.

The crude extracts were further investigated on the purification and identification by chromatographic method. Preliminary studies of paper chromatography are required to extract the compound from the fermented broth. The purpose of using paper chromatography is to know how to extract the bioactive compound from fermented broth by which solvent systems (Crueger and Crueger, 1989). In the study of paper chromatography, was carried out by four solvents such as 20 % NH_4Cl, *n-butanol* saturated with water, ethyl acetate -acetic acid -water (3:1:1) and ethyl acetate saturated with water were used for preliminary characterization of the compound. It was found that *n-butanol* is suitable for the extraction of the antibacterial metabolite. Then, preliminary extraction of the solvent was studied with *n-butanol* for the good extraction of the antibacterial compound. In this study, the extraction of the first supernatant was showed the best extraction of antibacterial metabolite.

Antibacterial metabolite extracted was adjusted to pH 5 - 10 with *n-butanol* in the ratio of 12:4 by using the method of James, 1998. The pH level of the growth medium has a marked effect on secondary metabolite production with synthesis fall rapidly either side of an optimal level. The hydrogen or hydroxyl ion concentration may have a direct effect on cell, or it may act indirectly by varying the degree of dissociation of substances in the medium. Therefore, the change of pH is also important for the enzyme activity of microorganisms, for the intermediate products, their dissociation and solubility (Rizk *et al*., 2007). It was found that the best antibacterial metabolite was extracted with *n-butanol* in the ratio of 12:4 at pH 7.0. Thus, the antibacterial metabolite could be extracted with *n-butanol* at pH 7.0. Thus, in this research pH 7.0 is the best for the production of the antimicrobial metabolite by *Stachybotrys* (Jain and Pundir, 2011).

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STUDY ON MORPHOLOGICAL, PHYSICOCHEMICAL INVESTIGATION AND ANTIMICROBIAL ACTIVITIES OF *SANSEVIERIA TRIFASCIATA* HORT. EX PRAIN. (NA-GAR-SET-GAMON)

Htay Htay Myint[1], Thin Thin Swe[2]

Abstract

The plant *Sansevieria trifasciata* Hort. ex Prain. (Na-gar-set-gamon) (or) snake plant belongs to family Asparagaceae It was naturally grown and found abundantly in Myanmar. They were collected from Dagon University, East Dagon Township, Yangon Region during the flowering and fruiting periods. The morphological characters, preliminary phytochemical test, physicochemical investigation and antimicrobial activities were studied. In morphological study, the plant was evergreen herb, stem less and rhizome horizontal. Preliminary phytochemical test showed the presence of alkaloid, α-amino acid, glycoside, carbohydrates, reducing sugar, phenolic compound, flavonoid, steroids and trace of terpenoids. In physicochemical investigation, the water soluble ash content of leaves was the most highest than the other solvents. In antimicrobial activities, the ethanol extract of leaves showed the most significant inhibition on *Vibrio cholerae*.

Keywords: Phytochemical test, Physicochemical test, Antimicrobial activities

Introduction

In Myanmar, there are two types of medicinal plants: wild plants and cultivated plants. Traditional medicine is an important part of human health care in developing countries.

Herbal medicine also called botanical medicine or phytopharmacy is a plant derived material or preparation with therapeutic or other human health benefits which contains either raw or processed ingredients from one or more plants. This is a major remedy in traditional medicine system, which is largely based on the use of vegetative and reproductive parts of the plants (Federico,

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1989). The medicinal plants, *Sansevieria trifasciata* Hort. ex Prain belonging to the family Asparagaceae, which are found abundantly in Myanmar.

Genus *Sansevieria* is native to tropical Africa; in Java, from the plains up to 1000, not rarely cultivated on a small scale, in gardens, fences and hedges also on or around groves (Backer, 1968). The family Asparagaceae comprises about 153 genera and some 2,500 species of flowering plants. Distributed nearly worldwide, the family is extremely diverse, and its members are united primarily by genetic and evolutionary relationships rather than morphological similarities (Melissa, 1993).

Sansevieria trifasciata Hort. ex Prain. grown of West Africa, it is cultivated as an ornamental pot plant. It is also distributed in Southern India. It grown dry region. Dry rocky and sandy places, lowlands and mid-country. Flowering seasons are January-June. Flowers fainty fragrant. Becoming depleted due to over-exploitation. English name of this plants is Bow-string hemp. Fibers extracted from the leaves were formerly used for making bow-strings and fishing lines. Now used in the Central Province for making reed mats and dusters. Roots used in indigenous medicine (Dassanayake and Clayton, 2000). *Sansevieria trifasciata* Hort. ex Prain. is called na-ga-set in Myanmar, snake plant, mother-in-law's tongue and bow-string hemp in English. This plant distributed in Yangon of Myanmar Region (Hundley and Chit Ko Ko, 1987 and Kress and Yin Yin Kyi, 2003).

In Myanmar, *Sansevieria trifasciata* Hort. ex Prain. root juice with honey is used for chronic cough and leaf juice treats mucous in throat for children. Leaf fibers used as rope. Leaf with white stripe is called Nagaset and leaf margin yellow is Nagakhoe (Ashin Nagathein, 1983). This plant has analgesic activity and antipyretic activity. Traditional used of this plant is the treatment of ear pain, swellings, boils, fever and inflammatory disordered (file://G:download/downloadflle-lhtm).

Several species are popular houseplants in temperate regions, with *Sansevieria trifasciata* Hort. ex Prain. is the most widely sold; numerous cultivars are available. The Chinese usually keep this plant potted in a pot

often ornamented with dragons and phoenixes. As a houseplant *Sansevieria* thrives on warmth and bright light, but will also tolerate shade. In Korea, potted *Sansevieria* is commonly presented as a gift during opening ceremonies of businesses or other auspicious events. *Sansevieria* use the crassulacean acid metabolism process, which absorbs carbon dioxide and releases oxygen at night. This purportedly makes them suitable bedroom plants. However, since the leaves are potentially poisonous if ingested, *Sansevieria* is not usually recommended for children's bedrooms. Some believe that having *Sansevieria* near children (such as in the study room) helps reduce coarseness, while others recommend placing pots near the toilet tank to counter the drain-down vibrations (file:///G:/ download/ Sansevieria. htm).

The aim of present research is to know the chemical compound and medicinal value of the plant. To fulfill this aim, the main objectives are (i) to verify the morphological characters of the plants (ii) to examine powdered sample of leaves for standardization of crude drug (iii) to investigate the qualitative analysis on phytochemical test (iv) to find out the presence or absence of chemical properties, physicochemical properties and (v) to examine the antimicrobial activities of leaves extracts.

Materials and Methods

Botanical Studies

The specimens used in this research were collected widely in Dagon University, East Dagon Township, Yangon Region. They were collected especially during the flowering and fruiting period from January to June in 2013. The collected fresh specimens of both vegetative and reproductive parts of the plants were identified by using literatures of Lawrences, 1964; Backer, 1965; Hundley and Chit Ko Ko, 1987; Dassanayake, 2000 and Kress *et al.*, 2003. Taxonomic descriptions were accompanied with the photograph of natural habitats, L.S of flower, T.S of ovary and parts of the plants with measurements.

Chemical Studies

The leaves of *Sansevieria trifasciata* Hort. ex Prain. were collected from Dagon University, East Dagon Township, Yangon Region. The leaves samples were washed with water and were cut slices by knife. Then these slices were dried at room temperature for 2-3 weeks. The dried leaves were pulverized by grinding with a blender to get fine powdered and stored in air tight container. For preliminary phytochemical test, the air-dried powdered of the leaves were tested for alkaloids, α-amino acid, glycoside, cyanogenic glycoside, carbohydrates, reducing sugar, starch, saponin, tannin, phenolic compound, flavonoid, steroids and terpenoids were carried out. The physicochemical properties solubility of powdered leaves were carried out using moisture content, total ash content, water soluble ash content, acid insoluble ash content, aqueous soluble matter content and various solvent such as methanol, ethanol, ethyl acetate, chloroform, petroleum ether and acetone.

Preliminary Phytochemical Test of Leaves of Sansevieria trifasciata Hort. ex Prain.

The preliminary phytochemical tests were carried out according to Vogel, 1956; British Pharmacopoeia 1968, Marini Bettolo *el. al.,* 1981; Robison 1983 and Central Council for Research in Unani Medicine, 1987.

Test for Alkaloid

One gram of powdered sample was boiled for about 20 minutes with 20ml of 10% HCl and filtered. The filtrate was divided into four portions and tested with Dragendroff's reagent, Sodium picrate, Wagener's reagent and Mayer's reagent. The precipitate formed an addition of the reagent indicated the presence of alkaloid (Robison, 1983).

Test for α-Amino acid

One gram of powdered sample was boiled with 10ml of distilled water for 20 minutes and then filtered. And then, a few drops of each filtrate was spotted on a filter paper using a capillary tube, allowed it to dry and sprayed

with ninhydrin reagent. The filter paper was dried at room temperature and then kept it in over at 110°C for a few minutes after which the purple colour appears due to the presence of α-amino acids (Marini Bettolo *et. al.,* 1981).

Test for Glycoside

One gram of powdered sample was heated in a glass test tube with 10ml of distilled water on the water-bath for 20 minutes. The mixture was filtered and 10% basic lead acetate solution was added drop-wise to the filtrate. Pale yellow precipitate was observed which showed the presence of glycoside (Marini Bettolo *et. al*.,1981).

Test for Cyanogenic glycoside

Two grams of powdered sample was boiled with 10ml of distilled water for 20 minutes and filtered. Then about 5 drops of concentrated sulphuric acid were added and sodium picrate paper was trapped in the neck of the test tube by means of a loosely closed cock. The resultant mixture was heated by using a spirit burner. Observation was made to see if the paper turned brick red which indicated the presence of cyanogenic glycoside (Marini Bettolo *et. al.,* 1981).

Test for Carbohydrate

One gram of powdered sample was boiled with 10ml of distilled water for 20 minutes and filtered. The filtrate was introduced into a test tube and a few drops of 10% α-naphthol was added shaken. The test tube was then inclined at an angle of 45° and concentrated sulphuric acid was added slowly along the side of the tube. A red ring was formed between the two layers, showing the presence of carbohydrate (Marini Bettolo *et. al.,* 1981).

Test for Reducing Sugar

One gram of powdered sample was boiled with 10ml of distilled water for 20 minutes and filtered. The filtrate was treated with Fehling's solution, then boiled about 20 minutes, it furnished green precipitates, indication the presence of a reducing sugar (Vogel, 1956).

Test for Starch

One gram of dried powdered sample was boiled with 10 ml of distilled water for about 20 minutes. It was then filtered and two drops of iodine solution were added to the filtrate. Blue black colour was formed which indicate the presence of starch (Marini Betolo *et. al.*, 1981).

Test for Saponin

One gram of powdered sample was boiled with 10 ml of distilled water for about 20 minutes and filtered. The filtered and the filtrate shaken vigorously with distilled water for a few minutes. Market forthing which lasted for about half an hour to take place, indicating the presence of saponin (Marini Betolo *et. al.*, 1981).

Test for Tannin

One gram of powdered sample was boiled with 10ml of distilled water for about 20 minutes and filtered. The filtrate was treated with a few drops of 1% ferric chloride solution. If a bluish black or yellowish brown colour resulted indicating the presence of tannins (Marini Betolo *el. al.*, 1981).

Test for Phenolic compound

One gram of powdered sample was boiled with 10 ml of distilled water for 20 minutes and filtered. The filtrate was treated with neutral *5%* ferric chloride solution, it gave deep blue colour, indicating the presence of phenol groups (Marini Betolo *et. al.*, 1981).

Test for Flavonoid

One gram of powdered sample was extracted with 95% ethanol for 20 minutes and filtered. Then, the ethanolic extract was treated with 5-10 drops of dilute hydrochloric acid which was followed by a small piece of zinc or magnesium. The solution was boiled for few minutes. The appearance of pink or brown colour indicates the presence of flavonoid (Robison, 1983).

Test for Steroids and Terpenoids

One gram of powdered sample was extracted with petroleum ether for 20 minutes and filtered. When the petroleum ether extract was dissolved in 1ml chloroform. The chloroform extract was treated with 3 drops acetic anhydride and concentrated sulphuric acid. The reenish colour was turns to blue green indicate the presence of steroids and deep pink of terpenoid (Central Council for Research in Unani Medicine, 1987).

Physicochemical Investigation of Leaves *Sansevieria trifasciata* Hort. ex Prain.

Physicochemical investigation was determined according to British Pharmacopoeia (1965) as follows.

Determination of Moisture Content

Five grams of powdered was weighed accurately in a beaker and dried in an oven at the temperature of 110°C for 1 hr. After drying the beaker was removed from the oven and cooled at room temperature and weighed. The procedure was repeated until a constant weight was obtained. Then, percentage of moisture content was calculated.

$$\% \text{ of moisture} = \frac{M-m}{W} \times 100$$

M = weight of sample of weighting beaker in g before drying

m = weight of sample and weighting beaker in g after drying

W = weight of taken in g.

Determination Weight of Total Ash

Ten grammes of powdered was weighed in a crucible and placed in a muffle-furnace at 500°C until substance turned into ash. After that, the crucible was cooled and weighted. This procedure was repeated until a constant weight was obtained and the percentage of total ash was calculated.

$$\% \text{ of total ash} = \frac{\text{M-m}}{\text{W}} \times 100$$

Determination of Water Soluble Ash

The total ash was boiled with 10ml of water for 15 mins. The insoluble matter was collected on an ashless filter paper, washed with water and ignited to a constant weight. This weight or insoluble matter was substrated from the weight of the total ash, to give weight of the water-soluble ash.

Determination of Acid Insoluble Ash

The total ash was boiled gently with 10ml of 6% dilute hydrochloric acid for 5 minutes and filtered using ashless filter paper. The insoluble matter was collected and washed with boiling water until free of acid. The acid insoluble residue was dried in an oven at 105° C and weighed. The percentage of the acid insoluble ash was calculated.

Determination of Soluble Matter Content in Different Solvents

Soluble matter content was determined by the method of British Pharmacopoeia, 1968. Five grams of powder was soaked with 50ml of distilled water in flask, closed for 72 hrs. The mixture was filtered and evaporated in a weighed beaker then placed on a boiling water-bath until it was completely evaporated. The percentage of soluble matter was calculated. Similarly the same procedure was repeated for the determination of aqueous, methanol, ethanol, ethyl acetate, chloroform, petroleum ether and acetone.

Antimicrobial Activities of Different Solvent Extracts from Leaves of *Sansevieria trifasciata* Hort. ex Prain.

Apparatus Used

Autoclave, clean bench, conical flask, hot air sterilizer, measuring cylinders, micropipettes, steam-drying oven, petridishes, pipettes, water bath and loops.

Test Microorganisms

The test organisms, used in this research work were obtained from the Central Research and Development Center (CRDC) for determination of antimicrobial activities.

Preparation of Plates for Antimicrobial Activities Test

The antimicrobial activities were performed by agar-well diffusion method. Nutrient agar was prepared according to method described by Cruikshank, 1975. Nutrient agar was boiled and 20-25 ml of the medium was poured into a test- tube and plugged with cotton wool and autoclaves at 121 °C for 15 minutes. Then the tubes were cooled down to 30-35°C and poured into sterilized petridishes and 0.01 ml of spore suspension were also added into the dishes. The agar was allowed to set for 30 minutes after with 5mm plate agar well was made with the help of sterilized cork borer. After that, about 0.1ml of sample was introduced into the agar-well and incubated at 37°C for 24-48 hrs. The inhibition zone appeared around the agar-well indicating the presence of anti-microbial activity. The extent of antimicrobial activity was measured from the zone of inhibition diameter. The results were shown in Table 2, Figures 2 to 4.

Results

Morphological Characters of *Sansevieria trifasciata* Hort. ex Prain.

Scientific name - *Sansevieria trifasciaia* Hort. ex Prain.

Myanmar name - Nagar-set-gamon

English name - Snake plant, Mother – in law's tongue, Bow-string hemp.

Family - Asparagaceae

Flowering and fruiting period - January to June

Part used - Leaves

Perennial herbs, evergreen. Stemless; rhizome horizontal; sympodial, producing leafy shoots at intervals, 0.5-1.0m in height, aerial shoots in a single clump. Leaves simple, tuft thick, upright fleshy to rigidly coriaceous, both surfaces shinning smooth, dark green, with numerous very conspicuous, light or greyish green irregularly confeined transverse bands, a narrow dark green margin, tapering to apex, acute, apiculate, linear-laceolate or ensiform and channeled, and 52.5-76.9cm in length and 3.5-5.5cm in breadth. Inflorescences (peduncle included) racemes, 51.0-57.0cm in length and 6.0-7.0cm in breadth penduncle length and breadth. Flowers in fassicles of 3-7, membranous bract, 5.0-7.0mm in length and 2.0-3.0mm in breadth, each flower with of a minute bract, 2.0-2.5mm in length and 0.5-1.0 mm in breadth; pedicel cylinder, 0.5-1.0mm in length and 0.5-0.8mm in breadth, actinomorphic, regular, bisexual, trimerous, hypogynous. Tapels 3+3, united at the base, tapel tube short 1.5-1.8mm in length and 2.0-3.0mm in breadth, cylindrical, pale yellowish green; limbs linear or narrowly lanceolate, revolute, pale greenish white, 1.5-2.0cm in length and 1.0-2.0mm in breadth, inferior. Stamens 3+3, epipetalous, filaments filiform, pale yellowish green, 1.9-2.1cm in long; anther dithecous, dorsifixed, longitudinal dehiscence, sagittate at base, 2.5-3.0mm in length and 0.3-0.5mm in breadth, inferior. Ovary 3-carples, 3.0-3.5mm in length and 1.0-2.0mm in breadth, 3 loculi, with anatropous ovule in each locule, axile placentation; style filiform, 2.0mm- 3.0mm in length, pale yellowish green, stigma 3 lobed, exserted, 1.0-1.5mm in diameter, superior. Fruit berry globose, orange in colour when ripe. Seeds broadly ovoid, with horny endosperm.

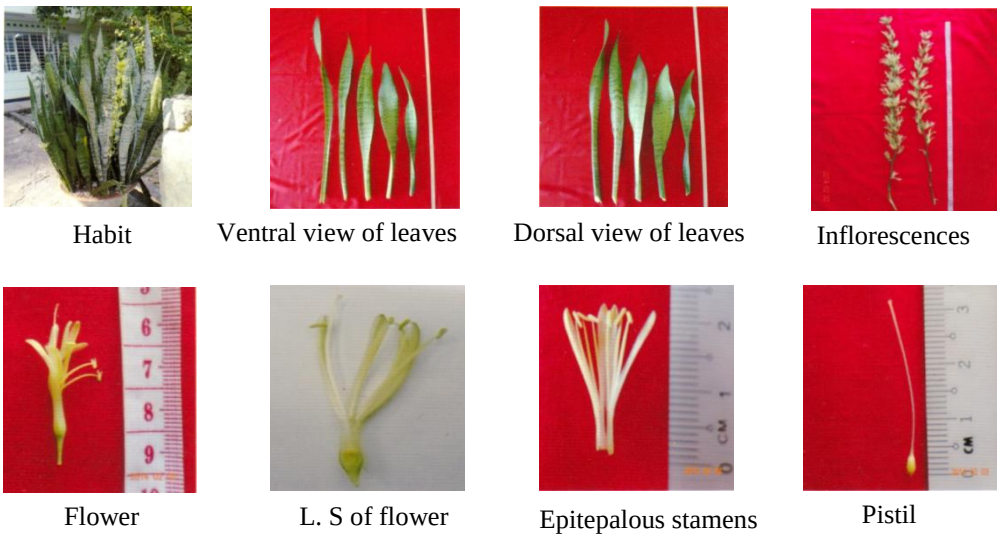


Figure 1. Morphological characters of *Sanseviveria trifasciata* Hort. ex Prain

Chemical Studies

Preliminary Phytochemical Test of Leaves from Sansevieria trifasciata Hort. ex Prain.

The results of preliminary phytochemical test of air-dried powdered leaves from *Sansevieria trifasciata* Hort. ex Prain. indicated that alkaloid, α-amino acid, glycoside, carbohydrate, reducing sugar, starch, phenolic compound, flavonoid, steroids and terpenoids (trace) are found to be present and cyanogenic glycoside, starch, saponin and tannin are absent. The results are shown in Table 1.

Table 1. Preliminary Phytochcmical Test of Leaves from *Sansevieria trifasciata* Hort. ex Prain.

No.	Test	Extract	Test reagent	Observation	Results
1.	Alkaloid	10% HCl	Dragendroffs reagent Sodium picrate solution Wagner's reagent Mayer's reagent	Orange ppt Yellow ppt Brown ppt White ppt	+ + + +
2.	α-amino acid	H_2O	Ninhydrin reagent	Purple spot	+
3.	Glycoside	H_2O	10% lead acetate solution	Pale yellow ppt	+++
4.	Cyanogenic glycoside	H_2O	H_2S0_4 (Conc:) & sodium picrate paper	No colour change	–
5.	Carbohydrates	H_2O	10% α-napthol + H_2S0_4 (Conc:)	Red ring	+
6.	Reducing sugar	H_2O	Fehling's solution	Greenish yellow ppt	+
7.	Starch	H_2O	Iodine solution	Brown ppt	–
8.	Saponin	H_2O	Distilled water	No foaming	–
9.	Tannin	H_2O	1% $FeCl_3$ solution	Brown ppt	–
10.	Phenolic compound	H_2O	5% $FeCl_3$ solution	Bluish black ppt	+ + +
11.	Flavonoid	95% Ethanol	Mg/HCL (Conc:)	Brown colour	+
12.	Steroids	Petroleum ether	Acetic anhydride	Green colour	+
13.	Terpenoids	Petroleum ether	Acetic anhydride and H_2S0_4 (Conc:)	Pink colour (trace)	(trace)

Key to the table (+ + +) = prominent (+) = present (-) = absent (ppt.) = precipitate

Physicochemical Investigation of Leaves of *Sansevieria trifasciata* Hort. ex Prain

The results of the physicochemical investigation, the moisture content were determined and recorded. The solubility of leaves powdered in

petroleumether, acetone, ethyl acetate, methanol, distilled water, ethanol and chloroform were carried out to determine the amount of total solids soluble in an individual solvent. *Sansevieria trifasciata* Hort. ex Prain. leaves were found to be significantly soluble in aqueous were highest than those of other solvents and the least soluble in petroleum- ether. The results were shown in Table 2.

Table 2. Physicochemical Examination of Leaves of *Sansevieria trifasciata* Hort ex Prain.

No.	Physicochemical characters	Average (%)
1.	Moisture content	30.39
2.	Total ash content	11.10
3.	Water soluble ash content	30.17
4.	Acid insoluble ash content	11.59
5.	Aqueous soluble matter content	3.18
6.	Methanol soluble matter content	2.92
7.	Ethanol soluble matter content	2.52
8.	Ethyl acetate soluble matter content	2.26
9.	Chloroform soluble matter content	1.26
10.	Petroleum ether soluble matter content	1.10
11.	Acetone soluble matter content	2.68

Antimicrobial Activities of Different Solvent Extracts of Leaves of *Sansevieria trifasciata* Hort. ex. Prain.

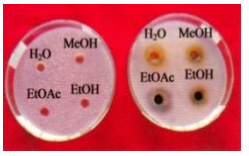
In this study, methanol, chloroform and petether extract of leaves showed activities against on nine test organisms. Aqueous extract of leaves did not showed against on *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Bacillus pumalis*. Ethanol extract of leaves showed activities against *Vibrio cholerae, Proteus mirabilis and Bacillus subtilis.* Ethyl acetate extract of leaves showed against on *Vibrio cholerae*, *Pseudomonas aeruginosa and Klebsiella pneumoniae.* The acetone extract of leaves did not showed against on *Pseudomonas aeruginosa.* Among them, ethanol extract of leaves showed the highest activity against on *Vibrio cholerae.* These results were shown in Table 2 and Figure 2.

Table 3. Inhibition Zone Exhibited by Different Solvent Extract of Leaves of ***Sansevieria trifasciata*** Hort. ex Prain.

No.	Microorganisms	Inhibitions Zone (mm)						
		H_2O	MeOH	EtOH	EtOAC	$CHC1_3$	P.E	Ace
1.	*Vibrio cholerae*	20 +	20 +	35 + + +	30 + +	15 +	15 +	10 +
2.	*Pseudomonas aeruginosa*	-	25 +++	-	10 +	15 +	15 +	-
3.	*Staphylococcus aureus*	15 +	15 +	-	-	20 +	15 +	10 +
4.	*Klebsiella pheumoniae*	20 +	20 +	-	15 +	15 +	10 +	12 +
5.	*Proteus mirabilis*	-	20 +	20 +	-	20 +	15 +	15 +
6.	*Candida albicans*	20 +	25 ++	15 +	-	15 +	10 +	12 +
7.	*Esherichia coli*	15 +	20 +	10 +	-	10 +	12 +	10 +
8	*Bacillus subtilis*	10 +	10 +	-	-	20 +	15 +	10 +
9.	*Bacillus pumalis*	-	20 +	15 +	-	15 +	13 +	10 +

Key to the table

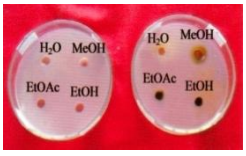
(+) = 10mm-20mm (++) = 21mm-30mm (+++) = 31mm above(5 mm) = Agar well



Control

Antimicrobial activity

Vibrio cholerae



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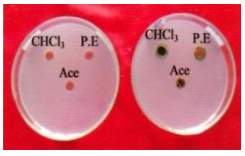
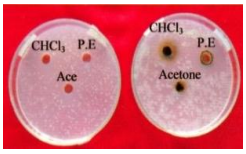


Antimicrobial activity

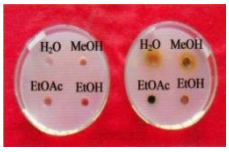
Pseudomonas aeruginosa

Control



Antimicrobial activity

Staphylococcus aureus



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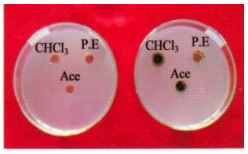

Antimicrobial activity

Klebsiella pneumoniae

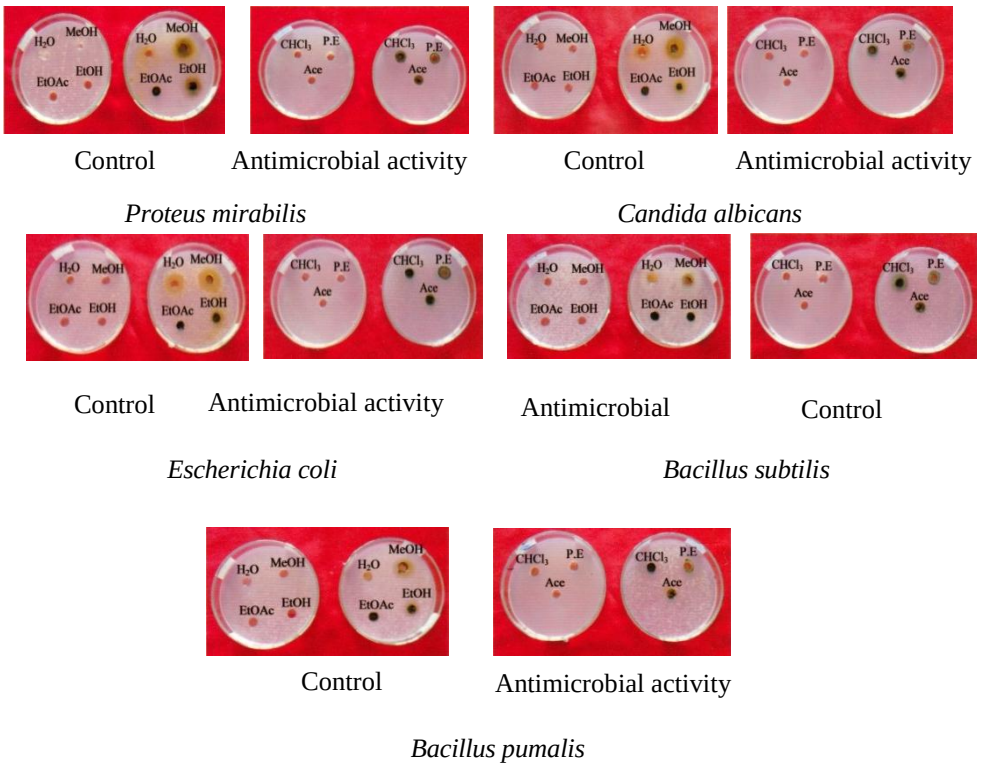

Figure 2. Antimicrobial activities of leaves of *Sansevieria trifasciata* Hort. ex Prain.

Discussion and Conclusion

In this research, the medicinal plant *Sansevieria trifasciata* Hort. ex Prain. belonging to family Asparagaceae has been studied. This plant was collected as wild and cultivated plants which are found abundantly in Dagon University, East Dagon Township, Yangon Region. In morphological studies, they are perennial herbs, evergreen; stemless, rhizome horizontal. Leaves

simple, tuft thick, upright fleshy to rigidly coriaceous, with light or grayish green transverse bands, dark green margin, linear-lanceolate. Inflorescences racemes, flowers in fassicles, actinomorphic, regular, bisexual, hypogynous. Tepal tube short, limbs linear. Epitepalous stamens. Ovary 3 carpels, one ovule in each locule, axile placentation. Fruit berry. Seed with horny endosperm. These results were agreed with Michael, 2004).

The preliminary phytochemical test was carried out on the powders of *Sansevieria trifasciata* Hot. ex Prain. According to these results, alkaloid, glycoside, carbohydrates, flavonoid and terpenoids were present the described by Trease and Evans, 2002, file:// G:/ download/downloadfile-1.htm.

In the physicochemical examination, the powdered leaves were soluble in seven solvents. Among them, the highest yield was obtained from aqueous extract of leaves and moderately soluble in petroleum ether.

In test of antimicrobial activities, the different solvent extracts of leaves inhibited *Vibrio cholerae, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia, Proteus mirabilis, Candida albicans, Escherichia coli, Bacillus subtilis* and *B. pumalis.* Methanol extract of leaves showed higher antimicrobial activities than other extract.

Therefore, the present study focused chemical compounds by using phytochemical test, physicochemical properties and antimicrobial activities of this plant which could be assumed to be beneficial for human health, especially in Myanmar traditional medicine.

Acknowledgements

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Website

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TAXONOMIC STUDY ON NINE SPECIES OF ANGIOSPERMAE IN YAN LAW GROUP OF VILLAGES, KYAING TONG TOWNSHIP

Tin Tin Maw[1]

Abstract

The present study deals with the members of Angiospermae growing in Yan law group of villages, Kyaing Tong Township. Some Angiospermae from Yan law group of villages has been collected, identified and then morphological characteristic were studied. In this study, 9 species belonging to 8 genera of 7 families were identified and systematically arranged according to APG III system, 2009 (Angiosperm Phylogeny Group) with colored plates. All species are dicotyledonous. Artificial key to the species, detail description of individual species has also been described. In addition, their flowering period, Myanmar names and English names were also described.

Keywords: Taxonomy, Yan law group of villages

Introduction

Kyaing Tong Township is situated in Golden Triangle of Eastern Shan State of Myanmar. Yan law group of villages is located in Kyaing Tong Township. Yan law group of villages is bounded by Kyaing Tong in the east, Loi lon group of villages in the west, Hiaw kwal group of villages in the south and Wout soung group of villages in the north. It lies between 21° 16' 20"-21° 17' 40" North Latitude and 99° 33' 50"-99° 35' 20" East Longitude. Yan law group of villages lies 806 meter above sea level. The area is about 40.65 kilometer square.

During the period from January to April 2018, an average monthly rainfall is 1.61 inches and 5 rainy days. This area almost gets no rain fall in February. The average maximum temperature is 29.53° C and average minimum temperature is 13° C. The coldest month of this area is February (10.6° C). The warmest month is March (32.4° C).The maximum percentage of

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humidity in January is 86 and the minimum percentage of humidity in February is 45.

Red-gray and yellow-grey sandy soils cover with mountain area and alluvial soils cover flat land and low land area.

Yan law group of villages is in the mountain deciduous forest region. The natural vegetation consists of herbs, shrubs, climbers, twiners, vines and woody trees. The families Rosaceae, Onagraceae, Loganiaceae, Acanthaceae, Lamiaceae, Plantaginaceae and Convolvulaceae are found in this area. The family Convolvulaceae (*Ipomoea triloba* L.) was commonly found in this area. The Loganiaceae (*Buddleja asiatica* Lour.) was rarely found in the study area.

In the present study 9 species belonging to 8 genera of 7 families under subclass Magnoliidae had been identified and fully described.

The aim and objectives of the present research are mainly to record the knowledge on the natural resources in study area, to get valuable information of Angiospermae to be used for other researchers and to provide invaluable taxonomic data and information in the compilation of flora of Myanmar in the future.

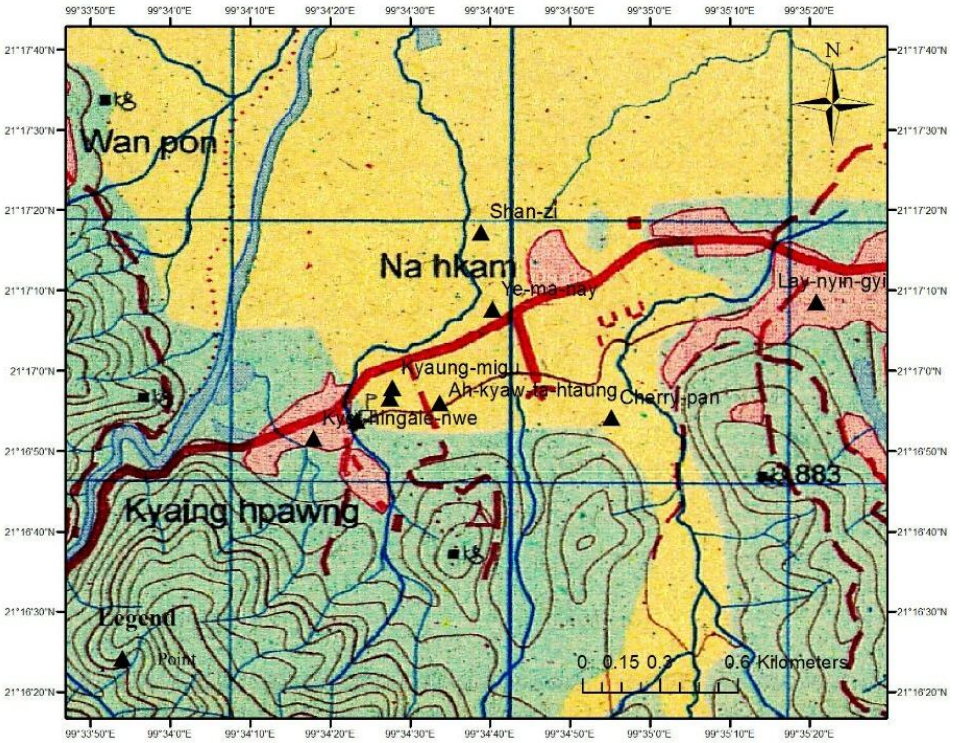

Figure 1. Location map of Yan law group of villages

(Source: Department of Geography, Bago University)

Materials and Methods

Some members of Angiosperm were collected from Yan law group of villages. The specimens were collected from January to April, 2018. The specimens were kept immediately into the plastic bags to identify and classify systematically. The collected specimens had been observed and noted in detail. In addition to construction of artificial key to the species, all the resulting species are systematically arranged into family according to APG III system, 2009 (Angiosperm Phylogeny Group). The specimens were recorded by photographs. The collected specimens were identified with the references of Flora of British India (Hooker, 1881-87), Flora of Java (Backer, 1965) and Flora of Ceylon (Dassanyake, 1980-2001).

Results

Table. List of the collected species (Subclass : Magnoliidae)

Super order	Order	Family	No.	Scientific name	Myanmar name
Fabids	Rosales	Rosaceae	1.	*Prunus cerasoides* D.Don.	Cherry-pan
			2.	*Prunus persica* (L.) Batsch	Shan-zi
Malvids	Myrtales	Onagraceae	3.	*Ludwigia octovalvis* (Jacq.) Raven	Lay-nyin-gyi
Lamids	Gentianales	Loganiaceae	4.	*Buddleja asiatica* Lour.	Kyaung-migu
	Lamiales	Acanthaceae	5.	*Lepidagathis hyalina* var. *semiherbacea* Clarke in Hook. f.	Unknown
		Lamiaceae	6.	*Gmelina arborea* Roxb.	Ye-ma-nay
		Plantaginaceae	7.	*Plantago major* L.	Ah-kyaw-ta-htaung
	Solanales	Convolvulaceae	8.	*Ipomoea triloba* L.	Unknown
			9.	*Merremia vitifolia* (Burm.f.) Hall. f.	Kyet-hingale-nwe

Taxonomic descriptions

1. Rosaceae (Juss. 1789)

1.1. *Prunus cerasoides* D.Don. Prod. Fl. Nepal. 239. 1825.

Myanmar name - Cherry-pan

English name - Indian Cherry

Flowering period - February to April

Deciduous trees, up to 12 m high, stems and branches terete, whitish, glabrous. Leaves simple, alternate; stipules laciniate, glandular, deciduous; petioles 0.7-1.5 cm long, with a pair of circular glands at the apex, grooved

above and provided with scales at the base; blades ovate or oblong lanceolate, 6-15 cm by 1.5-6 cm, more or less rounded or broadly cuneate at the base, sharply serrate along the margin, acuminate at the apex, membranous, glabrous. Inflorescences from lateral buds, fascicled or umbelled, often 3-flowered, glabrous; peduncles 0.5-1.5 cm long. Flowers white or pink, 2-3.5 cm in diameter, actinomorphic; bracts spatulate, 4 cm by 3 cm, toothed at margin; pedicels slender, 0.8-2 cm long. Calyx campanulate, slightly 5-lobed; lobes triangular, acute or obtuse, hypanthium tubular, widened towards the throat, 8-12 mm long, brownish red. Petals 5, free, obovate or suborbicular, 1 cm by 0.6-0.8 cm, pink, inserted at the mouth of hypanthium, erect. Stamens 30-35 in 2 rows, outer ones longer, adnate to the hypanthium; filaments filiform, variable in length; anthers dithecous, variable in length. Ovary inferior, ellipsoid, 1-celled, fleshy-juicy, glabrous, 2 ovules in the locule on pendulous placentae; style 0.5-1 cm long; stigma bifid. Fruits drupaceous, ellipsoid, 1.5 cm by 1 cm, yellowish red, glabrous. Stone bony, rugose and furrowed. (Figure 2. A)

Distribution - Temperate Himalaya, South India, Nepal, mountainous regions of South China, Burma, Thailand, North Vietnam and North Laos (Trivengadumt as cited in Dassanayake 1981). According to Kress and Yin Yin Kyi (2003), this species was distributing in Chin State, Kachin State, Magway Division, Mandalay Division and Shan State of Myanmar.

Specimen examined - Eastern Shan State; Kyaing Tong Township, Yan law group of villages, N 21° 16' 54.13", E 99° 34' 55.20", 824 meter, Dr. Tin Tin Maw, 4. 2. 2018, collected no. 2.

1.2. *Prunus persica* (L.) Batsch, Beytr. Entw. Pragm. Gesch. 1: 30. 1801.

Amygdalus persica L., Sp. Pl. 1: 472.1753.

Myanmar name - Shan-zi; Me-mon

English name - Paech

Flowering period - February to April

Deciduous trees, up to 6 m high; young twigs glabrous. Leaves simple, alternate, stipules linear, subulate, glandular, deciduous; petioles 0.5-1.5 cm long; blades broad, oblong-lanceolate, 7-15 cm by 1.5-5 cm, cuneate at the base with two sessile glands on the margin, uniformly crenate-serrate along the margin, long acuminate at the apex, membranous, glabrous. Flowers pink, 2.5-3 cm in diameter, actinomorphic; bracts ovate, tomentose outside; pedicels very short or sessile. Calyx campanulate, 5-lobed, oval-elliptic, obtuse, pubescent outside, hypanthium cupulate, 4-5 mm. Petals 5, free, orbicular or oblong, 1.5-2 cm by 1.2-1.5 cm, much expanded, concave, clawed, pink, showy. Stamens 20-40; filiment filiform, 1-2 cm long; anther dithecous. Ovary inferior, globoid, tomentose,2 ovules in the locule on pendulous placentae; style slender, 1-1.5 cm long, villous at base, glabrous above; stigma capitate. Fruits drupaceous, globoid, 3-5 cm in diameter, yellow or red, fleshy, tomentose. Stone deeply pitted and furrowed, very hard. (Figure 2. B)

Distribution - Native of China. Cultivated in temperate regions and in mountainous regions in tropical countries (Tirvengadumt as cited in Dassanayake 1981). Kress and Yin Yin Kyi (2003) noted that this species was cultivated in Myanmar.

Specimen examined - Eastern Shan State; Kyaing Tong Township, Yan law group of villages, N 21° 17' 17.24", E 99° 34' 38.92", 808 meter, Dr. Tin Tin Maw, 15.2.2018, collected no. 4.

2. Onagraceae (Juss. 1789)

2.1. *Ludwigia octovalvis* (Jacq.) Raven, Kew. Bull. 15: 476. 1962.

Oenothera octovalvis Jacq. Enum. Pl. Carib. 19. 1760.

Myanmar name - Lay-nyin-gyi

English name - Mexican primrose willow

Flowering period - April to July

Annual, robust herbs, sometimes suffruticose; stems and branches puberulent or densely villous. Leaves simple, alternate; stipules minute, subsessile; blades linear to narrowly ovate, 1-12 cm by 1-4 cm, cuneate at the base, entire along the margin, acute at the apex, puberulous or glabrous on both surfaces. Flowers axillary and solitary, yellow, about 2 cm in diameter, actinomorphic; bracteoles reduced or up to about 1 mm long. Calyx tubular, 4-toothed; tube just above the ovary, 1-1.5 cm long; teeth triangular, acute, shorter than tube. Petals 4, broadly obovate, 0.5-1.8 cm long, emarginate, yellow. Stamens 8, free, inserted; filaments filiform, equal, 1-1.5 mm long, greenish white; anthers dithecous, oblong, 1 mm long. Ovary inferior, linear, 1-1.5 mm long, tetralocular or pentalocular, with many ovules on the axile placentae; style short; stigma thick, slightly 4-lobed, subglobose. Capsules cylindric, 2-4 cm by 2.5-5 mm, pale brown with 8 dark brown ribs, glabrous or villous, many-seeded, irregularly dehiscent. Seeds obovoid, 0.5-0.8 mm long, brown with a white, corky. (Figure 2. C)

Distribution - Throughout the tropics, portions of the distribution undoubtedly due to spread of man, especially on Pacific Islands (Wagner as cited in Dassanayake 1995). Kress and Yin Yin Kyi (2003), treated this species was distributed in Bago Division, Mandalay Division, Sagaing Division, Shan State, Taninthayi Division, Yangon Division of Myanmar.

Specimen examined - Eastern Shan State; Kyaing Tong Township, Yan law group of villages, N 21° 17' 08.60", E 99° 34' 80.84", 810 meter, Dr. Tin Tin Maw, 10.4.2018, collected no. 9.

3. Loganiaceae (R. Br. ex Mart. 1827)

3.1. ***Buddleja asiatica*** Lour. Fl. Cochinch. 72. 1790.

Myanmar name - Kyaung-migu; Kyaung-migo

English name - Butterfly Bush

Flowering period - January to March

Perennial, erect, unarmed shrubs or small trees, 1.5-6 feet, stems and branches terete or subterete, laterally compressed at the apex, grey white or fulvous-tomentose. Leaves simple, opposite and decussate, stipules linear; petiole 2-5 mm long; blades oblong to narrowly lanceolate; 3.5-15 cm by 1.5-5.5 cm, attenuate at the base, entire or crenate or toothed along the margin, acuminate at the apex, dull dark green above, more or less densely white-tomentose beneath. Inflorescences terminal, erect, dense to rather lax, paniculate spike drooping at the tip; primary peduncles 20-35 cm long, ridgid to flaccid, narrow to rather thick. Flowers white, 5-8 mm in diameter, actinomorphic, fragrant; bracts liner, 2.5 mm long, pubescent. Calyx campanulate, 4-lobed, 2 mm long; lobes triangular, 1.5 mm long, acute at the apex, densely pubescent outside. Corolla salverform, 4-lobed, imbricate in bud, marcescent after anthesis, pubescent; tube straight or curved, 5.6 mm long, whitish cream, pubescent within, lobes sub-rounded, 2.5 mm long, subequal, overlapping to the left in bud, patent after anthesis, glabrous within. Stamens 4, free, inserted above the middle of the corolla-tube; anthers dithecous, ovate-oblong, subsessile, basifixed, pale yellow. Ovary superior, ovoid, 1 mm long, glabrous, bilocular, with numerous ovules in each locule on the axile placentae; style linear, glabrous; stigma thickened or 1-lobed. Fruits capsular, septicidally 2-valved, ellipsoid, 6-8 mm long, many seeded, glabrous. Seeds minute. (Figure 2. D)

Distribution - Malaya, Cochin-China, and China (Hooker 1885). According to Kress and Yin Yin Kyi (2003) stated that this species was widely distributed in Myanmar.

Specimen examined - Eastern Shan State; Kyaing Tong Township, Yan law group of villages, N 21° 16' 57.83", E 99° 34' 27.82", 820 meter, Dr. Tin Tin Maw, 12.3.2018, collected no. 6.

4. Acanthaceae (Juss. 1789)

4.1. ***Lepidagathis hyalina*** var. ***semiherbacea*** Clarke in Hook. f. Fl. Brit. Ind. 4. 521. 1885.

Myanmar name - Unknown

English name - Curved Lepidagathis

Flowering period - November to February

Perennial herbs, erect, up to 1m high; stems and branches subquadrangular, puberulous. Leaves simple, opposite and decussate, exstipulate; petioles fattened, 3-12 mm long; blades ovate-elliptic or ovate-lanceolate, 2.5-9 cm by 1.5-3.5 cm, obtuse at the base, entire along the margin, acute at the apex, tomentose on both surfaces. Inflorescences axillary or terminal dense spikes, 1-sided, usually clustered, peduncle 3-8 cm long. Flowers white, 7 mm in diameter, zygomorphic; bracts ovate-lanceolate, 5-9 mm long, sericeous without, glabrous within; bracteoles linear, 6-9 mm long, sericeous without, glabrous within. Sepals 5, unequal, one larger than others, linear-lanceolate, 5-8 mm long, mucronate, pubescent. Corolla distinctly 2-lipped, white with yellowish brown spots on the lower lip, glandular hairy without, puberulous within; tube 6 mm long, narrow at the base, slightly widened above, upper lip 2-lobed; lower lip 3-lobed. Stamens 4, didynamous, inserted; filaments filiform, 1-1.7 mm long, anthers dithecous, oblong, pale yellow. Ovary superior, oblongoid, 2.5-3 mm long, bilocular, with 2 ovules in each locule on the axile placentae; style filiform, 5-7 mm long, sparsely puberulous; stigma capitate. Capsules oblongoid, 5 mm by 1 mm, 2-4-seeded, glabrous. Seeds orbicular. (Figure 2. E)

Distribution - Endemic (Cramer as cited in Dassanayake 1998). Kress and Yin Yin Kyi (2003) recorded that this species was cultivated in Myanmar.

Specimen examined - Eastern Shan State; Kyaing Tong Township, Yan law group of villages, N 21° 16' 53.76", E 99° 34' 23.36", 830 meter, Dr. Tin Tin Maw, 27.1.2018, collected no. 1.

5. Lamiaceae (Martinov. 1820)

5.1. *Gmelina arborea* Roxb., Hort. Beng. 46. hyponyn. 1814.

Myanmar name - Ye-ma-nay

English name - White teak

Flowering period - March to April

Perennial, deciduous, unarmed trees, to 18 m high; stems and branches terete or quadrangular while young, stout, woody, solid, glabrous; barks smooth, pale ashy-grey or grayish yellow with blackish patches; internodes 3-6 cm long. Leaves simple, opposite and decussate; exstipulate, petioles cylindric, terete, 5-12 cm long, canaliculate above, reddish green, puberulent or glabrous; blades broadly ovate, 8-25 cm by 7-17 cm, cordate or truncate at the base, with 2 shining glands at the insertion of leaf, entire along the margin, acute to acuminate at the apex, coriaceous, tomentose or glabrous above, densely fulvous tomentellous with stellate hairs beneath. Inflorescences terminal paniculate cymes; penduncles terete, 4.5-8 cm long, stout, green, puberulous. Flowers yellow, about 3-3.5 cm in diameter, zygomorphic, large, showy, fragrant; bracts linear-lanceolate, 0.4-1 cm long, acute at the apex, yellowish green and turned to brown, glabrous within and densely tomentose without, caducous. Calyx broadly campanulate, equally 5-toothed, brown or brownish green without, green within, densely tomentose without, glabrous within, persistent; tube about 4 mm long, with 2 glands at the anterior side without; lobes ovate, 2 mm long, acute to acuminate at the apex. Corolla funnel-shaped, bilabiate, 5-lobed, brownish yellow without, bright yellow within; tube distinctly curved, 1.5-2.5 cm long, widen at the mouth of tube, tomentose without, glandular-hairy within, lobes unequal in size and shape, broadly ovate to orbicular, 1-2 cm by 1-1.5 cm, obtuse to acute at the apex, the upper lip shortly bifid, sparsely short-white ciliate, the lower lip 3-lobed with a central large one. Stamens 4, free, didynamous, slightly exserted, attached at the base of the widened part of corolla tube; filaments stout, 1.3-2 cm long, slightly thickened at the base, yellow, puberulous; anthers dithecous,

oblong-lanceolate, brown or black. Ovary superior, globoid, 3.5-4.5 mm long, green, glabrous, tetralocular, with one ovule in each locule on the axile placentae; style filiform, 1.5-2 cm long, slightly exserted, glabrous; stigma ligulate. Drupes ovoid or obovoid-pyriform, 1.2-1.8 cm in diameter, juicy, 1 or 2-seeded, orange-yellow when ripe, glabrous. Seeds oblong. (Figure 2. F)

Distribution - Native from Pakistan, Bhutan, and India east through Bangladesh, Burma, and Thailand to Indo-China, Malaya and Indonesia, north to southern China; introduced in many parts of tropical Africa, South America, and elsewhere (Harold Moldenke and Alma Moldenke as cited in Dassanayake 1983). According to Kress and Yin Yin Kyi (2003), this species was distribution in Bago Division, Kachin State, Mandalay Division, Shan State, and Yangon Division of Myanmar.

Specimen examined - Eastern Shan State; Kyaing Tong Township, Yan law group of villages, N 21° 17' 07.65", E 99° 34' 40.34", 816 meter, Dr. Tin Tin Maw, 30.3.2018, collected no. 8.

6. Plantaginaceae (Juss. 1789)

6.1. ***Plantago major*** L. Sp. Pl. 112. 1753.

Myanmar name - Ah-kyaw-ta-htaung; Se-gyaw-paung-ta-htaung

English name - Plantain

Flowering period - December to March

Perennial herbs, stems erect, to about 10 cm long. Leaves simple, alternate or radical, radical rosette, exstipulate, petioles channelled, 2-9 cm long, villous, sheathing at base, densely hairy when young; blades elliptic, broadly elliptic or ovate, 5-16 cm by 1.5-7.5 cm, more or less broadly cuneate at the base, entire or more often dentate along the margin, obtuse at the apex, glabrous or sparsely hairy, densely villous when young. Inflorescences axillary spike, peduncles erect or ascending, more or less arcuate, 5-30 cm long, furrowed, nearly glabrous; spike 5-20 cm long, with flowers more or less distant towards the base. Flowers greenish white, small, sessile,

actinomorphic; bracts elliptic, 1-2 mm long, green, pale and membranous along the margin, glabrous or ciliolate with short hairs, keeled. Calyx 4-lobed; lobes ovate, 2-2.5 mm long, subequal, green, pale and membranous at margins, glabrous, with keel extending to tip. Corolla salverform, 3-5 mm long, 4-lobed, tube oblong-ovoid, lobes lanceolate, narrowly ovate, acute reflexed between the sepals, glabrous. Stamens 4, exserted, epipetalous; filaments filiform 5 mm long; anthers dithecous, 1 mm long, white. Ovary superior, ovoid, bilocular, with many ovules in each locule on axile placenta; style simple; stigma bifid. Fruits capsular, ovoid, 5 mm long, attenuate at apex and truncate, purplish, glabrous. Seeds mostly many, ovoid-oblong or angular, 1-1.5 mm long, black-brown. (Figure 2. G)

Distribution - Also in Nilgiri Hills in Peninsular India, Himalayas, China (Dassanayake 1996). Kress and Yin Yin Kyi (2003) recorded that this species was distributing in Kachin State, Magway Division, Mandalay Division and Shan State of Myanmar

Specimen examined - Eastern Shan State; Kyaing Tong Township, Yan law group of villages, N 21° 16' 55.97", E 99° 34' 33.72", 822 meter, Dr. Tin Tin Maw, 5.2.2018. collected no. 3.

7. Convolvulaceae (Juss. 1789)

7.1. *Ipomoea triloba* L. Sp. Pl. 161. 1753.

Myanmar name - Unknown

English name - Little Bell; Threelobe morning glory

Flowering period - December to March

An annual, terrestrial, prostrate or twining herbs; stems cylindrical, solid, glabrous or pubescent. Leaves simple, alternate, exstipulate, petioles 1.5-13 cm long, sparsely tuberculate, glabrous; blades broadly ovate to orbicular, 2.5-8.5 cm by 2-7 cm, angular or rounded at the base, entire or coarsely dentate or deeply 3-lobed, obtuse or acute and mucronulate at the apex, glabrous or sparsely pilose. Inflorescences axillary, sub-umbelliform

cyme; peduncles 1.5-13 cm long, angular, minutely verruculose, glabrous. Flowers lavender or pinkish purple, 1.2-1.5 cm in diameter, actinomorphic; bracts 2, lanceolate to ovate-lanceolate, about 2 mm long; pedicels 2.5-9 mm long, angular, minutely verruculose, glabrous. Sepals 5, oblong to elliptic-oblong, subequal, 4.5-9 mm by 2.7-3 mm, entire and ciliate along the margin, obtuse to acute and mucronate at the apex, slightly reflexed, sparsely pubescent on the outer surface. Corolla funnelform, 5-lobed; tube 10-12 mm by 3 mm, the throat dark purple; lobes 8-10 mm long. Stamens 5, included; filaments subequal, 5-9 mm long, hairy and dilated at the base, white, anthers dithecous, ovate-oblong, white. Ovary superior, ovoid, 1-2 mm long, densely hirsute, bilocular, with 2-ovules in each locule on the axile placentae; style 8-10 mm long, included; stigma 2-lobed, globose, white. Capsules subglobose, 5-7 mm long, brown, bristly pubescent. Seeds 4 or fewer, subrotund, 3-3.5 mm long, black, glabrous. (Figure 2. H)

Distribution - An American species whose major region of distribution is the West Indian Island; now a pantropical vine found throughout Malaysia, the Pacific Islands and tropical Asia (Austin as cited in Dassanayake 1980). Kress and Yin Yin Kyi (2003) stated that this species was distributing in Yangon Division of Myanmar.

Specimen examined - Eastern Shan State; Kyaing Tong Township, Yan law group of villages, N 21° 16' 56.58", E 99°3 4' 27.49", 821 meter, Dr. Tin Tin Maw, 18.2.2018, collected no. 5.

7. 2. ***Merremia vitifolia*** (Burm. f.) Hall. f., Bot. Jahrb. Syst. 16: 552. 1893.

Convolvulus vitifolius Burm. f., Fl. Ind. 45. t. 18. f. 1. 1768.

Myanmar name - Kyet-hingale-nwe; Sa-pyit-nwe

English name - Grape leaf; wood rose

Flowering period - January to April

Annual, terrestrial, twining herbs; stems cylindrical, green to purplish green, glabrous or patently hirsute with white or fulvous hairs. Leaves simple;

alternate, exstipulate, petiole 2.5-16 cm long, cylindrical, green to purplish green, glabrous or patently hirsute; blades orbicular in shape, palmately 5-7 lobed, 5-19 cm by 3.5-17 cm, the lobes broadly triangular to lanceolate, broadly cordate at the base, weakly dentate to crenate along the margin, acuminate or acute to obtuse and mucronulate at the apex, green, sparsely to densely hairy on the lower surface, hairy or glabrous on the upper surface. Inflorescences axillary cyme of 1-7-flowers; peduncles 1.2-16 cm long, cylindrical, green to purplish green, glabrous or patently hirsute. Flowers bright-yellow, 4.5-5 cm in diameter, slightly zygomorphic, bracts 2, lanceolate, 1.3-2 mm by 1.2-1.5 mm; pedicels cylindrical, 1-1.8 cm long, thickened to the distil portion, glabrous or patently hirsute. Sepals 5, oblong to ovate-oblong, subequal, the outer two 1-1.5 cm by 3-5 mm, the inner three 1.2-1.8 cm by 5.5-6 mm, entire along the margin, obtuse or acute and mucronulate at the apex, reddish green to green, sparsely hirsute or glabrous, with glandular pellucid dots, accrescent in fruits. Corolla funnel-shaped, 5-lobed; tube gibbous on one side, 2.3-3.5 cm by 6-10 mm, bright yellow and paler towards the base; lobes 1.7-2.7 cm long, rounded to obtuse, bright yellow, glabrous. Stamens 5, inserted; filaments unequal, the shorter ones 3.5-5.5 mm long, the longer ones 1.2-1.5 cm long, hairy and dilated at the base; anthers dithecous, ovate-lanceolate to oblong, 3.5-4 mm long. Ovary superior, ovoid, 1-1.8 mm by 1-1.5 mm, glabrous, tetralocular with one ovule in each locule; style 0.8-1.8 cm long, inserted; stigmas 2, globose. Capsules subglobose to depressed-globose, 1.2-1.5 cm by 0.8-1.5 cm, straw-coloured, glabrous; pericarp papery. Seeds 4 or fewer, 4.5-6.5 mm by 2.5-4.5 mm, brownish black, glabrous. (Figure 2. I)

Distribution - Found from India and Ceylon to Indo-China eastward throughout Malaysia (Austin as cited in Dassanayake 1980). Kress and Yin Yin Kyi (2003) recorded that this species was reported from Myanmar.

Specimen examined - Eastern Shan State; Kyaing Tong Township, Yan law group of villages, N 21° 16' 52.56", E 99° 34' 17.96", 834 meter, Dr. Tin Tin Maw, 23.3.2018, collected no. 7.

An artificial key to the studied species:

1. Ovary inferior..... 2

1. Ovary superior..... 4

2. Tree; petals 5; stamens numerous; fruits drupaceous 3

2. Herb; petals 4; stamens 8; fruits capsular..... 3. *Ludwigia octovalvis*

3. Stipules lacinate; bracts spatulate; ovary glabrous; drupe less than 1.5 cm in diameter.....1. *Prunus cerasoides*

3. Stipules linear, not lacinate; bracts ovate; ovary tomentose; drupe more than 3 cm in diameter.....2. *Prunus persica*

4. Leaves alternate..... 5

4. Leaves opposite and decussate..... 7

5. Inflorescences spike; corolla 4-lobed.....7. *Plantago major*

5. Inflorescences cymose; corolla 5-lobed.....6

6. Leaf-blades 5-7-lobed, flowers bright yellow, more than 4 cm in diameter.....9. *Merremia vitifolia*

6. Leaf-blades 3-lobed; flowers pinkish purple, less than 2 cm in diameter.....8. *Ipomoea triloba*

7. Flowers actinomorphic; corolla 4-lobed; stamens equal.....4. *Buddleja asiatica*

7. Flowers zygomorphic; corolla 5-lobed; stamens didynamous..... 8

8. Tree; inflorescences cymose; fruits drupaceous.....6. *Gmelina arborea*

8. Herb; inflorescences spike; fruits capsular.....

.....5. *Lepidagathis hyalina* var. *semiherbacea*

Discussion and Conclusion

The present study deals with the plants growing in Yanlaw group of villages, Kyaing Tong Township. Totally, 9 species belonging to 8 genera of 7 families under subclass Magnoliidae had been studied in the present paper. All the species presented in this study are dicotyledonous plants.

The families in this research paper are Rosaceae,Onagraceae Loganiaceae, Acanthaceae, Lamiaceae, Plantaginaceae and Convolvulaceae under the subclass Magnoliidae. They are arranged according to the classification of APG III system, 2009.

Among the species in the present study, the species of *Ipomoea triloba* L. is commonly found in this area. The species of *Buddleja asiatica* Lour. is rarely found. Among the 9 species**,** *Buddleja asiatica* Lour. is shrub, *Prunus cerasoides* D.Don., *Prunus persica* (L.) Batsch and *Gmelina arborea* Roxb. are trees, the rest species are herbs. Except *Buddleja asiatica* Lour., *Lepidagathis hyalina* var. *semiherbacea* Clarke and *Gmelina arborea* Roxb. are opposite leaves and others are alternate. Flower of *Lepidagathis hyalina* var. *semiherbacea* Clarke, *Gmelina arborea* Roxb. and *Merremia vitifolia* (Burm.f.) Hall. f. are zygomorphic, but the rest species are actinomorphic. Except *Prunus cerasoides* D.Don., *Prunus persica* (L.) Batsch and *Ludwigia octovalvis* (Jacq.) Raven are inferior ovary, others are superior ovary. *Prunus cerasoides* D.Don. and *Prunus persica* (L.) Batsch. are pendulous placentation while the others are axile placentation. Fruits of *Prunus cerasoides* D.Don., *Prunus persica* (L.) Batsch. and *Gmelina arborea* Roxb. are drupaceous, but those of others species are capsular.

Prunus persica (L.) Batsch. is found in the study area used for edible. *Prunus cerasoides* D.Don. and *Gmelina arborea* Roxb. are used for ornamental plants. All 9 species are also medicinally important plants. *Ludwigia octovalvis* (Jacq.) Raven included the IUCN (International Union for the Conservation of Nature) Red list of threatened species.

According to the data collected, it can be noted that 9 species from 8 genera are distributing. The collected species are identified and described with comments on their scientific names, Myanmar names and coloured plates. It is hoped that this research of present investigation have contributed towards a better understanding of 9 species distributed in Yan law group of villages for its paper utilization in the other researchers in various field of study. Finally, it is also hoped that this research paper will provide invaluable taxonomic data and information in the compilation of flora of Myanmar in the future.

A. *Prunus cerasoides* D.Don. B. *Prunus persica* (L.) Batsch. C. *Buddleja asiatica* Lour.

D. *Buddleja asiatica* Lour. E. *Lepidagathis hyalina* F. *Gmelina arborea* Roxb.

var. *semiherbacea* Clarke

G. *Plantago major* L.

H. *Ipomoea triloba* L.

I. *Merremia vitifolia* (Burm. f.) Hall.f.

Figure 2

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INVESTIGATION OF TRACE ELEMENTS IN PLANT SPECIES IN TAUNGNITAUNG AREA, MANDALAY REGION

Sandar[1]

Abstract

There are about 74 plants, counted and collected in the study area. Among these plants, nine plant species with are the most abundant, are selected and contents or three elements in selected 9 species are analyzed by atomic absorption spectrophotometery methods. The biogeochemical data of selected 9 species was also investigated with the cooperation of geochemist from the Department of Applied Geology in order to know whether the selected plants were indicator plants or not. Based on the results, the experimental area can be categorized into 5 groups phytophysiogically. It was found that *Hyptis suaveolens* L. was more potent absorber in copper mineralization than other selected plant species. It is concluded *Hyptis suaveolens* L. can be defined as local indicator plant species as it indicates copper mineralization in the area.

Keywords: Soil Samples, Plant samples, Biogeochemial data, Plant community and Local indicator plant.

Introduction

Geobotany involves the visual identification of vegetation. It can also be stated in another way that it involves the visual investigation of particular species of plant communities which may indicate mineralization in the bedrock. Biogeochemical exploration techniques involve chemical analysis of plants have been used in many part of the world to identify mineral deposit. (Brook, 1972)

Geobotany is used to describe a form of mineral prospecting which relies on characteristic of vegetation to identify the location and extent of are bodies.

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Plants communities indicate either rock types or mineralization. Indicator plants have the advantage over plant communities in that they are likely to enable the mineralization to be located more exactly. Those plants which always indicate the presence of a definite elements are called universal indicators. (Canon, 1960)

Geobotanical investigation of plant cover types or communities indicates mineralization in the bedrock. Some botanical associations with mineralization had been known since at least the 8^{th} or 9^{th} centuries (Karpinsky, 1841)

Therefore, plants and plant associations could be used to characterize the geology of an area as well as they are related to the geologic environment.

The present study, plant collections , soil collections and plant community were studied. Among 74 plants, nine plant species to further investigation of concerning the mineralization and detection of indicator plants.

The aim and objectives of present study is to survey the correlation of soil mineralization and the concentration of accumulation of some mineral in the plant tissues. So as to select which one is the indicator plant in specific experimental area. To support the decision of indicator plants, the structure of plant community, the Biological Absorption Coefficient of different elements from the soil were detected. To study elements distribution in plants along the survey line of the study area. To Observe the plant community. To find different plant species Biological Absorption Coefficient. To determine an indicator plant of Taungnitaung Copper mineralized area.

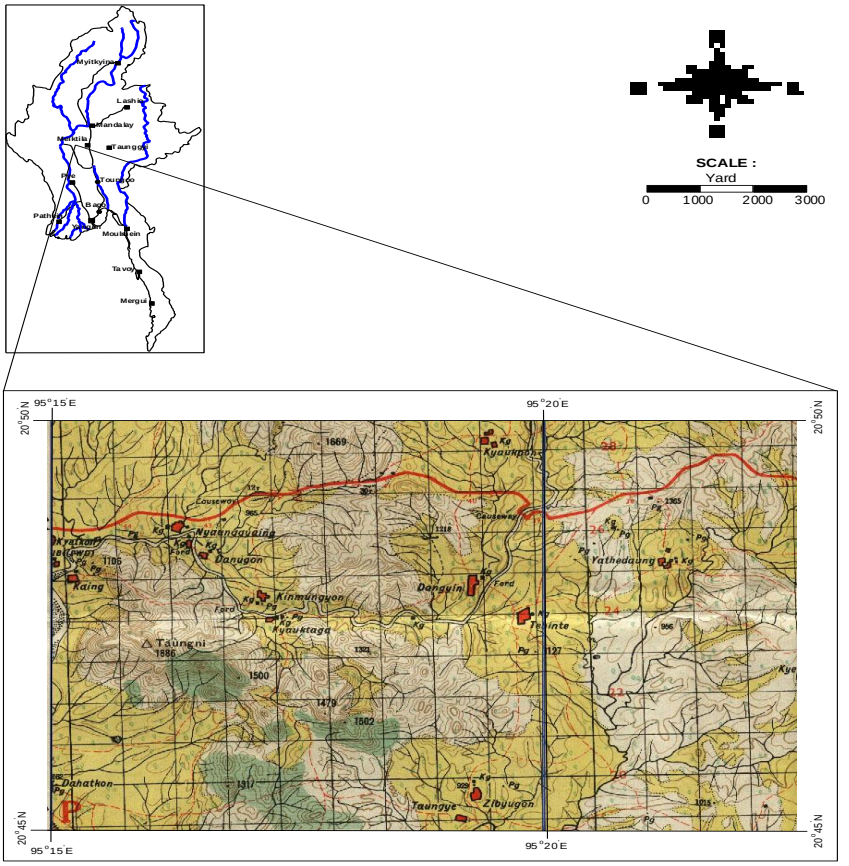

Figure 1. Location Map of Taungni Taung Area

Materials and Methods

Study Area

The study area lies within latitueds N 20° 47' 9" and N 20 ° 48' 12" and longitueds E 95 ° 15' 54" and E 95 ° 16' 48". Taungni Taung area is situated near Kaing village, Kyaukpadaung Township, Mandalay Division. This area is within the boundary of vertical grids 61 to 62 and horizontal grids 23 to 25 and is included partly in the following one inch topographic maps No.84, p/5. The peak elevation of Taungni Taung is about 575m above sea level. (Figure 1).

In the field area, belt transect method are using to carry out transect survey line which is perpendicular to the regional strike of lithologic units of study area. In belt transects consist of continuous serious of quadrats running across the profile of the area. Although the use of quadrats is most usual apporach in indicator plants. (Santra, 1993) Antoher different approach will be needed for studying plants growing over narrow are bodies. And the present study are selected by 23 quadrats for this research. Sample collection point along the mineralized area.

In each 15 m x 15 m quadrat, all plant samples were counted and marked and characteristic of nine plant species were identified among them 74 plant species. Leave samples of nine plant species were washed under running water and air drying is made before preparation for ashing plant specimens in oven. By using an atomic absorption spectrophotometer all geochemical samples were analyzed for Cu elements. Element uptake of plant species data were correlated with soil data.

Statistical Analysis. Box whisker plots are drawn after the nine selected plant species samples have been analyzed. Chemical data, BAC relationship, selected species are calculated and described by using microsoft excel and statistical data analysis software.

Results

Phytosociological Survey Mtheod

As a result of pereliminary phytosociological investigationin Taungni Taung area, the plant community of the study area can be classified into five groups. They are *Terminalia oliveri* Brands. *Tephorisa villosa* Pers. *Azadichrata indica* A.Juss. *Hyptis suaveolens*. L., *Eupatorium adoratum* L. community the following data.

1. *Terminalia oliveri* Brands. Community

   Height - 7 m to 12 m (ree layer)

   Cover% - 75% to 95% (tree layer)

Lithologic Unit - Andesite, Porous argillite, Silica rich

Subunit - Typical subunit

Tephorsia villosa Pers. subunit

Cassia tora L. subunit

Figure 2. *Terminalia oliveri* Brands. (Than) community

1(a). Typical subunit

Height	-	7 m to 12 m
Cover %	-	80 % to 95 % (tree)
Average No. of Species	-	13
Lithologic Unit	-	Andesite,Porous argillised

1(b). *Tephorsia villosa* Pers subunit

Differential species of subunit- *Tephorsia villosa* Pers

Height	-	3 m (shrub layer),
Cover %	-	40 % to 80 %, (shrub layer)
Average No. of Species	-	13
Lithologic Unit	-	Andesite, Porous argillised

Figure 3. *Tephorsia villosa* Pers (Me-yaing kalay)sub unit

1.(c) *Cassia tora* L. Sub unit

Differential species of subunit- *Cassia tora* L.

Height	-	2.5 m to 3 m (shrub layer),
Cover %	-	60 % to 80 %, (shrub layer)
Average No. of Species	-	16
Lithologic Unit	-	Porous argillised, Silica Rich

Figure 4. *Cassia tora* L.(Dan-gywe) sub unit

2 .*Tephorsia villosa* Pers. Community

Height	-	3m (shrub layer)
Cover %	-	40 % to 95 %
Lithologic Unit	-	Porous argillised, Silica Rich rock
Subunit	-	Typical, *Azadirachta indica* A.juss., *Hyptis suaveolens* L. and *Harrisonia perforata* Merr.

Figure 3. *Tephorsia villosa* Pers (Me-yaing kalay) cmmunity

2(a). Typical subunit

Height	-	3 m (shrub layer),
Cover %	-	85 % to 95 %, (shrub layer)
Average No. of Species	-	22
Lithologic Unit	-	Porous argillised rock

2(b). *Azadirachta indica* A.juss Sub unit

Different species of sub unit - *Azadirachta indica* A.juss

Height	-	12 m to 13 m (tree layer)
Cover %	-	80 % to 90 %, (tree layer)
Average No. of Species	-	17

Lithologic Unit - Porous argillised, Silica Rich rock

Figure 5. *Azadirachta indica* A.juss . (Tama)Sub unit

2(c) *Hyptis suaveolens* L. (Taw-pin sein) subunit

Height	-	3 m (shrub layer)
Cover %	-	60 % to 90 % (shrub)
Average No. of Species	-	14
Lithologic Unit	-	Porous argillised rock

Figure 6. *Hyptis suaveolens* L. (Taw-pin sein) subunit

2(d). *Harrisonia perforata* Merr. sub unit

Different species of sub unit	- *Harrisonia perforata* Merr., *Cassia tora* L.
Height	- 7 m to 15 m (trees layer)
Cover %	- 70 % to 95 %, (tree layer)

Average No. of Species - 20

Lithologic Unit - Porous argillised, Silica Rich rock

Figure 7. *Harrisonia perforata* Merr. (sugyin) sub unit

3. *Azadirachta india* A.juss. Community

Height - 8 m to 13 m (tree layer)

Cover % - 80 % to 95 % (tree layer)

Average No. of Species - 17

Lithologic Unit - Porous argillised, Silica Rich rock,

Figure 5. *Azadirachta india* A.juss. (Tama) community

4. *Hyptis suaveolens* L. Community

Height	-	2 m to 3 m (shrubs layer)
Cover %	-	50 % to 95 % (shrubs layer)
Lithologic Unit	-	Silica Rich rock, Andesite rock
Subunit	-	Typical subunit,
		Cassia tora L. subunit

Figure 6. *Hyptis suaveolens* L.(Taw-pin sein) community

4(a). Typical subunit

Height	-	3 m (shrub layer)
Cover %	-	85 % to 90 % (shrubs layer)
Average No. of Species	-	16
Lithologic Unit	-	Andesite rock

4(b). *Cassia tora* L. subunit

Height	-	2 m to 3 m (shrub layer)
Cover %	-	50 % to 95 % (shrubs layer)
Average No. of Species	-	16
Lithologic Unit	-	Silica rich rock, Andesite rock

5. *Eupatorium odoratum* L. Community

Height	-	2.5 m to 3 m (shrub layer)
Cover %	-	40 % to 95 % (shrubs layer)
Average No. of Species	-	9
Lithologic Unit	-	Silica rich rock, Porous argillised rock

Figure 8. *Eupatorium odoratum* L. (Bezat)community

Table 1. Biological Absorption Coefficient of BAC (Cu) in different plant species

Sample No	Hyp	Tec	Bam	Com	Cro	Mil	Tep	Pav	Cas
1	1.030	0.943	1.000	0.526	1.826	0.286	0.769	0.889	0.909
2	1.194	1.514	1.067	1.067	0.833	0.893	0.714	0.857	1.250
3	1.191	0.743	1.200	0.952	1.333	0.476	1.143	1.714	0.800
4	1.019	1.406	1.200	1.071	1.034	1.040	1.029	2.000	0.957
5	1.058	0.766	1.250	1.075	0.323	1.000	1.000	1.042	0.806
6	1.049	0.822	0.709	0.972	0.333	1.000	1.143	1.410	0.767
7	1.074	0.597	1.200	0.826	0.448	1.000	1.154	1.125	0.741
8	1.180	0.607	1.255	0.750	0.576	1.158	1.167	0.727	0.778
9	1.617	0.542							
10	1.915	0.382							
11	1.198	0.431							
12	1.892	0.342							
13	1.804	0.352							
1St Quartile	1.058	0.431	1.050	0.807	0.420	0.789	0.942	0.881	0.775
Median	1.191	0.607	1.180	0.962	0.705	1.000	1.086	1.083	0.803
3rd Quartile	1.617	0.822	1.213	1.068	1.109	1.010	1.146	1.486	0.921
GeoMean	1.251	0.683	1.094	0.883	0.701	0.786	0.999	1.154	0.864

Hyp = *Hyptis suaveolens* L.
Tec = *Tectona hamiltoniana* Wall.
Bam = *Bambusa bambos* L.
Com = *Combretum apetalum* Wall.
Cro = *Croton joufra* Roxb.
Mil = *Millettia brandisiana* Kur.
Tep = *Tephrosia villosa* Pers.
Pav = *Pavonia glechomfolia* A.Rich.
Cas = *Cassia tora* L.

Table 2. Comparison of mean value of BAC (Cu) and different plant species

Maximum	Minimum	Mean	Std.Dev	Std.Error	Index	Species
1.915	1.019	1.325	0.347	0.096	1 =	*Hyptis suaveolens* L.
1.514	0.342	0.727	0.c376	0.104	2 =	*Tectona hamiltoniana* Wall
1.455	0.909	1.185	0.188	0.067	3 =	*Bambusa bambos* L
1.075	0.526	0.905	0.194	0.069	4 =	*Combretum apetalum* Wall
1.826	0.323	0.838	0.535	0.189	5 =	*Croton joufra* Roxb
1.158	0.286	0.857	0.307	0.108	6 =	*Millettia brandisiana* Kur
1.167	0.714	1.015	0.180	0.064	7 =	*Tephrosia villosa* Pers
2.000	0.727	1.221	0.449	0.159	8 =	*Pavonia glechomfolia* A.Rich
1.250	0.741	0.876	0.168	0.059	9 =	*Cassia tora* L

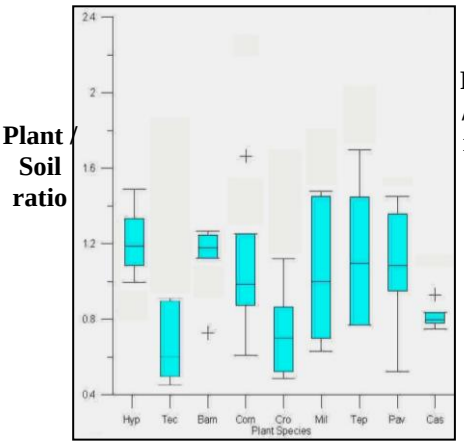

Figure 9. Medium value of Box and Whisker plot (Cu) in different species

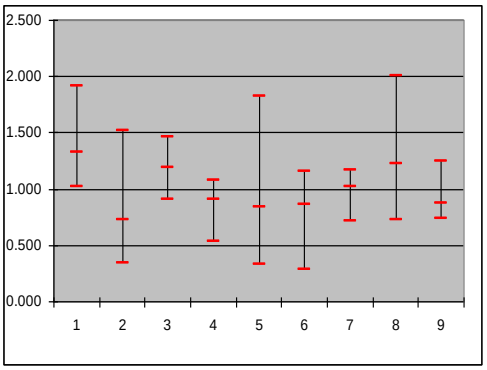

Figure 10. Comparison of mean value(Cu) in different species

Table 3. Atomic Absorption Spectrophotometer data of Nine Plant Species

Species	As (ppm)	Ag (ppm)	Cu (ppm)	Pb (ppm)	Zn (ppm)	Sb (ppm)
Hyptis suaveolens	0.709	0.986	170.125	31.000	16.875	0.076
Tectona hamiltoniana	0.236	1.154	96.625	48.000	22.000	0.512
Bumbusa bambos	0.005	0.074	29.250	41.375	66.875	0.073
Combretum apetalum	0.470	0.981	94.375	32.250	14.875	0.149
Croton joufra	0.664	0.680	112.500	43.750	17.625	0.253
Millettia brandisiana	0.541	0.558	95.000	32.250	14.625	0.164
Tephorsia villosa	0.536	0.696	68.750	33.750	16.000	0.111
Pavonia glechomifolia	0.854	1.003	47.500	32.875	19.500	0.106
Cassia tora	0.003	0.049	22.000	19.125	65.250	0.069

Discussion

The Study area is composed of three lighologic units, it observed. The lithologic units are observed as porous argillite rock, silica rock and andesite rock unit. (Han Sein, 2005).

Nature of plant community was classified into five groups, (i) *Terminalia oliveri* Brands (Than), which grows on all lithologic unit;(ii) *Tephorsia villosa* Pers, (meyaingkalay) which occurs abundantly on silica rich rock unit and porous argillite rock unit; (iii)*Azadirachata indica.* A Juss. (Tamar) which also occurs on all three lithologic units; (iv) *Hyptis suaveolens* (L.) Poit (Taw Pin Sein), which is observed only an andesite rock unit(v) *Eupatorium odoratum* L (Bezat) which grows on silica rich rock unit and porous argillite rock unit. To find the indicator plant in the study area, nine

plant species are selected depending on their the plant communities relative density and uptaking a elements from the biogeochemical classification data, statistical data, Atomic Absorption Spectrophotometer data.

The results of the biological absorption data, it is also found that the Cu value of Mean and Median of *Hyptis suaveolens* L. plant species are highest. Therefore, it can absorb Cu elements more than any other plant species. According to element concentration, biological absorption coefficient data *Hyptis suaveolens* L. is found to be the most uptakable species of all other nine selected species . Besides *Hyptis suaveolens* L. is observed as plant community which abundant develops on Andesite rock unit and absorbs Cu element most.

Amos *et.al* (1981), Shwe Thazin Kyi (1993) and Han Sein (2005) also pointed that Cu mineralization is occurred in the andesite rock unit. From the study of literature reviews, it is described that *Hyptis suaveolens* L. plant species, with their stunted growth, act as an indictor plant for copper rich soil. High copper concentration in some of their organs are observed. (Pal and Sindhupe, 1998).

Therefore, *Hyptis suaveolens* L. plant species is the most absorbent which uptake Cu elements in the study area.

Conclusion

To get the accurate result, nine plant species were selected in the study area. Biological absorption coefficient data *Hyptis suaveolens* L. is found to be the most Cu uptake species of all other nine selected species. The present study area were classified into five plant community group.

According to the laboratory test results, it can be interpreted that *Hyptis suaveolens* L. absorb Cu elements more than any other plant species. Therefore, *Hyptis suaveolens* L.is local indicator plant species in the study area.

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IN VITRO PROPAGATION OF *EUCALYPTUS CITRIODORA* HOOK. UNDER CONTROL OF SOME PHYTOHORMONES AND SUCROSE

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Abstract

In vitro propagation of *Eucalyptus citriodora* Hook. was carried out in the tissue culture laboratory of Vegetable and Fruit Research and Development Centre (VFRDC), Yemon, Hlegu Township, Yangon Region. In multiplication, the shoot apex and nodal segment of *Eucalyptus citriodora* Hook. were cultured in modified (Murashige and Skoog, 1962) MS medium containing various combinations of naphthalene acetic acid (NAA) and 6-benzylaminopurine (BAP). Among them, EuS_6 (NAA 0.75 + BAP 3.0 mg L^{-1}) showed the longest shoot length (2.25 cm) but maximum number of shoot (8.97) and maximum number of leaves (16.74) were obtained from EuS_5 (NAA 0.15 + BAP 3.0 mg L^{-1}). It is therefore EuS_5 (NAA 0.15 + BAP 3.0 mg L^{-1}) was regarded as the suitable treatment for shoot multiplication. *In vitro* shoot elongation, micro shoots (1 - 1.5 cm) were sub-cultured on MS medium added on BAP (0.1 mg L^{-1}) and the combination of BAP (0.1 mg L^{-1}) and (Gibberellic acid) GA_3 (0.1, 0.5 and 1.0 mg L^{-1}). Among them, BAP (0.1 mg L^{-1}) gave the longer shoot length than the combination of BAP and GA_3. Therefore, individual BAP treatment was assumed as a suitable treatment for *Eucalyptus* shoot elongation. In rooting experiment, half-strength MS medium supplemented with various concentrations of (indole-3-butyric acid) IBA (0, 0.5, 1.0 and 1.5 mg L^{-1}) and also supplemented with sucrose (0, 2, 4, 6 and 8 %) on elongated shoots (1.5 - 2.5 cm). The maximum number of root (1.7) was obtained from 2% sucrose.

Key words; *Eucalyptus citriodora* , Multiplication, NAA, BAP, GA_3

Introduction

Genus *Eucalyptus* belongs to the family Myrtaceae, which are mostly found in tropical regions. The native of this genus is the Australia (Logeswari

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and Kanagavalli, 2014). *Eucalyptus citriodora* Hook. is assumed as essential oil producing cultivar and also called Hnet-chauk in Myanmar (Krass et al., 2003). The oil of *Eucalyptus citriodora* Hook. extracted from leaves, has various medicinal benefits, as a stimulant, aphrodisiac, antispasmodic and antiseptic, used in the treatment of septic fevers, asthma, ulcers and spongy and bleeding gums. It is also reported as good for digestion as a nerve sedative and anti-malarian (Santos, 1997).

Eucalyptus is traditionally propagated through the seedling route. In such genetically diverse stocks, trees with the better qualities, such as a straight clear bole, disease and pest resistance, drought tolerance, fast growth, etc., occur at very low frequencies. Due to extensive cross pollination, seed progeny of superior trees do not maintain their superior characteristics. Various methods of vegetative propagation have been attempted, but most have resulted in failure, specifically when applied to explants from adult eucalypt tissues (Durand-Cresswell *et al*., 1982 ***In:*** Sharma and Ramamurthy, 2000). Although clonal propagation of *Eucalyptus* hasbeen achieved using many *in vitro* techniques, the establishment of cultures directly from mature trees or tissues still poses many problems, like difficulty in disinfestation, production of phenolics, rooting rates, etc. (Jones and Staden, 1997 ***In:*** Sharma and Ramamurthy, 2000). Eucalypt plantations raised using micropropagation techniques have been reported to yield higher biomass than seedling-derived plantations (Khuspe *et al.,* 1987 ***In:*** Sharma and Ramamurthy, 2000) if preceded by a selection of superior trees (Sharma and Ramamurthy, 2000).

Micropropagation through axillary proliferation and adventitious shoot proliferation on nodal explants has been successful (Cid *et al.,* 1999; Glocke *et al.,* 2006 ***In***: Cintra, 2007). The most common culture medium is MS medium (Murashige and Skoog, 1962) with a low auxin and cytokinin ratio is most commonly used for shoot multiplication (Watt *et al.,* 2003 ***In***: Cintra, 2007). To stimulate shoot elongation, gibberellic acid was added to some media (Cid *et al.,* 1999; Glocke *et al.,* 2006 ***In***: Cintra, 2007).

Several reports used different cytokinin and gibberellins combination to induce shoot elongation, mainly for genetic materials of difficult *in vitro* propagation (Brondani *et al.,* 2011).

The induction of adventitious roots is difficult in some *Eucalyptus* species
(*E. marginata* and *E. nitens*) but relatively easy in others (*E. camaldulensis*). *Eucalyptus globulus* is moderately easy to multiply, but difficult to root *in vitro* even when explants are taken from seedlings (Bennett *et al.,* 1994). Blomstedt *et al.* (1991) found that the frequency of rooting *E. regnans* F. Muell. *In vitro* was higher and callusing was prevented when a high IBA pulse (98 mM for 7 days in the dark) was applied instead of continual maintenance on 5 mM IBA. Le Roux and van Staden (1991) employed 9.8 mM IBA throughout rooting of a clone of *E. grandis* x *E. macarthurii*, plants and roots developed from callus at basal ends of shoots but these roots did not survive the hardening-off period. Muralidharan and Mascarenhas (1987) reported somatic embryogenesis in *Eucalyptus citriodora* on semisolid B5 medium supplemented with increased sucrose concentration (5%).

Regarding to the above facts, the germinated shoots are cultured on modified MS medium, the aim and objectives of NAA and BAP on formation of callus, to investigate the growth of nodal segments of *Eucalyptus citriodora* Hook. on different concentrations and combination of NAA and BAP, to study the shoot multiplication using by the plant growth regulators, and to study the effect of BAP and GA_3 on shoot elongation and to induced roots in the elongated shoots.

Materials and Methods

Study 1. Shoot multiplication of *Eucalyptus citriodora* Hook. using the combination of

NAA + BAP

The shoot multiplications of the germinated seedlings were conducted in the tissue culture laboratory of Vegetable and Fruit Research and Development Centre (VFRDC), Yemon, Hlegu Township, Yangon Region. The experiment was conducted in 2015.

Inoculation of shoot apex and nodal segment for shoot multiplication

Thirty days old shoot apex about 1 cm size and nodal segment of *in vitro* raised seedlings from germination treatments were excised in the culture chamber. These segments were cultured in modified MS medium containing various combinations of NAA and BAP, sucrose 30 g L^{-1} and agar 5 g L^{-1} at pH 5.8. Thirty mililiter medium was poured in a culture bottle. These cultures were maintained under 1000 - 1200 lux white fluorescent light for 16 hours light period and 8 hours dark period, temperature at 25 ± 2˚C and relative humidity 30 - 50%. A regular sub-culturing was carried out every 30 days to MS fresh medium. The treatments were MS basal medium supplemented with (NAA 0.15 + BAP 2.25) mg L^{-1}, (NAA 0.75 + BAP 2.25) mg L^{-1}, (NAA 1.50 + BAP 2.25) mg L^{-1}, (NAA 2.25 + BAP 2.25) mg L^{-1}, (NAA 0.15 + BAP 3.0) mg L^{-1}, (NAA 0.75 + BAP 3.0) mg L^{-1} and Control. Each treatment had 10 replications.

Data collection and statistical analysis

The number of shoot per cultured bottle, average shoots length, number of leaves were recorded. The collected data were analyzed using IRRISTAT software version 4.0 developed by International Rice Research Institute (IRRI), Los Baños, the Philippines.

Study 2. Shoots elongation of *Eucalyptus citriodora* Hook. in MS medium supplemented with combination of BAP + GA_3

The experiment was conducted in the laboratory of Vegetable and Fruit Research and Development Centre (VFRDC), Yemon, Hlegu Township, Yangon Region.

Inoculation of shoots for elongation

Shoot explants were obtained from the multiplicated shoots of the previous experiment. These shoots (1 - 1.5 cm size) were cultured on MS basal medium supplemented with 0.1 mg L^{-1} each of BAP and GA_3 for shoot elongation. In this experiment, 0.1 mg L^{-1} of BAP was combined with three concentrations of GA_3 (0.1, 0.5 and 1.0 mg L^{-1}), each had ten replicates were set up in completely randomized design (CRD).

Data collection and statistical analysis

The following characters were measured: number of shoots per cultured bottle, average shoots length, number of leaves, leaf width and leaf length. The collected data were analyzed using IRRISTAT software version 4.0 developed by International Rice Research Institute (IRRI), Los Baños, the Philippines.

Study 3. Rooting of multiplicated shoots of *Eucalyptus citriodora* Hook. using IBA and sucrose

The experiment was conducted in the laboratory of Vegetable and Fruit Research and Development Centre (VFRDC), Yemon, Hlegu Township, Yangon Region in 2015.

Rooting of multiplicated shoots by IBA treatments

In rooting, the explants were obtained from the elongated shoots of the previous experiment. The size of shoots 1.5 - 2.5 cm were cultured on half strength MS medium supplemented with different concentrations of IBA for rooting. In IBA treatment, four concentrations of IBA and each concentration with ten replications were set up in completely randomized design (CRD). However, Thirty seven (37) days after cultured, the contamination was observed. Therefore the next cultured of rooting had to establish in half strength MS medium supplemented with the same concentrations of IBA (0, 0.5, 1.0 and 1.5 mg L^{-1}). Each treatment had 10 replications.

Rooting of multiplicated shoots by sucrose treatments

In this experiment, 1.5 - 2.5 cm length shoots from the previous experiment were transferred to half strength MS medium supplemented with various concentration of sucrose (0, 2, 4, 6 and 8%) for rooting (followed by Muralidharan and Mascarenhas, 1987). Five concentrations of sucrose with ten replications each were set up in completely randomized design (CRD).

Culture medium preparation

The elongated shoots were cultured on half-strength MS medium supplemented with various concentrations and combination of IBA as well as different concentrations of sucrose. The pH was adjusted 5.6 ± 0.2. Thirty milliliter medium was poured in a culture bottle. These cultures were maintained for 16 hours light period and 8 hours dark period using 1000 - 1200 lux from 4 feet white fluorescent tubes. The cultures were also maintained at the temperature of 25 ± 2ºC and relative humidity of 30 - 50%.

Data collection and statistical analysis

The following parameters were measured: number of shoot per cultured bottle, average shoots length, number of roots and average roots length. The collected data were analyzed using IRRISTAT software version 4.0 developed by International Rice Research Institute (IRRI), Los Baños, the Philippines.

Results

Study 1. Shoot multiplication of *Eucalyptus citriodora* Hook. using the combination of

NAA + BAP

Shoot length

The developed shoot length was observed 14 days after culture the segments. However, the initial measurement of shoots did not carry out because of the minute size of developed segments. It is therefore the data collection was started 30 days after culture and it was done in every 3 weeks

intervals. The longest shoot length (2.25 cm) was obtained from EuS_6 (NAA 0.75 + BAP 3.0 mg L^{-1}) followed by 2.00 cm from EuS_3 (NAA 1.50 + BAP 2.25 mg L^{-1}). The shortest shoot length (1.38 cm) was observed from EuS_1 (NAA 0.15 + BAP 2.25 mg L^{-1}). EuS_7 (Control) had 2.01 cm which was lower than EuS_6 but higher than the other treatments. By statistics, the treatment means were not significantly different from each other (Table 1).

Table 1. Effect of NAA and BAP on shoot length of *Eucalyptus citriodora* Hook.

Treatment	Shoots length (cm)					
	30 DAI	51 DAI	72 DAI	93 DAI	114 DAI	Mean
EuS_1 (NAA 0.15 + BAP 2.25 mg L^{-1})	0.98	1.33	1.35	1.58	1.68	1.38
EuS_2 (NAA 0.75 + BAP 2.25 mg L^{-1})	0.98	1.42	1.67	2.07	2.32	1.69
EuS_3 (NAA 1.50 + BAP 2.25 mg L^{-1})	1.01	1.35	1.78	2.49	3.35	2.00
EuS_4 (NAA 2.25 + BAP 2.25 mg L^{-1})	0.98	1.29	1.17	1.73	2.03	1.44
EuS_5 (NAA 0.15 + BAP 3.0 mg L^{-1})	0.87	1.09	1.72	2.52	2.77	1.79
EuS_6 (NAA 0.75 + BAP 3.0 mg L^{-1})	1.33	1.42	1.84	2.44	4.2	2.25
EuS_7 (Control)	1.07	1.44	1.59	2.29	3.64	2.01
F-test	ns	ns	ns	ns	*	-
5 % LSD	0.40	0.49	0.64	1.19	1.58	-
cv %	22.10	20.70	22.50	31.20	31.10	-

DAI=Days after inoculation EuS=Shoot of eucalypt ns=non significan *=significant

Number of shoots

The maximum number of shoots (8.97) was observed from EuS_5 (NAA 0.15 + BAP 3.0 mg L^{-1}) followed by 8.03 from EuS_6 (NAA 0.75 + BAP 3.0 mg L^{-1}). The minimum number of shoot (3.19) was obtained from EuS_7

(Control). By statistics, the treatment means were highly significant from each other (Table 2).

Table 2. Effect of NAA and BAP on number of shoot of *Eucalyptus citriodora* Hook.

Treatment	Number of shoots					Mean
	30 DAI	51 DAI	72 DAI	93 DAI	114 DAI	
EuS_1 (NAA 0.15 + BAP 2.25 mg L^{-1})	4.00	5.33	6.33	7.67	8.83	6.43
EuS_2 (NAA 0.75 + BAP 2.25 mg L^{-1})	5.00	6.17	7.67	8.17	10.03	7.41
EuS_3 (NAA 1.50 + BAP 2.25 mg L^{-1})	3.83	5.50	7.50	9.00	11.90	7.55
EuS_4 (NAA 2.25 + BAP 2.25 mg L^{-1})	3.00	4.50	6.67	8.50	10.50	6.63
EuS_5 (NAA 0.15 + BAP 3.0 mg L^{-1})	6.50	6.77	8.43	10.30	12.87	8.97
EuS_6 (NAA 0.75 + BAP 3.0 mg L^{-1})	4.00	5.83	8.30	9.90	12.13	8.03
EuS_7 (Control)	1.00	2.13	2.93	3.87	6.00	3.19
F-test	**	**	**	**	**	-
5 % LSD	1.34	1.85	2.15	2.33	2.88	-
cv %	44.00	30.50	30.80	29.00	26.50	-

DAI = Days after inoculation EuS = Shoot of Eucalypt ** = highly significant

Number of leaves

The highest number of leaves (16.74) was obtained from EuS_5 (NAA 0.15 + BAP 3.0 mg L^{-1}) followed by 15.89 from EuS_6 (NAA 0.75 + BAP 3.0 mg L^{-1}). The lowest number of leaves (8.97) was observed from EuS_7 (Control). By statistics, the treatment means were not significantly different from each other (Table 3).

Table 3. Effect of NAA and BAP on number of leaves of *Eucalyptus citriodora* Hook.

Treatment	Number of leaves					
	30 DAI	51 DAI	72 DAI	93 DAI	114 DAI	Mean
EuS_1 (NAA 0.15 + BAP 2.25 mg L^{-1})	4.67	6.37	9.33	12.90	14.53	9.56
EuS_2 (NAA 0.75 + BAP 2.25 mg L^{-1})	7.67	11.33	13.17	16.33	20.00	13.70
EuS_3 (NAA 1.50 + BAP 2.25 mg L^{-1})	8.00	12.67	15.10	18.23	19.30	14.66
EuS_4 (NAA 2.25 + BAP 2.25 mg L^{-1})	7.00	9.00	12.83	16.73	20.00	13.11
EuS_5 (NAA 0.15 + BAP 3.0 mg L^{-1})	8.00	11.83	15.33	19.83	28.70	16.74
EuS_6 (NAA 0.75 + BAP 3.0 mg L^{-1})	7.00	11.30	15.53	19.77	25.87	15.89
EuS_7 (Control)	6.00	6.83	8.67	10.33	13.00	8.97
F-test	ns	ns	*	ns	**	-
5 % LSD	4.29	4.82	4.51	6.85	7.15	-
cv %	35.00	27.30	19.70	23.60	19.90	-

DAI = Days after inoculation EuS = Shoot of Eucalypt ns = non significant * = significant ** = highly significant

Study 2. Shoots elongation of *Eucalyptus citriodora* Hook. in MS medium supplemented with combination of BAP + GA_3

Shoot length

Shoot development was observed 14 days after culture. It is therefore the data collection was started 14 days after culture. Fifty-six days after inoculation, the longest shoot length (3.01 cm) was observed from EuE_1 (BAP 0.1 mg L^{-1}) followed by 2.90 cm was obtained from EuE_3 (BAP 0.1 + GA_3 0.5 mg L^{-1}). The shortest shoot length (1.38 cm) was produced from EuE_5 (Control). By statistics, the treatment means were highly significant from each other (Table 4).

Table 4. Effect of BAP and GA_3 on shoot length of *Eucalyptus citriodora* Hook.

Treatment	Shoot length (cm)				
	14 DAI	28 DAI	42 DAI	56 DAI	Mean
EuE_1 (BAP 0.1 mg L^{-1})	1.86	2.34	3.74	4.11	3.01
EuE_2 (BAP 0.1 + GA_3 0.1 mg L^{-1})	1.81	1.99	2.75	3.23	2.45
EuE_3 (BAP 0.1 + GA_3 0.5 mg L^{-1})	1.76	2.24	3.53	4.06	2.90
EuE_4 (BAP 0.1 + GA_3 1.0 mg L^{-1})	1.34	1.96	2.56	2.81	2.17
EuE_5 (Control)	1.11	1.26	1.41	1.74	1.38
F-test	**	**	**	**	-
5%LSD	0.37	0.44	0.42	0.53	-
cv %	21	20.6	13.6	15.1	-

DAI = Days after inoculation, EuE = Elongation of Eucalypt, ** = highly significant

Number of shoots

The maximum number of shoots (26.81) was produced from EuE_1 (BAP 0.1 mg L^{-1}) followed by 24.61 from EuE_2 (BAP 0.1 + GA_3 0.1 mg L^{-1}). The minimum number of shoots (7.32) was obtained from EuE_5 (Control). By statistics, the treatment means were highly significant from each other (Table 5).

Table 5. Effect of BAP and GA_3 on number of shoot of *Eucalyptus citriodora* Hook.

Treatment	Number of shoots				
	14 DAI	28 DAI	42 DAI	56 DAI	Mean
EuE_1 (BAP 0.1 mg L^{-1})	9.51	19.86	33.29	44.57	26.81
EuE_2 (BAP 0.1 + GA_3 0.1 mg L^{-1})	11.29	19.14	29.29	38.71	24.61
EuE_3 (BAP 0.1 + GA_3 0.5 mg L^{-1})	8.14	16.43	27.29	37.43	22.32
EuE_4 (BAP 0.1 + GA_3 1.0 mg L^{-1})	9.14	15.14	24.43	32.29	20.25
EuE_5 (Control)	4.71	6.29	8.00	10.29	7.32
F-test	*	**	**	**	-
5%LSD	4.02	3.63	4.42	4.21	-
cv %	42.60	21.40	16.40	11.70	-

DAI = Days after inoculation, EuE = Elongation of Eucalypt, * = significant, ** = highly significant

Number of leaves

The maximum number of leaves (40.43) was observed from EuE_1 (BAP 0.1 mg L^{-1}) followed by 37.36 from EuE_3 (BAP 0.1 + GA_3 0.5 mg L^{-1}). The minimum number of leaves (15.22) was obtained from EuE_5 (Control). By statistics, the treatment means were highly significant from each other (Table 6).

Table 6. Effect of BAP and GA_3 on number of leaves of *Eucalyptus citriodora* Hook.

Treatment	Number of leaves				
	14 DAI	28 DAI	42 DAI	56 DAI	Mean
EuE_1 (BAP 0.1 mg L^{-1})	15.57	28.14	48.29	69.71	40.43
EuE_2 (BAP 0.1 + GA_3 0.1 mg L^{-1})	11.86	23.00	44.00	67.71	36.64
EuE_3 (BAP 0.1 + GA_3 0.5 mg L^{-1})	11.71	23.29	44.43	70.00	37.36
EuE_4 (BAP 0.1 + GA_3 1.0 mg L^{-1})	12.00	21.00	37.29	55.71	31.50
EuE_5 (Control)	9.29	13.00	17.29	21.29	15.22
F-test	ns	**	**	**	-
5%LSD	4.86	5.82	8.68	8.04	-
cv %	36.4	24.3	20.6	12.8	-

DAI = Days after inoculation, EuE = Elongation of Eucalypt, ns = non-significant,

** = highly significant

Leaf width

The best results of leaf width (0.33 cm) was obtained from EuE_1 (BAP 0.1 mg L^{-1}) followed by 0.32 cm from EuE_3 (BAP 0.1 + GA_3 0.5 mg L^{-1}). The lowest leaf width (0.19 cm) was produced from EuE_5 (Control). By statistics, the treatment means were highly significant from each other. It was found that BAP alone support leaf expension but the addition of GA_3 inhibit leaf width expension (Table 7).

Table 7. Effect of BAP and GA_3 on leaf width of *Eucalyptus citriodora* Hook.

Treatment	Leaf width (cm)				
	14 DAI	28 DAI	42 DAI	56 DAI	Mean
EuE_1 (BAP 0.1 mg L^{-1})	0.26	0.29	0.34	0.43	0.33
EuE_2 (BAP 0.1 + GA_3 0.1 mg L^{-1})	0.21	0.21	0.26	0.29	0.24
EuE_3 (BAP 0.1 + GA_3 0.5 mg L^{-1})	0.24	0.26	0.33	0.46	0.32
EuE_4 (BAP 0.1 + GA_3 1.0 mg L^{-1})	0.13	0.20	0.21	0.24	0.20
EuE_5 (Control)	0.13	0.14	0.23	0.26	0.19
F-test	**	**	**	**	-
5%LSD	0.43	0.51	0.62	0.47	-
cv %	20.1	21	20.6	12.7	-

DAI = Days after inoculation EuE = Elongation of Eucalypt ** = highly significant

Leaf length

The longest leaf length (0.45 cm) was observed from EuE_3 (BAP 0.1 + GA_3 0.5 mg L^{-1}) followed by 0.42 cm from EuE_1 (BAP 0.1 mg L^{-1}). The shortest leaf length (0.32 cm) was achieved from EuE_4 (BAP 0.1 + GA_3 1.0 mg L^{-1}). The shortest leaf length (0.32) was also obtained from EuE_5 (Control). By statistics, the treatment means were highly significant from each other (Table 8).

Table 8. Effect of BAP and GA_3 on leaf length of *Eucalyptus citriodora* Hook.

Treatment	Leaf length (cm)				
	14 DAI	28 DAI	42 DAI	56 DAI	Mean
EuE_1 (BAP 0.1 mg L^{-1})	0.36	0.40	0.46	0.47	0.42
EuE_2 (BAP 0.1 + GA_3 0.1 mg L^{-1})	0.31	0.37	0.44	0.44	0.39
EuE_3 (BAP 0.1 + GA_3 0.5 mg L^{-1})	0.37	0.39	0.47	0.56	0.45
EuE_4 (BAP 0.1 + GA_3 1.0 mg L^{-1})	0.24	0.29	0.36	0.37	0.32
EuE_5 (Control)	0.24	0.27	0.34	0.41	0.32
F-test	**	**	*	**	-
5%LSD	0.74	0.77	0.91	0.79	-
cv %	21.9	20.3	20	15.9	-

DAI = Days after inoculation, EuE = Elongation of Eucalypt, * = significant, ** = highly significant

Study 3. Rooting of multiplicated shoots of *Eucalyptus citriodora* Hook. using IBA and sucrose

Rooting of multiplicated shoots by IBA treatments

Number of shoot

Thirty days after cultured number of shoot, shoot length, number of root and root length were recorded. The maximum number of shoot (17.40) was obtained from EuR_2 (IBA 0.5 mgL^{-1}) followed by 15.40 from EuR_4 (IBA 1.5 mg L^{-1}). The minimum number of shoot (14.20) was produced from EuR_3 (IBA 1.0 mg L^{-1}). The maximum number of shoot (21.70) was also obtained from EuR_1 (Control).

Number of root

The maximum number of root (12.20) was obtained from EuR_3 (IBA 1.0 mg L^{-1}). The second highest number of roots (10.90) was produced from EuR_4 (IBA 1.5 mg L^{-1}) followed by 7.80 from EuR_2 (IBA 0.5 mg L^{-1}). EuR_1 (Control) did not produce roots.

Shoot length

The longest shoot length (6.10 cm) was obtained from EuR_4 (IBA 1.5 mg L^{-1}) followed by 2.70 cm from EuR_2 (IBA 0.5 mg L^{-1}). The shortest shoot length (2.20 cm) was produced from EuR_3 (IBA 1.0 mg L^{-1}). EuR_1 (Control) possessed 4.70 cm which was shorter than EuR_4 but longer than EuR_2 and EuR_3.

Root length

The longest root length (1.20 cm) was obtained from EuR_4 (IBA 1.5 mg L^{-1}) followed by 1.00 cm from EuR_2 (IBA 0.5 mg L^{-1}) and EuR_3 (IBA 1.0 mg L^{-1}). EuR_1 (Control) did not produce roots.

Rooting of multiplicated shoots by Sucrose treatments

Thirty days after cultured, number of shoot, number of root, shoot length and root length in each treatment were collected. The maximum root length (2.48 cm) was observed in 4% sucrose treatment. The maximum number of root (1.67) was occurred in concentration of sucrose 2%.

Number of shoot

The maximum number of shoot (21.50) was obtained from EuC_2 (Sucrose 2%) followed by 10.30 from EuC_5 (Sucrose 8%). The minimum number of shoot (4.70) from EuC_3 (Sucrose 4%). EuC_1 (Control) had 13.00 which was lower than EuC_2 but higher than other treatments.

Number of root

The highest number of root (1.70) was obtained from EuC_2 (Sucrose 2%) followed by 1.20 from EuC_3 (Sucrose 4%). EuC_5 (Sucrose 8%) had the lowest number of roots (0.30). EuC_4 (Sucrose 6%) and EuC_1 (Control) have not developed any roots.

Shoot length

The longest shoot length (2.80 cm) was obtained from EuC_2 (Sucrose 2%). The second longest shoot length (1.30 cm) was observed from EuC_5 (Sucrose 8%) followed by 1.10 cm from EuC_3 (Sucrose 4%). The shortest shoot length (0.90 cm) was produced from EuC_4 (Sucrose 6%). EuC_1 (Control) had 2.70 cm which was lower than EuC_2 but higher than other treatments.

Root length

The longest root length (2.50 cm) was obtained from EuC_3 (Sucrose 4%) followed by (1.40 cm) from EuC_2 (Sucrose 2%). The shortest root lengths (0.30 cm) was produced from EuC_5 (Sucrose 8%). EuC_1 (Control) and EuC_4 (Sucrose 6%) were not produced root.

Table 9. Effect of IBA and sucrose on number of roots, root length, shoot length and number of shoots of *Eucalyptus citriodora* Hook.

Rooting	Shoot length (cm)	No. shoot	No. root	Root Length (cm)
EuR_1 (Control)	4.7	21.7	-	-
EuR_2 (IBA 0.5 mg L^{-1})	2.7	17.4	7.8	1
EuR_3 (IBA 1.0 mg L^{-1})	2.2	14.2	12.2	1
EuR_4 (IBA 1.5 mg L^{-1})	6.1	15.4	10.9	1.2

Rooting	Shoot length (cm)	No. shoot	No. root	Root Length (cm)
EuC_1 (Control)	2.73	6.33	-	-
EuC_2 (Sucrose 2%)	2.78	10.17	1.67	1.37
EuC_3 (Sucrose 4%)	1.13	4.67	1.17	2.48
EuC_4 (Sucrose 6%)	1.07	3.33	-	-
EuC_5 (Sucrose 8%)	1.33	6.00	0.33	0.27

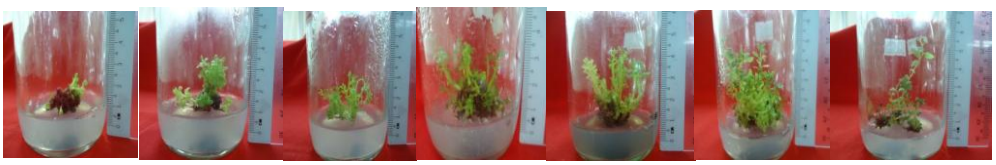

Figure 1. One hundred and fourteen days after culture of *Eucalyptus citriodora* Hook. on MS medium supplemented with various concentrations and combination of NAA and BAP for multiplication

Figure 2. Fifty-six days after inoculation of *Eucalyptus citriodora* Hook. on MS medium supplemented with various concentrations and combination of BAP and GA_3 for elongation

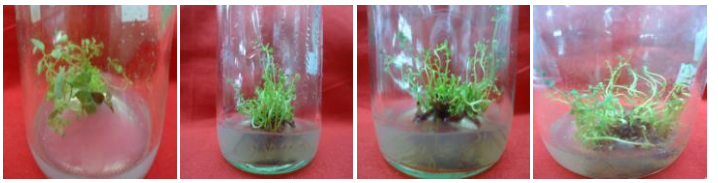

Figure 3. Thirty days after cultured, *in vitro* rooted plantlets of *Eucalyptus citriodora* Hook. on half MS medium supplemented with various concentrations of IBA for rooting

Figure 4. Thirty days old cultures of *Eucalyptus citriodora* Hook. on half MS medium supplemented with various concentrations of sucrose for rooting

Discussion and Conclusion

The 1 cm size 30 days old shoot apex and nodal segment of *in vitro* raised seedlings were excised in the culture chamber. These segments were cultured in modified MS medium containing various concentrations and combination of NAA and BAP. One hundred and fourteen days after inoculation (114 DAI), EuS_6 (NAA 0.75 + BAP 3.0 mg L^{-1}) possessed the longest shoot length (2.25 cm) but maximum number of shoot (8.97), and maximum number of leaves (16.74) were obtained from EuS_5 (NAA 0.15 + BAP 3.0 mg L^{-1}). In micropropagation, the responses of *Eucalyptus* species were varied according to concentration of plant growth regulators and also

depended on culture conditions. Gomes and Canhoto (2003 ***In:*** Brondani *et al.,* 2010) verified that 0.9 μM BAP (0.20 mg L^{-1}) combined with 0.05 μM NAA (0.01 mg L^{-1}) on half MS medium was the optimum concentration for *E. nitens* bud proliferation. Joshi *et al.* (2003 ***In:*** Brondani *et al.,* 2010) observed that *E. tereticornis* x *E. grandis* produced 20 to 25 buds per explant 150 days after cultured in MS medium supplemented with 1 mg L^{-1} BAP and 1 mg L^{-1} NAA. Similar results were obtained by Bisht *et al.* (1999 ***In:*** Brondani *et al.,* 2010) for *E. tereticornis* x *E. camaldulensis*, where the highest multiplication, regardless of clone, occurred after 120 days in MS medium supplemented with 1 mg L^{-1} BAP and 1 mg L^{-1} NAA. George *et al.* (2008 ***In:*** Brondani *et al.,* 2010) emphasized that excess cytokinin in the culture medium may be toxic to the explant, causing severe problems in the subsequent stages. Inclusion of BAP (1.0 - 10.0 mg L^{-1}) in the culture medium was essential for bud break and shoot multiplication of *R. hybrida* .

Bennett *et al.* (1994 ***In:*** Brondani *et al.,* 2010) recorded that BAP concentrations above 2.5 μM (0.56 mg L^{-1}) in MS medium reduced the average number of buds per explant of *E. globules*. Similar effects were verified by Bisht *et al.* (1999 ***In:*** Brondani *et al.,* 2010) for *E. tereticornis* x *E. camaldulensis*, who reported multiplication followed by elongation was occurred 120 days after cultured in MS medium supplemented with BAP and NAA. After 180 days in MS medium, all BAP and NAA treatments had elongation, producing shoots of 2.0 cm to 3.5 cm in length. During establishment, the lowest number of shoot bud production was 3 per explant and the highest 9 was obtained from EuS_5 (NAA 0.15 + BAP 3.0 mg L^{-1}). This treatment also had the highest number of shoot (8.97). It is therefore concluded that EuS_5 (NAA 0.15 + BAP 3.0 mg L^{-1}) can be assumed as the suitable treatment for shoot and leaves multiplication.

In the next step, the culture was carried out to find out the effect of various concentrations and combinations of BAP and GA_3 on *in vitro* shoot elongation of *Eucalyptus*. Micro shoots (1 - 1.5 cm) were sub-cultured on MS medium having BAP (0.1 mg L^{-1}) combined with various concentrations of

GA_3 (0.1, 0.5 and 1.0 mg L^{-1}). The study was monitored 14 days after cultured and measurement was done with respect to shoot length, number of shoots, number of leaves, leaf width and leaf length. The optimum shoots length response to the applied BAP and GA_3 were observed in pure BAP 0.1 mg L^{-1} treatment. Brondani (2011) reported that *Eucalyptus* hybrid H12 had the longest shoot length with medium supplemented with 0.07 mg L^{-1} BAP and 0.22 mg L^{-1} GA_3, with shoots on average of 1.25 cm in length when grown on half-strength MS medium. When full-strength MS medium was used, *Eucalyptus* hybrid H12 clones were 1.13 cm long with medium containing 0.08 mg L^{-1} BAP and 0.16 mg L^{-1} GA_3 . In this study, the longest shoot length was obtained from the explants cultured on the MS medium containing BAP (0.1 mg L^{-1}). After 56 DAI on MS medium containing BAP 0.1 mg L^{-1} had elongation, producing the shoot length of 3.01 cm. The maximum number of shoot was 26.81, the maximum number of leaves was 40.43 and the maximum leaf width was 0.33 cm and leaf length was 0.45 cm. Joshi *et al.* (2003) observed that the mean length of elongated shoots varied from 2.5 to 3 cm in length during 30 days when grown on MS medium supplemented with 1 mg L^{-1} BAP and 0.04 mg L^{-1} GA_3. Similar effects were verified by Bisht *et al*. (1999) for *E*. *tereticornis* x *E*. *camaldulensis*, who reported that multiplication followed by elongation at 120 days after culture on MS medium supplemented with BAP and NAA. After 180 days on MS medium, all BAP and NAA treatments had elongation, producing shoots of 2.0 cm to 3.5 cm in length. However, Graca and Mendes (1989) reported that shoot elongation was greatest when cultured on half-strength MS medium containing 0.1 mg L^{-1} BAP and 0.01 mg L^{-1} IBA. Addition of GA_3, at both concentrations, did not induce as much growth as culturing on half-strength MS medium alone. In my experiment, using the combination of BAP and GA_3 did not give the superior result as in the medium supplemented only BAP. Nevertheless, shoots were longer when cultured on medium containing GA_3, compared to those grown on MS medium alone. Le Roux and Van Staden (1991) reported that Gibberellic acid has been added to media to obtain shoot elongation. Franclet and Boulay (1982) the addition of activated charcoal to a mediu m containing GA_3 and

other growth regulators was reported to cause elongation and to recover the morphological characteristics of the species. Graca and Mendes (1989) the addition of activated charcoal and growth regulators reduced shoot elongation and caused leaf browning. The greater reduction of shoot growth was also observed when shoots were cultured on Goncalves medium containing 0.5 mg L^{-1} BAP and 1.0 mg L^{-1} IAA. Joshi *et al.* (2003) reported that in *Eucalyptus* F_1 hybrid (*Eucalyptus tereticornis* × *Eucalyptus grandis*), the elongation of shoots was achieved within 15 days when such rosette clump of buds were cultured on MS medium supplemented with Kn and GA_3 along with BAP. In the present study, individual BAP treatment to *Eucalyptus citriodora* Hook. gave the longer shoot length than the combination of BAP and GA_3. It is therefore concluded that BAP treatment was suitable for *Eucalyptus* shoot elongation.

The rooting experiment was carried out to find out the effect of various concentrations of IBA (0, 0.5, 1.0 and 1.5 mg L^{-1}) and sucrose (0%, 2%, 4%, 6% and 8%) on elongated shoots (1.5 - 2.5 cm). The maximum number of roots was observed in half strength MS medium supplemented with 1.5 mg L^{-1} IBA where the small calli are observed in IBA treatments. The same was also observed in 2% sucrose supplemented medium. For root initiation IBA, NAA and IAA were used at a level of 0.5 mg L^{-1} each (Joshi *et al;* 2003). Joshi *et al;* 2003 mentioned that highest number of roots (3.22 ± 0.32) was observed in 1 mg L^{-1} IBA while the lowest number of roots (1.16 ± 0.15) in 0.1 mg L^{-1} IBA. The maximum root length (5.66 ± 0.69 cm) was observed in lower concentration (0.1 mg L^{-1}) of IBA (Joshi, 2003). In the present study, the maximum number of root (12.2) was obtained from 1.0 mg L^{-1} IBA and longest root length (1.2 cm) from IBA 1.5 mg L^{-1}.

Forty days after cultured, the problem of micro propagation of *Eucalyptus* by IBA treatment were: firstly browning of explants from the cut ends, however, the callus formation was still developed. At subsequent days, leaf drying of the explants was observed. Finally, wilting and drying of the whole explants were resulted this IBA experiment. Therefore the application

of the different concentration of sucrose was carried out in this experiment. Sucrose is a necessary component in medium because explants *in vitro* are unable to photosynthesize effectively. Twenty to 60 g L^{-1} sucrose is most commonly used concentrations. Alternative sugars could be glucose, maltose, or lactose (Coffin *et al*., 1976). The results showed that the maximum root length (2.48 cm) was observed in concentration of 4% sucrose. The maximum number of root (1.67) was occurred in concentration of sucrose 2%.

Conclusion

NAA 0.15 + BAP 3.0 mg L^{-1} was suitable for shoot and leaf multiplication. BAP 0.1 mg L^{-1} gave the optimum shoot elongation. GA_3 has not significant effect on shoot elongation compared to BAP. IBA 1.0 mg L^{-1} gave the multiplicated roots but 1.5 mg L^{-1} of IBA gave the optimum root length. Sucrose 2% had the effect on number of root but 4% sucrose on root length.

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THE STUDY ON WILD ORCHIDS AT YEE- AYE RESERVED FOREST OF KALAW TOWNSHIP IN SOUTHERN SHAN STATE

Moe Sandar Shein[1]

Abstract

The present work is concerned with the study on wild orchids of natural habitat in Yee Aye Reserved Forest in Kalaw Township. The Yee Aye Reserved Forest is situated in Kalaw Township of Taunggyi District and also the southern west part and 5.5 miles distance from Kalaw city.In this recent study 6 genera and 17 species were recorded from recent study Yee Aye Reserved Forest type is Hill ever green forest type. The Yee Aye Lake located in the centre of Yee Aye Reserved Forest. Most of the wild orchids were collected around the area of this lake. Epiphytic genera namely ***Bulbophyllum, Coelogene, Dendrobium, Eria, Pholidota*** and ***Lusia*** *were* collected. Photographs have been taken to record habits of orchids in nature.The collected specimens were classified, indentified and described with colour photographs of their natural habitats and inflorescence. The morphological characters have been emphasized and artificial keys from the tribe to the species have been constructed and GPS location system.

Keywords: Wild Orchids, Yee Aye Reserved Forest, Epiphyte, Lithophyte, habitat, artificial keys.

Introduction

The family Orchidaceae are largest family among Angiospermae, Monocotyledonae. Some botanist estimated about 35000 orchids among flowering plants. Orchidaceae grow well throughout the world. They can thrive in tropical, subtropical and temperate regions except in ice capped regions and deserts. The most wild orchids have distributed various regions of Myanmar that is tropical, subtropical and temperate regions, especially they have grown in temperate regions. Now The study area is Kalaw Township in Taunggyi district of Southern Shan State. Kalaw township is located on the east by Shwe Nyaung Township, on the west by Thazi Township, on the south by Pin Laung Township, on the north by Pindaya Township, and it lies

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between North latitude 20°25′-21˚0′ and East latitude 96°20-97˚10′. There are various types of forest in this township that is hill evergreen forest, lower deciduous forest, Indine forest and mixed deciduous forest. Six reserved forest are controlled by Forest Department. Among them the invest gold area is Yee Aye reserved forest in the recent study.Which forest is Hill evergreen forest type (The hill evergreen forest is found in the north at altitudes of over 1,000 meters above sea level. In other regions they are found area of high altitudes.These type of forest is less dense than the tropical evergreen forest because it has less large trees.This type of forest too is quite cool because it is found at high altitudes. The hill evergreen forest is very important to the preservation of water sources.Trees are mainly shrubs mixed with some pines.Smaller plants in the forest include ground orchids and other tropical plants such as wild roses,violets and lilaes.In addition there are small plants that grown on the larger trees such as moss and orchids.(Hill evergreen forest<<pirun-Ku.ac.th>) where the wild Orchids have grown on the various plants.

Yee Aye reserved forest situated in Northern East of Pin Laung Township and North by Shwe Nyaung Township and Southern West part between Kalaw and Thazi Township 5.5 miles far from Kalaw city. The area of these forest is about 1952 acres and altitude of 1465 meters and lies between North latitude 20°36′ and East longitude 96°31′. Genus ***Bulbophyllum, Coelogyne, Dendrobium, Eria, Pholidota, Lucia*** have been found in this area. In this recent study, (2) Subfamilies belong to (3) Tribes (5) Subtribes (6) genera and (11) species have been collected from this study area including epiphyte and lithophytes .The classification and taxonomic description of collected specimens are provided with coloured photographic and artificial keys of genera and species are also constructed.

Methodology

The specimens were collected from Kalaw Township of Taunggyi District. All these specimens were colourful photographed to record their actual habitat and the nature of inflorescence. The collected specimens were classified according to Dresseler's classification (R.L. Dresseler's (1927) and identified by Seidenfaden (1992) Grant: B (1966): Nantiya Vaddhanaputi (2006) Hooker, J.D. (1954). Seidenfaden and Smitch (1965), Dassanayake, N.D. (1981), Flora of China Vol. I & II (2013) and Flora of Thailand Vol. XI & XII.Part I&II (2014) methods. Herbarium specimen well prepared and submitted to Botany Department Yangon University.

Results

In this paper (2) subfamily, (4) tribes, (5) subtribes (6) genera and (11) species have been collected from study area. According to Seidenfaden and Wood (1992)

Key to the Subfamily

1. Pollina soft, waxy, without stalk or with caudiculae only rarely with stipes. Anther erect and earlier ontogeny ---------- **(I)** ***Epidendroideae***
1. Pollinia catilagenous or bony, usually with stipes. Anther incumbent already from earliest stages in ortogeny often strongly deflexed at maturity. ---------- **(II)** ***Vandoideae***

I. Subfamily Epidendoideae

Subfamily	Tribe	Subtribe	Genus	Species	Myanmar name
Epdiendrodeae	Coelogyneae	Coelogyninae	*Coelogyne*	*tenasserimensis*	ငွေနှင်းဖြူ ရှည်သေး
				nitata	ငွေနှင်းဖြူ မျိုးကွဲ
			Phholidota	*arfticulata*	None
			Eria	*pannea*	နတ်သမီးပန်း
				amica	နတ်သမီးပန်း
			Dendrobium	*cariniferum*	မဟာဒေဝီ
				Tortile	သူယောင်ပန်း
			Bulbophyllum	*siamense*	သင်းကြယ်
				refractum	သင်းပန်ကာ
				secundum	သင်းနက် မျိုးကွဲ
				blepharistes	None

Key to the Genera of Subtribe Coelogyninae

1. Pseudobulb large. Lip not saccate with papillose. Column long and slender. ---------- **1. *Coelogyne***

1. Pseudobulb slender. Lip saccate at the base without papillose. Columns hort. ---------- **2 .*Pholidota***

1. *Coelogyne* Lindl.

Sympodial epiphyte with creeping rhizome. Pseudobulb oblong ovate. Leaves two alternate. Inflorescence from the base of the pseudobulb. Sometime terminal, with persistant peduncular bracts. Flower medium. Sepals

and petals free. Lip trilobe. Column long and slender with large wings around the stigmatic surface. Anther two cells. Pollinia 4.

Key to the species of genus *Coelogyne*

1. Flower yellow. Midlobe of lip broadly flat with two long lateral keels on blade, reddish brown patch on mesochile and dark brown at the base**.** ---------- 1. ***C. tenasserimensis***

1. Flower pure whit. Midlobe of lip oblong acute with three crenulate on tubercle and reddish yellow eyes. ---------- 2. ***C nitata***

1.1. *Coelogyne tenaserimensis* Seiden.f.

Habit

Inflorescence

Flower

1.1 *Coelogyne tenaserimensis* Seiden.f.

Epiphyte. Pseudobulb compressed with deeply ridges. Leaves oblong acuminate in pairs.Inflorescence with 4-5 flowers,erect from the base of the pseudobulb, closed persistent sheath at the base. Flower yellow, about 2.5 cm acros. Sepal 3 equal, oblong acute, yellow, margin extrose, about 1.5 cm long 0.5 cm wide. Petals lanceolate, acute, back ward, yellow. Lip trilobed, side lobes triangular obtuse, small erect, yellow, midlobe broadly flat, much wavy on hypochile, two straight long lateral keels on the blade, yellow with reddish orange block on mesochile and dark brown at the base. Column pale yellow, curved with red strip. Pollinia 4.

Myanmar Name - Ngwe Hinn Phyu Shay Thaye (ငွေနှင်းဖြူ ရှည်သေး)

Occurrence - Myanmar, Kalaw Township, Yee-Aye reserved forest. N 20˚ 34'', E 96˚ 30', 1415 alt.

Distribution - N E India, Burma, Thailand (Seidenfaden, 1992)

Ecology - Epiphyte, Hill evergreen forest. Flowering period April

1.2. *Coelogyne nitada* Lindl.

Habit

Inflorescence

Flower

1.2. *Coelogyne nitada* Lindl. Coll. Bot. 33: Gen. and Sp: 40: Fol. Orchit

***C. ocellata* Lindl. In Wall. Cat. 1953.**

Epiphyte. Pseudobulb subglobose, dark green. Leaves oblong acute, glabrous. Inflorescence erect with 3-5 flowers.Flower pure white,4.00 cm across. Sepals 3 oblong lanceolate, white with nerves equal, expended. Petals lanceolate acute, pure white with district nerved.Lip trilobed, side lobes rounded with yellow spot, midlobe ovate acute, crenate keels white with 3crenulate on tubercle in reddish yellow eyes. Column pale green, long curved with wings. Pollinia 4.

Myanmar Name - Ngwe Hinn Phyu Myo Kwe (ငွေနှင်းဖြူ မျိုးကွဲ)

Occurrence - Myanmar, Kalaw Township, Yee- Aye reserved forest. N 20˚ 35", E 96˚ 31'

Distribution - NW Himalaya eastwards to Yunnan (**Seidenfaden**, 1992)

Ecology -Epiphyte, Hill evergreen forest, 1417 alt. Flowering perid March- April

2. *Pholidota* Lindl.

Epiphyte or lithophyte, pseudobulb cylindrical and slender.Leaves arising from the top of pseudobulbs, ovate-oblong lanceolate, apex acute, shortly petiole. Inflorescence terminal from top of pseudobulbs, rachis often conspicuous zigzag fashion. Flower small to large, floral bract distinct, cymbiform. Sepals concave. Lip sessile at the base of the column, saccade. Colum very short with wide wing around the anther. Colum foot absent. Anther 2 chamber. Pollinia 4, wary, globose.

2.1. ***Pholidota articulator* Lindl.**

Habit

Inflorescence

Flower

2.1. *Pholidota articulator* Lindl. in Wall. Cat 1992. And Gen &Sp-Orch 1830:30

***Pholidota griffthii* HK. F, Le PL. T.1881. 1889**

Epiphyte.Pseudobulb,long cylindrical with grooved.Leaves two, oblong lanceolate with prominent veins, slightly coriaceous. Inflorescence terminal, short, erect. Flower creamy green, about 1.00 cm across; floral bracts cymbiform about 1.00 cm long 0.8 cm wide. Dorsal sepal and lateral sepals are equal in size and shape, ovate acute, concave, not expended, 0.7 cm long and 0.5 cm wide. Lateral petals are smaller than the sepals, ovate acute. Lip saccate at hypochite with 5 longitudinal keels, expended at epichile, which bears bilobed, each lobe rounded, recurved, broader than the long, slightly twisted, pale yellow patch at base. Colum shortly clavaterasellum triangular. Pollina 4 wavy.

Myanmar Name	-	Kwyet-hme-pan-myo-kywe (ကြွက်မြီးပန်း မျိုးကွဲ)
Occurrence reserved	-	Rare species, Myanmar, Kalaw Township, Yee-Aye forest. N 20˚ 36" 2.5", E 96˚ 32' 1.3",
Distribution	-	India, Nepal, Bhutan, Bangladesh, South China, Myanmar, Indochina, Peninsular, Malaysia, Boneo, Java Sumatra (**Floral of Thailand** 2014, Volume 12, Part two). (**Flora of China 2014**). Myanmar, Java Malaya (**Holttun R.E**. 1969)
Ecology period	-	Epiphyte, Hill evergreen forest. 1412 meter. Flowering May to November

3. *Eria* Lindl.

Epiphyte with erect. Pseudobulbs are tufted, crowded on creeping root stock. Stem short sometimes branching. Leaves coriaceous articulate, duplicate, thin or fleshy, sessile or petiolate, glabrous sometimes hairy. Inflorescences raceme, terminal or lateral, one or many flowered. Flower

small, resupinate. Lip trilobe sessile on the column foot. Column large or short. Pollinia 8 with caudical. Only one genus ***Eria*** of Subtribe Eriinae was collected in study area.

Key to the species of Genus *Eria*

1. Pseudobulb absent, leaves terete, floral bract very small. Flower yellowish orange. Lip concave with grandular callus---------- 1. ***E pannea***

1. Pseudobulb fusiform,leaves oblong acute. Flower pale green with purple strip. Lip broder than the long three keels on mesochile. --------2.***E. amica***

3.1. *Erica pannea* Lindl

Habit

Inflorescence

Flower

3.1. *Eria pannea* Lindl. Bot, Reg.28:64, Misc 79.1942.

***E nivosa* Ridl., J.Nat. Hist. Siam Soe 4:116, 1921.**

Epiphyte. Stem very short, about 3.00-5.00 cm high with long rhizome. Leafy stem arise on equal apart on the rhizome. Leaf terete, yellowish green, flattened at the base, about 3.00-4.00 cm long and 0.5 cm wide. Inflorescence raceme erect, 3-4 flowers, pedunele very short. Flower small, yellowish orange hairy, about 0.8 cm across, resupinate. Sepals 3, dorsal sepal oblong acute, about 0.7 cm long and 0.5 cm wide inner surface yellow, white hairy outside. Petals 3, lateral sepals shorter than the sepals, linea; mentum long, obtuse. Lip oblongobtuse, brownish black, concave with glandular callus on lip, column very short. Pollima 8.

Myanmar Name - Nat Tha Mee Pan (နတ်သမီးပန်း)

Occurrence - Myanmar, Kalaw Township, Yee-Aye reserve forest N 20˚ 35", E 96˚ 31" (Chin, Taninthayi (Kress *et al*., 2003)

Distribution - Malaya, Indonisia (**Seidenfaden,** 1992.)

Ecology - Epiphyte, Hill evergreen forest, 1366 m alt. Flowering period April

3.2. *Eira amica* Rchb.f.

Habit

Inflorescence

Flower

3.2. *Eria amica* Rchb.f. Van-orch. 2:162 1870-A.Dkerr 1969

***Eria confuse* HK-j. Ic. Pl-T.1850**

Epiphyte. Stem fusiform with several nodes about 10.00 cm in height. Leaves alternate, oblong acute, thin, leaf. Sheath overlapping at the base about 8.00 cm long 2.00 cm wide, entire, both surfaces glabrous. Inflorescence raceme, axillary, many-flowered, peduncle suberect. Flower greenish yellow, resupinate, about 1-2 cm long and 2.00 cm wide, floral bract cymbiform, large, deflexed, incurved, pale green, persistent about 0.8 cm long and 0.6 cm wide, glabrous sepal 3, dorsal sepal oblong acute, glabrous about 0.7 cm long 0.4 cm wide light greenish yellow with three purple lines, lateral oblong-obtuse, sepals fuse at the base forming mentum obtuse. Petals 3, lateral petals

narrow, greenish yellow with purple lime. Lip trilobed, side lobes small, semicircular front edge, midlobe broader than long with retuse at apex, reddish purple, keels on mesochile. Column short, pale yellow, stout. Pollinia 8. Operculum pale yellow.

Myanmar Name - Nat Tha Mee Pan (နတ်သမီးပန်း)

Occurrence - Myanmar, Kalaw Township, Yee- Aye reserved forest.

N 20˚ 35'' 12', E 96˚ 31', (Chin, Taninthayi (**Kress** *et al*.,2003)

Distribution - NW Himalaya eastwards to China and Taiwan (**Seidenfaden**,1992)

Ecology - Epiphyte, Hill evergreen forest, 1366 m alt. Flowering period,April

4. *Dendrobium* Sw.

Pseudobulb more or less elongated cylindrical, leafy pseudobulb at stems. Leaves bifoliate, alternate. Flowers are lateral, fascicle or racemes. Sepals and petals nearly uniform in shape.Two lateral sepals are longer than the other, adhere commonly to the side of the column, usually prolong into a blunt spur. Pollinia 4 in pairs side by side, quite free. Anther two cells.

Key to the species of Genus *Dendrobium*

1. Inflorescence suberect with 2-3 flowers. Flower rose purple. Sepals and petals oblong waved spirally twisted. Lip creamy yellow with dark purple stripe at the base. ---------- **1. *D. tortile***

1. Inflorescence subterminal with 2-3 flowers. Flower whitish orange. Petals broder than the sepals with key on the adaxil.Midlobe of lip rounded, slightly endulate with four fimbricate keys on the mesochile.---------- **2. *D. cariniferium***

4.1. *Dendrobium tortile* Lindl.


Habit


Inflorescence


Flower

4.1. *Dendrobium tortile* Lindl.In Gard-Chron. 1847, 797.

Epiphyte. Pseudobulbsub fusiform compressed, erect, clavate furrowed narrow at the base. Leaves narrow retuse, glabrous. Inflorescence pseudoterminal, pendulous with 2-3 flowers, peduncle short. Flower showy, rose purple with long pedicle, about 6.00 cm across, mentum conical, purple. Dorsal and lateral sepal purple, oblong, undulate and twisted, equal in size, about 4.00 cm long 1.00 cm wide. Laeral petals as long as sepals, equal in size and shaped. Lip subsaccate with a dilated everted erose limb, creamy white with light purple veins on blade, dark purple lime at hypochile, pubescent on both sides. Column short, about 1.00 cm long 0.2 cm wide, green. Operculum purple with papillose. Pollina 4, oblong, yellow.

Myanmar Name - Thu Young Pan (သူယောင်ပန်း)

Occurrence - Myanmar, Kalaw Township, Yee- Aye reserved forest.
N 20˚ 35' 6.11", E 96˚ 32' 45",

Distribution - NE India, Andamans, Thailand and Malaya (Seidenfaden, 1992), Myanmar (Grant's, 1966) Report from Myanmar (**Kress** *et al*., 2003)

Ecology - Epiphyte, Hill evergreen forest. 1420m. Flowering period March

4.2. *Dendrobium cariniferun* HG. Reichanbach, Gard.


Habit


Inflorescenc


Flower

4.2. *Dendrobium cariniferun* HG. Reichanbach, Gard. Chron. 1869: 611: 1896

***Callista carinifera* (H.G Reichenbach) Kurtze.**

Pseudobulb erect, fusiform with fine black hairy sheaths, about 10-15.00 cm long, 4-5 cm wide. Leaves oblong acute with densely hair on abaxial surface. Inflorescence pseudoterminal, 2-3 flowers with short peduncle. Flower yellowish orange, 5.00 cm across, fragrant, thickly texture,Dorsal sepals ovate lanceolate, lateral sepals obliquely ovate triangular with strong keels on the back.Mentum as long as the dorsal sepal corniformacute, incurved.Lateral petals obovate acute.Lip trilobed, embracing the column, orange inside, side lobes rounded crenate, midlobe obovate, margin irregularly notched, excurved at apex, creamy yellow with 5 distinct shortly fimbricate keels on epichile creamy yellow with orange inside. Column short, about 0.5 cm long 0.2 cm wide. Operculum subspherical, white. Pollinia 4.

Myanmar Name - Maha Deiwi (မဟာဒေဝီ)

Occurrence - Myanmar, Kalaw Township, Yee- Aye reserved forest.N 20˚ 36' 2.5", E 96˚ 31' 37", Chin, Kachin,Shan, Taninthayi (Kress *et al*., 2003)

Distribution - NE India, Laos, Myanmar, Thailand, Vietnam (**Flora of Ihina**, 2010) Report from Myanma (**Kress** *et al*., 2003), Assam,Myanmar, Thi, Malasia, Indionisia, Phillippines (Seidenfaden,1992)

Ecology -Epiphyte, Hill evergreen forest. 1400 m, alt. Flowering period-March-April

Only one genus ***Bulbophyllum*** of Subtribe Bulbophylllinae was found in study area.

5. Bulbophytllum Thou.

Pseudobulb close or distinct, vary in size its top carrying a single leaf, only rarely two. Inflorescence one to many flower arising at the base of the pseudobulb. Flowers single or closed head flower much varying in size from small to quite large. Sepals equal or lateral sepals much larger than the dorsal, joined to the column foot to form mentum, petals always smaller than the sepals. Lip almost nearly mobile, usually fleshy, tongue-shaped, straight or curved, papillose or warty. Column short with conspicuous wings, column foot curved forward. Pollinia 4.

Key to the species of Genus *Bulbophyllum*

1. Inflorescence with many flowers. Lateral petals, spathulate with long papillose. ---------- **1. *B. secundum***

1. Inflorescence subumbellate with a few flowers. Lateral petals nearly circular with fat dark purple fibrates at apex ---------- **2. *B. blepharistes***

5.1. *Bulbophyllum securdum* HK.f.

Habit

Inflorescence

Flower

Petals

5.1. *Bulbophyllum securdum* HK.f. Fl.Brit. India 5: 764, 1890

Epiphyte, pseudobulb close depress, conic to lenticular on rhizome. Leaves elliptic, obtuse acute with petiole.Inflorescence erect, elongated with 8-20 flowers, about 15-20.00 cm long and 0.2 cm wide, floral bract triangular, acuminate. Flower resupinate, second, pale green with dull reddish or purple. Sepals 3 equal, dorsal sepal ovate acute, hooded lateral sepals triangular, pale green with reddish purple at the base, ciliolate, fleshy with furrow in the centre. Petals 3, Lateral petals obovate to spathulate, about 0.2 cm long and 0.1 cm wide, papillose toward tip, margin ciliate, apex rounded to obtuse, very smaller than the sepals, black lip purple oblong, fleshy, finely papillose with margin ciliate, slightly concave on hypochile with deeply cleft and basal callus reddish brown tubercle on mesochile, about 0.4 cm long 0.2 cm wide. Column triangula.Pollinia 4 long.

Myanmar Name - Thazin-Net (သဇင်နက်)

Occurrence - Myanmar, Kalaw Township, Yee- Aye reserved forest. 20˚ 36", E 96˚32'

Distribution-Himalaya,Thailand,Yunnan (**Seidenfaden**,1992), India, Myanmar, Nepal, Thailand, Vietnam (**Floral of China**, 2008)

Ecology -Epiphyte, Hill evergreen forest, 1412 mm alt. Flowering period March-April

5.2. *Bulbophyllum blepharistes* Rchb. f.

Habit

Inflorescence

Flower

5.2. *Bulbophyllum blepharistes* Rchb. F. Flora: 278, 1872-A.DKess 1969:

***Cirrhopetalumspicatum* Gagnep. Bull Mus. Parisz. S.22(3): 402. 1950**

***Cirrhopetalumblepharistes* Uook. F. 1896**

Tripudianthesblepharistes (Kchbf.) Sglach.Kres 2007.

Epiphyte, pseudobulb is yellow, obovate, slightly 4 angles in column ovoid, about 2-5 cm long 2.00 cm wide. Leaves oblong ovate with cleft apex two leaves, bifoliate, glabrous. Inflorescence subumble on long scape with a few flowers about 8-9.00 cm long with brown peduncle, rising from the leafless pseudobulbs. Flower greenish yellow about 3.00 cm long 2-3 cm wide. Dorsal sepal oblong acute 1.5 cm long 0.5 cm wide, pale green with brown stripes lateral sepals, obliquely triangular about 3.00 cm long 1.5 cm wide, lower surfaces of edges join forming narrow ellipse, with purple stripe at the base. Lateral petals nearly circular with fat dark purple fimbricate at apex. Lip about 6-7 cm across. Lip tongue-shaped with groove, obtuse, yellow

side lobes, curved backward outside. Column pale yellow with purple spot at the base and yellow horn. Pollinia 4 in pairs.

Myanmar Name - None

Occurrence - Myanmar,Kalaw Township,Yee-Aye reserved forest.N 20˚ 36",E 96˚ 31'

Distribution- Myanmar, Thailand (**Seidenfaden**, 1992), Rare species, Feb, 8, 2018, JJveam, Brachartha Rchbf. *http.*Thaninthayi (**Kress** *et al*, 2003)

Ecology - Epiphyte, tropical deciduous forest, Hill evergreen forest.1367 m alt. Flowering period – November

II.Subfamily.Vandoideae

Subfamily	Tribe	Sutribe	Genus	Species	Myanmar Name
Vandoideae	Vandeae	Sarcanthinae	*Lucia*	*Psyche* Rchb.f.	none

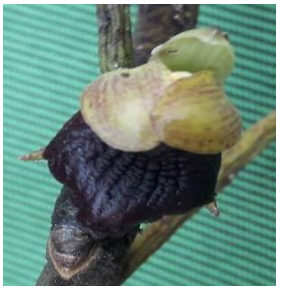
6. *Lucia*. Gaud

Epiphyte. Stem long. Leaves terete. Pseudobulb absent.The root vermiform. Flower spicate on a short, dense scape very short and thickened. Sepals and petal free, equal or petals larger than the sepals. Lip fleshy, fixed immovably to the base of column, distinctly divided by grove into a basal hypochile and epichile, basal part more or less hollow, sometime in the distinct side lobes, apical part usually longer, often wrinkled grooved longitudinally. Colum short, foot absent. Pollinia 2, with short broad stipe.

6.1. *Lucia pshyche* Rchb.f.

Habit

Inflorescenc



Flower

6.1. *Lucia pshyche* Rchb.F. in Box.Xeit.1863, 98, in Gard Chron 1963, 342.

***Lucia loosenis* Guill Bull. Mus. Paris 2.5. 35 (6): 651 (1963).**

Monopodial epiphyte.Stem long with ridged. Pseudobulb absent.Leaves terete, elongated obtuse, dull green with violet blotch.Inflorescence one to two flowers, perforating the leaf sheath. Flower solitary, large, about 2.00 cm long 1.3 cm wide. Dorsal sepals ovate obtuse, slightly incurved, greenish white, lateral sepals ovate obtuse, fleshy margin entire. Lateral petals linea spathulate longer than the sepals. Lip broadly oblong, ovate retuse, dark purple with trace, side lobes tessellate, convex, about 1.8 cm long 1.5 cm wide, glabrous. Column short and stout, white. Operculum white and smooth. Pollinia 2, stigmatic surface concave. Ovary inferior.

Note : ***Lucia pshyche* Rchb.f.** was revealed as native in Myanmar (**Holttum**, 1964)

Myanmar Name - None

Occurrence - Myanmar, Kalaw Township, Yee-Aye reserved forest.

N 20˚ 35" 55.8", E 96˚ 3' 45",

Distribution - Myanmar (Native), Thailand (**Holttum**, 1964) (**Seidanfadan**, 1992) (**Grand's,** 1996) Mon, Thaninthayi (**Kress** *et al*, 2003)

Ecology - Epiphyte, Hill evergreen forest. 1412 m alt. Flowering period- May

Discussion

This paper based on some collected wild orchids specimens. The present list is (2) subfamily, (3) tribe, (5) subtribe, (6) genera and (17) species. The subfamily Epidendroideae includes (2) tribe, (4), subtribe, (6) genera and (11)species. Genus ***Coelogyne, Polidata, Eria, Dndrobium ,Bulbophyllum*** and ***Lucia*** have been collected from study area. In recent study, two species of genus ***Coelogyne*** was collected in this study area that is ***Coelogyne tenasserimensis* Seidenf**. which possess its midlobe broadly flat, much wavy on hypochile, straight long lateral keels on blade, and reddish orange on mesochile dark brown at the base ***C. nitada* Lindl.** which distinguished character is pure white flower, sidelobes of lip with yellow sport and midlobe with 3 crenulate on tubercle in reddish yellow eyes. Only one species of genus ***Pholidota*** *is* ***Pholidota articulate* Lindl**. possess saccate lip with 5 longitudinal keels and expended at epichile. Two species genus ***Eria*** are ***E.pannea* Lindl.,** ***E.*** *and* ***amica* Rchb.**f.**, *Eria pannea* Lindl.** has tereat leave, pseudobulb absent, flower yellowish orange and saccate lip with grandular yellow callus. ***Eria amica* Rchb.**f. posses pale green flower with purple stripe and yellow midlobe with three keels on mesochile.Two species of genus ***Dendrobium*** are ***D. tortile* Lindl., and *D. cariniferum* Rchb.f**., ***D. tortile* Lindl.** which distinct character is spirally twisted waxy oblong sepals and petals. ***D. cariniferum* Rchb.f.** contains long incurved mentum, sepals with keeled on the back and rounded midlobe slightly undulate with four fimbriate keels on mesochile. Two species of genus ***Bulbophyllum*** are ***B.secundum* HK.f.,** and ***B. blepharistes***. **Rchb.f. *B.secundum* HK.f**. has small spathulate

lateral petals with long black papillose. ***B. blepharistes***. **Rchb.f.** possess subumbellate erect inflorescence, lateral sepals obliquely triangular, lower surface of edges join forming narrow ellipse, lip purple tongue-shaped with groove.

In subfamiluy Vandoideae, (1) tribe, (1) subtribe and only one genus was collected from study area. Only genus of subtribe Sarcarthinae under tripe vandeae is genus ***Lucia***. Only species of genus ***Lucia*** is ***Lucia psyche*** which diagnostic character is one flower inflorescence, lip broadly ovate dark purple, retuse, convex with tubercle line. In this paper, all collected species are epiphyte and lithophytes.

C**onclusion**

In recent study some species of ***Eria pannea, Eria amica, Bulbophyllum secumdum , B. blepharistes,*** and ***Lucia psyche* Rchb.f.** were only found in Chin, Mon, Thaninthayi, Kachin by (Kress *et al*, 2003) but these species are also found in Kalaw Township of Taunggyi District in Southern Shan State. Among them ***Lusia psyche,Rchb.f.*** is native in Myanmar. (**Holttum,1964**) and ***B.blepharistes*** Rchb.f. is becomed rarely to find nowaday (www.orchids species.com) and also found the different character of leaves in species ***Eria pannea* Lindl.** There are a lot of genus *Bulbophyllum* in this study area for the future study. Today wild orchids are gradually disappeared by human activity and some are disappeared completely by invalid trade to neighboring countries. Therefore all nationality must be protect the living jewels by strong forest law for natural resource of Myanmar.So the orchidologist have to find out continuously and help partially for Flora of Myanmar, and report to government for protection of our living jewels.

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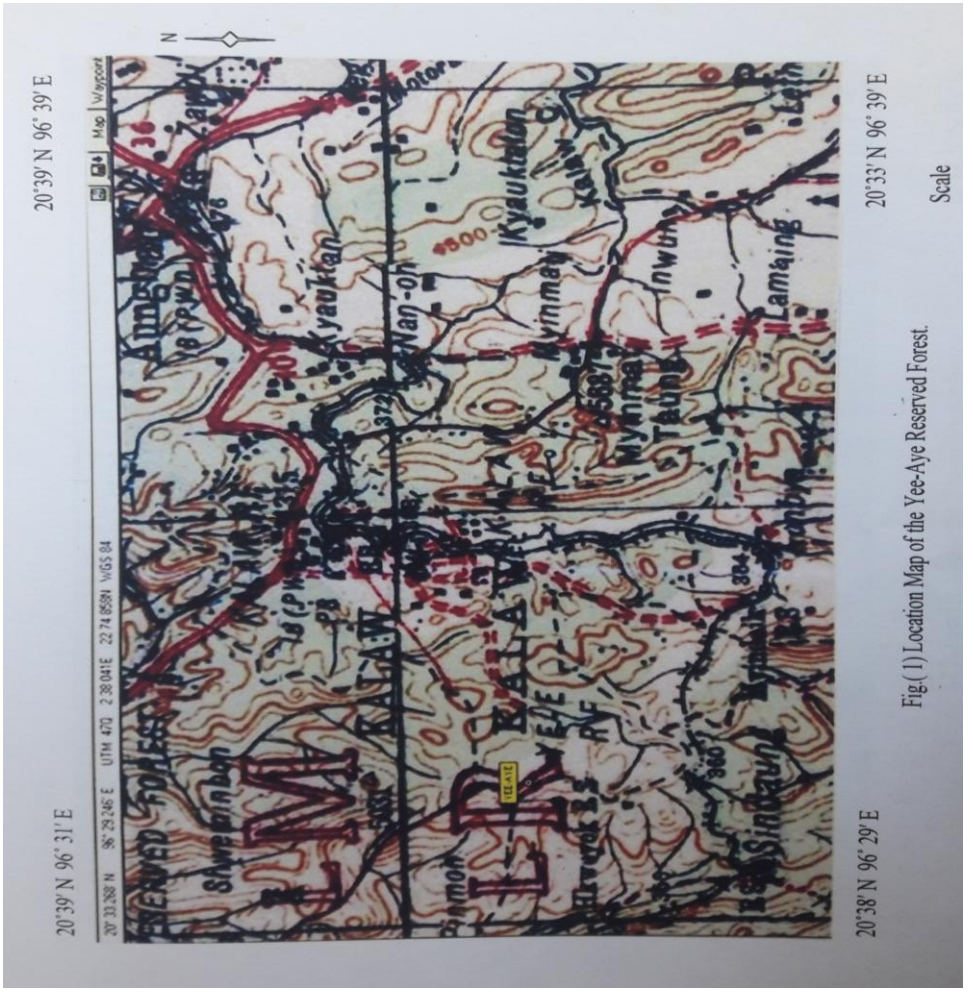

Fig.(1) Location Map of the Yee-Aye Reserved Forest.

EFFECTS OF THE SYSTEM OF RICE INTENSIFICATION (SRI) WITH GREEN MANURE ON GROWTH AND YIELD OF SOME COMMERCIAL RICE *ORYZA SATIVA* L. VARIETIES FROM KAWA TOWNSHIP, BAGO REGION

Yin Mar San[1], Soe Soe Aung[2], Ohnmar[3]

Abstract

This study was carried out to study the performance of the System of Rice Intensification (SRI) on the growth and yield of selected rice varieties by comparing the traditional cultivation method and SRI method. This study also determined the SRI and green manure application on three commercial rice varieties: Sinthukha, Shwewharhtun and Manawthukha. In traditional method, the spacing of 20 × 15 cm was followed and 25 day-old seedlings were used whereas in SRI cultivation method, the wider spacing of 25 × 25 cm was followed and the 8 to12 day-old seedlings were used. Although large numbers of seedlings were needed for traditional method, a small amount of seedlings were needed for SRI method. The results indicated that all the plant characters and grain yields were significantly affected by the seedling ages. The maximum grain yields, 6912.71kg ha^{-1} were observed in 8 day-old seedlings (T_3) under SRI method and the minimum, 4561.05 kg ha ha^{-1} were observed in 25 day-old seedlings (T_1). Among the three varieties, Shwewharhtun (V_2) gave the highest grain yields. There were no interaction between the seedling ages and the rice varieties.

Key words: System of Rice Intensification (SRI)

Introduction

Rice which is to be transplanted into lowland puddle soil must first be nursed on seedbeds. In traditional rice cultivation, 25 to 30 day-old seedlings were used to transplant (Soe Paing Oo and Mar Mar Kyu, 2012). The traditional method of paddy cultivation demanded for more water, increased cost of inputs including heavy amount of chemical fertilizers, pesticides and less returns producing negative effect on the livelihoods of the farmers.

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In the traditional paddy cultivation method, farmers adopt unscientific methods to address some of the problems in the paddy cultivation. Methods like aged nursery, difficult way of relocating seedlings, transplanting bunch of seedlings, less spacing, stagnating water and applying more chemical inputs were basically have behavior on yield and productivity. The tendency to apply less time for cultivation also has contributed to the problem (Upendra, 2015).

SRI is a sort of management method that raises productivity of land, labor and capital. Researchers have demonstrated that SRI is a model of sustainable agriculture that reduces inputs, conserves water, improves soil structure and increases yield. It mainly emphasizes on careful transplanting of younger seedling at a wider spacing, which ensures more root growth and profuse tillering (CARM-DAKSH, 2008).

The SRI method seems to solve the problems with the traditional method of paddy cultivation. The SRI method allows paddy plant to have normal growth with less water. SRI makes use of the naturally available organic manure as inputs. This allowed farmers to use organic manures available on the farm at low cost, and thus be able to meet a significant portion of the crops nutrient demand and to improve soil fertility (Pandian *et al*., 2011).

The utility of green manure for increasing soil productivity has been recognized from early times in some rice-growing areas, particularly China, India, and Southeast Asia. The potential benefits are many. Green manure can increase soil nitrogen content, concentrate phosphorous and significantly increase the available phosphate content in the soil, maintain and renew soil organic matter, and improve soil structure and physical characteristics (Bin, 1983).

This study was carried out with the following objectives: to study the performance of the System of Rice Intensification (SRI) on growth and yield of selected rice varieties; to compare traditional cultivation method and SRI method; to determine the appropriate seedlings age of three commercial rice

varieties and to minimize the chemical fertilizer by using the green manure and distribute the effective knowledge of SRI method for rice cultivation.

Materials and Methods

Experimental site and materials

The tested varieties which were grown from July to October 2017 as monsoon rice at the Farm of Thabyu Village, Kawa Township, Bago Region, by using SRI method and traditional rice cultivation method. The three commercial rice varieties used in the experiment were: V_1 = Sinthukha, V_2 = Shwewarhtun and V_3 = Manawthukha.

Collection and Preparation of green manure plants

The well-ripen seeds of *Samanea saman* (Jacq.) Merr. (Kalarkokko) were collected around the Thabyu village, Kawa Township. After treating the pre-germination test, 300 seeds for each plot were soaked in water for 24 hours and incubated 12 hours. Then, the seeds were broadcasted into ploughed experimental plots. Sprinkling water was using to be succeeded the germination.

Preparation of the experimental plot

The split plot design with 4 replications was used in this experiment. Individual plot size was 3.35 square meters and it consisted of 7 rows with 7 plants at the spacing of 25 × 25 cm under SRI and 20 × 15 cm under traditional method. The spacing between each block was 3 feet and each replication was 2 feet.

Preparation process of green manure fertilizer

After 45 days of planting, *Samanea saman* (Jacq.) Merr. (Kalarkokko) plants which used as green manure were thoroughly mixed and buried in the pots for monsoon rice cultivation. Application of 10 days interval, the green manure plants have already been decomposed into organic matter for nitrogen sources.

Seedbed preparation for raising seedlings

To transplant different seedling ages of three rice varieties at the same date, nine seedbeds for each seedling age were separately prepared in July 2017. The size of one seedbed was 1.5 × 1.5 sq. ft and raise above the original soil level. Seedbed was covered with plastic sheet and then lay down the mixture of cow dung manure and soil to get the soil layer of 5 cm. Seed rate on each seedbed was 2.5 g for SRI method and 7.5 g for traditional method. Seeds were covered by rice ash to prevent moisture losses and birds. Irrigation was done whenever necessary.

Methods

Transplanting procedure

In the field experiment, seedlings were transplanted with the age of 25 days for traditional method, the 10 and 8 days for SRI (System of rice intensification method). One single seedling per hill was used for SRI method and a bunch of three seedlings per hill were used for traditional method. Weed control was done on each 15 days interval of hand weeding and the weeds buried in soil as biomass organic matter. Adequate protection measures were given against pest and diseases.

Data collection

Four random plants of each variety were taken from each plot and the collected data were plant height (cm $plant^{-1}$), root length (cm $hill^{-1}$), number of tillers $hill^{-1}$, number of effective tillers $hill^{-1}$, number of spikelet $panicle^{-1}$, filled grain percent (%), 1000 grains weight (gm $hill^{-1}$) and grain yield (kg ha^{-1}).

Statistical analysis

The data were subjected to analysis of variance designed in two factors factorial in RCBD (Split-plot Design) by using the IRRISTAT (Version 4.0.2). Factor A was assigned in three rice varieties (V_1 = Sinthukha, V_2 = Shwewharhtun and V_3 = Manawthukha) and Factor B was assigned in

treatments (T_1 = 25 day-old seedlings, T_2 = 10 day-old seedlings and T_3 = 8 day-old seedlings). Treatment means were separated and compared with Least Significant Difference (LSD) test at 5 % level of significant.

Grain yield

Grain yield (t/ha) = panicle number m^{-2} × spikelet number $panicle^{-1}$ ×Percent of filled spikelet × 1000 grains weight (g) × 10^{-5}

(Yoshida, 1981)

Results

Plant height

There was highly significant on rice varieties at transplant stage and reproductive stage (Table 1). In vegetative stage, there was no significant effect on varieties. Means plant height of Shwewharhtun (V_2) was higher than those of Sinthukha (V_2) and Mannawthukha (V_3) rice varieties. According to the seedling ages, plant height was observed from 97.93 cm to 101.73 cm at vegetative stage and 133.8 cm to 135.4 cm at reproductive stage. And reproductive stage, the 8 day-old seedlings was the highest plant height in reproductive stage. The 10 day-old seedlings and the 25 day-old seedlings were the same in plant height. At the same seedling ages, plant height was the highest in Shwewharhtun variety.

Root length

Root lengths were highly significant different among the varieties at their transplant and reproductive stages (Table 2). Maximum average root length was achieved in Shwewharhtun variety 140.70 cm at all three stages while the minimum root length was observed in Sinthukha variety 130.22 cm. Highly significant differences were found in root lengths of all seedling ages at the vegetative stage. Root lengths of the 8 day-old seedlings were higher than those of the 10 and 25 day-old seedlings. There was no interaction between varieties and seedling ages on root length at reproductive.

Number of tillers and effective tillers

Tiller number was no significantly differences among varieties (Table 3). But among the seedling ages, there was highly significant. Maximum tiller numbers 27.08 were achieved in the 8 day-old seedlings while minimum tiller numbers 16.95 were observed in the 25 day-old seedlings at their vegetative stages. Effective tiller numbers were observed ranged from 11 to 16 among treatments. The 8 day-old seedlings had the highest effective tillers and followed by the 10 day-old seedlings. The 25 day-old seedlings had the lowest effective tiller numbers. In all treatments, effective tiller numbers of young seedling ages were higher than old seedling ages. There was no interaction between varieties and seedling ages on number of tillers.

Numbers of spikelets

Numbers of spikelet per panicles were not significantly differences in all varieties. Shwewharhtun variety was the lowest spikelet numbers although spikelet numbers of Sinthukha and Manawthukha varieties were not considerably different. Spikelet numbers ranged from 128.23 to 136.51 among the different seedling ages. The spikelet numbers per panicle of the 8, 10 and 25 day-old seedlings were not significantly different. Although, the maximum spikelet numbers were obtained from the 8 day-old seedlings, the 25 day-old seedlings had the minimum spikelet numbers. No interaction was observed between varieties and seedling ages (Table 4).

Filled grain percent

Mean effect of varieties and seedling ages on filled grain percent were shown in Table 5. Filled grain percent were not significantly differences among varieties and seedling ages. The highest filled grain percent (93.28%) was resulted from Manawthukha variety and the second highest (92.06%) was found in Sinthukha variety. According to the seedling ages, filled grains percent was found to be ranged from 91.15 % to 93.12% in all seedling ages. The higher filled grains percent was resulted from the10 day-old seedlings and the lower filled grains percent was found in the 25 day-old seedlings. There

was no interaction between varieties and seedling ages on filled grains and unfilled grains percent.

1000 grains-weight

Mean effect of varieties and seedling ages on the 1000 grains-weight were highly significant differences in Table 6. The highest 1000 grains-weight were observed in Shwewharhtun and followed by Manawthukha variety. There was no significantly difference among seedling ages. The1000 grains-weight ranged from 22.16 to 22.58 gm. The 1000 grains-weight of all seedling ages were nearly the same. In this study the 8 and 10 day-old seedlings were observed maximum 1000 grains-weight (22.58 gm) and the 25 day-old seedlings achieved minimum 1000 grains-weight (22.16 gm). No interaction between variety and seedling age was detected.

Grain yield

There was no significant effect of varieties on grain yield (Table 7). The average yield of Shwewharhtun variety was significantly higher than those of Sinthukha and Manawthukha varieties. Grain yield ranged from 4561.05 kg ha^{-1} to 6912.71 kg ha^{-1}. It was observed that maximum grain yield in each variety was resulted from the 8 day-old seedlings. Grain yield were generally decrease as the seedling ages were increased. The second highest grain yield was observed in the 10 day-old seedlings and the lowest was the 25 day-old seedlings. There was no interaction between the seedling ages and varieties.

Table 1. Mean effects of seedling ages on plant height of three rice varieties

Factors	Plant height (cm plant^{-1})		
	Transplant stage	Vegetative stage	Reproductive stage
Factor A: Varieties			
V_1	22.74	93.65	130.22
V_2	24.36	109.73	140.70
V_3	22.09	95.44	132.13
F-test	**	ns	**
LSD (5%)	0.66	11.78	4.53
CV (%)	3.40	14.10	4.40
Factor B: Seedling ages			
T_1	30.77	101.73	133.80
T_2	20.19	97.93	133.80
T_3	18.27	99.15	135.43
F-test	**	ns	ns
LSD (5%)	0.66	11.78	4.53
CV (%)	3.40	14.10	4.40
Factor A × Factor B Varieties × Seedling ages			
V_1T_1	31.80	86.88	127.20
V_1T_2	19.28	94.94	132.20
V_1T_3	17.15	99.13	140.90
V_2T_1	30.45	116.50	138.75
V_2T_2	22.17	105.31	142.45
V_2T_3	20.45	107.38	133.32
V_3T_1	30.05	101.81	131.40
V_3T_2	19.03	93.56	131.65
V_3T_3	17.20	90.94	130.11
F-test	**	ns	ns
LSD (5%)	1.15	20.40	7.84
CV (%)	3.40	14.10	4.40

**$p \leq 0.01$, ns = non significant.

Table 2. Mean effects of seedling ages on root length of three rice varieties

Factors	Root length (cm hill$^{-1)}$		
	Transplant stage	Vegetative stage	Reproductive stage
Factor A: Varieties			
V_1	2.27	39.42	14.81
V_2	3.22	63.60	14.83
V_3	2.70	57.63	14.78
F-test	**	**	ns
LSD (5%)	0.29	2.72	1.689
CV (%)	12.7	6.10	13.60
Factor B: Seedling ages			
T_1	2.84	40.73	13.79
T_2	2.78	60.30	14.69
T_3	2.56	59.63	15.95
F-test	ns	**	ns
LSD (5%)	0.29	2.73	1.69
CV (%)	12.70	6.10	13.60
Factor A × Factor B Varieties × Seedling ages			
V_1T_1	2.78	58.00	14.43
V_1T_2	1.66	59.38	14.19
V_1T_3	2.38	58.88	15.81
V_2T_1	2.92	64.50	13.44
V_2T_2	3.38	62.44	14.88
V_2T_3	3.38	63.88	16.19
V_3T_1	2.85	57.69	13.50
V_3T_2	3.33	59.62	15.00
V_3T_3	1.93	56.13	15.86
F-test	**	**	ns
LSD (5%)	0.50	4.72	2.93
CV (%)	12.70	6.10	13.60

**$p \leq 0.01$, ns = non significant.

Table 3. Mean effects of seedling ages on tillering of three rice varieties

Factors	Number of Tillers and Effective tillers (hill^{-1})		
	Vegetative stage	Reproductive stage	
Factor A: Varieties	tiller	Tiller	effective tiller
V_1	22.28	22.29	15.16
V_2	25.34	23.39	13.59
V_3	20.79	21.27	13.45
F-test	ns	ns	ns
LSD (5%)	4.13	3.67	1.75
CV (%)	21.60	19.70	15.00
Factor B: Seedling ages			
T_1	16.95	15.5	11.12
T_2	24.39	24.81	15.15
T_3	27.08	26.6	15.95
F-test	**	**	**
LSD (5%)	4.13	3.67	1.75
CV (%)	21.60	19.70	15.00
Factor A × Factor B Varieties× Seedling ages			
V_1T_1	13.36	16.56	11.56
V_1T_2	24.87	21.38	15.50
V_1T_3	28.62	28.94	18.43
V_2T_1	21.43	16.06	12.00
V_2T_2	26.31	27.62	14.88
V_2T_3	28.31	26.50	13.88
V_3T_1	16.06	14.00	9.78
V_3T_2	22.00	25.43	15.07
V_3T_3	24.31	24.37	15.60
F-test	ns	ns	ns
LSD (5%)	7.15	6.37	3.05
CV (%)	21.60	19.70	15.00

**$p \leq 0.01$, ns = non significant

Table 4. Mean effects of seedling ages on number of spikelets panicle $^{-1}$ of three rice varieties

Factors	Number of Spikelets (panicle^{-1})
Factor A: Varieties	
V_1	134.25
V_2	128.61
V_3	135.19
F-test	ns
LSD (5%)	7.95
CV (%)	7.20
Factor B: Seedling ages	
T_1	128.23
T_2	133.31
T_3	136.51
F-test	ns
LSD (5%)	7.95
CV (%)	7.20
Factor A × Factor B Varieties × Seedling ages	
V_1T_1	129.41
V_1T_2	138.75
V_1T_3	134.60
V_2T_1	120.29
V_2T_2	127.25
V_2T_3	138.31
V_3T_1	135.02
V_3T_2	133.96
V_3T_3	136.61
F-test	ns
LSD (5%)	13.76
CV (%)	7.20

ns = non significant

Table 5. Mean effects of seedling ages on filled grains percent of three rice varieties

Factors	Filled grains (%)
Factor A: Varieties	
V_1	92.06
V_2	91.69
V_3	93.28
F-test	ns
LSD (5%)	1.70
CV (%)	2.20
Factor B: Seedling ages	
T_1	91.15
T_2	93.12
T_3	92.77
F-test	ns
LSD (5%)	1.70
CV (%)	2.20
Factor A × Factor B Varieties × Seedling ages	
V_1T_1	89.37
V_1T_2	92.69
V_1T_3	94.14
V_2T_1	91.77
V_2T_2	92.82
V_2T_3	90.50
V_3T_1	92.32
V_3T_2	93.85
V_3T_3	93.66
F-test	ns
LSD (5%)	2.94
CV (%)	2.20

ns= no significant

Table 6. Mean effects of seedling ages on 1000 grains-weight of three rice varieties

Factors	1000 Grains-weight (gm)
Factor A: Varieties	
V_1	21.00
V_2	24.17
V_3	22.17
F-test	**
LSD (5%)	0.95
CV (%)	5.10
Factor B: Seedling ages	
T_1	22.16
T_2	22.58
T_3	22.58
F-test	ns
LSD (5%)	0.95
CV (%)	5.10
Factor A × Factor B: Varieties × Seedling ages	
V_1T_1	21.12
V_1T_2	21.13
V_1T_3	21.11
V_2T_1	23.00
V_2T_2	23.00
V_2T_3	25.01
V_3T_1	21.02
V_3T_2	23.11
V_3T_3	21.16
F-test	ns
LSD (5%)	1.65
CV (%)	5.10

**$p \leq 0.01$, ns = non significant.

Table 7. Mean effects of seedling ages on grain yield of three rice varieties

Factors	Grain Yield (kg ha^{-1})
Factor A: Varieties	
V_1	6090.93
V_2	6157.25
V_3	6039.04
F-test	ns
LSD (5%)	531.36
CV (%)	10.40
Factor B: Seedling ages	
T_1	4561.05
T_2	6813.46
T_3	6912.71
F-test	**
LSD (5%)	531.36
CV (%)	10.40
Factor A × Factor B: Varieties × Seedling ages	
V_1T_1	4428.32
V_1T_2	6972.73
V_1T_3	6871.73
V_2T_1	4993.80
V_2T_2	6536.56
V_2T_3	6941.40
V_3T_1	4261.03
V_3T_2	6931.08
V_3T_3	6925.01
F-test	ns
LSD (5%)	920.35
CV (%)	10.40

**$p \leq 0.01$, ns = non significant

Discussion and Conclusion

The maximum mean plant height was observed in Shwewharhtun variety at vegetative and reproductive stages. The plant height of young seedling ages was higher than those of old seedlings. The maximum means root length, 63.60 cm was observed in 10 day-old seedlings under SRI method and the minimum means length 59.63 cm was observed in 25 day-old seedlings under traditional method. Rabenandrasana (2000) reported that the success of SRI is based on the synergistic development of both the tillers and root system where there is vigorous root growth, the plant grows fuller and taller; consequently more access to nutrients and water for tiller and seed development.

The maximum tiller and effective tiller were observed in the 8 day-old seedlings at their vegetative and reproductive stages. The minimum tiller and effective tiller were observed in the 25 day-old seedlings. The result indicated that tiller performance in young seedling is higher than in old seedling. Thit Thit Soe (2008) stated that the tiller numbers were greater in spacing of 25 × 25 cm than spacing of 15 × 15cm at the same seedling ages.

Filled percent in SRI method was greater than in traditional method. Under SRI, the average filled grain percent was 93.12 % while 91.15 % was observed in traditional method. Enhanced growth parameters might have helped in better filling of spikelets. In the present study, 1000 grains weight was not significant among treatments. Aidei and Beighley (2006) reported that cultivation methods didn't have such effect on 1000 grains weight.

According to the results, SRI plots produced significantly higher grains yield than traditional cultivated plots. At the spacing 25 × 25 cm, the 8 day-old seedlings (T_3) had achieved the maximum grain yield and followed by the 10-day old seedlings (T_2) and the 25-day old seedlings (T_1). It is therefore recommended that for rice variety under SRI practice, the optimum transplanting spacing that gives maximum yield is 25 × 25 cm (Reuben *et al.*, 2016).

By comparing the growth and yield of the three commercial rice varieties, under SRI and traditional method, Shwewharhtun variety gave the highest grain yield. Measurements of crop performance, SRI practice were significantly more successful than non-SRI practice (traditional method). It is concluded that the SRI method of cultivation is more advantageous to the paddy farmers than compared to traditional method as the reduction in cost of cultivation, higher yields obtained per acre and lesser seed rates used in sowing.

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PHYTOCHEMICAL INVESTIGATION AND ANTIMICROBIAL ACTIVITIES OF *KAEMPFERIA GALANGA* L.

Phyo Moh Moh Zin[1], Khin Cho Cho Oo[2]

Abstract

The medicinal plant *Kaempferia galanga* L.is locally known as kun-sar-gamone belonging to the family Zingiberaceae collected from ka-wa Township, Bago Region. Preliminary phytochemical tests, physicochemical properties and elemental analysis were carried out by using the powdered samples of the rhizomes. According to the physicochemical properties, the samples were more soluble in water than the other solvents. In the result of elemental analysis, the concentration of arsenic was found to be 0.00009%. The concentration of elements were studied by using Atomic Absorption Spectrometer (AAS) at Universities Research Centre (URC). In addition, nutritional values of the rhizomes were examined at the Food Industries Development Supporting Laboratory (FIDSL). Fats, fibers, proteins and carbohydrates were observed as nutritional contents. High content of carbohydrate was also found. Antimicrobial activities of various solvent extracts of rhizomes from *Kaempferia galanga* L. were investigated at Pharmaceutical Research Department (PRD) by using agar-well diffusion method with nine pathogenic microorganisms. Ethylacetate extract showed that most significant activity against *E.coli* and *Proteus mirabilis*.

Keywords: *Kaempferia galanga* L., Qualitative and Quantitative analysis, Antimicrobial Activities.

Introduction

The Zingiberaceaefamily occur mainly in the tropics and subtropics. Asia and consists of over 40 genera and about 1,000 species (Dassanayake, 1983).An indigenous plant, *Kaempferia galanga* L. belongs to family Zingiberaceae. *Kaempferia galanga L.*, is known as “Kun-sar, gamone” in Myanmar, “galangal (or) “*Kaempferia”* in English, “Shajiang” in China, “Chekur” in Malaysia, “Chandramula” in India and “Prohom in Thailand.

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This family is rhizomatous herbs with simple and distichous leave. In Peninsular Malaysia and Indonesia the leaves and rhizomes are chewed as an expectorant for coughs and sore throat or pounded and used in poultices or lotions, they are often used as an ingredient of children's medicines and tonics (De Padua *et al*., 1999).

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are chemical compounds that occur naturally in the medicinal plants (phyto means "plant" in Greek), leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals may have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds(Wadood *et al*., 2013).

Characterization of physicochemical properties attained strong interest in the pharmaceutical research area and is now a standard method. It is one of the key challenges to develop a pharmaceutical active ingredient into a drug, which combines biological activity with an appropriate physiochemical profile. Poor solubility in aqueous media is one of the major hurdles in the drug development process (Kerns *et al*., 2008).

Nutritive value of plant has its own importance. Carbohydrates fats and proteins form the major portion of the diet, while minerals and vitamins form comparatively a smaller but never a less important part. The rhizome is rich in essential oil and is being used for the treatment of cold, headache, expectorant, diuretic, stomachic, coughs and asthma (Rajendra *et al*., 2011).

The antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against

drug resistant microbial pathogen. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Harbone and Boxter, 1995).

The aim of the research work is to explore the Myanmar medicinal plants and to promote the Myanmar traditional medicine scientifically. To achieve the aim, the objectives are to verify both vegetative and reproductive part of the plant, to perform the qualitative and quantitative analysis of rhizomes, to analyze the nutritional values, to find out the antimicrobial activities of various solvent extracts of rhizomes from *Kaempferia galanga* L.

Materials and Methods

Morphological study of *Kaempferia galanga* L.

Collection and identification

The plant specimens of *Kaempferia galanga* L. were collected from Ka-wa Township, Bago Region during the flowering and fruiting periods from June to October, in the year 2013. For morphological study, the specimens were measured, described in detail and identified with the help of available literatures (Hooker, 1894; Backer, 1968; Dassanayake, 1983; Wu Delin and Kai Larsen; 2000). The specimens such as habit, leaves, inflorescences and flowers were recorded with the photographs. Herbarium specimens were prepared and kept in the Herbarium, Department of Botany, University of Yangon.

Qualitative analysis of powdered rhizomes from *Kaempferia galanga* L.

Preliminary phytochemical investigation on rhizomes from *Kaempferia galanga* L. was carried out to examine the plant constituents. The powdered rhizomes of *Kaempferia galanga* L. was tested qualitatively for the presence or absence of alkaloid, α-amino acid, carbohydrate, starch, reducing sugar, cyanogenic glycoside, glycoside, phenolic compound, saponin, tannin,

flavonoid, steroid and terpenoid. According to the methods of Marini Bettolo *et al*., (1981), Central Council for Research in Unani Medicine (1987) and Trease and Evans (2002), the investigation of phytochemcial studies was applied. The results were as shown in Figure (1) and Table (1).

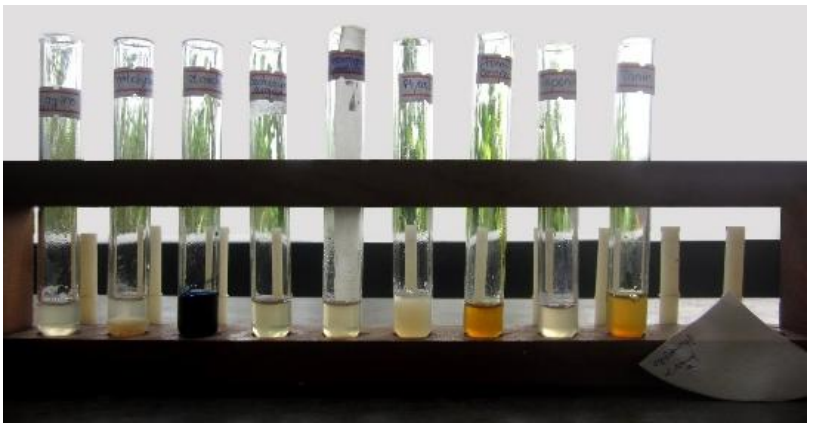


Figure 1. Phytochemical Test

Quantitative analysis ofpowdered rhizomes from *Kaempferia galanga* L.

In the quantitative analysis, moisture content, total ash, acid soluble ash, water soluble ash and various solvents such as ethanol, petroleum ether, methanol, ethyl acetate, chloroform, acetone and distilled water soluble contents were carried out according to the method of British Pharmacopoeia (1968).

Elemental analysis of powdered rhizomes from *Kaempferia galanga* L. by using Energy Dispersive X-Ray Fluorescence spectrophotometer (EDXRF)

The relative concentration of elements inpowdered rhizomes of *Kaempferia galanga* L.were analyzed by using Energy Dispersive X-Ray Fluorescence (EDXRF) spectrophotometer, Shimadzu Co. Ltd, Japan, at the

Physics Department, University of Mandalay. The parameters of each part of the spectrophotometer are as follows:

Detector Type	Si (Li) detector
Liquid N_2 Supply	Only during measurement
Detection area	10 mm^2
Resolution	Less than 155eV (MnKa 1500Hz)

The EDX-700 Shimadzu spectrometer can detect a wide range of the elements from aluminium (Al) to uranium (U). The required data can be produced in a few minutes and it has a high degree of resolution for the spectrum evaluation. The powdered sample was pressed into pellet by a hydraulic press of 4 tons. The pellet was used in the EDX-700. Shimadzu Spectrophotometer which produced the X-ray spectrum, consisting of the respective elements. The spectrum evaluation was carried out by the use of the built in elemental analysis software.

Detection of heavy metals by using Atomic Absorption Spectrophotometric (AAS) analysis of powdered rhizomes from *Kaempferia galanga* L.

Some minerals namely sodium, calcium, potassium, copper, chromium, magnesium, iron, zinc and manganese were quantitatively analyzed by Perkin and Elmer Analyst 800 spectrophotometer. Ten grams of powdered sample was placed in a weighed crucible and heated in a Muffled furnace at 500°C to achieve completely ash. About 0.5 g of ash was filtered with 300 meshes and digested in 5 ml of concentrated hydrochloric acid. The solution was evaporated overnight to dryness in air and the residue was leached in a water bath treated with 5 ml of hydrochloric acid mixture at a temperature of about 70°C for 30 min. The solution was stirred by using vortex mixer. These solutions were decanted and the clear solution was made up to 100 ml with deionized water. Ten ml of the resultant solution was

pipette accurately and made up to 100 ml with deionized water again the solutions were stand overnight and then were aspirated on an atomic absorption a spectrophotometer. Atomic Absorption Spectrophotometer measured the atomic vapor produced from a sample solution by light from a Hollow Cathode Lamp (HCL) and Electrode less Discharge Lamp (EDL) that emitted characteristic light wavelength of an element. The light was absorbed by the atoms of the element present in the flame. The degree of absorption was measured by photomultiplier tube.

Nutritional values of powdered rhizomes from *Kaempferia galanga* L.

The rhizomes of *Kaempferia galanga* L. were evaluated for its nutritive value at Food Industries Development Supporting Laboratory (FIDSL), Yangon. The nutritional value had been undertaken according to the method of Association of Official Analytical Chemists (AOAC) (Horwitz, 1980).

Antimicrobial activities of various solvent extracts of rhizomes from *Kaempferia galanga* L.

Apparatus

Autoclave, beaker, bottle, conical flask, clean bench, cotton wool, hot air sterilizer, loops, measuring cylinders, micropipettes, steam-drying oven, petridishes, pipette and water bath.

Microorganisms

The solvent extracts of rhizomes were tested against nine pathogenic microorganisms by using agar-well diffusion method. In the test microorganisms *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albicans, Escherichia coli, Vibrio cholerae, Klebsiella pneumoniae and Proteus mirabilis* were included. The test was conducted at the Pharmaceutical Research Department (PRD).

Procedure for antimicrobial activity

The study of antimicrobial activities was performed by agar-well diffusion method and nutrient agar was prepared according to the method of Cruickshank (1975). Nutrient agar was boiled and 20 - 25 ml of the medium was poured into each test tube and plugged with cotton wool and sterilized at 121 °C for 15 minutes in an autoclave. Then, the tubes were cooled down to 30-35°C and the content was poured into sterilized petridishes and 0.1 - 0.2 ml of test organism was also added into the dishes. The agar was allowed to set for 2 - 3 hours. Then, 10 mm plate agar-well was made with the help of sterilized agar-well borer. After that, about 0.2 ml of sample was introduced into the agar-well and incubated at 37 °C for 24 hours. The inhibition zone appeared around the agar-well, indicating the presence of antimicrobial activity. The diameter of the inhibition zones were measured with the help of transparent ruler, at the diameter zone of inhibition including the agar well.

Table 1. Types of microorganisms and their diseases

No	Type of microorganism	Diseases
1.	*Bacillus subtilis*	Diarrhoea and food poisoning
2.	*Staphylococcus aureus*	Pneumonia, skin infections and food poisoning
3.	*Pseudomonas aeruginosa*	Urinary tract infection, gastrointestinal infection, inflammation
4.	*Bacillus pumalis*	Food poisoning and eye infections
5.	*Candida albicans*	Vaginal infection and skin infection
6.	*Escherichia coli*	Urinary tract infections, diarrhoea and dysentery
7.	*Vibrio cholera*	Vomiting, diarrhoea
8.	*Klebsiella pneumonia*	Pneumonia, urinary tract infections
9.	*Proteus mirabilis*	Wound infections, urinary tract infection and pneumonia

(Cruickshank, 1975)

Results

Morphological characters of *Kaempferia galanga* L.

Scientific name - *Kaempferia galanga* L.

Myanmar name - Kun-sar-gamone

English name - Sand ginger, Aromatic ginger

Family - Zingiberaceae

Herbs with aromatic rhizome, rhizomes yellowish white inside, fragrant. Leaves opposite and distichous, simple, the lamina broadly elliptic to slightly orbicular, 12.0-15.5cm long and 10.0-13.1cm wide, the bases cuneate, the margins entire, the tips acuminate, both surfaces glabrous; shortly petioles; leaf-sheath open,10.0 -23.0 cm long and 0.35 - 0.5 cm wide. Inflorescence terminal, compact spike; bracts lanceolate, 5.8-6.0 cm long and about 1.0 cm wide. Flower white with violet center, 5.6- 5.8 cm long and 2.6-2.8 cm wide, complete, bisexual, irregular, zygomorphic, trimerous, epigynous; sepals (3), synsepalous, tubular, 2.0-2.3 cm long and about 0.3 cm wide, spathaceous splitting, white; petals (3), synpetalous, tubular, the tubes 3.6-3.8 cm long and about 0.3 cm wide, the lobes 1.6-1.8 cm long and 0.25-0.35 cm wide, white; stamens 1+ $(2)^{st}$+2^{st}, epipetalous, 1 fertile stamen4.5mm long and 5.0mm wide , 2 - outer staminodes fused to form a labellum, 17.0mm long and 18.0mm wide, white with violet center and 2- inner lateral petaloid free staminodes, 12.0 mm long and 8.0 mm wide , filaments grooved, exserted, anthers dithecous, introrse, dorsifixed, longitudinal dehiscence; ovary inferior, ovoid, 2.5 mm long and 2.0 mm wide, tricarpellary, syncarpous, trilocular, axile placentation, the style long and slender, 37.0mm long and 0.5 mm wide the stigma capitate as shown in Figures (2 to 18).Fruits and seeds not seen. Flowering and fruiting time is June to October.

Morphological characters of *Kaempferia galanga* L.

Figure 2. Plants in natural habit

Figure 3. Close-up view of plant

Figure 4. Habit

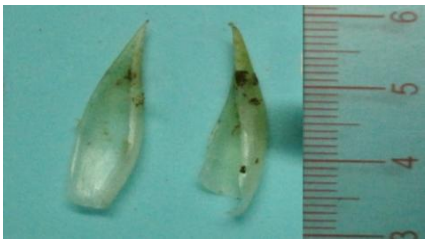
Figure 5. Rhizome

Figure 6. T.S of rhizome

Figure 7. Ventral view of leaves

Figure 8. Dorsal view of leaves

Figure 9. Bract with flower



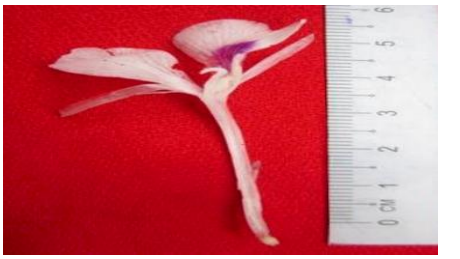


Figure 10. Bract

Figure 12. Calyx

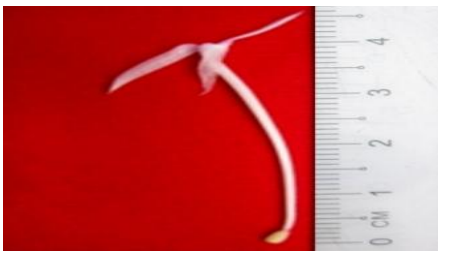
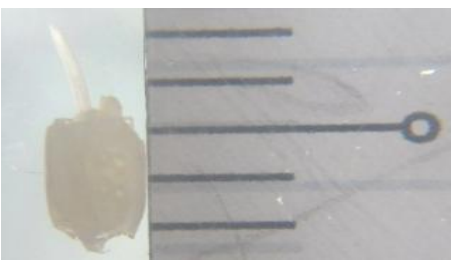


Figure 13. Corolla

Figure 14. Corolla with petaloid staminodes and fertile stamen

Figure 15. Pistil



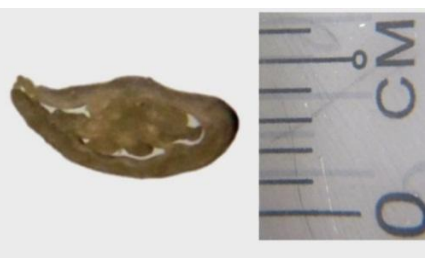


Figure 16. L.S of ovary

Figure 17. T.S of ovary

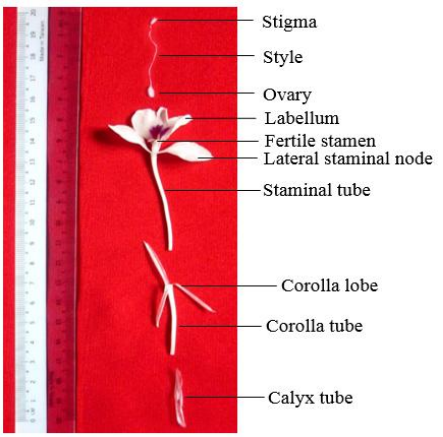


Figure 18. Floral parts

Qualitative analysis of powdered rhizomes from *Kaempferia galanga* L.

In preliminary phytochemical test, the presence or absence of alkaloid, α-amino acids**,** carbohydrate, starch, reducing sugar, cyanogenic glycoside, glycoside, phenolic compound, saponin, tannin, flavonoid, steroid and terpenoid were observed in the rhizomes. α-amino acids and cyanogenic glycoside were absent. The results were shown in Table (2).

Table 2. The qualitative analysis of powdered rhizomes from *Kaempferia galanga* L.

No	Test	Extract	Test Reagents	Observation	Result
1	Alkaloid	1%HCL	(1)Mayer's Reagent (2)Wagner's Reagent (3)Dragendroff's Reagent	White ppt Brown ppt Orange ppt	+ + +
2	α-amino acids	H_2O	Ninhydrin solution	No change in colour	-

3	Carbohydrate	H_2O	10% α-naphthol+conc-H_2SO_4	Red ring	+
4	Starch	H_2O	I_2KI solution	Blue color	+++

No	Test	Extract	Test Reagents	Observation	Result
5	Reducing sugar	H_2O	Benedict's solution	Brick red ppts	+
6	Cyanogenic glycoside	H_2O	(1)Conc-H_2SO_4 acid (2)Sodium picrate paper	No change in color	-
7	Glycoside	H_2O	10%lead acetate solution	White ppts	+
8	Phenolic compound	H_2O	Ferric chloride	Deep blue color	+
9	Saponin	H_2O	Distilled water	Frothing	+
10	Tanin	H_2O	Ferric chloride	Deep blue color	+
11	Flavonoid	EtOH	(1)Mg turning (2)Conc HCL acid	Pink color	+
12	Steroid	P.E	Acetic anhydride+conc- H_2SO_4	Blue green color	+
13	Terpenoid	P.E	Acetic anhydride+conc- H_2SO_4	Deep pink color	+

(+) present (-) absent ppt (precipitate)

Quantitative analysis of powdered rhizomes from *Kaempferia galanga* L.

In physicochemical properties moisture content, total ash, acid insoluble, water soluble ash and solubility in different solvents of rhizomes of *Kaempferia galanga* L. were observed. According to this result, the solubility of the powdered rhizomes in water soluble matter content was to be highest and moderately soluble in methanol as shown in Table (3).

Table 3. The quantitative analysis of powdered rhizomes from *Kaempferia galanga L.*

No	Physicochemical characters	Average(%)
1	Moisture content	7.57
2	Total ash content	8.01
3	Water soluble ash content	0.4
4	Acid insoluble ash content	0.7
5	Ethanol soluble matter content	30.0
6	Methanol soluble matter content	10.0
7	Pet-ether soluble matter content	20.0
8	Ethyl-acetate soluble matter content	20.0
9	Chloroform soluble matter content	25.0
10	Acetone soluble matter content	30.0
11	Water soluble matter content	**35.0**

Table 4. Elemental analysis of powdered rhizomes from *Kaempferia galanga* L.

No	Symbol	Element	Concentration value rhizomes (%)
1.	Cd	Cadmium	0.0006
2.	P	Phosphorus	0.2564
3.	Ca	Calcium	0.1645
4.	K	Potassium	2.616
5.	Cu	Copper	0.00051
6.	Fe	Iron	0.07039
7.	Pb	Lead	0.00009
8.	Mn	Manganese	0.02189
9.	S	Sulfur	0.04701
10.	Zn	Zinc	0.00266
11.	Hg	Mercury	0.00014
12.	Cr	Chromium	0.00084
13.	As	Arsenic	0.00009

Elemental analysis of powdered rhizomes from *Kaempferia galanga* L. by using Energy Dispersive X-ray Fluorescence (EDXRF)

In order to determine the heavy toxic metals and macronutrient elements in plant samples, qualitative elemental analysis was performed by EDXRF method at the Universitie's Research Centre, University of Yangon. Pellets of samples (2.5 cm diameter) were first made by using a pellet making machine. X-ray spectrometer permits simultaneous analysis of light element to heavy element (Griken *et .al*., 1986). Energy dispersive X-ray fluorescence spectrometer (Shimadzu EDX-700) can analyze the elements from AL to U under vacuum condition. X-ray fluorescence uses X-rays to excite an unknown sample. The individual elements comparising in the sample remit their own characteristic X-rays. They are detected by using semiconductor detector [Si (Li)] that permits simultaneous analysis of multi elements within the sample. In this way, EDX-700 spectrometer determines the elements that are present in the sample. It can perform two kinds of quantitative analysis: the Fundamental Parameter (FP) Method and the Calibration Curve Method. In the (FP) method, theoretical results can be calculated even when standard sample are not available. It can be applied to most samples but the accuracy must be checked in advance. In the calibration curve method, experimental results can be obtained by using standard sample. Although Limited sample can be applied, the accuracy is high. In the present study, the FP method was applied for the elemental analysis. The results were reported and discussed in Figure (19) and Table (4).

SPECTRO X-LabPro — Job Number: XRF2014

Preset Sample Data

Sample Name	**Rhizome**	Dilution Material	M_HWC
Description		Sample Mass (g)	5.0000
Method	FP-Pellets-121997ne1	Dilution Mass (g)	1.0000
Job Number	XRF2014	Dilution Factor	0.8333
Sample Type	Pressed tablet, 32 mm	Sample rotation	No
Sample State	Pellet_32	Date of Receipt	01/10/2014 12:57:41
Sample Status	A X X A X X X A	Date of Evaluation	01/10/2014 12:58:06

Results

The error is the statistical error with 1 sigma confidence interval

Z	Symbol	Element	Norm. Int.	Concentration	Abs. Error
13	Al	Aluminum	132.0450	0.1626 %	0.0028 %
14	Si	Silicon	387.7035	0.2459 %	0.0015 %
15	P	Phosphorus	1236.4361	0.2564 %	0.0007 %
16	S	Sulfur	992.6904	0.04701 %	0.00011 %
17	Cl	Chlorine	2302.1015	0.2938 %	0.0006 %
19	K	Potassium	1683.8357	2.616 %	0.004 %
20	Ca	Calcium	155.1122	0.1645 %	0.0010 %
22	Ti	Titanium	14.9347	0.01182 %	0.00034 %
23	V	Vanadium	5.8083	0.00337 %	0.00024 %
24	Cr	Chromium	8.6737	0.00084 %	0.00005 %
25	Mn	Manganese	141.7020	0.02189 %	0.00018 %
26	Fe	Iron	757.0658	0.07039 %	0.00024 %
27	Co	Cobalt	0.9702	< 0.00030 %	(0.0) %
28	Ni	Nickel	3.4475	0.00007 %	0.00001 %
29	Cu	Copper	16.9398	0.00051 %	0.00002 %
30	Zn	Zinc	118.3588	0.00266 %	0.00002 %
31	Ga	Gallium	0.3105	< 0.00005 %	(0.0) %
32	Ge	Germanium	0.0000	< 0.00005 %	(0.0) %
33	As	Arsenic	9.4628	0.00009 %	0.00001 %
34	Se	Selenium	5.3297	0.00005 %	0.00001 %
35	Br	Bromine	99.1746	0.00081 %	0.00001 %
37	Rb	Rubidium	488.7517	0.00237 %	0.00001 %
38	Sr	Strontium	91.3289	0.00040 %	0.00001 %
39	Y	Yttrium	23.8606	0.00010 %	0.00001 %
40	Zr	Zirconium	0.0000	< 0.00010 %	(0.0) %
41	Nb	Niobium	0.0000	< 0.00010 %	(0.0) %
42	Mo	Molybdenum	1.8134	0.00007 %	0.00002 %
47	Ag	Silver	0.0000	< 0.00020 %	(0.0) %
48	Cd	Cadmium	2.6547	0.00006 %	0.00002 %
50	Sn	Tin	6.8609	0.00045 %	0.00006 %
51	Sb	Antimony	4.6696	0.00037 %	0.00007 %
52	Te	Tellurium	2.6345	< 0.00030 %	(0.0) %
53	I	Iodine	3.3498	0.00021 %	0.00008 %
55	Cs	Cesium	0.0000	< 0.00040 %	(0.0) %
56	Ba	Barium	5.2036	0.00132 %	0.00050 %
57	La	Lanthanum	0.0000	< 0.00020 %	(0.0) %
58	Ce	Cerium	0.0000	< 0.00020 %	(0.0) %
72	Hf	Hafnium	9.9867	0.00057 %	0.00003 %
73	Ta	Tantalum	6.6298	0.00032 %	0.00003 %
74	W	Tungsten	0.0000	< 0.00010 %	(0.0) %
80	Hg	Mercury	6.9467	0.00014 %	0.00001 %
81	Tl	Thallium	4.6311	0.00006 %	0.00001 %
82	Pb	Lead	4.9869	0.00009 %	0.00002 %
83	Bi	Bismuth	5.6595	0.00011 %	0.00001 %
90	Th	Thorium	1.0414	< 0.00010 %	(0.0) %
92	U	Uranium	9.7474	< 0.00010 %	(0.0) %
		Sum of concentration		3.91 %	

Figure 19. EDXRF data of relative elements contents of the powdered rhizomes from *Kaempferia galanga* L.

Detection of heavy metals by using Atomic Absorption Spectrometric (AAS) analysis of powdered rhizomes from *Kaempferia galanga* L.

The content of heavy metals were analysed by using AAS, measured in the unit of mg/L. According to AAS, arsenic, cromium, lead and mercury were not found detected. The results were described in details as shown in Table (5).

Table 5. Elemental analysis of powdered rhizomes from *Kaempferia galanga* L.by using AAS

No	Elements	PPM
1	Arsenic(As)	ND
2	Cadmium(Cd)	0.007
3	Cromium(Cr)	ND
4	Lead(Pb)	ND
5	Mercury (Hg)	ND

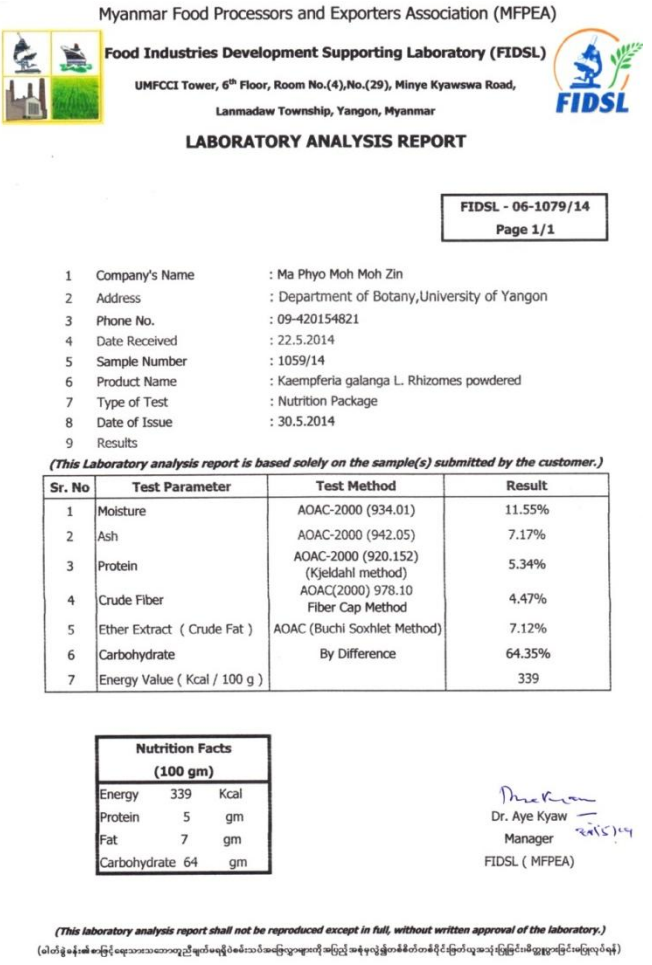
N.D= Not Detected

Nutritional values of powdered rhizomes from *Kaempferia galanga* L.

The nutritional contents such as protein, fiber, fat, carbohydrate of rhizomes from *Kaempferia galanga* L. was analysed at Food Industries Developed Supporting Laboratory (FIDSL). Among them, carbohydrate was found to the highest content. The results were shown in Figure (20) and Table (6).

Table 6. Nutritional value of powdered rhizomes from *Kaempferia galanga* L.

No	Type of Nutrients	Content(gm)
1	Protein	5.34
2	Fat	7.12
3	Carbohydrate	**64.35**
4	Energy Value (Kcal/100g)	339



Myanmar Food Processors and Exporters Association (MFPEA)

Food Industries Development Supporting Laboratory (FIDSL)

UMFCCI Tower, 6th Floor, Room No.(4),No.(29), Minye Kyawswa Road,
Lanmadaw Township, Yangon, Myanmar

FIDSL

LABORATORY ANALYSIS REPORT

FIDSL - 06-1079/14
Page 1/1

1	Company's Name	: Ma Phyo Moh Moh Zin
2	Address	: Department of Botany,University of Yangon
3	Phone No.	: 09-420154821
4	Date Received	: 22.5.2014
5	Sample Number	: 1059/14
6	Product Name	: Kaempferia galanga L. Rhizomes powdered
7	Type of Test	: Nutrition Package
8	Date of Issue	: 30.5.2014
9	Results	

(This Laboratory analysis report is based solely on the sample(s) submitted by the customer.)

Sr. No	Test Parameter	Test Method	Result
1	Moisture	AOAC-2000 (934.01)	11.55%
2	Ash	AOAC-2000 (942.05)	7.17%
3	Protein	AOAC-2000 (920.152) (Kjeldahl method)	5.34%
4	Crude Fiber	AOAC(2000) 978.10 Fiber Cap Method	4.47%
5	Ether Extract (Crude Fat)	AOAC (Buchi Soxhlet Method)	7.12%
6	Carbohydrate	By Difference	64.35%
7	Energy Value (Kcal / 100 g)		339

Nutrition Facts (100 gm)		
Energy	339	Kcal
Protein	5	gm
Fat	7	gm
Carbohydrate	64	gm

Dr. Aye Kyaw
Manager
FIDSL (MFPEA)

(This laboratory analysis report shall not be reproduced except in full, without written approval of the laboratory.)

Figure 20. Nutritional values of powdered rhizomes from *Kaempferia galanga L.*

Antimicrobial activities of various solvent extracts of rhizomes from *Kaempferia galanga* L.

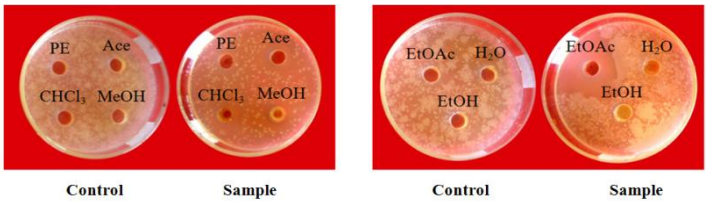
Antimicrobial activity was studied with 70% pet-ether, chloroform, methanol, acetone, ethyl acetate, ethanol, aqueous extract. Agar-well

diffusion method was used to determine the zone of inhibition of microbial growth at particular concentration of various extracts are as shown in Tables (7) and Figures (21 to 23).

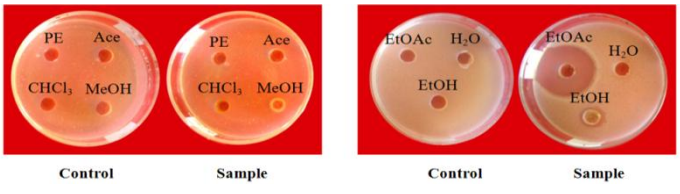
Table 7. Antimicrobial activity against nine test microorganisms by using various solvent extracts of rhizomes from *Kaempferia galanga* L.

Extracts	Test Organisms								
	Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus pumalis*	*Candida albicans*	*Escherichia coli*	*Vibrio cholerae*	*Klebsiella pneumoniae*	*Proteus mirabilis*
Pet-ether	18mm	18mm	15mm	11mm	-	-	-	-	12mm
$CHCl_3$	16mm	20mm	13mm	12mm	11mm	-	-	-	19mm
MeOH	17mm	20mm	15mm	14mm	12mm	-	-	-	30mm
Acetone	20mm	-	13mm	13mm	12mm	-	16mm	-	20mm
EtOAc	35mm	34mm	36mm	45mm	36mm	50mm	45mm	-	50mm
EtOH	-	22mm	15mm	12mm	12mm	14mm	-	-	22mm
H_2O	-	-	-	-	-	-	-	-	-

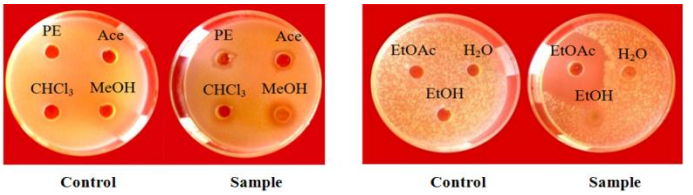
Agar well-10mm (-) Absent



Bacillus subtilis

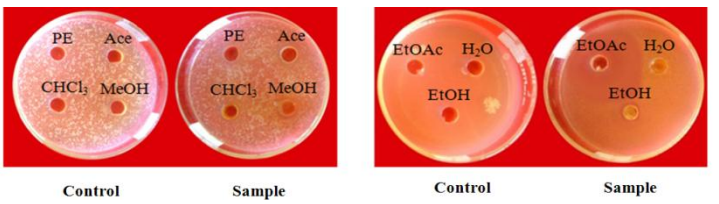


Staphylococcus aureus

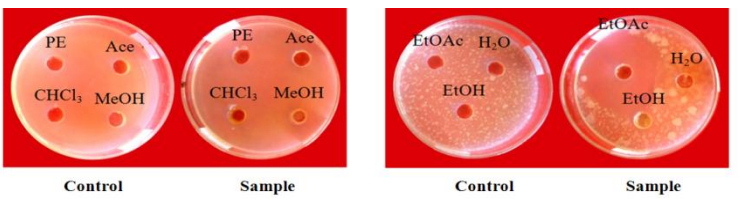


Pseudomonas aeruginosa

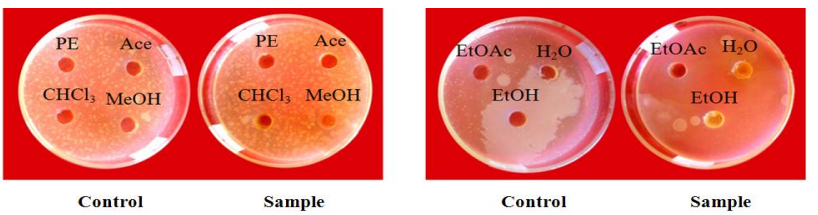
Figure 21. Antimicrobial activity of various solvent extracts of rhizomes from *Kaempferia galanga* L.



Bacillus pumalis



Candida albicans



Escherichia coli

Figure 22. Antimicrobial activity of various solvent extracts of rhizomes from *Kaempferia galanga* L.

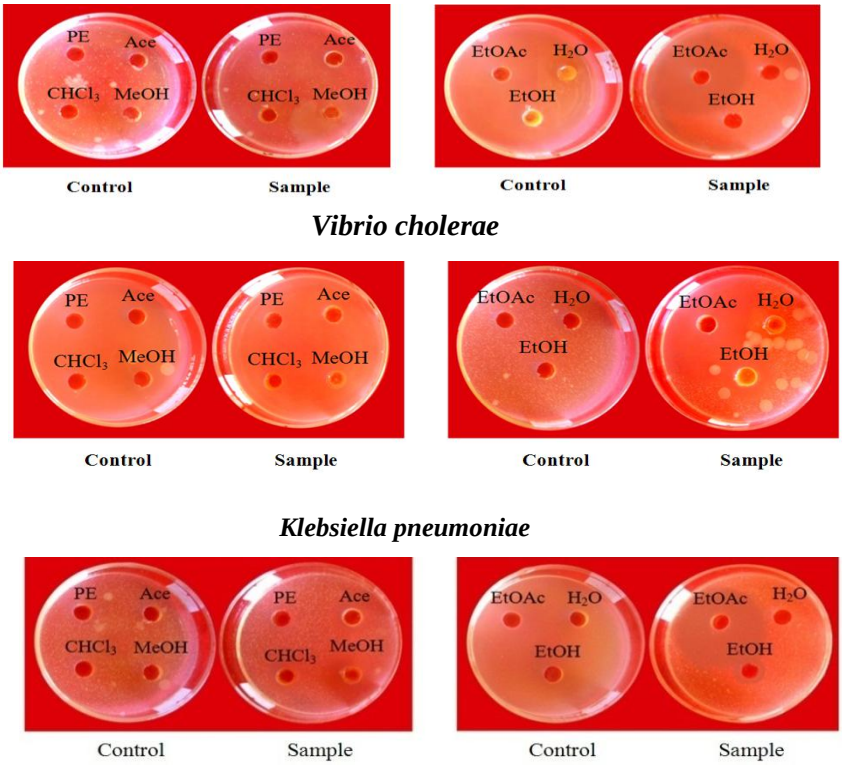


Figure 23. Antimicrobial activity of various solvent extracts of rhizomes from *Kaempferia galanga* L.

Discussion and Conclusion

Kaempferia galanga L. (Kun-sa-gamone) is a medicinal plant belongs to the family Zingiberaceae.In the present research, the outstanding characters of *Kaempferia galanga* L. are rhizomatous herbs with yellowish white. Leaves are opposite and distichous and broadly elliptic lamina with shortly petiole and leaf-shealth. Inflorescence compactly spike, white with violet center flower. Sepals are (3), tubular, white and spathaceous splitting. Petals are(3),

tubular with reflexed lobes, white. Stamens are $1+(2^{st})+2^{st}$, epipetalous, one fertile stamens, white with violet center labellum, lateral petaloid staminodes. Ovary is tricarpellary, trilocular, axile placentation and stigma capitate. These characters were agreement with those given by Lawrence (1964), Ridley and Hutchinson (1967), Dassanayake (1983) and Jiang ke (2000).

In this research, alkaloid, carbohydrate, starch, reducing sugar, glycoside, phenolic compound, saponin, tannin, flavonoid, steroid and terpenoid were detected. However α-amino acid and cyanogenic glycoside were not foundof rhizomes from *Kaempferia galanga* L. According to these results, *Kaempferia galanga* L. can be concluded as a plant rich in primary and secondary metabolites. In physicochemical examination, the percentage of soluble matters were calculated and found to contain the different yield contents. The powdered sample of *Kaempferia galanga* L. was mostly soluble in water. Experiments were carried out to find out moisture content, total ash, acid insoluble-ash, water soluble ash content and solubility matter in different solvents. The highly soluble solvent can be used for the extraction of active constituents.

Elemental analysis of the rhizomes were investigated by using EDXRF; Phosphorus (P), Potassium (K), Sulphur (S), Iron (Fe) and Calcium (Ca), were macroelements and Zinc (Zn), Copper (Cu), Molybdenum (Mo), Chlorine (Cl) and Nickel (Ni) were microelements. Among them, potassium was found to be the highest percentage. In this research, toxic elements, arsenic, chromium, lead, mercury were not found in rhizomes except cadmium according to Atomic Absorption Spectrometric Analysis. According to the result, nutritional contents of carbohydrate is high in the rhizomes of *Kaempferia galanga* L. carbohydrates are essential part of any diet. Carbohydrates provides the body with the energy it needs and are a good source of many vitamins and minerals (http://www.nutritional values, Human nutrition.com).

In antimicrobial activities of different solvents extracts were tested on nine pathogenic microorganisms by using agar-well diffusion method. Especially, ethyl-acetate extract was more effective than other solvents extracts but aqueous extract did not show antimicrobial activity. Antimicrobial activities of various solvents extracts did not effect on *Klebsiella pneumonia.* Kochuthressia *et.al*., (2012) revealed that various extracts such as ethanol, methanol, pet-ether, chloroform, aqueous extract showed antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholera, Candida albicans* respectively.

Acknowledgements

I wish to express my deep gratitude to Pro Rector Dr. San San Aye, Mawlamyine University, for her advices and kind suggestions. I must especially express my deep gratitude to Professor & Head Dr. Aye Pe, Professor Dr. Thandar Aye, Department of Botany, University of Yangon, for providing the departmental facilities and their invaluable suggestions. I would like to express my deepest gratitude to my supervisor Dr. Khin Cho Cho Oo, Associate Professor, Department of Botany, Dagon University for her overall supervision, in valuable opinions and collaboration in every aspect of my research.

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MORPHOLOGICAL AND MOLECULAR EVIDENCE OF NATURE HYBRIDIZATION BETWEEN TWO RELATED SPECIES, *FICUS RELIGIOSA* AND *FICUS RUMPHII* IN MYANMAR

Lum Tsai[1] , Jun Yokoyama[2]

Abstract

Ficus is a well known as a genus with a specific plant-insect relationship. All fig species have their own species-specific pollinators fig wasps (Agaonidae, Chalcidoidea, Hymenoptera). Seed dispersal are mediated by generally various kinds of vertebrates. While such ecological characteristics is considered to be associated with a range of gene flow, studies of the genetic structure of *Ficus* plants in tropical Asia have not been well understood in detail. In this study, it is focused on two species of monoecious figs, *Ficus religiosa* and its closely related species *Ficus rumphii*, which are widely distributed in Southeast Asian countries including Myanmar. These two species are generally found in the same environmental condition and also morphologically look like similar and sometimes difficult to distinguish from the morphological outstanding. To determine the genetic differentiation and the level of interspecific hybridization, using Amplified Fragment Length Polymorphism (AFLP) molecular marker is applied. As a result, the genetic differentiation between species and individuals of mixed genotype has been shown. It is considerable that amount of hybrid individuals were present and hybridization were occurred in both directions. Interspecific hybridization of the genus *Ficus* has been thought to be very rare. The results indicated that effects of interspecific hybridization in *Ficus* to its evolution could not be ignored. In addition, it is expected that the study was the first basic information about the genetic diversity of *Ficus* and the results affect the decision of the plant protection in Myanmar.

Keywords: AFLP, Hybridization, Morphological, *F. religiosa*, *F. rumphii*, Myanmar

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Introduction

The figs (*Ficus*, Moraceae) are one of the largest genera of woody flowering plants with about 800-850 species of free standing trees, epiphytes, shrubs, creepers, climbers and stranglers in tropical and subtropical regions worldwide (Corner 1965; Bailey Hortorium 1976). Roughly half of the known *Ficus* species are monoecious, and the rest are functionally dioecious (Berg 2003; Ronsted et al., 2008). Genus *Ficus* is well known to have extremely specialized symbiotic relations, one to one obligate mutualism with fig-wasps. The interactions between figs and fig-wasps (Agaonidae, Chalcidoidea, Hymenoptera) are special plant-pollinator relationships that are highly species-specific (Janzen 1979; Herre et al., 1999; Machado et al., 2001). In relation to the mutualism, *Ficus* plants are well characterized by hanging a flower on the inside wall of spherical inflorescence called a syconium. Generally, 4 different kinds of flowers can be found in the syconia (long-styled female flowers, short-styled female flowers, male flowers and neuter flowers). The only insect which carries pollen to the blooming flower in the inflorescence is the fig-wasp. Females of fig-wasps enter syconia and lay eggs with pollination to flowers. An important aspect of pollination symbiotic relationship of fig/fig-wasp is that a phytophagous insect (seed parasite) is a pollinator at the same time, and this point is greatly different from other relationships between plants and pollinator insects (Ramirez 1974; Wiebes 1979; Yokoyama and Iwatsuki 1998; Herre et al., 1996; Anstett et al., 1997).

There are about 85 species of fig in Myanmar (Kress et al., 2003). The fruits of them are important food sources for many animals including human beings. Some fig tree is planted for ornamental purposes, especially in landscaping due to its aesthetic shape and form. It is also commonly planted in gardens and along roadsides. Some species, as *F. religiosa*, is mostly planted near Buddhist temples as it is referred to as sacred trees in India. Hindus associate the tree with fertility in woman. It is also an important host to lac insects. The tree is also sacred in Myanmar Culture. The Buddha is said to

have gained enlightenment under a sacred fig called the Buddhi Tree (Bawdi-Nyaung).

The *F. religiosa* L. and *F. rumphii* Blume are generally known to be monoecious species, the male, female and gall flowers are borne together in the same syconium and large woody deciduous trees. It can be found in woodland areas with other trees. Flowering and fruiting are observed all year round. These two species are widely distributed in Myanmar. Sometimes, both species are growing together with the same environmental conditions up to elevations 5,000 ft. All of every parts of the tree are useful for as a traditional medicine for various ailments and mature stem-trunks are using as fuels. Generally, these two species are morphologically very similar and complicated (Yamazaki 1983). Although, they are similar natures, they still have different in their characteristics. Pollination is made by species-specific fig wasps and seed dispersal is achieved by birds and mammals.

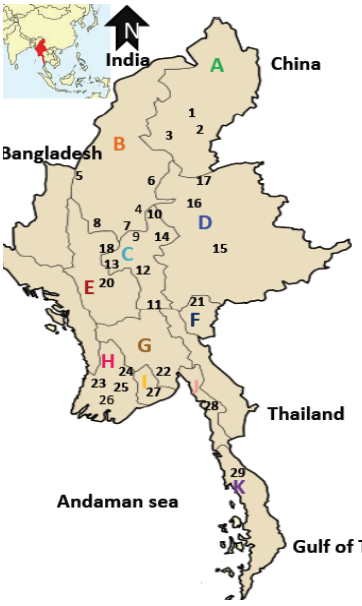
F. religiosa is the famous and essentially important species includes Myanmar (Bawdi-Nyaung) and some neighboring countries throughout the Southeast Asia because this species are well known about the religious sacred tree from the ancient times. Nowadays, this species is grown as an ornamental plant around the world. *F. religiosa* is pollinated by fig-wasps pollinator *Plastyscapa quadraticeps* (Galil & Eisikowitch 1968). The mainly native countries are India, Nepal, Chad, Thailand, Myanmar and Southwest China, Vietnam and other Southeast Asia. The related species *F. rumphii* is distributed in India and Malay Peninsula and the Malucca and another described that they occurred in the dry lower slopes of the mountains Panjab; Northern, Western, and Central India; Assam, Myanmar and Malay island (Corner 1965). The local name of *F. rumphii* is Nyaung-Phyu. The pollinating fig wasp is still not known for *F. rumphii*. Breeding systems, pollen and seed dispersal, and life-form are played important roles for the formation of genetic structure and geographical distribution of plant populations. In the cases of the plant species, the ranges of pollen and seed dispersal are greatly influenced by the media, and it is thought that the degree of the influence depend upon what kind of mediator or vectors is used for the dispersion.

There are a lot of studies by many researchers upon morphology, mating systems, or coevolution of figs and their species-specific pollinating wasps (Yokoyama 1995, 2003; Zavodna et al., 2005; Chen et al., 2008; Wang et.al., 2009; Azuma et al., 2010; Lomascolo et al., 2010; Ghara& Borges 2010). However, the studies upon population genetic structure in detail are rarely conducted, especially in Southeast Asia where the species diversity of figs is extremely high. Here, we performed population genetic structure of two closely related species pairs of figs, *F. religiosa* and *F. rumphii* by using AFLP molecular technique. Among the molecular marker technique, AFLP is a novel polymerase chain (PCR) base assay for DNA finger printing and polymorphism detection. In this study, we found extensive hybridization in both species. This findings suggested that hybridization plays an important role of the evolution of *Ficus*, and, at the same time, there is a big question that how they maintain the species identification in the situation that the interspecific hybridization is common. Moreover, genetic relationship among *Ficus* species from Myanmar and other countries will be expected.

Materials and Methods

Samples collection

In this study, a total of ca. 600 individuals of *F.religiosa* and *F. rumphii* were randomly collected from 28 different populations throughout the Myanmar in June 2012 and March 2013 (Figure 1). 374 individuals from these 600 individuals were genetic analysis. The collection sites were geo-referenced by using Global Positioning System (GPS). For each population, young leaves were collected from each individual, with an interval > 50m between individuals. Firstly, sampling plants were separated by morphological observation. Secondly, some leaves are wrapped by newspaper and pressed for preservation of specimens. After that, the leaves were measured individually for morphological data. One or two leaves are put into a plastic bag containing silica gel for storage until use.



Locality & Population No.	Individual No.
A.Kachin state	
1.Myitkyina(MKA)	62
2.Waimaw(WM)	18
3.Monyin(MN)	3
B.Sagaing division	
4.Sagaing(SG)	17
5.Kale(KL)	4
6.Shwebo(SB)	7
7.Myaung(M)	2
8.Moywa(MY)	8
C.Mandalay division	
9.Mandalay(MDL)	46
10.Pyi Oo Lwi(POL)	16
11.Naypyidaw(NPD)	17
12.Meiktila(MTL)	1
13.Bagan(PG)	7
14.Taungpyone(TP)	4
D.Shan state	
15.Taunggyi(TG)	27
16.Lashoi(LS)	2
17.Kutkai(KK)	3
E.Magway division	
18.Pakukku(PKK)	5
20.Yenanchaung (YNC)	14

Locality & Population No.	Individual No.
F.Kayah state	
21.Loikaw(LK)	5
G.Bago division	
22.Bago(BG)	15
H.Ayeyarwady division	
23.Pathein(PT)	9
24.Danuphyu(DNP)	2
25.PantanawPTN)	4
26.Myaung Mya(MM)	3
I.Yangon division	
27.Yangon(YG)	36
J.Mon state	
28.Mawlamyaing	19
K.Tanintaryi division	
29.Dawei(DW)	18

Figure 1. Sampling locations for the genetic studies of *F. religiosa* and *F. rumphii* in Myanmar.

Table 1. Geographic coordinates of sampling locations in Myanmar.

State & Division	Site ID	Latitude	Longitude	Elevation
Kachin	MKA	25°22'59"N	97°24'00"E	158m(518ft)
	WM	25°21'00"N	97°25'59"E	165m(541ft)
	MN	24°46'59"N	96°22'00"E	278(912ft)
Sagaing	SG	21°52'43"N	95°58'46"E	60m(196ft)
	KL	23°10'59"N	94°04'00"E	102m(334ft)
	SB	22°34'09"N	95°41'53"E	111m(364ft)
	M	21°50'54"N	95°25'28"E	73m(239.5ft)
	MY	22°06'30"N	95°08'30"E	82m(269ft)
Mandalay	MDL	21°58'29"N	96°05'00"E	63m(206ft)
	POL	222°01'59"N	96°28'00"E	1090m(3576ft)
	NPD	19°44'59"N	96°07'46"E	108m(354ft)
	MTL	20°52'39"N	95°51'30"E	244m(800ft)
	PG	21°12'02"N	94°53'11"E	95m(312ft)
	TP	21°58'29"N	96°05'00"E	63m(206ft)
Shan	TG	20°47'00"N	97°02'00"E	1400m(4590ft)
	LS	22°56'00"N	97°45'00"E	836m(2746ft)
	KK	23°27'00"N	97°56'00"E	1400m(4600ft)
Magway	PKK	21°20'00"N	95°05'00"E	48m(156.8ft)
	YNC	24°10'60"N	94°52'60"E	139m(459ft)
Kayah	LK	19°40'27"N	97°12'33"E	872m(2860ft)
Bago	BG	17°20'12"N	96°28'46"E	4m(13ft)
Ayeyarwady	PT	16°14'58"N	97°57'58"E	12m(39.2ft)
	DNP	17°22'00"N	95°27'00"E	11m(39ft)
	PTN	16°59'00"N	95°28'00"E	7m(26ft)
	MM	16°36'00"N	94°55'60"E	11m(39ft)
Yangon	YG	16°48'19"N	96°09'21"E	13m(42ft)
Mon	MLM	16°29'29"N	97°37'32"E	30m(98ft)
Tanintaryi	DW	14°04'59"N	98°12'00"E	5m(16ft)

Figure 2. Appearances of *F. religiosa* L.(left) and *F. rumphii* Blume (right).


Observations of ogy

Morphological observations have been done during the sample collecting in the fields. General observation of leaf type of individuals, conditions of tree trunk and their environmental condition and ecosystem characteristics were marked. Five parts of each individual leaf are measured for principal component analysis (PCA) data. Then identifying with the previous morphological and taxonomic literature about *Ficus* and researching data of other neighboring countries in Mandalay University, Myanmar.

DNA Isolation and AFLP analyses

DNA extractions were performed by using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Extracted DNA was frozen at -20°C until use. AFLP (Amplified Fragment Length Polymorphism) analyses were performed using the Ligation and Preselective Amplification Module and selective primers (Applied Biosystems, Foster City, CA) following the manufacturer's protocol (Vos et al., 1995). Initially, 41 selective primer pairs were tested on 20 individuals to determine those producing the most informative and strong profiles. Finally, three primer pairs, EcoRI-CAT/MseI-ACT, EcoRI-CAA/MseI-AGC, and EcorI-CAA/MseI-AGG, were used for selective amplifications. The final amplification products were electrophoresed with the GeneScan™ 500 Rox™dye Size Standard (Applied Biosystems) using an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems). We evaluated the peak pattern of the AFLP band using the analysis software GeneMapper (Applied Biosystems) in the range of 50-350 bp. Based on the AFLP data, an assignment test using Bayesian clustering was conducted using Structure 2.3.3 (Pritchard et al.,

2000; Falush et al., 2007). An analysis was then performed with 25,000 iterations following 25,000 replications of burn-in period. Based on the AFLP data, the genetic structure analysis with assignment tests were done by analysis software Structure 2.3.1 (Pritchard et al., 2000; Falush et al., 2007). For the assignment tests, a calculation of ΔK to estimate the appropriate cluster number was also conducted (Evanno et al., 2005).

Results

Morphological Observation

Generally, *F. religiosa* and their closely related species *F. rumphii* are actually difficult to distinguish from the morphological outlook, but, in the field observation, this two species are clearly separated by the part of leaf-tip length (aristate part was longer in *F. religiosa* than *F. rumphii*, sometimes acuminate but not aristate) and base of leaf-blade (cordate in *F. religiosa* and truncate in *F. rumphii*) (Fig. 3). Due to the principal component analysis (PCA), the widest part of the leaves tended to come more basal position in *F. religiosa* than in *F. rumphii*. But leaf size variation of two species largely overlapped (Fig. 4). This situation may reflect geographic differentiation and/or interspecific hybridization.

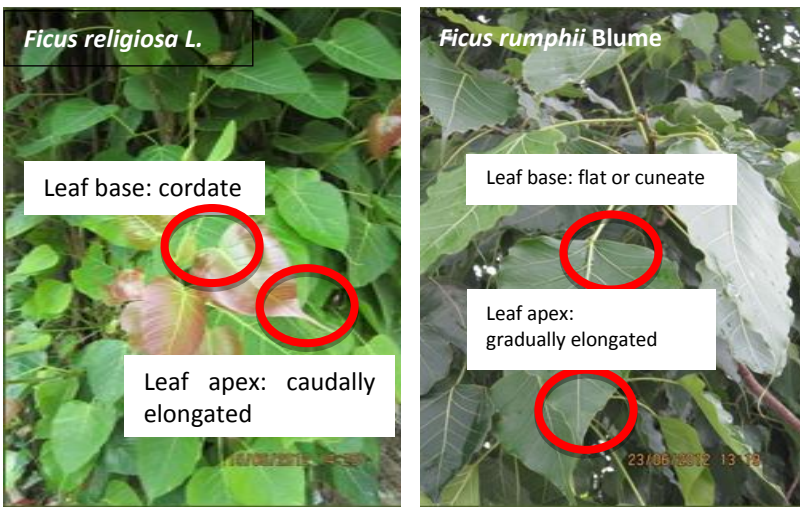


Figure 3. Morphologically difference part of *F. religiosa* and *F. rumphii*.

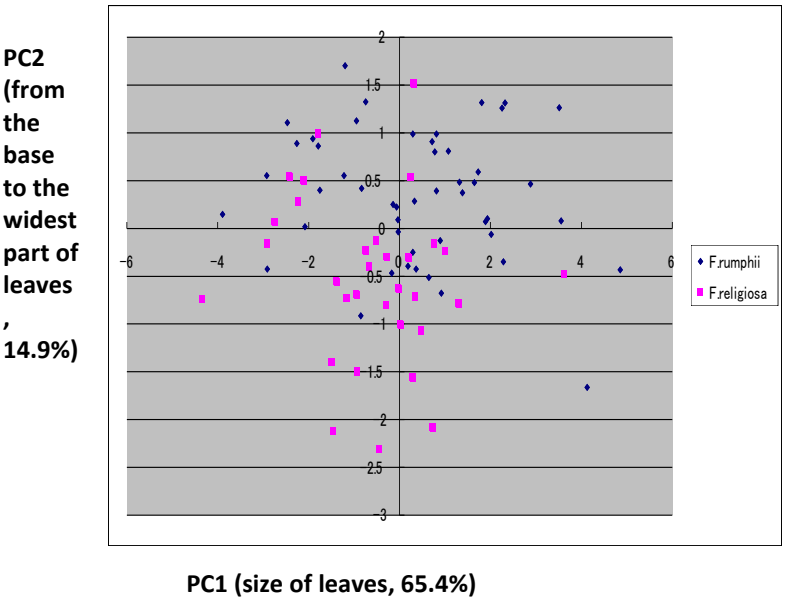


Figure 4. Morphological differences between leaves of *F.religiosa and F. rumphii*.

AFLP Analysis

First, the assignment test was performed with all 374 individuals of *F. religiosa* and *F. rumphii* together and analyzed as K=2. As the result, *F. religiosa* and *F. rumphii* were well divided from each other, suggesting genetic distinction between species (Fig. 5). However, it was shown that many admixture genotypes between the two species also occurred. When we defined the hybrid individuals as 0.2 or more membership value for the less frequent cluster, a total of 42 individuals out of 374 were shown as hybrid individuals. These hybrid individuals were found throughout in the sampled area and morphological characteristics of the were quite variable (Table. 2 and Fig. 6).

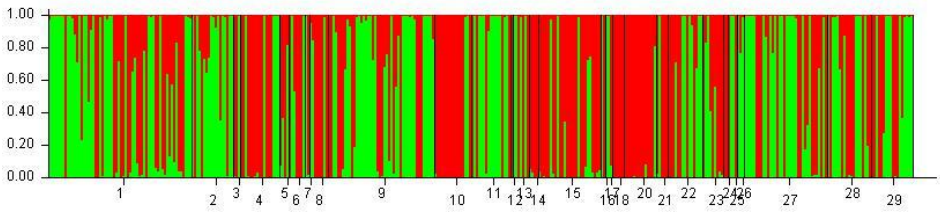


Figure 5. A result of assignment test for the combined data of *F. religiosa* and *F. rumphii*, two genetic clusters (K=2). Numbers under the bar plot indicate individual ID and vertical bar is indicated to interspecific hybridization ratio. Two clusters were as follows: (1) *F. religiosa* (red) and (2) *F. rumphii* (green).

Table 2. Summary of individuals assigned as the mixed genotype between *F. religiosa* and *F. rumphii* based on the AFLP analysis. Letters and numbers in parenthesis indicate minor genetic cluster and its frequency. (R-red gene type %, G-green gene type %)

No.	POP No.	Hybrid INDV/siteID	No.	POP No.	Hybrid INDV/siteID
1	1	MKA 20(R. 0.35)	22	9	MDL 27(R.0.35)
2	1	MKA 22(G. 0.25)	23	9	MDL 31(R.0.38)
3	1	MKA25(R.0.50/G.0.50)	24	9	MDL 32(R.0.35)
4	1	MKA 37(R.0.30)	25	9	MDL 36(R.0.50, G.50)
5	1	MKA 42(R.0.38)	26	15	TG 2(G.0.38)
6	1	MKA 43(R.0.35)	27	15	TG 4(R.0.26)
7	1	MKA 47(R.0.20)	28	15	TG 5(R.0.25)
8	1	MKA 56(R.0.40)	29	22	BG 13(R.0.25)
9	1	MKA 58(R.0.42)	30	22	BG 23(R.0.28)
10	1	MKA 6(R.0.20)	31	23	PT 2(R.0.20/18)
11	2	WM 13(R.0.20)	32	23	PT 4(G.0.40)
12	2	WM 15(R.0.25)	33	23	PT 6(R.0.47)
13	2	WM 16(R.0.40)	34	26	MM 2(R.0.25)
14	2	WM 17(R.0.25)	35	27	YG 20(R.0.23)
15	2	WM 4(G.0.40)	36	27	YG 33(G.0.20)
16	5	KL 2(G.0.38)	37	27	YG 34(G.0.38)
17	5	KL 4(R.0.20)	38	27	YG 6(R.0.38)
18	6	SB 3(R.0.45)	39	28	MLM 14(R.0.38)
19	8	MY 2(R.0.20)	40	28	MLM 8(G.0.20)
20	9	MDL 16(R.0.38)	41	29	DW 15(G.0.30)
21	9	MDL 2(G.0.20)	42	29	DW 5(G.0.38)

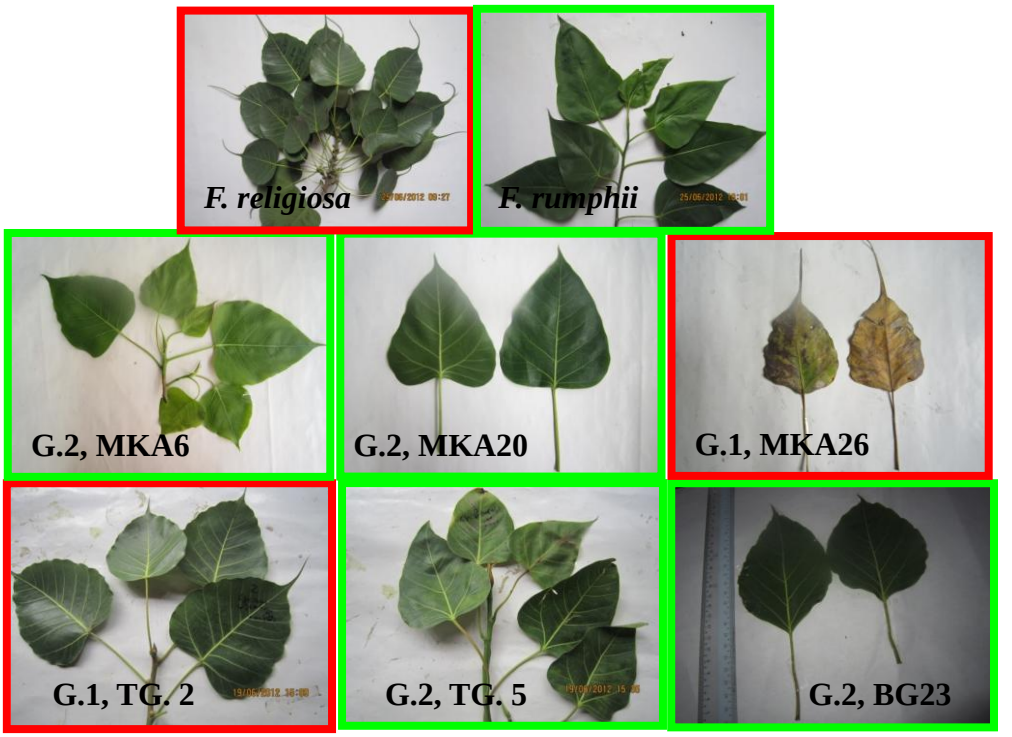


Figure 6. Representative individuals with hybrid genotypes between *F. religiosa* and *F. rumphii*

In the next step, *F. religiosa* and *F. rumphii* were analyzed separately to confirm the genetic structure of each species. The results of *F. religiosa* (195 individuals) showed that three genetic types were recognized (K=3, Fig. 7). Many individuals of the population 1 (MKA), population 9 (MDL) and population 27 (YG) had the least frequent genetic cluster (indicated as blue in Fig. 7). MKA and MDL were the populations where first and second highest numbers of hybrid individuals were recorded (Table. 6). This fact indicated that the genetic cluster represented introgressant fraction of hybrid genotypes. Almost all individuals of the population 20 (YNC), population 8 (MY) and population 10 (POL) were composed of one of the frequent genetic cluster (indicated as red in Fig. 7). However, overall, each population of *F. religiosa* was genetically admixture of individuals of two frequent genetic clusters.

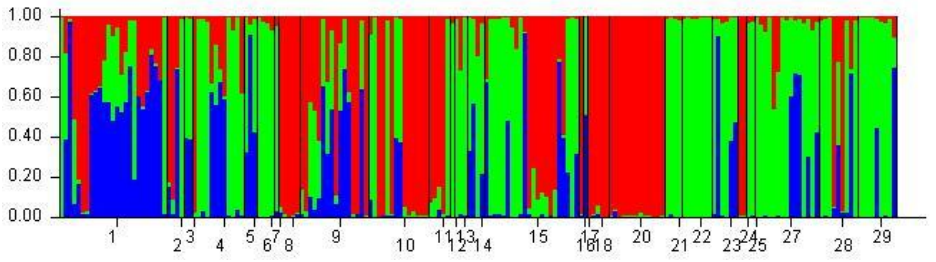


Figure 7. A result of assignment test for the data of *F. religiosa*, three genetic clusters (K=3). Numbers under the bar plot indicate individual ID.

Similarly, the result of *F. rumphii* (179 individuals) showed the presence of two genetic clusters (Fig. 8). In population 9 (MDL) and population 1 (MKA), individuals with the less frequent genetic cluster (indicated as red in Fig. 8) were abundant. These two populations was also shown that the least frequent genetic cluster of *F. religiosa* was observed in many individuals (Fig. 8). Thus, the results indicated that the less frequent genetic cluster of *F. rumphii* is also an introgressant fraction of hybrid genotypes. From these two results of assignment tests, both species were genetically mixed extensively in both directions.

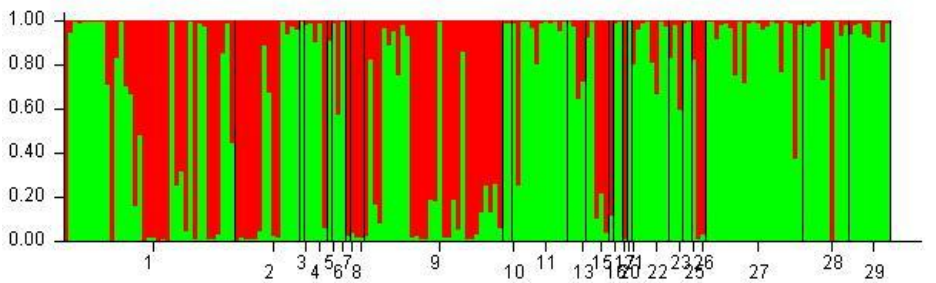


Figure 8. A result of assignment test for the data of *F. rumphii*, two genetic clusters (K=2). Numbers under the bar plot indicate individual ID.

Discussion and Conclusion

Two monoecious fig species were examined in this study, *F. religiosa* and *F. rumphii*, were distinguished from each other by leaf morphology. The morphological differences between them were indicated previously by some authors (Corner 1965; Yamazaki 1983) and were confirmed in this study. From the result of the assignment test, genetic differentiation of two species was obvious though these two species were closely related and sympatric throughout the territory of Myanmar. On the other hand, the interspecific hybridization between those two species was also found in this study. Previous study, *F. pumila* L. and relative species *F. thunbergii* Maxim. In Japan hybrid individuals were also found, although few, and hybridization pattern is unidirectional introgression from *F. thunbergii* to *F. pumila*. Characteristics of the hybridization conditions were, however, different form those of the case between *F. pumila* and *F. thunbergii* (Lum Tsai *et al.*, 2015). First, the hybridization between *F. religiosa* and *F. rumphii* in Myanmar was extensively found in many populations. Second, these two species hybridized and introgressed from both directions.

For the first point, factors affected the extensive hybridizations were unclear. However, general flowering phenology of monoecious figs may be influenced in this situation. Flowering and fruiting were generally synchronized in a single individual but not synchronized between individuals. Therefore, there are at least some flowering (receptive) and fruiting (releasing fig-wasps) individuals in a given population of monoecious figs. In this phenological feature, fig-wasps sometime fail to find receptacle figs when the give population of monoecious fig are small (Bronstein et al., 1990). In the situation, individuals of closely related species may be recipients of heterospecific fig-wasps. Occurrence of *F. religiosa* is often affected by human activities and individuals are tended to be isolated from others. The habitats of *F. rumphii* are natural forests but these habitats are recently destructed and fragmented. These situations in both species, combined together, may be basis of extensive hybridization between the two species.

For the second point, introgression occurred in the particular populations though morphological characteristics were rather stable for both species. One of the reasons may be the simple genetic basis of characteristic leaf forms for each species. However, there are no instances of genetic basis of leaf morphology in figs and we do not have information to confirm this hypothesis. On the other hand, morphological identities of species were sometimes maintained even introgressions present in some extent (Tsukaya et al., 2003; Yamaji et al., 2007). The case of two monoecious fig species in Myanmar may be the similar case to those previous studies. Although the influence to morphological features are rather small, these types of introgression should be affected the evolution of given plant groups by introducing genetic sources of different origins and the case of *Ficus* is also not an exception.

The case of *F. religiosa* and *F. rumphii* in Myanmar; generally, both species are morphological very similar in the field observation. These two species were genetically distinguished into two types, even though large number of mixed individuals are existing between both species. Life history is almost the same in both species for such as flowering and fruiting pattern and growing environments. Therefore, under these ecological and environmental conditions, fig and fig-wasp obligate mutualism may be breakdown between *F. religiosa* and *F. rumphii*.

Monoecious fig trees are commonly with large canopies and fig-wasps can fly for long distance. Syconia of the *F. religiosa* and *F.* rumphii are small, therefore easy to feed and carry far away for various birds including small species. These factors were contributed genetic homogenization among populations of *F. religiosa* and *F. rumphii*. It is needed more information about the introgressive hybridizations of figs and further analyses for the nature of introgression in detail should bring more accurate pictures of the evolution and diversification in genus *Ficus*.

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STUDY ON THE FERMENTATION CONDITIONS OF ENDOPHYTIC FUNGUS, MZF-2 FROM *MOMORDICA CHARANTIA* L.

May Zin Myo[1], Zar Zar Yin[2] , Kay Thi Mya[3]

Abstract

Isolation of endophytic fungi was done by surface sterilization procedure from Cucurbiticeae family, *Mormordica charantia* L. (Kyet-hinga), *Cephalandra indica* Naud. (Kinmon), *Lagenaria siceraria* (Mol.) Standl. (Bu) and *Luffa acutangula* (L.) Roxb. (Hkawe). Agar well diffusion method was used for assay performance with ten kinds of test organisms. All isolated fungal strains showed antimicrobial activity except MZF-8, 11, 12, 13 and 15. Among them fungus MZF-2 isolated from *Momordica charantia* L. was screened for further investigations based on the results of maximum inhibition against *Candida albicans* NITE 09542. Different fermentation parameters were studied and included fermentation period, inoculum size, effect of various carbon sources and nitrogen sources, fermentation media, pH, temperature and agitation condition. 5th day and 4% size were found to be optimum time period of fermentation and inoculum size. In the growth of carbon sources, MZF-2 were excellent growth on corn and potato as well as malt extract showed the excellent result for nitrogen sources. Maximum antifungal activity was obtained when fermentation medium was supplemented with carbon source as glucose and peptone as nitrogen source. Concerntration of fermentation medium (FM-7) ingredients like each 1.0 g peptone, glucose and sucrose proved to be the best fermentation medium. And maximum bioactive metabolite productions occur in pH of 6, temperature at 25˚C and shaking culture with 100rpm speed.

Keywords: antimicrobial activity, antifungal metabolites.

Introduction

Endophytes are defined as microorganisms which inhabit inside of healthy plant tissues and are now considered as ubiquitous symbionts of plants from their surprisingly common detection from many species. Common

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endophytes include a variety of bacteria, fungi and actinomycetes and they can be isolated from wild or cultivated crops of either the monocots or dicots (Petrini, 1986).

Among the microbial group of the most frequently isolated endophytes are the fungi. Cucurbiticeae family is a major source of medicinal agents since ancient time. Both plants and fungi are known for producing a large number of chemically diverse secondary metabolites. The secondary metabolite is obtained by fermentative process. During fermentation, the organisms produce the antibiotic material, which can then be isolated for use as a drug (Fenical, 1993). Fermentation is a complex process, it not only depends on the performance and fermentation medium, also requires the suitable environmental conditions such as inoculation volume, medium capacity, fermentation time, temperature, agitation rate and initial pH . These factors may affect the antibiotics production (Martin and Demain, 1980).

World health problems caused by drug-resistant bacteria and fungi are increasing as a result an intensive search for newer and effective antimicrobial agents is needed. Endophytic fungi have received attention of the scientific community due to their capacity to produce novel bioactive compounds (Strobel *et al*., 2004). Therefore, the present study was carried out the isolation and fermentation studies for antifungal compounds produced from selected fungus. The aim and objectives of this study were to isolate the endophytic fungi and optimization of production parameter for antifungal metabolites.

Materials and Methods

Study area and collection of plant samples

These plant materials, *Momordica charantia* L.,*Cephalandra indica* Naud., *Langenaria siceraria* (Mol.) Standl. and *Luffa acutangula* (L.) Roxb. were collected from Pathein Township, Ayeyarwady Region from June to August, 2016. The identification of these plants were referred by (Flora of Hong Kong, 2009 and Hundley and Chit Ko Ko, 1987).

Isolation of endophytic microorganisms (Espinosa-Garcia, F. J. & J. H. Langenhein. 1991)

In the isolation procedure of endophytic microorganisms, the leaves were washed in running tap water for 15 minutes and were cut into about 0.3 cm pieces. Then, these parts were sterilized by soaking in 95% ethanol for 15 minutes. And again, these parts were cut into smaller pieces and dried on sterilized tissue paper. After drying these pieces were placed on Low Carbon Agar (LCA) medium plate and incubated at room temperature. When hypal tips grow out, they were transferred into Potato Glucose Agar (PGA) medium.

Screening for antimicrobial activities (NITE 2005)

The isolated fungi were grown on PGA medium at room temperature for 5 days. After incubation period, these fungi inoculated into the seed medium (glucose 0.5 g, peptone 0.3 g, yeast extract 0.3 g, $MgSO_4 7H_2O$ 0.01 g, K_2HPO_4 0.01 g, $CaCO_3$ 0.01 g, DW 100 mL at pH 6.5) for 3 days at room temperature. After three days, the seed medium (2%) was transferred into the fermentation medium (glucose 1.0 g, peptone 0.5 g, yeast extract 0.5 g, $MgSO_4 7H_2O$ 0.01 g, K_2HPO_4 0.01 g, $CaCO_3$ 0.01 g, DW 100 mL at pH 6.5) and carried out for 3- 10days and evaluated the antimicrobial activity by agar well diffusion method.

Screening of antimicrobial activity by agar well method (Collins, 1965)

1 day old culture test broth (0.2 mL) was added to 25 mL warm assay medium (glucose 1.0 g, peptone 0.3 g, KNO_3 0.1 g, DW 100 mL, agar 1.8 g) and thoroughly mixed and poured into plate. After solidification, the agar was left to set . Cork borer was used to make the wells (8 mm in diameter). And then, the fermented broth (20 µL) was carefully added into the well and incubated at room temperature for 24-48 hours. The diameter of the zones of inhibition around each well measured and recorded after 24-48 hours incubation.

Test organisms

Agrobacterium tumefaciens NITE 09678, *Aspergillus paraciticus* IFO5123, *Bacillus subtilis* IFO 90571, *Candida albicans* NITE09542 , *E. coli*

AHU5436, *Micrococcus luteus* NITE83297, *Pseudomonas fluorescens* IFO94307, *Saccharomyces cerevisiae* NITE52847, *Salmonella typhi* AHU 7943 and *Staphylococcus aureus* AHU8465, were obtained from NITE (National Institute of Technology and Evaluation, Kisarazu, Japan).

Study on the effects of sizes of inoculum on fermentation (Crueger, W., & Crueger, A. 1989)

The selected fungus MZF-2 was grown on PGA medium for 5 days at room temperature. After 5 days incubation period, this fungus was inoculated into 100mL seed medium. For the size of inoculum, seed culture (1%, 2%, 3%, 4%, 5%) were transferred into the each flask of 100 mL fermentation medium. All fermentation media were carried out 5 days and antifungal activity was investigated by agar well diffusion method.

Effect of carbon and nitrogen sources

Various carbon sources such as glucose, fructose, sucrose, galactose, dextrose, lactose, xylose, maltose, glycerol, soluble starch, tapioca powder, corn and various nitrogen sources as asparagine, KNO_3, malt extract, meat extract, NH_4Cl, NH_4NO_3. $NaNO_3$, $(NH_4)_2SO_4$, Peanut cake, Peptone, yeast extract, and urea were employed.

Effect of glucose and peptone concentration on fermentation medium

In this study six types of carbon concentration, fermentation medium FM1- glucose 1.5 g, FM2- glucose 2 .0 g, FM3- glucose 2.5 g, FM4- glucose 3.0 g, FM5- glucose 3.5 g, FM6- glucose 4.0 g were used . As well as six types of nitrogen concentration, fermentation medium FM7-peptone 1.0 g, FM8- peptone 1.5 g, FM9- peptone 2 .0 g, FM10- peptone 2.5 g, FM 11-peptone 3 .0 g, FM12- peptone 3.5 g were applied.

Effect of pH (Furtado *et al*., 2005)

The optimization of pH of the fermentation broth for antifungal metabolite production was done by carrying out the fermentation at seven different pH values viz. 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0. For each pH value,

desired pH by using either 0.1M NaOH or 0.1 M HCl was adjusted into fermentation medium.

Effect of incubation temperature (Cazar *et al*., 2004)

The optimization temperature for antifungal metabolite production was carried out at six different incubation temperatures viz. 20, 25, 30, 35, and 40 and 45°C. The fermentation medium was carried out 5 days and antifungal activity was studied by agar well diffusion method.

Comparision of static culture and shaking culture (Hassan and Bakhiet *et al*., 2017)

250 mL conical flask containing 100 mL of the fermentation medium was incubated on the shaker (100 rpm) for 5 days. At the same time, another those fermentation medium was incubated under static condition without shaking. These shaking culture and static culture were compared by using agar well diffusion method.

Results

Isolation of endophytic fungi

A total of fifteen fungi were isolated from four selected species of Cucurbitaceae family. Isolated fungi were designated as MZF. Two isolates MZF-1 and 2 were obtained from *Momordica charantia* L. and the other strains (MZF 3 and 4) were isolated from *Cephalandra indica* Naud. Another strains (MZF 6-15) were obtained from *Luffa acutangula* (L.) Roxb. and MZF-5 was isolated from *Lagenaria siceraria* (Mol.) Standl.These results were shown in Table 1 and Figure 1.

Table 1. Isolated fungi

Scientific Name	Myanmar Name	English Name	Fungi
Momordica charantia L.	Kyet-hinga	Bitter gourd	MZF-1, MZF-2
Cephalandra indica Naud.	Kinmom	Wild snake gourd	MZF-3, MZF-4
Lagenaria siceraria (Mol.) Standl.	Bu	Bottle gourd	MZF-5
Luffa acutangula (L.) Roxb.	Hkawe	Ridge gourd	MZF-6 to MZF-15

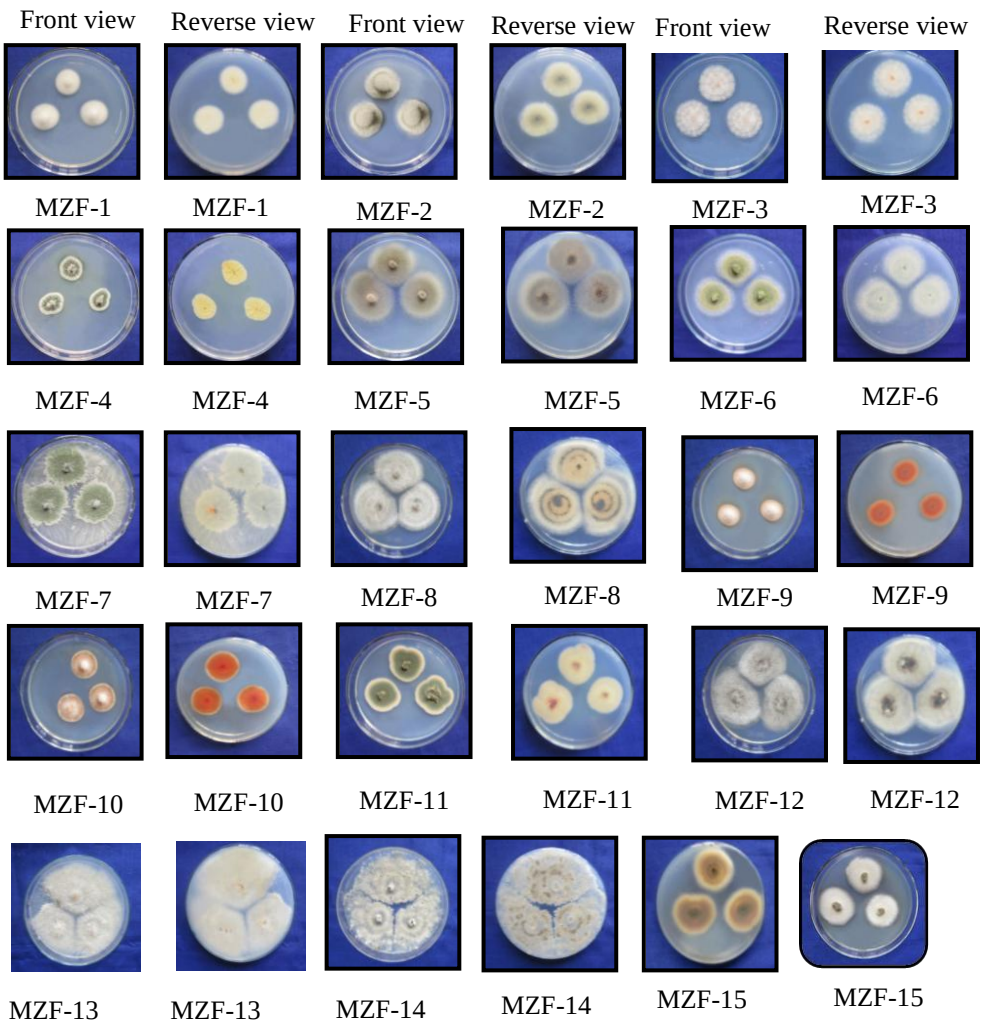


Figure 1. Morphologies of isolated fungi on PGA medium

Antimicrobial activities of isolated fungal strains

Ten isolated fungi (MZF-1, 2, 3, 4, 5, 6, 7, 9, 10 and 14) had antimicrobial activity and remaining five isolates (MZF- 8, 11, 12, 13,15) could not produce antimicrobial metabolites. MZF- 1 (21.77 mm) and MZF-4 (21.70 mm) in 5 days as well as MZF-7 (20.23 mm) and MZF- 14 (20.09 mm) in 6 days fermentation period played the highest activities on *Agrobacterium tumefaciens.* MZF-2 (23.95 mm) and MZF-9 (19.86 mm) had the best activities on *Candida albicans* in 5 days fermentation period. MZF- 3 (20.00 mm) and MZF-6 (18.96 mm) were the strongest activity against *Escherichia coli* in 6 days and 7 days fermentation period. MZF-5 (22.17 mm) and MZF-10 (19.19mm) showed the significant inhibitory zone on *Micrococcus luteus* in 5 day fermentation period. These results were displayed in Figure 2. Among them, antifungal activity of isolated fungus MZF-2 showed the maximum inhibitory zone against *Candida albicans.*

Table 2. Zone of inhibition (in mm) of isolated fungus MZF-2

Fermentation period	**Test organisms**									
	1	2	3	4	5	6	7	8	9	10
3 day	_	_	_	20.78	15.53	_	_	_	16.32	17.11
4 day	_	_	_	22.18	15.97	_	15.46	_	16.30	16.69
5 day	10.31	_	_	23.95	16.94	_	15.86	_	14.31	16.00
6 day	10.86	_	_	23.61	16.97	_	14.95	_	14.00	15.43
7 day	11.75	_	_	23.18	14.74	_	14.47	_	13.27	14.46
8 day	11.24	_	_	22.93	14.52	_	15.85	_	13.21	_
9 day	11.24	_	_	21.86	13.59	_	15.13	_	13.20	_
10 day	11.23	_	_	21.84	13.57	_	15.10	_	13.70	_

1 – *Agrobacterium tumefaciens*
2 – *Aspergillus paraciticus*
3 – *Bacillus subtilis*
4 – *Candida albicans*
5 – *Escherichia coli*
6- *Micrococcus luteus*
7 – *Saccromyces cereviciae*
8 – *Salmonella typhimurium*
9 – *Staphylococcus aureus*
10- *Pseudomonas fluorescence*

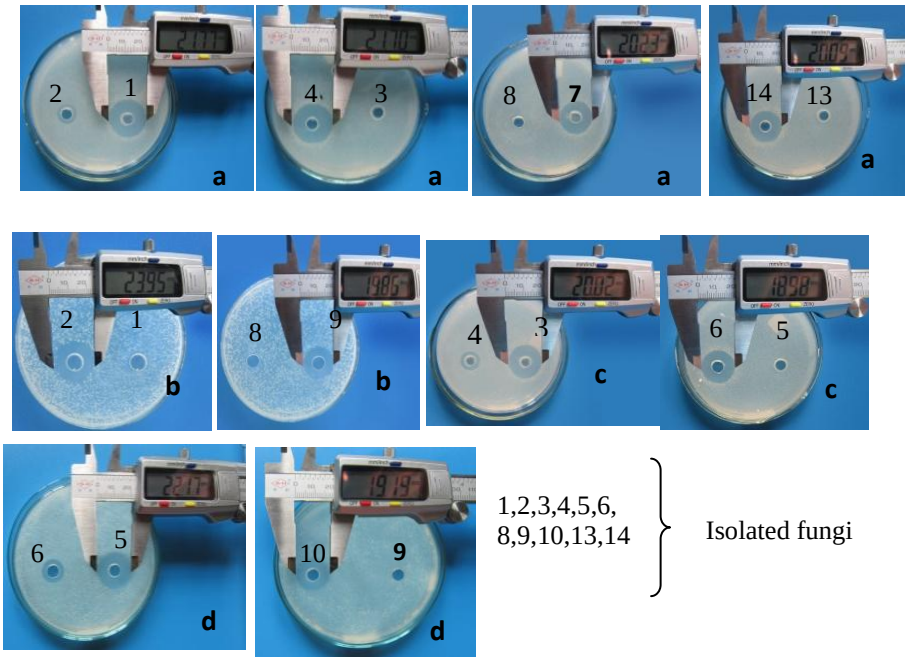


Figure 2. Anitmicrobial activity of isolated fungi against
(a) *Agrobacterium tumefaciens*
(b) *Candida albicans*
(c) *Escherichia coli*
(d) *Micrococcus luteus*

The effect of size of inoculum on the fermentation

In this research work, the effect of size of inoculum was studied by using 1% to 5 % inoculum. Using 4% inoculum showed significantly higher (25.54mm) than others, followed by 3% and 5% (22.32mm and 21.87 mm) respectively. Minimum inhibition zone was observed by using 1% (19.93mm) and 2% (20.81mm).

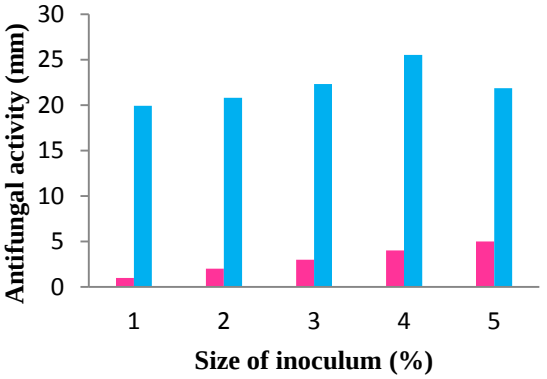


Figure 3. The effect of size of inoculum

Carbon and nitrogen sources utilization for growth

The carbon sources from corn and potato were excellent growth, moderate growth on glucose, sucrose, glycerol, soluble starch and tapioca powder, while other six carbon sources showed poor results. The excellent growth were found on malt extract, poor results on NH_4Cl, NH_4NO_3. $NaNO_3$, $(NH_4)_2SO_4$, urea and the left six nitrogen sources were good growth.

Table 3. Growth of MZF-2 on carbon and nitrogen sources

Carbon sources	Growth	Nitrogen sources	Growth
Glucose	Moderate (4.0cm)	Asparagine	Moderate (3.6cm)
Fructose	Poor (2.0cm)	KNO_3	Moderate (4.0cm)
Sucrose	Moderate (3.6cm)	Malt extract	Excellent (5.5cm)
Galactose	Poor (2.3cm)	Meat extract	Moderate (4.5cm)
Dextrose	Poor (1.6cm)	NH_4Cl	Poor (1.7cm)
Lactose	Poor (1.8cm)	NH_4NO_3	Poor (2.4cm)
Xylose	Poor (2.0cm)	$NaNO_3$	Poor (1.8cm)
Maltose	Poor (1.8cm)	$(NH_4)_2SO_4$	Poor (2.8cm)
Glycerol	Moderate (4.0cm)	Peanut cake	Moderate (3.6cm)
Soluble starch	Moderate (3.5cm)	Peptone	Moderate (3.5cm)
Tapioca powder	Moderate (3.5cm)	Yeast extract	Moderate (3.7cm)
Corn powder	Excellent (4.7cm)	Urea	Poor (2.0cm)
Potato	Excellent (4.5 cm)	_	_

1cm-2cm = poor, 3cm- 4cm = moderate , >4 = excellent

Effect of carbon and nitrogen utilization on fermentation

The significant inhibition zone (24.09 mm, 23.81mm, and 22.04 mm) were obtained in glucose, sucrose and corn as amended media. Dextrose (22.00mm), xylose (20.80 mm), glycerol (20.51mm), and soluble starch (20.18 mm) showed moderate inhibition zone. Glactose (17.32 mm), maltose (18.54 mm), tapioca powder (12.84 mm) and potato (17.38 mm) were regarded as poor inhibition zone. Similarly, the addition of peptone displayed the greatest activity (22.91mm), followed by KNO_3 (22.38 mm), asparagine (22.32 mm), yeast extract (21.94 mm), meat extract (21.70 mm) and $NaNO_3$ (20.53 mm). There were no activities when fructose and lactose were used as carbon source and malt extract, NH_4Cl, NH_4NO_3, $(NH_4)_2SO_4$ and peanut cake were used as nitrogen source. These results were shown in Figure 4 and 5.

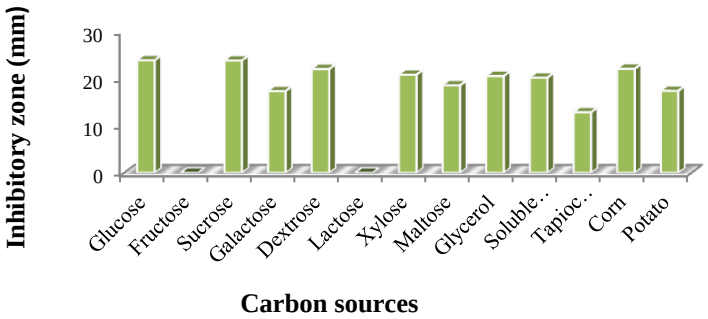


Figure 4. Effectof carbon utilization on fermentation

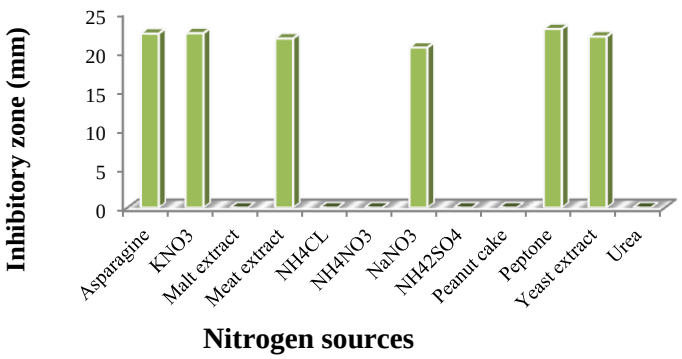


Figure 5. Effect of nitrogen utilization on fermentation

Effects of carbon and nitrogen concentration on fermentation medium

Various glucose concentrations were tested 1.5 g, 2.0 g, 2.5 g, 3.0 g, 3.5 g and 4.0 g. Glucose of 2.5 g concentration (FM 3) showed remarkable result 24.40 mm followed by glucose 2.0 g (FM 2) 23.92 mm and 1.0 g glucose (FM 1) 23.80 mm. Peptone concentrations (1.0 g, 1.5 g, 2.0 g, 2.5 g, 3.0 g, 3.5 g) were also studied. The addition of peptone at concentration of 1.0 g (FM 7) resulted in a maximum antifungal activity 25.42 mm, followed by (FM 8) in peptone 1.5 g 23.79 mm and (FM 9) in peptone 2.0 g 20. 00mm. There were no activity on concentration of peptone 3.0 g and 3.5 g. These datas were described in Figure 6 and 7.

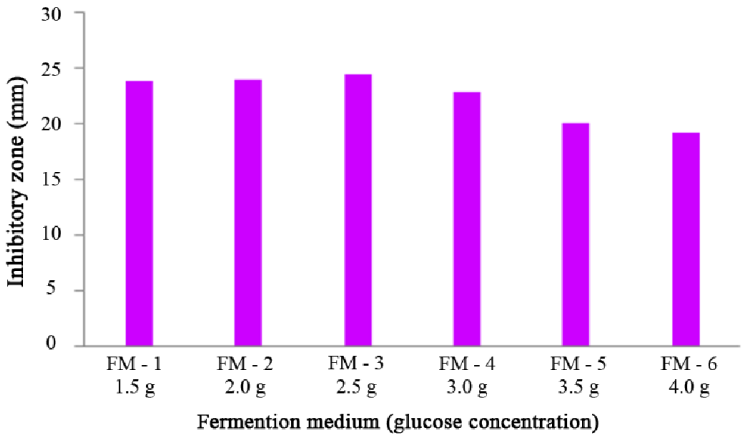


Figure 6. Antifungal activity of MZF-2 on fermentation medium with various glucose concentration

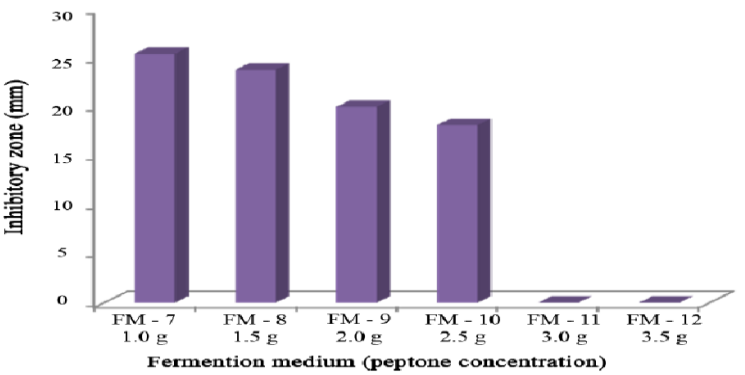


Figure 7. Antifungal activity of MZF-2 on fermentation medium with various peptone concentration

Effect of pH and temperature

The effect of pH and temperature were tested with different pH levels (pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) and different temperature ranges (20˚C, 25˚C, 30˚C, 35˚C, 40˚C and 45˚C). Maximum inhibitory zone was occurred in pH 6 (25.04 mm) and it was followed by pH 5 (22.53 mm) , pH 7 (21.90 mm) and pH 4 (21.03 mm). Under base conditions, minimum inhibitory zone was observed at pH 8, 9, 10 (19.85 mm, 18.52 mm and 17.60 mm) respectively. Maximum antifungal activity was recorded at 25˚C (24.62 mm) , followed by 30˚C (22.99 mm) and 35˚C (20.87 mm). And the antifungal activity completely inhibited at 45˚C. There was a gradual decrease in antifungal production when the temperature was increased from 25 ˚C to 40˚C.

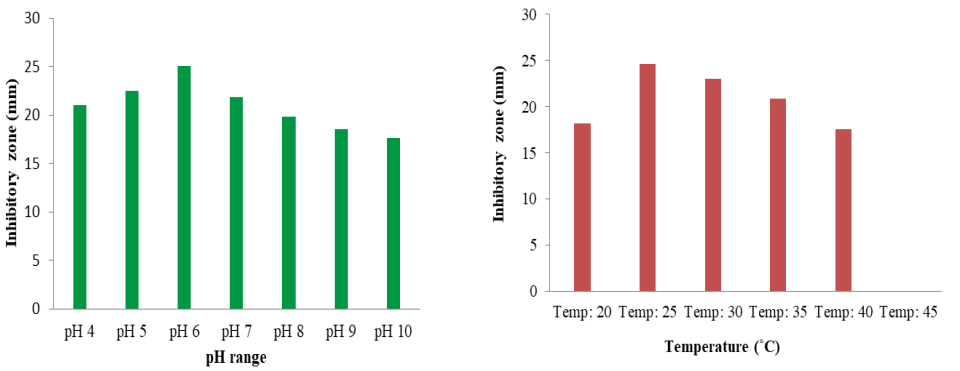
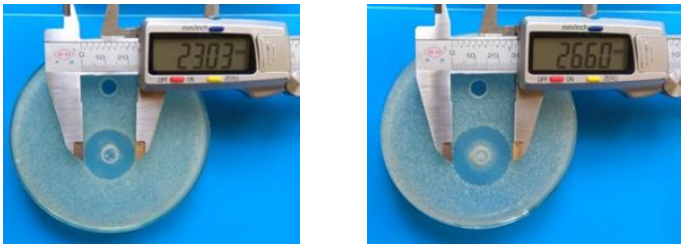


Figure 8. Effect of different pH and temperature

Comparision of static culture and shaking culture

When comparing the static culture and shaking culture of fermentation medium, antifungal activity from shaking culture is better than (26.60 mm) than that of static culture (23.03 mm).



Static culture Shaking culture

Figure 9. Comparisom of static culture and shaking culture

Discussion and Conclusion

Endophytic fungi from medicinal plants are relatively unexplored as potential source of novel species and novel natural products for medicinal and commercial exploitation. In present study, a total of fifteen endophytic fungi were isolated and screened for antimicrobial metabolite production. Ten isolated fungi (MZF-1, 2, 3, 4, 5, 6, 7, 9, 10 and 14) could display the antimicrobial activity inhibiting the test organisms and the remaining five isolates (MZF- 8, 11, 12, 13,15) could not produce antimicrobial metabolites. Similarily, Sarika *et al*., 2014 isolated total eight endophytic fungi from Cucurbiticeae family, *Momordica charantia* L. and those endophytic fungi showed maximum inhibition zone against *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis* and *Staphylococcus aureus*.Among the potent strains, antifungal activity of isolated fungus MZF-2 , isolated from *Momordica charantia* L. showed the maximum inhibitory zone of 23.94 mm against *Candida albicans* NITE 09542 in 5 days fermentation period.Therefore selection of MZF-2 was carried on further experiments.

Modifying fermentation parameters such as time, temperature, pH, and nutrients can help expanding the range of secondary metabolites (Pfefferle *et al*., 2000) . In determining the most suitable size for production antifungal compounds, 4% inoculum size reached the highest activity (25.54 mm) so that 4% size of inoculum regarded as the most suitable size. In investigation of the effect of carbon and nitrogen, addition of corn powder and potato as carbon

source were excellent growth and maximum inhibition zone reached up (24.09 mm and 23.81mm) in glucose and sucrose, followed by corn (22.04 mm). Addition of potato resulted excellent growth of the fungus but less bioactive metabolite production (17.38mm). Suja, 2013 aslo reported that zone of inhibition, 24 mm was obtained in sucrose supplemented media. Excellent growth and the maximum production of antifungal metabolite in MZF-2 was observed in the presence of malt extract and peptone (22.91 mm) as nitrogen source. The supplement of malt extract showed excellent growth of the fungus but did not show antimicrobial activity.

Fermentation medium (FM-7) showed significant result by using peptone (1.0 g), glucose (1.0 g) and sucrose (1.0 g). It has been reported that generally addition of glucose enhances the metabolite production, but its significance in many fermentation process decreases because at higher concentration, it has inhibitory effect (Hutter *et al*., 1982). Similarly, addition of peptone above 1 % decreased antifungal metabolite production in selected strain MZF-2. Therefore FM-7 was chosen as a selected fermentation medium. Maximum antifungal activity was found at pH 6 as the diameter of zone of inhibition was 25.04 mm. Similar result had been reported earlier by Nishihara *et al*., 2001 during the production of FR198248, a new antiinfluenza agent at pH value between 6.3 to 6.4 from *Aspergillus terreus.* Maximum inhibitory activity was recorded at the incubation temperature of 25˚C (24.62 mm). Antifungal metabolite production increased with the increase in temperature from 20 to 25 ˚C. However, as temperature was increased from 25 to 40 ˚C, there was a decline in antimicrobial metabolite production. According to these results, 25 ˚C is the most suitable temperature for the antimicrobial metabolite production. These results were in agreement with Jain and Pundir, 2011. In this study, under shaking culture, the diameter of inhibitory zone was more higher than under static culture. The result was in agreement with the description of Hassan *et al*., 2017 that the antibacterial compounds production of *Aspergillus fumigatus* showed the best result with 100 rpm shaking speed.

The present study concluded that the optimum conditions required for the production of bioactive metabolites by selected endophytic fungus MZF-2 were determined and metabolites showed better antifungal activity against human pathogen, *Candida albicans*.

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TAXONOMIC STUDY ON THIRTEEN FERNS AND FERN ALLIES FROM HTEE SE KHAR WATERFALL AREA, KAYAH STATE

Ei Ei Moe[1], Nu Nu Htwe[2] and Soe Myint Aye[3]

Abstract

The taxonomic study on ferns and fern allies from Htee Se Khar Waterfall area, Loikaw Township have been undertaken. The study area is situated between North Latitude 19°52' and 19°53' and East Longitude 97°14' and 97°15'. All the species were collected from June to October 2017. The 13 species belonging to 11 genera and 8 families were included. Most of the ferns are found as terrestrial and epiphytes on the trunk of tree. *Selaginella braunii* Baker, *Selaginella ciliaris* (Retzius) Spring, *Adiantum capillus-veneris* L., *Adiantum caudatum* L., *Cystopteris fragils* (L.) Bernh, *Pteris vitata* L., *Ampelopteris prolifera* (Retz) Copel, *Cyclosorus interruptus* (Wild) Hito are found as terrestrial species. *Drynaria sparsisora* (Desv.) T. Moore, *Microsorum punctatum* (L.) Copel, *Pyrrosia nuda* (Gies) Cheng and *Psilotum nudum* L.P. Beavu are found as epiphytic species, *Equisetum ramosissimum* var. *altissimum* Bir is found as semi-aquatic species. All the collected species are described with figures of photographs. Artifical key of the collected plant, comparable characteristics of the species was conducted.

Keywords: Taxonomic study, ferns and ferns allies, Loikaw Township, Kayah State,an artificial key

Introduction

Pteridophytes (ferns and fern allies) are called as reptile group of plants and are one of the earliest groups of vascular plants. A fern is a kind of plant which produces spores in sporangia borne in patches on the surfaces or edge of a leaf. The patch of sporangia is called a sorous; the presence of sori that can recognize a fern. The pteridophytes constitute a significant part of the earth's plant diversity and being the second largest group of vascular plant

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communities. Pteridophytes are represented by about 305 genera, comprising more than 10,000 species all over the world. About 191 genera and more than 1000 species are reported from India (Joseph & Thomas 2015).

Fern sporophytes are common and very distinctive plants in the vegetation of many parts of the world while a gametophyte is quite inconspicuous. The general sexual life cycle of ferns is characterized by the alternation of two generations consisting of a prominent sporophyte plant and a much smaller but independent plant, the gametophyte. (Sharpe *et al.* 2010).

Spores are formed in sporangia. In eusporangiate ferns, sporangia are formed from a group of cells, which is the plesiomorphic state. Eusporangia are found in all other vascular plants, except in the leptosporangiate ferns, where the sporangium develops from a single cell into a structure with a stalk, wall and spores. Leptosporangiate ferns form a clade that includes the bulk of fern species, but eusporangiate ferns are composed of several independent groups (Christenhusz & Chase 2014).

Pteridophytes have had a long history on the earth. They probably had their maximum development during the carboniferous and started dwindling in numbers and luxuriance thereafter, till the present times when other than the ferns, only seven living genera are now available. These are: *Psilotum*, *Tmesipteris*, *Equisetum*, *Lycopodium* (in the conservative sense), *Phylloglossum*, *Selaginella* and *Isoetes*). The rest are extinct and represented by fossils (Khullar 2000).

There is no detail information of Ferns and their allies in Htee Se khar Waterfall. The Ferns and fern allies are widely distributed and its taxonomic information is still needed to be recorded in Htee Se Khar Waterfall. There were between Shan and Kayah border and the present research area work have forecasted on that area.

The aim and objectives of the study are to classify and identify the fern species, to record their distribution and morphological characteristics, and to provide the knowledge on the natural resources in the study area.

Materials and Methods

Ferns and fern allies were collected from Htee Se Khar Waterfall area in Loikaw Township of Kayah State. The study area is situated between East longitude 97º 14' and 97º 15' and between North latitude 19º 53' and 19º 52', having 899 m above the sea level in elevation, in Loilin lay village area. The specimens were collected from June to October 2017. The members of terrestrial, epiphytes and semi-aquatic species were included. Plant collection and preservation technique of De Vogel 1987 is used to make herbarium.

The plants were pressed directly in the plant press and plant habit, especially the sori bearing surfaces are recorded by coloured photographs.

The Literature that have been used for identification are Noteboom (1959), Beddome (1969), and Winter & Amoroso (2003). The valid scientific names have been used by checking in index of international plant name. The classification systems and arrangement followed to Smith *et al*. (2006). Location map of study area was shown in figure 1. The specimens were mounted together with a label of field data on herbarium sheets which will be deposited at the Herbarium of Botany Department, University of Mandalay for references and further scientific studies.

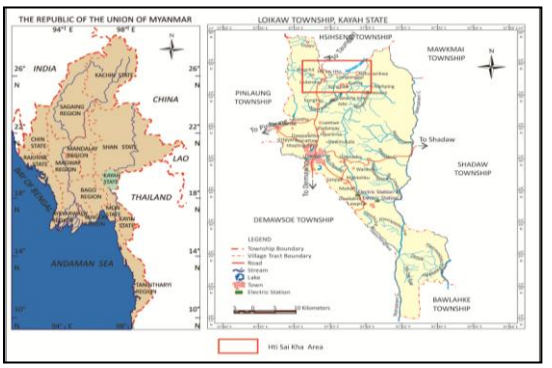

Figure 1. Location map of Htee Se Khar Waterfall Area in Kayah State

Results

In the present study, altogether 13 species of 11 genera belonging to 8 families were collected in Htee Se Khar Waterfall area.

Table 1. The list of collected species

Class	Order	Family	Species
1.Lycopodiospida	1. Lycopodiales	1. Selaginellaceae	1. *Selaginella braunii* Baker.
			2. *Selaginella ciliaris* (Retzius) Spring.
2.Equisetopsida	2. Equisetales	2. Equisetaceae	3. *Equisetum ramosissimum* var. *altissimum* Bir
	3. Psilotales	3. Psilotaceae	4. *Psilotum nudum* L.P. Beavu.
3.Polypodiopsida	4. Polypodiales	4. Adiantaceae	5. *Adiantum capillus-veneris* L.
			6. *Adiantum caudatum* L.
		5. Cystoteridaceae	7. *Cystopteris fragils* (L.) Bernh.
		6. Pteridaceae	8. *Pteris vittata* L.
		7. Thelypteridaceae	9. *Ampelopteris prolifera* (Retz) Copel.
			10. *Cyclosorus interruptus* (Wllid) Hito.
		8. Polypodiaceae	11. *Drynaria sparsisora* (Desv) T. Moore.
			12. *Microsorum punctatum* (L.) Copel.
			13. *Pyrrosia nuda* (Gies.) Ching

Table 2. Comparable attributed characteristics of ferns and fern allies of Htee Se Khar Waterfall area

No.	Botanical name	Habit			Rhizome or caudex			Ramenta	
		Terr es-trial	epip-hyte	semia-quatic	prostrate	erect	colour	shape	colour
1.	*Selaginella braunii* Baker.	+			nil	+		nil	
2.	*Selaginella ciliaris* (Retzius) Spring	+			nil			nil	
3.	*Equisetum ramosissimum* var. *altissimum* Bir			+		+	dark brown	nil	
4.	*Psilotum nudum* L. P. Beavu.		+				dark brown	nil	
5.	*Adiantum capillus-veneris* L.	+			creeping				pale brown
6.	*Adiantum caudatum* L.	+			creeping				dark brown
8.	*Pteris vittata* L.	+				+		linear lanceolate	brownist green
9.	*Ampelopteris prolifera* (Retz.) Copel.	+			creeping			ovate-lanceolate	brownish green
10.	*Cyclosorus interruptus* (Wild). Hito.	+			creeping			linear lanceolate	yellowish brown
11.	*Drynaria sparsisora* (Desv.) T. Moore.		+		creeping		brown	ovate oblong	brownish black
12.	*Microsorum punctatum* (L.) Copel		+		creeping		brown	triangular	brown
13.	*Pyrrosia nuda* (Gies.) Cheng.		+		creeping		dark brown	lanceolate	pale yellow

d

Botanical name	Stipe Colour	Stipe Hair	Frond morphic		Frond	
			mono-	di-	simple	uni
braunii Baker.	yellowish green	nil	+			
ciliaris (Retzius) Spring.	greenish yellow	nil	+			
ramosissimum var.altissimum	green to greenish	glabrous	+			
udum L.P.Beavu.	green	glabrous	+			
capillus-veneris L.	black	glabrous	+			
caudatum L.	dark brown	glabrous				+
fragile (L.) Bemh.	brown	shiny scales	+			
ta L.	brownish green	densely scaly	+			+
is prolifera (Retz) Copel.	plae green	glabrous	+			+
interruptus (Wlid). Hito.	dark brown	glabrous	+			+
parsisora (Desv) T. Moore.	yellowish brown	glabrous		+		
n punctatum (L.) Copel.	strawed colour	glabrous	+		+	
ada (Gies.) Cheng.	pale green	densely	+		+	

Table 2. Continued

No.	Botanical name	Frond						Sporangia	Sp
		Pinnae		Texture				No. of annulus	colour
		pin-nate	pinna-tifid	membr-anous	thin	coria-ceous	firm		
1.	*Selaginella braunii* Baker.			herbaceous					yellowish orange
2.	*Selaginella ciliaris* (Retzius) Spring.								yellow to orange
3.	*Equisetum ramosissimum* var.altissimum Bir					+		nil	bright green
4.	*Psilotum nudum* L.P.Beavu.							nil	lemon yellow
5.	*Adiantum capillus-veneris* L.				+			20	Reddish brown
6.	*Adiantum caudatum* L.	+				+		17	brown
7.	*Cystopteris fragils* (L.) Bemh.		+				+	14	dark brown
8.	*Pteris vittata* L.	+		herbaceous				25	greenish yellow
9.	*Ampelopteris prolifera* (Retz) Copel.					+		16	yellowish brown
10.	*Cyclosorus interruptus* (Wlid). Hito.		+	characteous		+		16	yellowish brown
11.	*Drynaria sparsisora* (Desv) T. Moore.		+					19	Pale yellow
12.	*Microsorum punctatum* (L.) Copel.					+		14	pale yellow
13.	*Pyrrosia nuda* (Gies.) Cheng.					+		16	yellowish brown

+ = Present

Figure 2 A. Habit B. Frond with Sori C. Sporangium D. Spores of *Selaginella braunii* Baker

Figure 3. A. Habit B. Frond with Sori C. Sporangium D. Spores of *Selaginella ciliaris* (Retzius) Spring.

Figure 4. A. Natural Habit B. Habit C. Strobilus D.Spore of *Equisetum ramosissimum* var. *altissimum* SS. Br

Figure 5. A Habit B. Frond with Sori C. Sporangium D. Spores of *Psilotum nudum* L.P. Beavu.

Figure 6. A. Habit B. Frond with Sori C. Sporangium D. Spores of*Adiantum capillus-veneris* L.

Figure 7. A. Habit B. Frond with Sori C. Sporangium D. Spores of *Adiantum caudatum* (L.)

Figure 8. A. Habit B. Frond with Sori C. Sporangium D. Spores of *Cystopteris fragils* Linn.

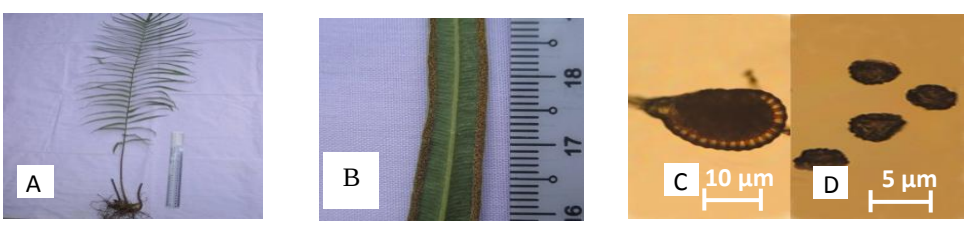

Figure 9. A. Habit B. Frond with Sori C. Sporangium D. Spores of Spore of *Pteris vittata* L.

Figure 10. A. Habit B. Frond with Sori C. Sporangium D. Spores of *Ampelopteris prolifera* (Retzius.) Copel.

Figure 11. A. Habit B. Frond with Sori C. Sporangium D. Spores of *Cyclosorus interrupts* (Wild.) Hito.

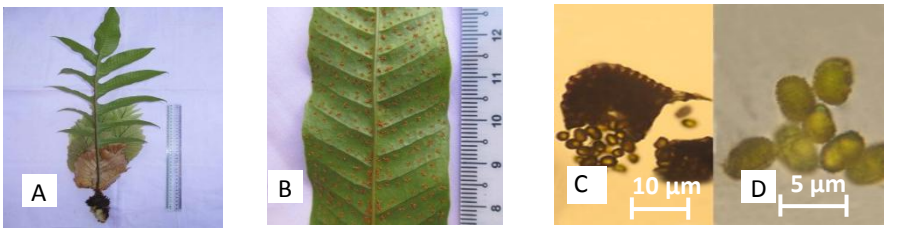

Figure 12. A. Habit B. Frond with Sori C. Sporangium D. Spores of *Drynaria sparsisora* (Desv.) Moore.

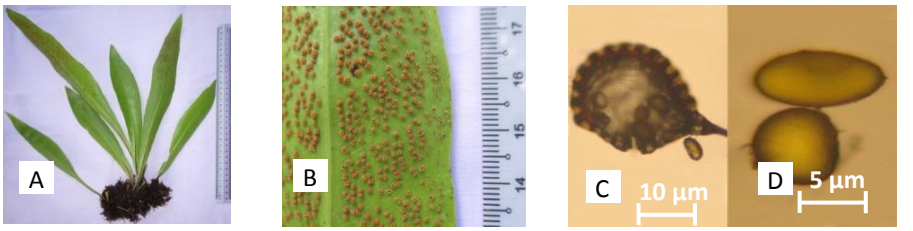

Figure 13. A. Habit B. Frond with Sori C. Sporangium D. Spores of *Microsorum punctatum* (L.) Copel.,

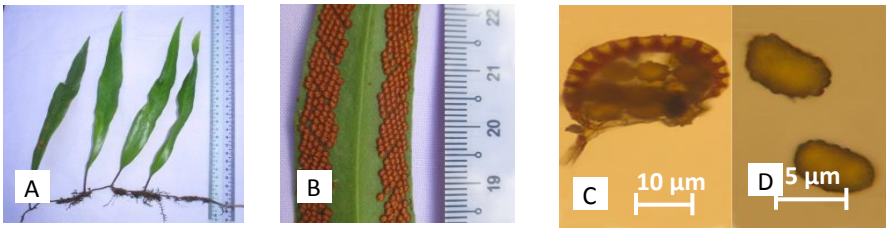

Figure 14. A. Habit B. Frond with Sori C. Sporangium D. Spores of *Pyrosia nuda* (Gies.) Cheng.

An Artificial Key to the Studied Species

1. Reproductive organs in strobilous or synangia ---------- 2
1. Reproductive organs in sorus ---------- 5
   2. Leaves scaly ---------- 3
   2. Leaves well-developed ---------- 4
3. Plants semiaquatic; stem hollow; sporangia on cone-like strobilous -----
   ---------- 3. *Equisetum rammosissimum*
3. Plants creeping; epilethic or epiphytic; stem solid; sporangia forming in synangia ---------- 4. *Psilotum nudum*
   4. Rhizophores absent ---------- 1. *Selaginella barunii*
   4. Rhizophores present ---------- 2. *Selaginella cilaris*
5. Plant epiphytic ---------- 6
5. Plant terrestrial, creeper or climber ---------- 7
   6. Frond dimorphic ---------- 11. *Drynaria sparsisora*
   6. Frond monomorphic ---------- 8

7. Stipe glabrous; laminae ovate to linear elliptic; spores pale-yellow ---------- ----------12. *Microsorum punctatum*

7. Stipe densely hairy; laminae oblong-lanceoalte; spores yellowish-bro---------- ----------13. *Pyrrosia nuda*

8. Indusia absent or false-indusiate ---------- 9

8. Indusia present ---------- 10

9. Sori borned on basal, united veinlets; proliferous buds common in axile of pinnae ----------9. *Ampelopteris prolifera*

9. Sori borned on reflex margin of the lobe; proliferous bud absent ---------11

10. Fronds unipinnate; annuli about 17-celled ---------- ---------- 6. *Adiantum caudatum*

10. Fronds bipinnate; annuli about 20-celled ---------- ---------- 5. *Adiantum capillus veneris*

11.Rhizome short-creeping; ramenta linear; pale brown; stipes hairy at base ---------- 12

11.Rhizome long-creeping; ramenta linear-lanceolate; yellowish brown; stipes glabrous –---------- 10. *Cyclosorus interrupts*

12. Sporangia 75.0 µm long and 70.0 µm in diameter; annuli 14-celled; sproes sub-globoid ---------- 7. *Cystopteris fragils*

12. Sporangia 87.5 µm long and 70.0 µm in diameter; annuli 20-celled; spores globose----------8. *Pteris vittata*

Discussion and Conclusion

The present research deals with the taxonomic study on ferns and fern allies growing in Htee Se Khar Waterfall area in Loikaw Township of Kayah State. It has been observed that the totally 13 species belonging to 11 genera and 8 families were distributed. The resulting species found in the present research belong to the order Lycopodiales, Equisetales, Psilotales and Polypodiales.

The growing habits of the plant vary in the studied area. The 8species such as *Selaginella braunii* Baker., *Selaginella ciliaris* (Retzius) Spring.,

Adiantum capillus-veneris L., *Adiantum caudatum* L., *Cystopteris fragils* (L.) Bernh., *Pteris vittata* L., *Ampelopteris prolifera* (Retz.) Copel., and *Cyclosorus interruptus* (Wild). Hito were growing as terrestrial. The 4 species such as *Drynaria sparsisora* (Desv.) T. Moore., *Microsorum punctatum* (L.) Copel., *Psilotum nudum* L.P. Beavu and *Pyrrosia nuda* (Gies.) Cheng. were growing as epiphyte. The specie of *Equisetum ramosissimum* var. *altissimum* Bir was growing as semiaquatic.

The rhizophores are borned from base to upper part of the main stem in *Selaginella ciliaris* (Retzius) Spring and rhizophore absent in *Selaginella braunii* Baker. These charcters similar to those stated by Xianchum *et al;* (2013).

The rhizome of *Adiantum capillus-veneris* L., *Adiantum caudatum* L., *Ampelopteris prolifera* (Retz) Copel., *Cyclosorus interruptus* (Wlid). Hito., *Drynaria sparsisora* (Desv) T. Moore., *Microsorum punctatum* (L.) Copel. and *Pyrrosia nuda* (Gies.) Cheng were creeping and the rhizome of *Selaginella braunii* Baker., *Equisetum ramosissimum* var.altissimum Bir, and *Pteris vittata* L. were erect.

The frond of *Drynaria sparsisora* (Desv) T. Moore. was dimorphic and the remaining 12 species were monomorphic. These characters were agreed with Winter & Amoroso (2003).

The various colour of stipe were found in variable. Those were yellowish green colour in *Selaginella braunii* Baker., greenish yellow colour in *Selaginella ciliaris* (Retzius) Spring, green to greenish colour in *Equisetum ramosissimum* var. *altissimum* Bir, green colour in *Psilotum nudum* L. P. Beavu., black colour in *Adiantum capillus-veneris* L., dark brown colour in *Adiantum caudatum* L., brown colour in *Cystopteris fragils* (L.) Bernh., brownish green colour in *Pteris vittata* L., dark brown colour in *Cyclosorus interruptus* (Wild). Hito., yellowish brown colour in *Drynaria sparsisora* (Desv.) T. Moore., strawed colour in *Microsorum punctatum* (L.) Copel., pale green colour *Pyrrosia nuda* (Gies.) Cheng.. These characters were agreed with Tagawa & Iwatsuki (1989).

Family Adiantaceae consists of 2 genera and 2 species were found in Htee Se Khar Waterfall area. The genus *Adiantum* can be easily distinguished from other genera by its fan-shaped to parallelogram-shaped leaflets and the edges usually toothed when sterile. The main characters of the *Adiantum* family are; ferns of small to moderate size, fronds variously divided, sori without indusial. These characters were agreed with Holttum (1960).

Equisetum ramosissimum var *altissimum* Bir and *Psilotum nudum* L. P. Beavu. Possessing jointed hollow stem and bearing nodes and internodes. Spores are isosporous and mixed with the elators. Their strobilus is cone-like. In *Psilotum*, the synangia borne adaxially to the projections, glabrous, green at first, yellow when mature with lemon-yellow or paler spores. These finding were agreed with Winter & Amoroso (2003).

The present research provide valuable information for researchers in various fields of study. This study will fullfil the required information of ferns and fern allies in Htee Se Khar Waterfall area and this research will be partially accomplished the ferns flora of Kayah state in Myanmar.

Acknowledgements

We would like to express my gratitude to Dr Thanda, Professor and Head, Department of Botany, Meiktila University, for her permission, invaluable advice and providing all the department facilities to do this research.

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MULTIPLICATION OF MYCORRHIZA INOCULUM FROM FIVE WEED PLANTS AND ITS EFFECT ON *Lactuca sativa* L.

Han Su Kyi[1], Ko Tin[2]

Abstract

The present study is concerned with investigation of the mycorrhiza on the lettuce plants not only to determine their growth but also for the multiplication of mycorrhiza inoculum. Mycorrhiza spores were collected from five weed plants in Mawlamyine University Campus during June to December, 2014 by using floating adhesion technique and wet sieving method. Mycorrhiza, collected from five selected weeds plants were mixed with sterile soil and inoculate on lettuce plants. Spores density and mycorrhizal colonization from rhizosphere of lettuce plants were recorded in every two weeks. Among five selected weed plants, the maximum rate of spore number and the highest colonization percent of mycorrhiza on lettuce plants were isolated from *Eclipta alba* (L.) Hassk. (Kyeik hman). Mycorrhiza isolated from *Eclipta alba* (L.) Hassk. (Kyeik hman) were used as inoculum and subjected into multiplication procedure. The polyethylene bag experiments were conducted on *Lactuca sativa* L. (lettuce). There are five treatment namely soil 5 kg soil (T_1), natural mycorrhiza 1 kg + soil 4 kg (T_2) , natural mycorrhiza 1 kg + biocomposer 0.5 kg + soil 4 kg (T_3), natural mycorrhiza 1 kg + cow dung 0.5 kg + soil 4 kg (T_4) and commercial mycorrhiza fertilizer (MF) 15 g + soil 5 kg (T_5) in polyetylene bag culture were used in the study. According to the result, the highest mycorrhiza inoculum potential (MIP) index were also found in T_2 (10.50) and T_4 (10.50).

Keywords: Weed, mycorrhizal colonization, mycorrhizal fertilizer, spores**,** *Eclipta alba* (L.) Hassk.

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Introduction

Mycorrhiza refers to an association between plant roots and soil borne fungi that colonize the cortical tissue of plant roots during period of active growth (Smith and Read 1997). Soil microorganism plays a major role in nutrients cycling and plant growth in contrast the chemical fertilizers, organic manures and are less expensive and increase productivity without harming the environment. It is therefore important to use vesicular-arbuscular mycorrhizal (VAM) fungi as biofertilizer. Mycorrhizal fungi are associated in the roots of most species and effectively increase the volume of soil that can be explored by the plant (Morton and Benny, 1990). Biocomposer is one of the organic fertilizers which are widely used in Myanmar Agriculture Service (MAS). It contains many organic nutrients. Biocomposer is the product of many basic sugar cane bubble waste (MAE, 2006).

Cowdung has high percentage of nitrogen and potassium, which plays an important role in a accelerating the translocation of photosynthesis from the leaves and shoots to the tuberous roots for bulking (Forbe and Watson, 1994)

Lettuce plants are planted just only for consuming as a vegetable. The leaves rich in vitamins and minerals are popularly used as salad. Lettuce has been cultivated for more than 2,500 years. The Romans grew many varieties, and it became widely appreciated in Asia and Europe (Grigson, 1978).

Present study, natural mycorrhiza, cormmerical mycorrhiza and biofertilizer were treated on cultivation of lettuce plants.

Material and Methods

Source of mycorrhiza

Natural mycorrhiza were collected from five weed plants such as *Eclipta alba* (L.) Hassk., *Mimosa pudica* L., *Phyllanthus urinaria* L., *Tridax procumbens* L., *Urena lobata* L. in Mawlamyine University Campus during June to December, 2014 by floating adhesion technique (Sutton and Barron, 1972) and by wet sieving method (Gerdemann and Nicolson, 1963). The

collected spores were identified by their, size, shape, colour and hyphal attachment according to Smith, 1997 and Miyasaka *et al.*, 2003.

Experimental Site

A polyethylene bag experiment was conducted in Taunggyi University, Shan State from September to October, 2015.

Preparation of Soil

Soils are sterilized by furan (fungicide) and maintained it at least 48 hours. Sterilized soil and selected natural mycorrhiza were mixed with 3:2 ratio.

Multiplication of mycorrhiza inoculum in different fertilizers used

Treatment 1- 5 kg (Soil)

Treatment 2- 1 kg (NM) + 4 kg (Soil)

Treatment 3- 1 kg (NM) + 0.5 kg (Biocomposer) + 3.5 kg (S0il)

Treatment 4- 1 kg (NM) + 0.5 kg (Cow Dung) + 3.5 kg (Soil)

Treatment 5- 15 g (Commercial Mycorrhiza Fertilizer) + 5 kg (Soil)

Quantification of Mycorrhiza fungi propagules (Mycorrhizal inoculum potential (MIP) Assay Methods, Bracker, 1999)

The MIP assay measures the percentage mycorrhizal colonization in a host plant over time, after the host plant has been grown in a series of inoculum and root colonization is estimated after 2 to 6 weeks. MIP technique is less complex and time consuming.

$$MIP = \frac{\text{Primary ingress + Secondary spread}}{2}$$

MIP = Mycorrhizal inoculum potential index

Primary ingress = % of infection (4 weeks) - % of infection (2 weeks)

Secondary spread = % of infection (6 weeks) - % of infection (4 weeks)

Figure 1. Soil mixture of natural VAM (4:1 Kg)

Figure 2. Soil mixture of biocomposer with VAM (3.5: 0.5: 1 Kg)

Figure 3. Soil mixture of cowdang with VAM (3.5: 0.5: 1 Kg)

Figure 4. Soil mixture of commercial with VAM (5 Kg per 15 g)

Figure 5. Preparation of polyethylene bags for planting

Estimation of VAM root colonization and collection of spores

Spores were collected from the rhizosphere soil by floating adhesion technique (Sutton and Barron, 1972) and by wet sieving method (Gerdemann and Nicolson, 1963). AM root colonization in the hosts were studied and caculated by using grid-line method (Newman, 1966). The total percentage of root colonization were determined in the following formula:

$$\text{Root colonization (\%)} = \frac{\text{Intersection of infected roots}}{\text{Total number of intersection roots}} \times 100$$

Results

In the present study, mycorrhiza were collected from five weed plants, such as *Eclipta alba* (L.) Hassk., *Mimosa pudica* L., *Phyllanthus urinaria* L., *Tridax procumbens* L., and *Urena lobata* L. The collected mycorrhiza spores and root colonization of five weed plants were shown in Tables 1and 2. Number of mycorrhiza spores and colonization percent of different mycorrhiza inoculated lettuce plants were shown in Figures 7-11 and Tables 3 and 4 Multiplication of VAM with different treatments on *lactuca sativa* L. were shown in Figures 12-16 and Tables 5 and 6.

Table 1. Determination of colonization percent on roots during June to December,2014

No	Scientific Name	June	July	Aug	Sep	Oct	Nov	Dec
1	*Eclipta alba* (L.) Hassk.	67%	79%	57%	55%	61%	77%	70%
2	*Mimosa pudica* L.	42%	46%	39%	32%	46%	50%	53%
3	*Phyllanthus urinaria* L.	54%	50%	44%	45%	54%	53%	56%
4	*Tridax procumbens* L.	51%	54%	30%	50%	49%	55%	53%
5	*Urena lobata* L.	59%	63%	45%	47%	57%	61%	57%

Table 2. Comparison of spores from rhizophere soil during July to December, 2014

No	Scientific Name	June	July	Aug	Sep	Oct	Nov	Dec
1	*Eclipta alba* (L.) Hassk.	50	67	75	100	160	81	89
2	*Mimosa pudica* L.	30	50	25	40	64	52	34
3	*Phyllanthus urinaria* L.	35	48	58	45	65	59	50
4	*Tridax procumbens* L.	41	54	60	58	75	65	75
5	*Urena lobata* L.	39	63	65	67	75	69	65

Identification of VAM Fungal Spores (Smith and Read, 1997; Miyasaka, 2003)

The collected spores were identified by their, size, shape, colour and hyphal attachment. Collected spores from *Eclipta alba* (L.) Hassk. (Kyeik-hman) were determined as Glomus.

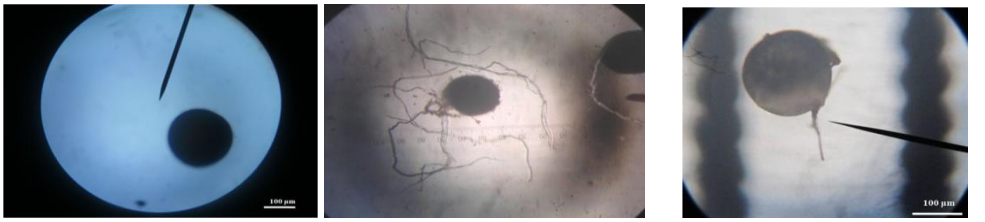
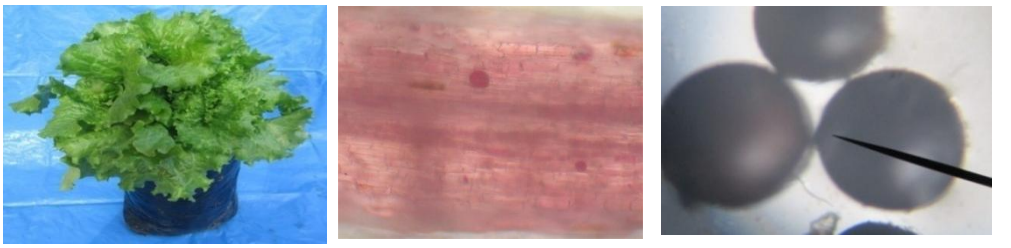


Figure 6. Morphological characters of collected VAM spores

Size	- 100 –310 μm
Colour	- reddish brown to black
Shape	- globose
Surface ornamentation	- smooth
Suspensor cell	- terminally on a bulbous cell present
Vesicle	- present



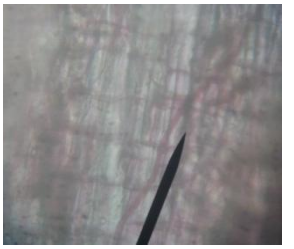
(A) Innoculated lettuce plant growing by Mycorrhiza isolated from *Eclipta alba* (L.) Hassk.

(B) Infected vesicle in root X 100

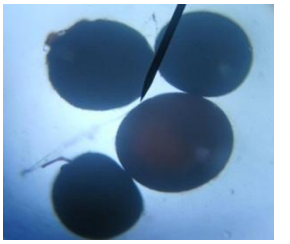
(C) Collected spores

Figure 7. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere

(A) Innoculated lettuce plant growing by Mycorrhiza isolated from *Mimosa pudica* L.



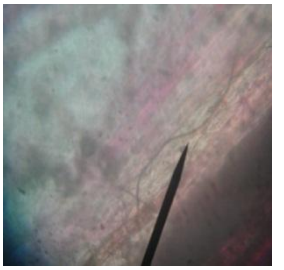
(B) Infected root (X 100)



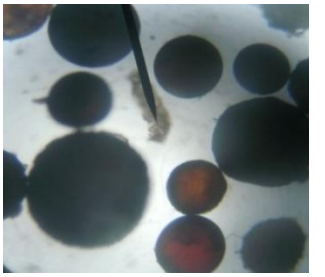
(C) Collected spores

Figure 8. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere

(A) Innoculated lettuce plant growing by Mycorrhiza isolated from *Phyllanthus urinaria* L.



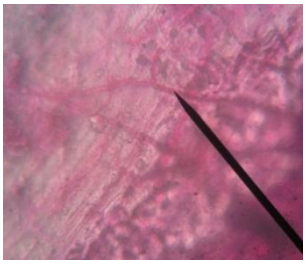
(B) Infected root (X 100)



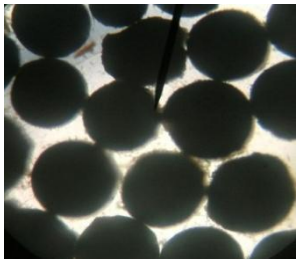
(C) Collected spores

Figure 9. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere

(A) Innoculated lettuce plant growing by Mycorrhiza isolated from *Tridax procumbens* L.



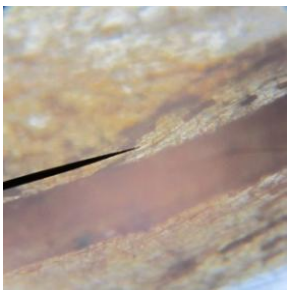
(B) Infected root (X 100)



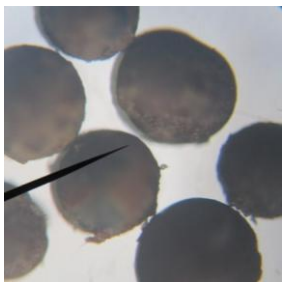
(C) Collected spores

Figure 10. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere

(A) Innoculated lettuce plant growing by Mycorrhiza isolated from *Urena lobata* L.



(B) Infected root (X 100)



(C) Collected spores

Figure 11. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere

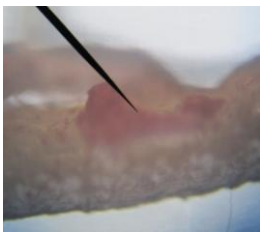
Table 3. Summarized infection percent of different mycorrhiza on letttuce plants

	% of infection				
	Eclipta alba (L.) Hassk.	*Mimosa pudica* L. .	*Phyllanthus urinaria* L. .	*Tridax procumbens* L.	*Urena lobata* L.
2 weeks	**61**	37	46	48	55
4 weeks	**69**	41	50	51	58
6 weeks	**71**	51	61	59	63

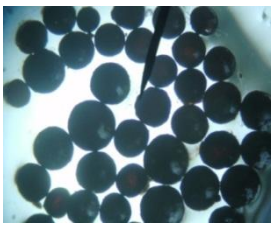
Table 4. Summarized different mycorrhiza spores number on lettuce plant

	Number of spores				
	Eclipta alba (L.) Hassk.	*Mimosa pudica* L. .	*Phyllanthus urinaria* L. .	*Tridax procumbens* L.	*Urena lobata* L.
2 weeks	**41**	29	31	37	29
4 weeks	**61**	38	49	42	38
6 weeks	**75**	45	52	48	45

Treatment - 1



(B) Infected root (X 100)



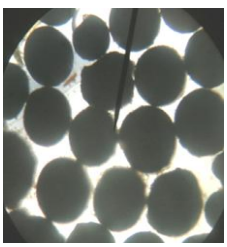
(C) Collected spores

Figure 12. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere. Treatment-1 (control)

Control

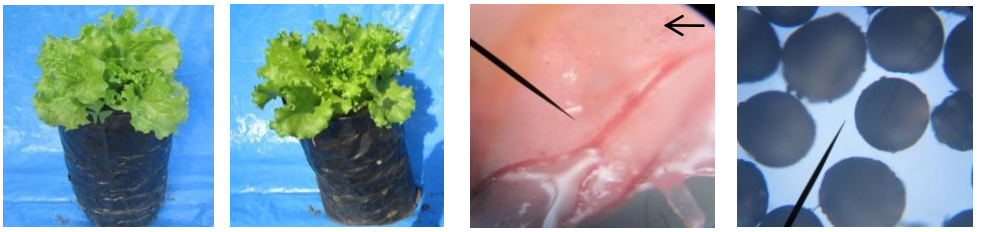
Treatment - 2

(B) Infected vesicle in root (X 100)



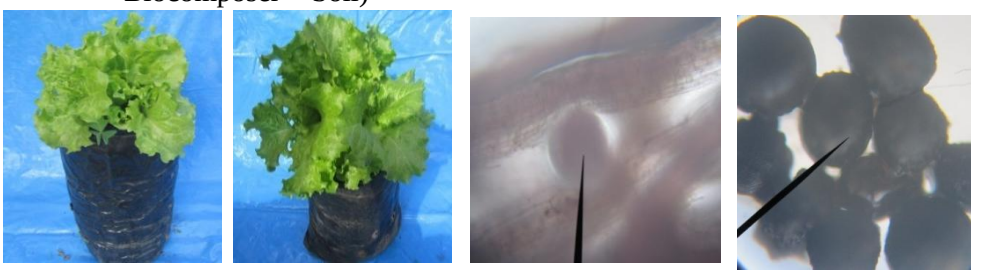
(C) Collected spores

Figure 13. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere. Treatment-2 (Natural Mycorrhiza + Soil)



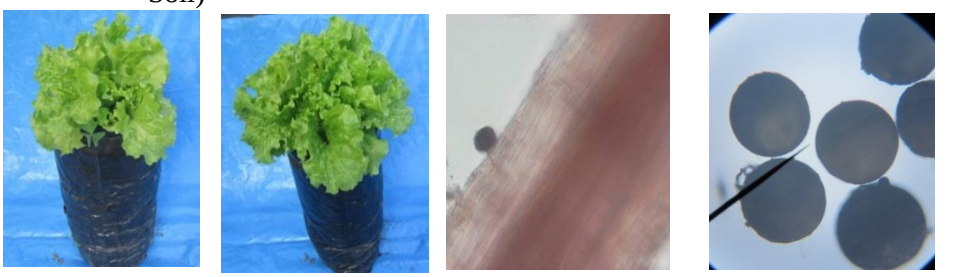
Control Treatment - 3 (B) Infected root (X 100) (C) Collected spores

Figure 14. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere. Treatment-3 (Natural Mycorrhiza + Biocomposer + Soil)



(A) Control Treatment - 4 (B) Spore forming inroot (X 100) (C) Collected spores

Figure 15. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere . Treatment-4 (Natural Mycorrhiza + Cow dung + Soil)



(A) Control Treatment - 5 Infected root and spore attachment X 100 (C) Collected spores

Figure 16. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere. Treatment-5 (Mycorrhiza Fertilizer + Soil)

Table 5. Summarized on the infection percent of different treatments

% of infection					
	T1 (Control)	T2 (Natural mycorrhiza + Soil)	T3 (Natural mycorrhiza + Biocomposer + Soil)	T4 (Natural mycorrhiza + Cowdung + Soil)	T5 (Commercial Mycorrhiza Fertilizer+ Soil)
2 weeks	44	62	56	53	59
4 weeks	50	72	59	63	69
6 weeks	52	83	69	74	79

Table 6. Summarized on the number of spores of different treatments

Number of spores					
	T1 (Control)	T2 (Natural mycorrhiza + Soil)	T3 (Natural mycorrhiza + Biocomposer + Soil)	T4 (Natural mycorrhiza + Cowdung + Soil)	T5 (Commercial Mycorrhiza Fertilizer+ Soil)
2 weeks	35	**73**	41	65	52
4 weeks	39	**80**	58	**69**	62
6 weeks	44	**88**	69	**82**	79

Table 7. Index of Mycorrhizal inoculum potential (MIP) on *Lactuca sativa* L.

Treatment	**MIP index**
T_1	4.00
T_2	**10.50**
T_3	6.50
T_4	**10.50**
T_5	10.00

Discussion and Conclusion

In this research, mycorrhiza were collected from five weed plants: *Eclipta alba* (L.) Hassk., *Mimosa pudica* L., *Phyllanthus urinaria* L., *Tridax procumbens* L., and *Urena lobata* L. These mycorrhiza were introduced into *Lactuca sativa* L. The maximum rate of spore number and the highest colonization percent of VAM on lettuce plants were isolated from *Eclipta alba* (L.) Hassk. (Kyeik hman). Root colonization percent were calculated into MIP assay method, because this techniques is simple and it can estimate the long term survival of VAM in the host (Bracker, 1999).

In these experiment, highest index of MIP were found in the application of natural mycorrhiza 1 Kg + soil 4 Kg (T_2) and natural mycorrhiza 1 kg + cow dung 0.5 Kg + soil 3.5 Kg (T_4) on lettuce cultivation. These findings were agreed with Hawkins and George (1999).

It was correlated with the number of spores in rhizosphere. Maximum rate of spore number were also found in T_2 and T_4. It was very possible, because other variable of measuring soil infectivity have been described (Table 6). Plenchette (1982) described an experiment whereby soil infectivity can be related from host infectivity relationship.

In fact VAM are obligate symbionts and therefore cannot be multiplied on laboratory media apart from a living host (Habte, 2000). According to present research, *Lactuca sativa* L. (lettuce) plant was suitable habitat for VAM, isolated from *Eclipta alba* (L.) Hassk. (Kyeik hman). Best multiplication of VAM, isolated from *Eclipta alba* (L.) Hassk. (Kyeik hman) can be obtained by mix with sterilized soil in 1:4 ratio.

In summarized, among the weed plants, *Eclipta alba* (L.) Hassk. (Kyeik hman) should be chosen as tracking plants for the multiplication of VAM in cultivation of lettuce plants. Furthermore, natural mycorrhiza 1 Kg + soil 4 Kg (T_2) and natural mycorrhiza 1 Kg + cow dung 0.5 Kg + soil 3.5 Kg (T_4) should be applied on the lettuce cultivation to be produced high MIP (Mycorrhizal Inoculum Potential) for the utilization of biofertilizer.

In conclusion, the utilization of organic fertilizers instead of inorganic fertilizers should be used in the growing plants to promote the organic farming system and to produce the high yield and more organic products of foods.

Acknowledgements

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PRELIMINARY PHYTOCHEMICAL INVESTIGATIONS OF LEAVES AND PSEUDOBULBSOF *DENDROBIUM APHYLLUM* (ROXB).C.FICHER

Kyawt Kyawt Khing[1], Khin Myo Thant[2]

Abstract

In this research, the studied plants were Myanmar medicinal orchids *Dendrobium aphyllum* belong to the family orchidaceae and locally known as Phayaungpanthit-khwa. This paper conducted morphological character, histological character and phytochemical constituents of the leaves and pseudobulbs of *Dendrobium aphyllum* (Roxb).C.Ficher were studied.The present studied plants were collected from South Dagon Township, Yangon Division during flowering period from December, 2017 to February, 2018. Fresh plants were grown in home garden. In morphological study, the collected plants were classified and identified with the help of available literatures. The plants were epiphytic herbs, evergreen stem, inflorescence raceme; flowers lavenda. In histological studied free hand section of the leaves and pseudobulbs of fresh specimens were prepared by using the blade and examined by the help of microscope. Anomocytictype of stomata was abundant in lower surface and absent on upper surface. Vascular bundles of leave and pseudobulbs were collateral and closed type. In phytochemical test, the powdered samples were examimed by the method of (Trease and Evens,1989; British pharmacopoeia 1968) . Leaves and pseudobulbs were dried and powdered using the homogenizer. The preliminary phytochemical investigation revealed the presence of, α-amino acid, carbohydrates, reducing sugar, glycoside, terpenoids and steroids but tannins, alkaloid, saponin, phenolic compound, flavonoid, starch were not detected in the plant.

Keywords: Dendrobium aphyllum phytochemicals.

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[2.] Associate Professor, Department of Botany, Myeik University

Introduction

Dendrobium is one of the largest genera in orchidaceous family. The genus is comprised of about 1,100 species , and 150 species have been indentified in Thiland. The stems of several *Dendrobium* species, locally knows as 'Shi-hu', have been used in Traditional ChinessMedicine for reducing fever, nourshing the stomach and promoting the saliva production.InThiland, the dired stems of *D.draconis* have been used in blood tonic effect in the form of the tea. Recent pharmacological investigation have shown that Dendrobium plants prossess a wide variety biological activities including cytotxic, antioxidative, antimaiarial, antifibrotic,hypoglycemic activities. (www.acgpubs.org).

Dendrobium aphyllm (Roxb).C.Ficher is an orchid found in most collection. It is also known as the 'hooded-orchid' because of the cone shaped leave of the flower. It is attractive and easily cultivated and has long, pendulous stems that become lefless in the resting period and, for afew week durningthe spring. It carries numerous, pinkish violet, fragrant fiowers with a pale yellow or whitish lip.(www. phyhojournal com.)

Dendrobium species of orchidaceae family has been credited as traditional medicine of the centuries in Asia, Europe and Australia countries with more than 1,100 species (Rosa, 2010). There are records of some species of *Dendrobium* used for medicinal purposes during ancient China during 2800 B.C (Hedge & Ingalhalli,1988). Currently total of 74 species of *Dendrobium* plants found in China and about 30 species of them are used intradition or folk medicine as antipyretic, eyes remedy, imunoregulator and as anti aging agent.(www.pakbs.org.PJ).

Dendrobium pierardii (or) *aphyllum* or *cucullatum* is an orchid found in most collection. It is also know as the 'hooded orchied' because of the core shaped lip of the flower. Racemes almost inflorescences, 1-3 flowered bundled from old leaves with fallen leaves for leaves, flowers spreading, pendulous. Sepals white purplish red above or sometimes entire lilac, petals; 2-3 cm long, 9-10 mm wide.beautiful pale pink flowera, with strong violet

fraglance, on long hanging canes. Also known as *Dendrobium aphyllum* or *pieradii*.Warm growing winter blooming.Blooms in the month of March/April. The plant *Dendrobium aphyllum*(Roxb).C.Ficher , petals much broader than the sepals, lip involate almost throughout the length; stem rather thin, pendulous; leaves ovate-oblong-lanceolate,acute,rather thin, native to South East Asia; in Java cultivated as an ornamental (Backer,1968).In this research, morphological characters, microscopical features of fresh and dried powder of leaves and pseudobulbs of *Dendrobium aphyllum*(Roxb).C.Ficher were have been undertaken. The aim of the present study was to identify the plant *Dendrobium aphyllum*(Roxb).C.Ficher by using vegetative and floral parts to investigate the histological characters of leaves and pseudobulbs and to investigate and revealed the phytochemical analysis of leaves and pseudobulbs.

Materials and Methods

The plants *Dendroium aphyllum*(Roxb).C.Ficher were collected from South Dagon Township,Yangon Division during the flowering period from December2017 to February 2018. The classification and identification of species was made by using the Backer,1963. The leaves and pseudobulbs of *Dendroium aphyllum*(Roxb).C.Ficher were used to study the histological characters. The fresh specimens were prepared by using the blade . Free hand sections of specimens were studied under the microscope. The collected leaves and pseudobulbs of *Dendrobium aphyllum*(Roxb).C.Ficher were repeatedly washed with tap water and finally with pure water. The sliced samples were dried under shades for three weeks. Dried samples were powdered by the grinding machine and were stored in air tight containers for the phytochemical investigation.Phytochemical analysis concerned with the presence or absence of alkaloids, α-amino acid, carbohydrates, reducing sugar, glycoside, phenolic compound, starch, saponins, tannins, steroids, terpenoids and flavonoids were investigated by the methods of British Pharmacopoeia, 1965 and Trease and Evans, 1989. Preliminary

phytochemical examination was carried out in the Department of Botany, Yenanchaung Degree College. These results were shown in Table 1.

Result

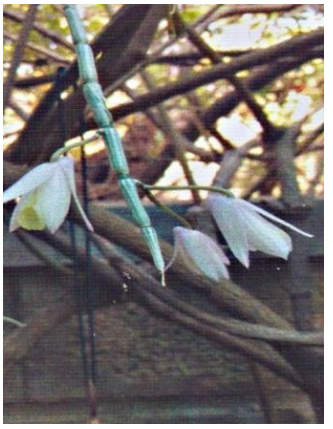
1. Morphological characters of *Dendrobium aphyllum*(Roxb). C. Fischer.

Sympodial epiphyte, ever green stem long, clubs-shaped 20-30cm long 2.00cm wide, large upwards and tapering, pear-shaped. Leave oblong lanceolate, acute about 6.00cm to 8cm long and 2.00cm to 3.00cm wide, margin entire and the tip acute both surface glabrous.Inflorescence, raceme, ovate 2.5 cm long 1.5cm wide, slightly lavenda with faintly purple tip. Labellum lateral upper portion of the pseudobubs, 2 to 3 flower per node, peduncle very short cylindrical, green, lavenda, resupincte, slightly fragrant, pedicel cylindrical, pale purple. Flower bracteate acute, dull white, glabrous, sepals 3 lavenda, oblong lanceolate acute, fused of the base, forming mentum lavenda, obtuse. Petals 3, 2 lateral petals oblong with deeply yellow in centre and purple at pointed end, finely pubescent on the upper surface, column short with stripe.Anther white, terminal, and celled, pollinia 4 in pairs. The stigmatic surface concaves, ovary inferior. Fig. 1-2.

A. Habit

B. Leaves

Figure 1. Habit and leaves of *Dendroium aphyllum* (Roxb). C. Ficher.



C. Inflorescence

D. Flower

Figure 2. Inflorescences and flower of *Dendrobium aphyllum* (Roxb). C. Ficher.

2. Histological characters of *Dendroium aphyllum*(Roxb).C.Ficher

2 (a) Microscopically characters of leaves and pseudobulbs of *Dendroium aphyllum*(Roxb).C.Ficher

Lamina

Insurface view, the epidermal cells of both surfaces were parenchymatous and thin walled .The cell walls of the upper surfaces and lower surfaces were polygonal to barrel in shape.Stomata were present on the lower surfaces and absent on the upper surface. They were anomocytic type.

In transverse sections of leaves, the cuticle layer of the upper surface and lower surface were thin. The upper epidermal cells were polygonal to barrel shape. The lower epidermal cells were similar in shape and size. The mesophyll cells were not differentiated into palisade and spongy parenchyma. The mesophyll cell consists of 8-10 layers of cells, which were oval to circular in shape and loosely arranged. They contained numerous chloroplasts.Small vascular bundles were present. The phloem cap and xylem cap were made up of three layer of sclerenchymatous cell . Each bundle was surrounded by a compact layer of thick-walled sclerenchymatous sheath, distinct from the

neighboring cells. *Dendroium aphyllum*(Roxb).C.Ficher was composed of annular and spirally thickened vessels, tracheids, fibres and xylem parenchyma. Phloem was composed of sieve-tubes, companion cells and phloem parenchyma cell Figure.(A and B).

Midrib

In surface view, the epidermal cells of both surfaces were parenchymatous and elongated along the length of the midrib. In transverse section, the epidermal cells of both surfaces were polygonal to rectangular in shape. The parenchyma cells were 4-5 layers in thickness above the vascular bundle and 2-3 layers in thickness below the vascular bundle. They were thin-walled and rounded to oval in shape.

The vascular bundle was more or less rounded in outline. Each vascular bundle was surrounded by a compact layer of sclerenchymatous cells known as the bundle sheath in layers. The cells were thick-walled, and lignified.

Vascular bundles of midrib were collateral and close type, the xylem cells are hexagonal, thick-walled, and lignified, composed of vessels, tracheids, fibers, and xylem parenchyma. The phloem cells were thin-walled and composed of sieve-tube, companion cells and phloem parenchyma cells Figure (C).

Pseudobulb

In surface view of the epidermal cells were rectangular and compactly arranged, stomata were absent.

In transverse section, the pseudobulbs were circular in shaped. Epidermal cell were round to oval in shaped and uniseriate. Ground tissue consists of large and small parenchymatous cells. Air cavities were conspicuous.

All vascular bundles were associated with sclerotic sheath. Sclerotic sheath at phloem was 2-3 layered where as xylem was single layered. Large and small

vascular rbundles were distributed in ground tissue. Vascular bundles were collateral and close type. The vessels of xylem were spiral, annular, sieve-tube, sclariform and pitted vessel Figure. (Dand E).

Diagnostic features of powdered leaves and Pseudobulbs of *Dendroium aphyllum*(Roxb).C.Ficher

Sensory characters

Light yellow green , characteristic odour .

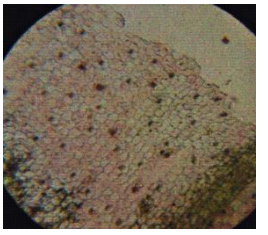
Macroscopical characters

The leaves were oblong and Lanceolate acute, margin entire and the tip acute both surface glabrous .

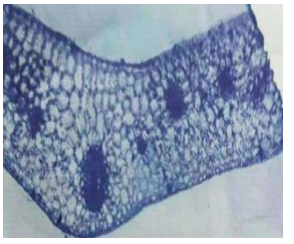
Microscopical characters

Fragments of epidermal cells ,fibres , unicellular trichome , trachied , spiral vessel , pitted vessel and sclereiform vessel were observed in powdered leaves and pseudobulbs .

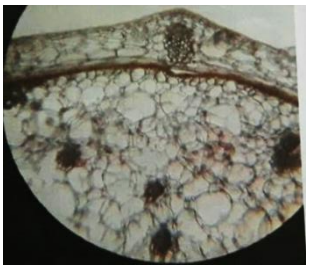
A. Upper surface of lamina



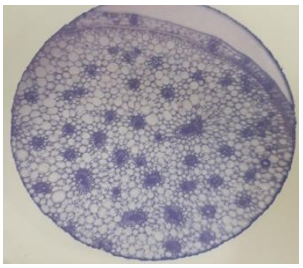
B. Lower surface of lamina



C. T.S of midrib



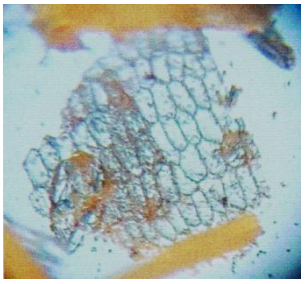
D. Pseudobulbs



E. Pseudobulbs

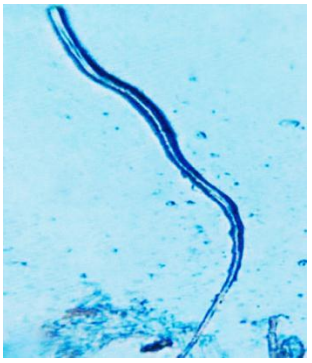
Figure 3. Internal structural of leaves and pseudobulbs of *Dendrobium aphyllum* (Roxb). C. Ficher

A. Powdered leaves

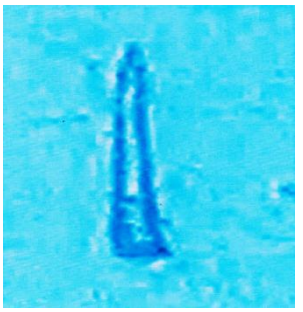


B. A fragmentof epidermal cell

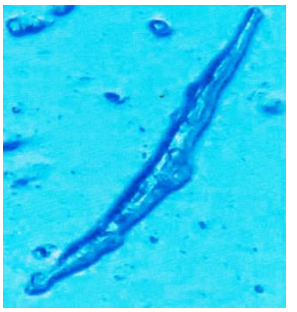
C. Fibers



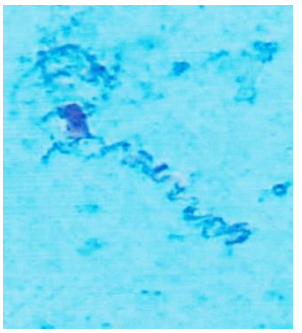
D. Fibers



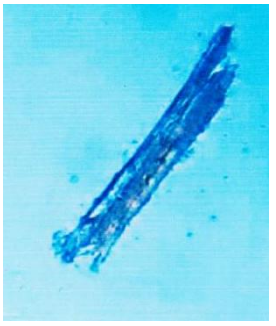
E. Unicellular uniseriatetrichome



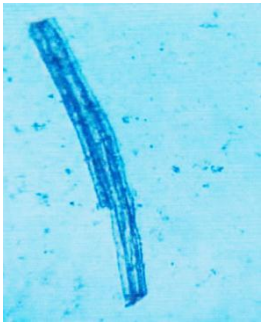
F. Tracheid



G. Spiral vessel



H. Pitted vessel



I. Sclereiform vessel

Figure 4. Microscopical structures of powdered leaves and pseudobulbs of *Dendrobium aphyllum* (Roxb). C. Ficher

3. Preliminary phytochemical investigation of leaves and pseudobulbs of *Dendroium aphyllum*(Roxb).C.Ficher

The preliminary phytochemical examination of the leaves and pseudobulbs of *Dendroium aphyllum* (Roxb).C.Ficher indicated the presence of carbohydrate, glycoside, α-amino acid, steroid, terpendoids , starch and reducing sugar. The results were shown in Table 1.

Table 1. Phytochemical constituent of leaves and pseudobulbs of *Dendroium aphyllum*(Roxb).C.Ficher

No.	Type of compound	Results	
		Leaves	Pseudobulbs
1.	Alkaloid	-	-
2.	Carbohydrates	+	+
3.	Glycoside	+	+
4.	Phenolic compound	-	-
5.	α-amino acid	+	+
6.	Saponin	-	-
7.	Tannin	-	-
8.	Flavonoid	-	-
9.	Steroid	+	+
10.	Terpenoid	+	+
11.	Reducing sugar	+	+
12.	Starch	+	+

(+) = presence (-) = absence

Discussion and Conclusion

In this research, the morphological characters , anatomical character and phytochemical study from leaves and pseudobulbs of *Dendroium aphyllum* (Roxb).C.Ficher have been carried.

Dendroium aphyllum(Roxb).C.Ficher belongs to the family orchidaceae. It is commonly known as Phayaungpan-thit-khwa in Myanmar . Sympodial epiphyte, ever green stem long, the, leaves oblong acute, margin entire and both surface glabrous; inflorescence, raceme, lateral upper portion

of the pseudobubs; flowers, peduncle very short cylindrical, green, lavenda, resupinate, slightly fragrant, pedicel cylindrical, pale purple, glabrous, sepals lavenda, oblong lanceolate acute, fused of the base, forming mentum, petals 3, 2 lateral petals oblong ovate, lavender with faintly purple tip. Labellum is deeply yellow in centre and purple at pointed and finely pubescent on the upper surface, column short, with stripe . Anther green yellow, terminal and two celled ;pollinia 4 in pairs. The stigmatic surface concaves, ovary inferior. These characters were agreement with those mentioned by Hooker, 1961; Backer, 1965; Moe Sandar Shein, 2008 and Mya Mya Than, 2008.

In these microscopical studies, the epidermal cells of both surfaces of leaves are polygonal in shaped . Anomocytic type of stomata was present on lower surface and absent on upper surface. This finding agreed with those mention by Metcalf 1960; Pandey ,2001.

Mesophyll cell is homogeneous, not differentiated into palisade and spongy parenchyma. It consists of barrel and circular to oval shaped cells with intercellular space. Assimilatory cells are rich with chloroplast. This finding agreed with those mention by Metcalf , 1960;Pandey ,2001 .

Vascular bundles are arranged in single series with layer, in midrib large vascular bundle at the center. Small and large vascular bundles on either side it. This finding agreed with those mention byMetcalf , 1960, Pandey ,2001 . The phloem cap present. Xylem comprises tracheids and fibres. Phloem consists of sieve tube elements, companion cells, fibers and parenchymatous cell. This finding agreed with those mention by Metcalf , 1960; Pandey ,2001.

The preliminary phytochemical examination of the leaves and pseudobulbs of *Dendrobium aphyllum* (Roxb).C.Ficher showed that the presence of carbohydrate, glycoside, α-amino acid, steroid, terpendoids,starch and reducing sugar. These findings in this research agreed with that mention by the literature of (British Pharmacopeia, 1965.

For this reason, plants containing secondary metabolites are very important to us as potential ingredient of herbal and many modern medicines.

In conclusion, the study focused on the morphological characters and phytochemical characters. The morphological characters were useful information in identification of the plant. Phytochemical finding in this rsearch could also be useful in medicinal plant research and production of drugs from plants.

For further research programme, pharmacological activities should be carried out concerning *Dendrobium aphyllum* (Roxb).C.Ficher , possess many medicinal values and other bioactive compounds should be isolated from plants parts.

Acknowledgement

I would like to express my sincere gratitude to Professor Dr. Tint Khine Aye , Head of Department of Botany Yenanchaung Degree collage for her kind help with very effective suggestion , advice and encouragement throughout the research work . My warm thanks are also extend to Dr. Khin Myo Thant , Associate Professor , Department of Botany , Myike University , for her kind help in this work .

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TAXONOMIC STUDY ON TEN WILD MUSHROOMS FROM LOIKAW TOWNSHIP IN KAYAH STATE

Au Au Khaing[1], Ohnmar Htwe[2] and Soe Soe Aung[3]

Abstract

The taxonomic studies on wild mushrooms from Loikaw Township, Loikaw District in Kayah State have been undertaken. The study area is located between N' 19º 14' 22" - 19º 59' 45" and E' 97º 7' 0.9" - 97º 31' 33". The wild mushrooms were collected from June to September, 2017. The 10 species of 9 genera belonging to 6 families and 3 order were collected, preserved, classified, identified and described. The collected species were identified as *Coprinus disseminatus* (Pers.) Gray, *Macrolepiota konradii* (Huijsman ex. P. D. Orton) M. M. Moser, *Amanita caesarea* (Scop.) Per., *Amanitopsis vaginata* (Bull.) Roze., *Hygrocybe ceracea* (Sowerby) P. Kumm., *Termitomyces schimperi* (Pat.) R. Heim, *Boletus pulverulentus* Opat., *Lactarius clarkeae* Cleland., *Lactarius volemus* (Fr.) Fr. and *Russula virescens* (Schaeff.) Fr. The growing habitats of *Coprinus disseminatus* (Pers.) Gray. was on the decayed woods and the others were on the soil. All species were edible. An artificial key to the studied species was constructed and presented.

Keywords: Taxonomic of wild mushrooms, Loikaw Township, Kayah State, edible.

Introduction

Mushrooms are fungi, generally considered to be lower forms of life, belonging to plant kingdom. There are about 45,000 known species of fungi and about 2000 of them are considered edible (Nair 1990). Mushrooms of one kind or another are to be found at almost every season but they occur in greatest abundance after showery weather in the months of July, August and September. Among the fungi, commonly known as mushrooms are the puffballs, club fungi, coral fungi, hedgehog fungi, truffles, trembling fungi,

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morels, stinkhorns, tube-bearing fungi and the gilled fungi or agarics. Fungi possessing no chlorophyll, must like animals, depend for their nourishment upon living or dead organic matters (Thomas 1948).

Fungi belong to the class of plants known as Cryptogams. The vegetable kingdom is divided into two great groups: one, flowering plants or phanerograms, which is characterized by forming seeds, the other, flowerless plants, cryptograms, which reproduced by spores (Ramsbottom 1923). Most fungi will grow between 0º and 35ºC, but optimum temperatures lies in the range of 20-30ºC. The ability of many fungi withstand extremely low temperatures as low as -195ºC. (Alexopoulos 1962).

Fungi are classified according to the way in which the spores are arranged. Basidiomycetes include mushrooms, toadstools, bracket fungi, fairly clubs. Basidiomycetes are characterized by having their spores borne, usually in fours, on the outside of basidia. Ascomycetes include morals, truffles, cup fungi, ergot and are characterized by having their spores usually in eights, borne within asci (Ramsbottom 1923).

The Basidiomycota contains at least 30,000 different species worldwide and includes many of our most familiar fungi. Fungi were not made of cellulose, like plants, but of chitin. They did not contain chlorophyll and could not use sunlight to convert carbon dioxide into sugars (Roberts & Evan 1950). Some basidiomycetes produce one or more other types of spore in addition to basidiospores. There are about 525 genera and 13,500 species (Smith 1979).

In Myanmar, mushrooms of Karen State was studied by Ku Yin Myint (1983). In 1987, Thida Saint presented mushrooms of Taunggyi and Kalaw areas. In 2010, Kyi Kyi Win studied on the systematic studies of mushrooms in Pyay District and phytochemical investigation of *Dictyophora indusiata* (pers) Fish. In 2014, Khin Sandi Pyone Cho presented the taxonomic study on mushrooms growing in Mandalay. In 2015, Aye Aye Maw presented taxonomic studies on wild mushrooms from Monywa District. Taxonomic studies on wild mushrooms from Southern Shan State was studied by Ohnmar Htwe (2017). Although many researchers had done the wild mushrooms flora

in Myanmar, the taxonomic studies on wild mushrooms have not been undertaken in Kayah State. Therefore, this study was carried out for this research work.

The aim and objectives of this study were to collect, classify and identify the morphological characteristics of the wild mushrooms from Loikaw Township, to study their detailed taxonomic characteristics and distribution, and to fulfill the scientific information in the compilation of the mushroom flora in Myanmar.

Materials and Methods

The study areas of wild mushrooms from Loikaw Township, Kayah State were situated between 19º 14' 22" N to 19º 59' 45" N Latitude and 97º 7' 0.9" E to 97º 31' 33" E longitude. The elevation of Loikaw is 899 meter above the sea level.

The fresh specimen of wild mushrooms were collected from Htee Se Khar Waterfall, Kyauk Taung village, Padangay village and Law Pi Ta village of Loikaw Township, Kayah State from June to September, 2017. The wild mushrooms which were growing on grassland, meadows, decomposing organic matters, hollow and rotten tree trunks were collected.

All the fresh specimens were recorded with photographs to get their actual habit and noted their fruiting characteristics. The collection, preservation and the spores print tecnique were followed by Krieger & Schaffer (1967) and Pacionic (1981). The fleshy matured specimens were selected for the preparation of spores print. The stipe was firstly removed by cutting it off as close as possible to the point of attachment of cap. It is obtained by placing a cap with the hymenium facing down on a sheet of white paper or a piece of glass-slide. A blow can then serve as a cover after a few hours, a layer of the spores was deposited. Finally real colour of the spores was determined by spores print.

The collected specimens were preserved in Formalin-Acetic acid-Alcohol (FAA) by the ratio of 5: 5: 90. Some of the dried specimens were placed in plastic bags and plastic bottles. Classification and identification of

the collected specimens were done by referring the literature; Bessey (1952), Krieger & Schaffer (1967), Coker & Couch (1969), Keizer (1998). An artificial key to the studied species was also constructed and presented. The herbarium specimens were numbered and deposited at the herbarium room of Department of Botany, University of Mandalay for the references and other scientific studies.

Results

Ten species of 9 genera belonging to 6 families and 3 orders were collected from four study areas of Loikaw Township in Kayah State. The morphological and spores characters of those species were classified and identified. The list of collected species and their comparable morphological characteristics were presented in Table 1- 2 and Figure 1-10.

Table 1. List of collected wild mushroom species from Loikow Township

Class	Sub-Class	Order	Family	No.	Scientific Name
Basidio-mycetes	Homobasidio-mycetidae	Agaricales	Agaricaceae	1.	*Coprinus disseminatus* (Pers.) Gray
				2.	*Macrolepiota konradii* (Hujjsman ex. P.D. Orton) M. M. Moser
			Amanitaceae	3.	*Amanita caesarea* (Scop.) Per.
				4.	*Amanitopsis vaginata* (Bull.) Roze.
			Hygrophoraceae	5.	*Hygrocybe ceracea* (Sowerby) P. Kumm.
			Lyophyllaceae	6.	*Termitomyces schimperi* (Pat.) R. Heim
		Boletales	Boletaceae	7.	*Boletus pulverulentus* Opat.
				8.	*Lactarius clarkeae* Cleland.
				9.	*Lactarius volemus* (Fr.) Fr.
		Russulales	Russalaceae	10.	*Russula virescens* (Schaeff.) Fr.

Table 2. Comparable morphological characteristics of wild mushrooms from Lowikaw Township

No.	Scientific Name	Growing Habitat	Edible/ Inedible	Cap			Gills / Pores	
				Colour	Shape	Umbonate	Colour	Attachment
1.	*Coprinus disseminatus* (Pers.) Gray	decay woods	edible	white to pale grey	campanulate	present	grayish-brown	free
2.	*Macrolepiota konradii* (Hujjsman ex. P.D. Orton) M. M. Moser	soil	edible	white	expanded	sligthly present	white	free
3.	*Amanita caesarea* (Scop.) Per	soil	edible	orange-red	expanded convex	absent	yellow	free
4.	*Amanitopsis vaginata* (Bull.) Roze.	soil	edible	leaden-brown	expended	absent	white	free
5.	*Hygrocybe ceracea* (Sowerby) P. Kumm.	soil	edible	pale-orange	convex	absent	pale-yellow	free
6.	*Termitomyces schimperi* (Pat.) R. Heim	soil	edible	white	convex expanded	present	white	free
7.	*Boletus pulverulentus* Opat.	soil	edible	dark-brown	convex	absent	yellow	adnate
8.	*Lactarius clarkeae* Cleland.	soil	edible	orange-brown	convex to depressed	absent	creamy-white	Adnate to decurrent
9.	*Lactarius volemus* (Fr.) Fr.	soil	edible	orange-brown	convex with depression	absent	Golden-yellow brown	decurrent
10.	*Russula virescens* (Schaeff.) Fr.	soil	edible	dull green	globose	absent	white	free

Table 2. Continued

No.	Scientific Name	Stipe				Spore			
		Shape	colour	hollow/ solid	annulus or ring	Colour	shape	texture	size (µm)
1.	*Coprinus disseminatus* (Pers.) Gray	slender	white	hollow	absent	dark-brown	elliptic	smooth	6-7.2×4.8-4.8
2.	*Macrolepiota konradii* (Huijsman ex. P.D. Orton) M. M. Moser	equal	white	hollow	present	white	ovoid	smooth apical germ pore	8.4-11.4×6-7.2
3.	*Amanita caesarea* (Scop.) Per	unequal	yellow	hollow	present	white	elliptic	smooth	6-7.2×6-6
4.	*Amanitopsis vaginata* (Bull.) Roze.	equal	white	hollow	absent	white	globose	smooth	8.4-12×6-8.4
5.	*Hygrocybe ceracea* (Sowerby) P. Kumm.	slender	white	hollow	absent	white	elliptic	smooth	8.4-10.8×4.8-6
6	*Termitomyces schimperi* (Pat.) R. Heim	unequal	white	solid	present	pink	elliptic	smooth	6-7.2×3.6-4.8
7.	*Boletus pulverulentus* Opat.	equal	reddish brown	solid	absent	olive-brown	fusiform	smooth	7.2-9.6×4.8-6
8.	*Lactarius clarkeae* Clenland.	unequal	orange-brown	solid	absent	white	globose	rough	6-8.4×6-7.2
9.	*Lactarius volemus* (Fr.) Fr.	equal	orange brown	solid	absent	white	globose	rough	7.2-7.2×7.2-7.2
10	*Russula virescens* (Schaeff.) Fr.	unequal	white	solid	absent	white	globose	smooth	6-7.2×4.8-6

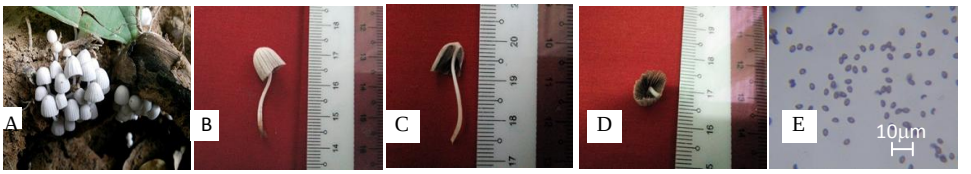

Figure 1. *Coprinus disseminatus* (Pers.) Gray. (A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

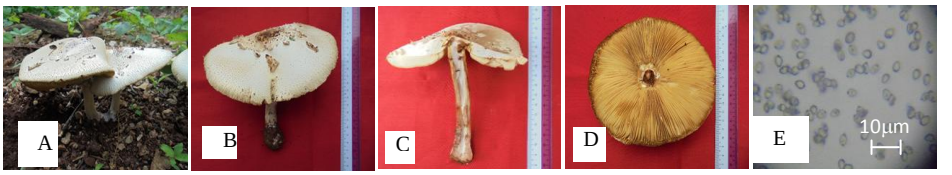

Figure 2. *Macrolepiota konradii* (Hujjsman ex. P.D. Orton) M. M. Moser (A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

Figure 3. *Amanita caesarea* (Scop.) Per. (A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

Figure 4. *Amanitopsis vaginata* (Bull.) Roze. (A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

Figure 5. *Hygrocybe ceracea* (Sowerby) P. Kumm. (A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

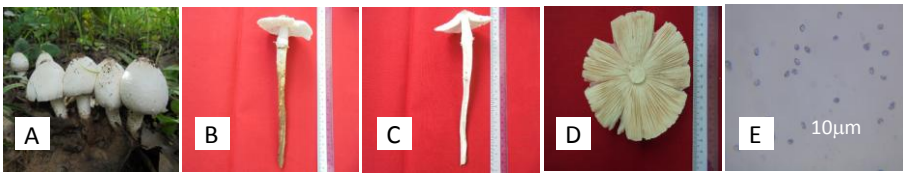

Figure 6. *Termitomyces schimperi* (Pat.) R. Heim. (A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

Figure 7. *Boletus pulverulentus* Opat. (A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

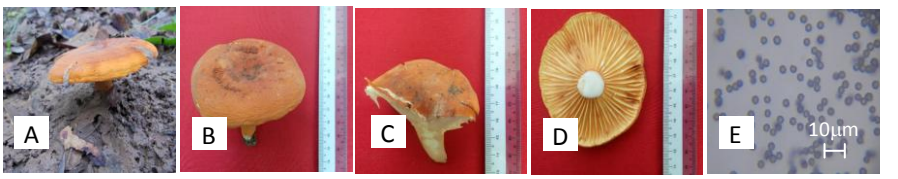

Figure 8. *Lactarius clarkeae* Cleland. (A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

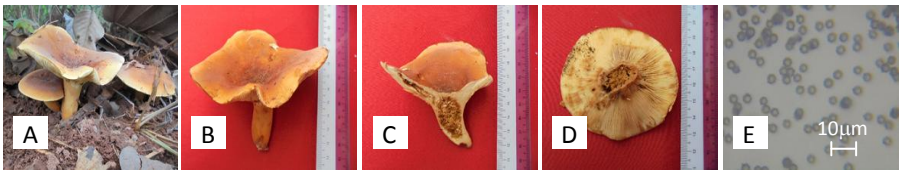

Figure 9. *Lactarius volemus* (Fr.) Fr. (A. Growing habitat, B. Fruiting body ilateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

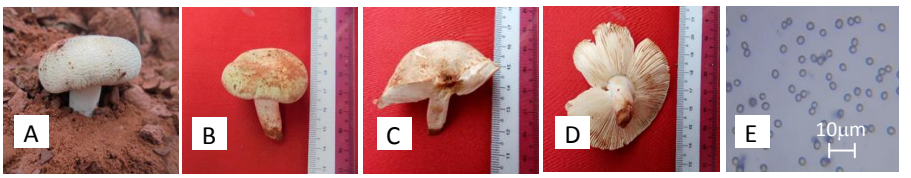

Figure 10. *Russula virescens* (Schaeff.) Fr. (A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

An Artificial Key to the Studied Species

1. Stipe hollow ---------- 2
1. Stipe solid ---------- 6
   2. Umbo present ---------- 3
   2. Umbo absent ---------- 4
3. Cap campanulate; gills white to pale grey ----1. *Coprinus disseminates*
3 Cap expended; gills white ----------9. *Macrolepiota konradii*
   4. Ring present; stipe yellow---------- 3. *Amanita caesarea*
   4. Ring absent; stipe white ---------- 5
5. Spore globose; cap leaden brown ---------- 4. *Amanitopsis vaginata*
5. Spore elliptic; cap pale orange ---------- 5. *Hygrocybe ceracea*
   6. Ring present; spore elliptic ----------6. *Termitomyces schimperi*
   6. Ring absent; spore fusiform and globose ----------7
7. Cap orange brown; spore rough ----------8
7. Cap dark-brown and dull green; spore smooth ----------9
   8 Stipe unequal; gills creamy white ----------8. *Lactarius clarkeae*
   8. Stipe equal; gills golden yellow brown --------4. *Lactarius volemus*
9. Cap convex; gills yellow, adnate ----------7. *Boletus pulverulentus*
9. Cap globose; gills white, free ----------10. *Russula virescens*

Discussion and Conclusion

In the present study, the taxonomic studies on ten species of wild mushrooms from Loikaw Township in Kayah State were undertaken. The fresh specimen of wild mushrooms were collected from Htee Se Khar Waterfall, Kyauk Taung village, Padangay village and Law Pi Ta village, Loikaw Township from June to September, 2017.

Among them, 9 species were gill mushrooms type and 1 species, *Boletus pulverulentus* Opat. was pore mushrooms type. In Loikaw Township, the 4 species collected from Htee Se Khar Waterfall were *Coprinus disseminatus* (Pers.) Gray, *Amanita caesarea* (Scop.) Pers., *Lactarius volemus* (Fr.) Fr. and *Russula virescens* (Schae. ff.). One species, *Amanitopsis vaginata* (Bull.) Roze. was collected from Kyauk Taung village. *Hygrocybe ceracea* (Sowerby) P. Kumm, *Termitomyces schimperi* (Pat.), R. Heim, *Boletus pulverulentus* Opat. and *Lactarius clakeae* Cleland. were collected from Padangay village. *Macrolepiota konradii* (Hujjsman ex. P. D. Orton) M. M. Moser was collected in Law Pi Ta village.

The growing habits of the fruiting bodies vary in the studied species. The 4 species such as *Amanita caesarea* (Scop.) Pers., *Amanitopsis vaginata* (Bull.) Roze., *Lactarius clakeae* Cleland. and *Russula virescens* (Schaeff.) Fr. were growing as the single fruiting bodies. The 6 species such as *Coprinus disseminatus* (Pers.) Gray, *Macrolepiota konradii* (Hujjsman ex. P. D. Orton) M. M. Moser, *Hygrocybe ceracea* (Sowerby) P. Kumm., *Termitomyces schimperi* (Pat.) R. Heim, *Boletus pulverulentus* Opat. *Lactarius volemus* (Fr.) Fr. were growing as the aggregated fruiting bodies. The present findings were agreed with the solitary and group growing habits which mentioned by Phillips (2006).

The 9 species such as *Macrolepiota konradii* (Hujjsman ex P. D. Orton) M. M. Moser, *Amanita caesarea* (Scop.) Per, *Amanitopsis vaginata* (Bull.) Roze, *Hygrocybe ceracea* (Sowerby) P. Kumm., *Termitomyces schimperi* (Pat.) R. Heim, *Boletus pulverulentus* Opat., *Lactarius clarkeae* Cleland., *Lactarius volemus* (Fr.) Fr. and *Russula virescens* (Schaeff.) Fr. were growing

on the soil. One species, *Coprinus disseminatus* (Pers.) was growing on the decayed woods. These findings were agreed with Koon (1990).

The 3 wild mushrooms species, *Coprinus disseminatus* (Pers.) Graw, *Macrolepiota konradii* (Hujjsman Ex. P. D. Orton) M. M. Moser, and *Termitomyces schimperi* (Pat.) R. Heim, were present the umbo on the cap. The others 7 species, *Amanita caesarea* (Scop.) Pers, *Amanitopsis vaginata* (Bull.) Roze., *Hygrocybe ceracea* (Sowerby) P. Kumm., *Boletus pulverulentus* Opat., *Lactarius clakeae* Cleland, *Lactarius volemus* (Fr.) Fr. and *Russula virescens* (Schae. ff.) Fr. were absent umbo on the cap.

Various cap shapes were also observed in this study areas. These were campanulate in *Coprinus disseminatus* (Pers.) Gray; ovate and expanded in *Macrolipiota konrandii* (Hujjsman ex. P. D. Orton) M. M. Moser.; expanded convex in *Amanita caesarea* (Scop.) Per.; bell-shaped to expaneded in *Amanitopsis vaginata* (Bull.) Roze.; convex expanded in *Termitomyces schimperi* (Pat.) R. Heim; convex in *Hygrocybe ceracea* (Sowerby) P. Kumm. and *Boletus pulverulentus* Opat., convex to centre depressed in *Lactarius clarkeae Cleland*, and convex with depression in *Lactarius volemus* (Fr.) Fr. and globose to convex in *Russula virescens* (Schaeff.) Fr. These findings were agreed with Largent (1973).

Gills were free in *Coprinus disseminatus* (Pers.), Gray, *Macrolepiota konradii* (Hujjsman ex. P. D. Orton) M. M. Moser, *Amanita caesarea* (Scop.) Pers., *Amanitopsis vaginata* (Bull.) Roze., *Hygrocybe ceracea* (Sowerby) P. Kumm., *Termitomyces schimperi* (Pat.) R. Heim. and *Russula virescens* (Schaeff.) Fr. The pores of *Boletus pulverulentus* Opat. were adnate and the gills of *Lactarius clarkeae* Cleland. were adnate to decurrent and *Lactarius volemus* (Fr.) were decurrent. These findings were agreed with Phillips (2006).

The stipe shapes were equal in *Macrolepiota konradii* (Hujjsman ex. P. D. Orton) M. M. Moser, *Amanitopsis vaginata* (Bull.) Roze, *Boletus purverulentus* Opat. and *Lactarius volemus* (Fr.) Fr. The stipe shapes of *Termitomyces schimperi* (Pat.) R. Heim., *Amanita caesarea* (Scop.) Pers.,

Lactarius clarkeae Cleland, *Russula virescens* (Schaeff.) Fr. were unequal. *Coprinus disseminatus* (Pers.) Gray, and *Hygrocybe ceracea* (Sowerby). P. Kumm were slender. The hollow stipes were observed in *Coprinus disseminatus* (Pers.) Gray, in *Macrolepiota konradii* (Hujjsman ex. P. D. Orton) M. M. Moser, *Amanita caesarea* (Scop.) Pers., *Amanitopsis vaginata* (Bull.) Roze. and *Hygrocybe ceracea* (Sowerby) P. Kumm. The remaining 5 species were solid stipes.

The spores colour were dark-brown in *Coprinus disseminatus* (Pers.) Gray, pink in *Termitomyces schimperi* (Pat.) R. Heim., olive brown in *Boletus pulverulents* Opat. and white in *Macrolepiota konradii* (Hujjsman ex. P. D. orton) M. M. Moser, *Amanita caesarea* (Scop.) Pers, *Amanitopsis vaginata* (Bull.) Roze., *Hygrocybe ceracea* (Sowerby) P. Kumm., *Lactarius clakeae* Cleland, *Lactarius volemus* (Fr.) Fr. and *Russula virescens* (Schae. ff.) Fr. These findings were agreed with Moore and Sullivan (2014). All of the studied species were edible. These findings were agreed with Groves (1979).

Some wild mushroom species from this Loikaw areas were also found in Karen State, Mon State, Southern Shan State, Mandalay area, Taungyi and Kalaw areas, Pyay District and Monywa Distinct. These are *Coprinus disseminatus* (Pers.) Gray, *Amanita caesarea* (Scop.) Per., *Lactarius volemus* (Fr.) Fr., *Russula virescencs* (Schaeff.) Fr. in Karen State (Ku Yin Myint 1983); *Coprinus disseminates* (Pers.) Gray. and *Amanitopsis vaginata* (Bull.) Roze. in Thaton District, Mon State (Thandar Soe 2013); *Coprinus disseminatus* (Pres.) Gray, *Aminita caesarea* (Scop.) Per., *Amanitopsis vaginata* (Bull.) Roze., *Lactarius volemus* (Fr.) Fr. and *Russula virescens* (Schaeff.) Fr. in Southern Shan State (Ohnmar Htwe 2017); *Coprinus disseminatus* (Pers.) Gray, *Amanita caesarea* (Scop.) Per., *Amanitopsis vaginata* (Bull.) Roze., *Termitomyces schimperi* (Pat.) R. Heim, *Lactarius volemus* (Fr.) Fr., *Russula virescens* (Schaeff.) Fr. in Taungyi and Kalaw areas (Thida Saint 1987); *Termitomyces schimperi* (Pat.) R. Heim. in Mandalay (Khin Sandy Phyone Cho 2014); *Coprinus disseminatus* (Pers.) Gray, *Amanita caesarea* (Scop.) Per., *Amanitopsis vaginata* (Bull.) Roze., *Termitomyces schimperi* (Pat.) R. Heim, in Pyay District (Kyi Kyi Win 2010) and *Coprinus*

disseminatus (Pers.) Gray, *Amanita caesarea* (Scop.) Per., *Termitomyces schimperi* (Pat.) R. Heim. and *Lactarius volemus* (Fr.) Fr. in Monywa District (Aye Aye Maw 2015).

Therefore, it would be concluded that the present study was one of the systematic records of wild mushrooms to be used by researchers in various fields of studies. This study will be provided the partial fulfillment of the information on the wild mushrooms distribution in Loikaw Township, in Kayah State and will be beneficial to accomplish the mushroom flora in Myanmar.

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MORPHOLOGICAL AND ANATOMICAL CHARACTERISTICS OF *CAMELLIA SINENSIS* (L.) KUNTZE FROM TWO DIFFERENT LOCALITIES

Yi Yi Naing[1], Win Win Khaing[2], Thein Kywe[3]

Abstract

Morphological and anatomical characteristics of *Camellia sinensis* (L.) Kuntze belonging to family Theaceae were studied at Department of Botany, University of Mandalay. The specimens were collected from Naung Cho Township of Northern Shan State and Mogok Township of Mandalay Region from June to October, 2017. The morphological and anatomical characteristics of leaves, stems and roots were studied, described, discussed and their photographs and photomicrographs were also presented. In morphological characteristics, the sizes of leaves, petioles and the numbers of petals were showed variable from one locality to another. The anatomical characteristics showed the differences in thickness of cell layers, the numbers and sizes of vascular bundles of leaves, stems and roots. The sclereids were observed in the ground tissues of petioles and midribs. The vascular bundles of stems were collateral type and roots were radial type and polyarch. The morphological and anatomical characteristics are useful in species confirmation and certain identification.

Keywords: *Camellia sinensis* (L.) Kuntze, Morphology, Anatomy.

Introduction

The family Theaceae comprises 19 genera and 600 species. The only genus economically important in the family is *Camellia* which includes about 120 species. These species were found in Cambodia, China, North East India, Indonesia, Southern Japan, South Korea, Laos, Malaysia, Myanmar, Nepal, Philippines, Thailand, Vietnam (Wu *et al.* 2007).

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In Myanmar, tea production is throught to have originated from the Palaung people on the Shan Plateau, parts of which are more than 6000 feet above sea level (So Pyay Thar 2016). Tea plant is grown in the Northern Shan State, Southern Shan State and Sagaing Region. Myanmar tea differs in quality according to the cultivation and processing (Kyaw Kyaw Sann 2009).Laphet, tea is a popular and important in Myanmar culture (Pyie Phyo Maung *et al*. 2012).The chemical components of green tea chiefly include polyphenols, caffeine and amino acids. Tea also contains flavonoids and has anti-oxidant properties. Tea flavonoids reduce inflammation, which have antimicrobial effects and prevent tooth decay (Tariq *et al.* 2013).

The systematic study on *Camellia sinensis* (L.) Kuntze and their detailed anatomical structure were performed in the present research work.The main objectives of this research are to study the morphological and anatomical characteristics of leaves, stems and roots of *Camellia sinensis* (L.) Kuntze from two different localities and to provide the specific information of morphological and anatomical characteristics for certain identification.

Materials and Methods

Camellia sinensis (L.) Kuntze belonging to family Theaceae were collected from Naung Cho Township, Northern Shan State and Mogok Township, Mandalay Region, from June to October 2017. The collected specimens were studied and identified in the Department of Botany, University of Mandalay with the help of literatures (Hooker 1875, Backer 1946 and Dassanayake 1996).

For anatomical study, the small portions of the specimens were cut into the 15-25 μm thick sections by using a rotary microtome. The dehydration, infiltration, embedding, staining and mounting were made according to Johansen's method (1940).

Results

1. Morphological Studies

1.1 *Camellia sinensis* (L.) Kuntze. Trudy Im. S. 10: 195. 1887.

Family	:	Theaceae
Scientific Name	:	*Camellia sinensis* (L.) Kuntze.
Common name	:	Tea
Myanmar name	:	Laphet
Flowering period	:	August to November

1.1 Morphological characters of *Camellia sinensis* (L.) Kuntze from two different localities (Figure 1)

Perennial shrubs or small trees, 3 - 5 m in high from Naung Cho and 5-8 m in high from Mogok. Stems terete, stout, leaf scar presents. Leaves

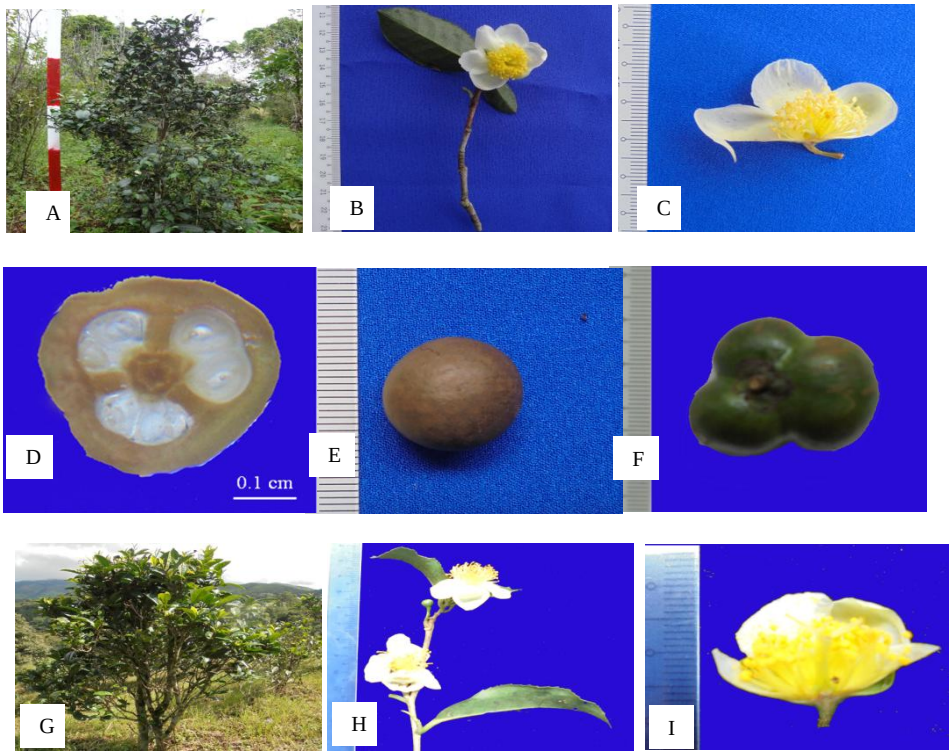

Figure 1. Morphological characters of *Camellia sinensis* (L.) Kuntze from two different localities
A. Habit, **B.** Inflorescence, **C.** L.S of flower from Naung Cho
D. T.S of ovary, **E.** Fruit, **F.** Seed from Naung Cho
G. Habit, **H.** Inflorescence, **I.** L.S of flower from Mogok
J. T.S of ovary, **K.** Fruit, **L.** Seed from Mogok

simple, alternate, exstipulate, petioles 0.3 - 0.8 cm by 0.2 - 0.3 cm from Naung Cho and 0.2 - 0.8 by 0.2 - 0.3 cm from Mogok; leaf blades ovate or oblong elliptic, dark green; glabrous on both surfaces; serrate along the margin; cuneate at the base; acuminate at the apex. Inflorescences axillary cluster of cyme; 1 - 3 flowered from two different localties; peduncles short, dark green and glabrous. Flowers bisexual, actinomorphic, hypogynous, creamy white or pale yellow, showy, fragrant; pedicels short, dark green, glabrous; bracts small, caduceous. Sepals 5, concave, dark green, glabrous on both surfaces, persistent. Petals 5 - 7 from Naung Cho and 6 - 7 from Mogok, obovate, free in two whorls. Stamens numerous, free, filament filiform; anthers dithecous, dorsifixed. Carpels 3, fused; ovary superior, 1 or 2 ovules in each locule on the axile placentae, silky tomentose; styles stout, trilocular or sometimes bilocular, 1 or 2 ovules in each locule on the axile placentae; styles stout, glabrous; stigmas trifid. Capsules woody, subglobose. Seeds rounded or plano-convex, pale brown, nonendospermic.

2. Anatomical Studies

2.1 Internal structure of the leaves of *Camellia sinensis* (L.) Kuntze from two different localities (Figure 2 and 3)

Petiole

In transverse section, the petioles of *Camellia sinensis* (L.) Kuntze studied were shield-shaped in outline, with two prominent wing. Distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System: Composed of epidermal cells. In transverse section, epidermis 1 - layered on both surfaces, the cell barrel in shape, compact, outer and inner walls convex, anticlinal walls straight.

Ground Tissue System: Composed of collenchymatous and parenchy-matous tissues. Collenchymatous tissue below the adaxial epidermis, 2 to 5 - layered from Naung Cho and 2 to 6 - layered from Mogok, the cells oval or rounded in shape; collenchymatous tissue above the abaxial epidermis, 1 to 4 - layered from Naung Cho and 2 to 6 - layered from Mogok; parenchymatous cells above the vascular bundle, 9 to 15 - layered from Naung Cho and 9 to 26 - layered from Mogok, the cells oval or rounded in shape; parenchymatous cells below the vascular bundle, 4 to 15 - layered from Naung Cho and 9 to 15 - layered from Mogok, the cells oval in shape.

Vascular Tissue System: A large vascular bundle embedded in the ground tissue, crescent-shaped in outline, collateral type, xylem on the adaxial side and phloem on the abaxial side; phloem 6 to 10 - layered from Naung Cho and 5 to 8 - layered from Mogok. Phloem composed of sieve tubes element,

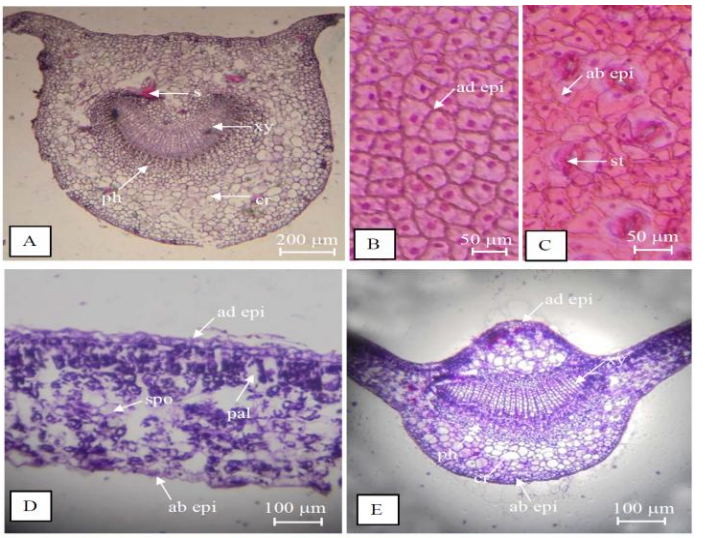

Figure 2. Internal structure of leaf of *Camellia sinensis* (L.) Kuntze from Naung Cho Township

A. T.S of petiole. **B.** Adaxial surface view of lamina. **C.** Abaxial surface view of lamina. **D.** T.S of lamina. **E.** T.S of midrib.
(ab epi = abaxial epidermal cell, ad epi = adaxial epidermal cell, cr = cortex, pal = palisade parenchyma cell, ph = phloem, s = sclereid, spo = spongy parenchyma cell, st = stoma, xy = xylem)

companion cells, fibers and phloem parenchyma. Xylem 6 to 10 - layered from Naung Cho and 4 to 8 - layered from Mogok. Xylem composed of vessel elements, tracheids, fibers and xylem parenchyma.

2.3 Internal structure of the stems of *Camellia sinensis* (L.) Kuntze from two different localities (Figure 4 and 5)

In transverse section, the stems of *Camellia sinensis* (L.) Kuntze were circular in outline, 1925.0 - 3500.0 µm in tangential diameter, 1825.0 - 2850.0 µm in radial diameter from Naung Cho and 2200.0 - 2450.0 µm in tangential diameter, 2175.0 - 2425.0 µm in radial diameter from Mogok.

Dermal Tissue System: In transverse section, epidermis 1 - layered, the cells oval to barrel in shape, 7.5 - 15.0 µm in length and 7.5 - 17.5 µm in width from Naung Cho and 12.5 - 22.5 µm in length and 7.5 - 17.5 µm in

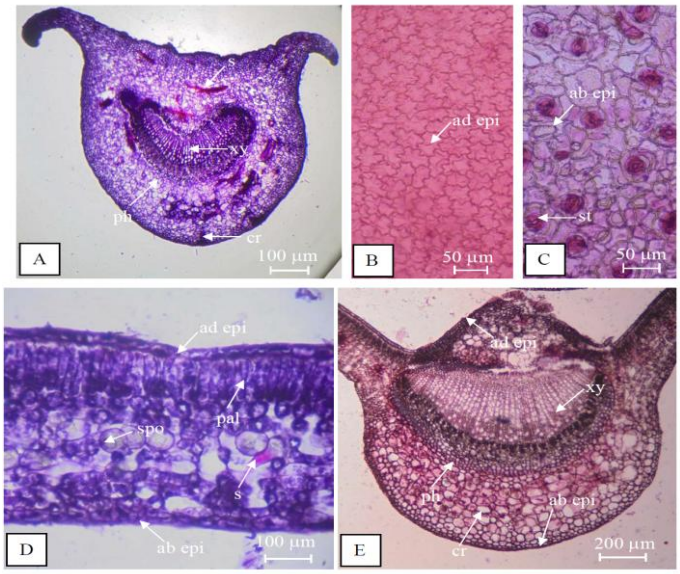

Figure 3. Internal structure of leaf of *Camellia sinensis* (L.) Kuntze from Mogok Township

A. T.S of petiole. **B.** Adaxial surface view of lamina. **C.** Abaxial surface view of lamina. **D.** T.S of lamina. **E.** T.S of midrib. **F.** Vessel element. **G.** Tracheid. **H.** Fiber.
(ab epi = abaxial epidermal cell, ad epi = adaxial epidermal cell, cr = cortex, pal = palisade parenchyma cell, ph = phloem, s = sclereid, spo = spongy parenchyma cell, st = stoma, xy = xylem)

width from Mogok, outer and inner walls convex, anticlinal walls straight.

Ground Tissue System: Composed of cortex, endodermis, pericycle and pith. The cortex differentiated into outer collenchymatous tissue and inner parenchymatous tissue. The outer collenchymatous tissues composed of 2 to 6 - layered from two different localities. The inner parenchymatous tissues composed of 4 to 9 - layered from two different localities, intercellular space present. Endodermis and pericycle were indistinct. Pith composed of

parenchymatous cell, thin - walled, the cells oval or rounded or polygonal in shape, thin-walled, intercellular space present.

Vascular Tissue System: Vascular bundles embedded in the ground tissue, the bundles arranged in a continuous circular ring, collateral type, 175.0 - 222.5 µm in thick from Naung Cho and 120.0 - 400.0 µm in thick from Mogok; phloem outer and xylem inner. Phloem composed of sieve tubes elements, companion cells, fibers and phloem parenchyma. Xylem arranged in radial rows, 2 to 7 - layered from Naung Cho and 5 to 10 - layered from Mogok, the layers 25.0 - 150.0 µm thick from Naung Cho and 82.5 - 170.0 µm thick from Mogok, the cells rounded or polygonal in shape, 10.0 - 35.0 µm in length, 7.5 - 27.5 µm in width from Naung Cho and 10.0 - 25.0 µm in length 12.5 - 30.0 µm in width from Mogok. Xylem composed of vessel elements, tracheids, fibers and xylem parenchyma.

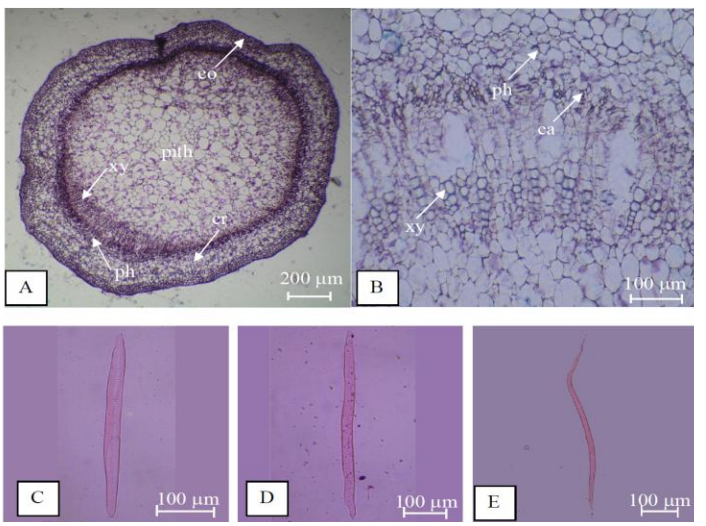

Figure 4. Internal structure of stem of *Camellia sinensis* (L.) Kuntze from Naung Cho Township
A. T.S of stem showing an outline **B.** Close up view of vascular bundle **C.** Vessel element **D.** Tracheid **E.** Fiber
(ca = cambium, co = collenchyma cell, cr = cortex, epi = epidermis, ph = phloem, xy = xylem)

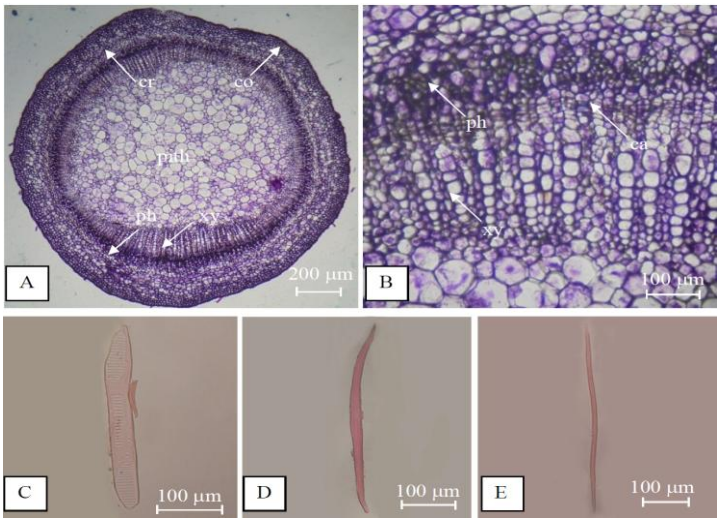

Figure 5. Internal structure of stem of *Camellia sinensis* (L.) Kuntze from Mogok Township
A. T.S of stem showing an outline **B.** Close up view of vascular bundle **C.** Vessel element **D.** Tracheid **E.** Fiber
(ca = cambium, co = collenchyma cell, cr = cortex, ph = phloem, xy = xylem)

2.5 Internal structure of the roots of *Camellia sinensis* (L.) Kuntze from two different localities (Figure 6)

In transverse section, the roots of *Camellia sinensis* (L.) Kuntze were circular in outline, 2575.0 - 3000.0 µm in tangential diameter, 2550.0 -3375.0 µm in radial diameter from Naung Cho and 2000.0 - 3300.0 µm in tangential diameter, 2125.0 -3250.0 µm in radial diameter from Mogok.

Dermal Tissue system: The epiblema is crushed and the initiation of periderm is formed.

Ground Tissue System: Composed of cortex, endodermis, pericycle and pith. Cortex composed of homogenous parenchyma cell, 5 to 7- layered, the cells barrel or polygonal in shape from two different localities. Endodermis and pericycle indistinct. Pith absent.

Vascular Tissue System: Vascular bundles radial type, polyarch, rays cells occur between the xylem and phloem cells. Phloem distributed at the periphery of the xylem. Phloem composed of sieve tubes elements, companion cells, fibers and phloem parenchyma. Xylem arranged as a continuous cylinder, 375.0 -2250.0 µm thick from Naung Cho and 850.0 -1825.0 µm thick from Mogok, the cells polygonal or rounded in shape. Xylem composed of vessel elements, tracheids, fibers and xylem parenchyma.

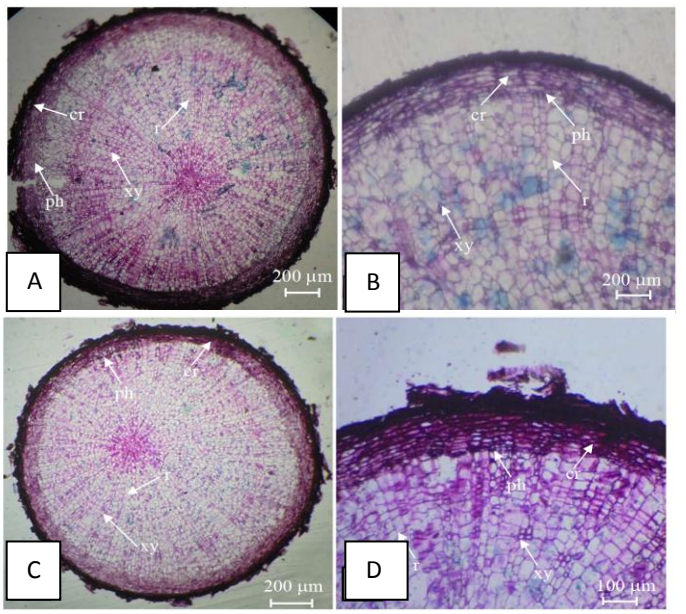

Figure 6. Internal structure of root of *Camellia sinensis* (L.) Kuntze from two different localities
A. T.S of root, **B.** Portion of T.S of root from Naung Cho
C. T.S of root , **D.** Portion of T.S of root from Mogok
(cr = cortex, en = endodermis, pe = pericycle, ph = phloem, r = ray cell, xy = xylem)

Discussion and Conclusion

The morphological and anatomical characteristics of *Camellia sinensis* (L.) Kuntze from Naung Cho Township, Northern Shan State and Mogok Township, Mandalay Region were studied. The plants were shrubs or small

trees. The leaves were simple, alternate, margin serrate and exstipulate from the two different localities. These characters were agreed with Wu *et al*. (2007). The sizes of leaves were variable from one locality to another. The sizes of leaves from Naung Cho were smaller than the leaves of Mogok. The sizes of petioles were also slightly different from one another.

Inflorescences were axillary cluster of cymes. These characters were agreed with Yun-fei & Nian-he (2007) and Wu *et al*. (2007). The sizes of flowers were slightly different. Sepals were 5, concave, dark green, glabrous on both surfaces, persistent. These characters were agreed with Backer (1934). Petals obovate, free in two whorls, the outer whorls smaller than the inner, green patches present at the tip of outer petals, glabrous. These characters were agreed with Wu *et al*. (2007). The number of petals were 5 - 7 from Naung Cho and 6 - 7 from Mogok. Fruits were woody, subglobose and seeds rounded or plano-convex from the two different localities. These characters were agreed with Dassanayake (1996) and Mahmood *et al*. (2010).

In anatomical characteristics, the transverse section of petioles, laminae, midribs, stems and roots were composed of dermal tissue system, ground tissue system and vascular tissue system. The transverse sections of petioles were observed in shield-shaped from two different localities. The size of cell, the number and thickness of cell layers were differed from one another. The sclereids were found in collenchymatous and parenchymatous cell of petioles from the two different localities and more abundance in Mogok Township. The vascular bundles were collateral type and crescent shaped. These characters were agreed with Metcalf and Chalk (1950).

In surface view of laminae, dermal tissues composed of 1 - layered of epidermal cells and cuticle thin on both surfaces. Anomocytic types of stomata present on abaxial surface of lamina from two different localities, and these characters were agreed with Metacalf and Chalk (1950) and Duarte (2006).

In transverse sections of laminae, the thickness was different from one locality to another. The number of palisade and spongy mesophyll layers were

differed from each other, 1- or 2 - layered from Mogok Township and 1 - layered from Naung Cho Township. These characters were agreed with Metcalf & Chalk (1950) and Kalra *et al.* (2013).

The transverse sections of midribs were observed semicircular-shaped in outline. The numbers, sizes of cells and thickness of cell layers were different from one locality to another. The sclereids were found in the cortex of midribs and stem. The vascular bundle was crescent-shaped from two different localities. These characters were agreed with the Metcalf & Chalk (1950) and Kalara *et al.* (2013).

In transverse section, the stems were observed circular-shaped in outline from two localities. The size of stems, the number and thickness of cell layers were slightly differed from one locality to another. Endodermis and pericycle were indistinct. Piths were cellular large, thin walled parenchymatous cell from two different localities. The vascular bundles of stems were collateral type, phloem outward and xylem inward these characters were agreed with Metcalf & Chalk (1950).

The transverse sections of roots were circular-shaped in outline from two different localities. The size of roots were slightly different from one locality to another. The sizes, the numbers and thickness of cell layers were different from one locality to another. The cortex composed of homogenous parenchyma cells. Vascular bundles were radial type and polyarch from two different localities. These characters were agreed with Metcalf & Chalk (1950).

In conclusion, the morphological and anatomical characteristics of *Camellia sinensis* (L.) Kuntze from two different localities were similar in structure. The size of leaves and flowers, number and size of petals were different from one locality to another. The size of cells, the number and thickness of cells layers were also different from one locality to another. It is hope that these finding will be useful in the identification and classification of the species. In addition, tea and tea leaf were popular and economic important

in Myanmar and this research will provide the requirements of valuable information on *Camellia sinensis* (L.) Kuntze in Myanmar.

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MORPHOLOGICAL AND ANATOMICAL CHARACTERISTICS OF *COCCINIA GRANDIS* (L.) VOIGT. AND *CUCUMIS TRIGONUS* ROXB. IN MANDALAY REGION

Theingi Kyaw[1] and Yin Aye[2]

Abstract

Morphological and anatomical characteristics of *Coccinia grandis* (L.) Voigt. and *Cucumis trigonus* Roxb. belonging to the family Cucurbitaceae were studied at Department of Botany, University of Mandalay. The specimens were collected from Chanmyathazi and Ngazun Townships of Mandalay Region from June to October, 2017. The morphological characteristics of the two species were annual, climbing or creeping herbs. Leaves were simple, alternate and exstipulate. Inflorescences were axillary or solitary cymes with unisexual, actinomorphic and epigynous flowers. In the anatomical characteristics, the anomocytic types of stomata present on both surfaces of laminae of the two species. The vascular bundles of the two species were bicollateral type in petioles, midribs and stems. The vascular bundles of the roots were radial type, and tetrarch in *Coccinia grandis* (L.) Voigt., and polyarchin *Cucumis trigonus* Roxb. The morphological and anatomical characteristics of the two species are useful in identification of the plant.

Keywords: *Coccinia grandis* (L.) Voigt., *Cucumis trigonus* Roxb., Morphology, Anatomy.

Introduction

The family Cucrubitaceae belonging to the order Cucurbitales, class Magnoliopsida. The family Cucurbitaceae is one of the most important in the Angiosperm taxa (Gabbar 2015). Cucurbitaceae family consists of two subfamilies and eight tribes. Two subfamilies are Cucurbitoideae and Zanonioideae. These tribes are Benincaseae, Cucurbiteae, Joliffieae, Melothrieae, Schizopeponeae, Sicyeae, Trichosantheae and Zanonieae

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(Heywood *et al.* 2007). In Myanmar, 26 genera and 69 species of Cucurbitaceae were recorded by Kress *et al*. (2003).

Coccinia grandis (L.) Voigt. is a dioecious plant. Fruit is a smooth, bright red, ovoid to ellipsoid berry 2.5 – 6 cm (Muniappan *et al*. 2009). All the parts of the plant used for liver diseases, diabetes mellitus, antimicrobial, asthma, ulcer, urinary tract diseases, allergy, bronchitis (Moideen *et al*. 2011).

Cucumis trigonus Roxb. is commonly known as bitter gourd. The fruit is shown to possess various activities such as antidiabetic activity, hepatoprotective activity, cardioprotective activity. The fruit pulpis bitter, acrid, thermogenic, anthelmintic, liver tonic, cardio tonic, appetizer, expectorant and intellect promoting (Subarayan & Thangaraj 2014).

The morphology and anatomy of the three species of Cucurbitaceae family was studied Kyaw San (1995). Mya Mya (1997) also reported a comparative morphology and anatomical study on two species of *Luffa*. Nwe Ni Tin (2009) described Taxonomy and pollen morphology of the Cucurbitaceae, Begoniaceae and Passifloraceae from Upper Myanmar. Some workers have described the morphological and anatomical characteristics of some members of Cucurbitaceae in Myanmar. However, the anatomical characteristics of *Coccinia grandis* (L.) Voigt., *Cucumis trigonus* Roxb. has not been done in Mandalay Region. Therefore, this study was performed for this research work.

The main objective of this study is to describe the morphological and anatomical characteristics of the two species, to compare the anatomical characters among the members of tribe Benincaseae and to get anatomical information that can be fulfill the need of systematic studies on the tribe Benincaseae.

Materials and Methods

The specimens of *Coccinia grandis* (L.) Voigt. were collected from Aung Pin Le' village (21° 57' 00.42" N and 96° 7' 48.03" E), and *Cucumis trigonus* Roxb. were collected from Nawaratt village (21° 41' 21.39" N and 96° 42' 46.57" E), Ngazun Township, Mandalay Region from June to October, 2017. The collected specimens were studied and identified by using the literature of Dessanayake (1999) and Nian- he (2007), at the Department of Botany, University of Mandalay.

For anatomical study, the small portions of the specimens were cut into the 15-25 μm thick sections by using a rotary microtome. The dehydration, infiltration, embedding, staining and mounting were made according to Johansen's method (1940).

Results

1. Taxonomical Studies

1.1 *Coccinia grandis* (L.) Voigt. Hort. Suburb. Calcutt. 59. 1845. (Figure 1)

Bryonia grandis L., Mant. 1:126. 1767.

Coccinia indica Wight & Arn, Prodr. Fl. Penins. Ind. Or. 1: 347. 1834; FKH 112. 1912.

Family : Cucurbitaceae

Scientific name : *Coccinia grandis* (L.) Voigt.

Local name : Kin-mon

Perennial, dioecious, climbing herb, stem and branch 5-angular or slender, solid, green, glabrous; tendril simple, about 1.5 cm long, green, pubescent. Leaves simple, alternate, exstipulate, blades broadly ovate or cordate, glabrous on both surfaces, gland dotted; cordate at the base, margin

entire or denticulate along the margin, obtuse at the apex. Inflorescence axillary cyme 1 to 3 flowers, cluster in staminate, solitary in pistillate. Flowers unisexual, actinomorphic, showy, white, ebracteate, pedicellate; pedicelate. Calyx campanulate, 5 – lobed, lobes linear, pale green, glabrous. Corolla campanulate, 5 – lobed, lobes ovate lanceolate, strongly nerved, light green on the base, pubescent. Staminate flowers, stamen 3, free, connate at the base; filaments connate 3.8 to 5.8 mm long, greenish white, pubescent; anther dithecous, conduplicate, basifixed, yellow. Pistillate flower, ovary inferior, oblongoid, many ovules, three parietal placentatae; style long; pale yellow; stigmas 3, each bifid, pale green. Fruits pepo, oblong- ovoid, 4.8 cm by 3.0 cm, indehiscent, smooth, fleshy, green, with white stripes when young, bright red when mature, sweet. Seeds many, compressed, smooth, white.

1.2 *Cucumis trigonus* Roxb. Hort. Beng. 70. 1814. (Figure 1)

Family : Cucurbitaceae

Scientific name : *Cucumis trigonus* Roxb.

Local name : Kasit

Annual, monoecious or dioecious, stout, climbing or creeping herb, stem and branches 5-angular, green, scabrous; tendrils simple, glabrous near the tip, green, scabrous. Leaves simple, alternate, exstipulate; blade suborbicular, palmately 5 lobes, deeply cordate at the base, dentate along the margin, acute at the apex. Inflorescence axillary or solitary cymes. Flower unisexual, actinomorphic, epigynous, yellow, 2.6 cm across at anthesis, bracteolelate, ebracteolate; pedicels of staminate flower cylindrical, pale yellowish green, pubescent. Calyx campanulate, 5 – lobes; lobes ovate, pale yellowish green, pubescent without. Corolla campanulate, 5 – lobed, lobes elliptic obovate, yellow, pubescent within and without; tube short, glabrous, entire along the margin. Staminate flowers, stamens 3, inserted on the mouth of the corolla tube; filament free, short, pale yellow, glabrous; anther dithecous. Pistillate flowers, ovary inferior, ovoid, unilocular with many

ovules on the three parietal placentae; style short; stigmas 3. Fruits pepo, indehiscent, obtusely ellipsoid or trigonous, 3.0 – 5.5 cm by 2.0 to 3.5 cm; yellow when ripe, smooth, glabrous. Seeds many, compressed, corrugate, smooth.

2. Anatomical Studies

2.1 Internal structure of the leaves of *Coccinia grandis* (L.) Voigt. (Figure 2)

Petiole

In transverse section, the petiole of *Coccinia grandis* (L.) Voigt. studied were cordate-shaped in outline.

Dermal Tissue System: In transverse section, both upper and lower epidermis 1- layered, the cells barrel or oval or rounded in shape.

Ground Tissue System: Differentiated into outer collenchymatous and inner parenchymatous tissues. Outer collenchymatous tissue composed of 3-to 6-layered, the cells polygonal in shape; parenchymatous layers lying internal to collenchymatous cells, 3- to 9- layered, the cells polygonal in shape.

Vascular Tissue System: Vascular bundles embedded in ground tissue, occurred in 9 groups, bicoalletral type and open,7 large bundles and 2 small bundles, each bundle surrounded by pericycle 6- to 8- layered, the cells polygonal in shape. Phloem lying on both side of xylem separated by cambium, phloem 5- to 9- layered, the cells rounded or oval in shape. Phloem composed of sieve tubes elements, companion cells, fibers and phloem parenchyma; xylem lying between the phloem, xylem 2- to 5-layered, the cells polygonal in shape, 24.0 – 48.0 μm in length. Xylem composed of vessel elements, tracheids, fibers and xylem parenchyma.

Lamina

In transverse section, the lamina of *Coccinia grandis* (L.) Voigt. studied are dorsiventral with reticulate venation, 300.0 – 360.0 μm thick.

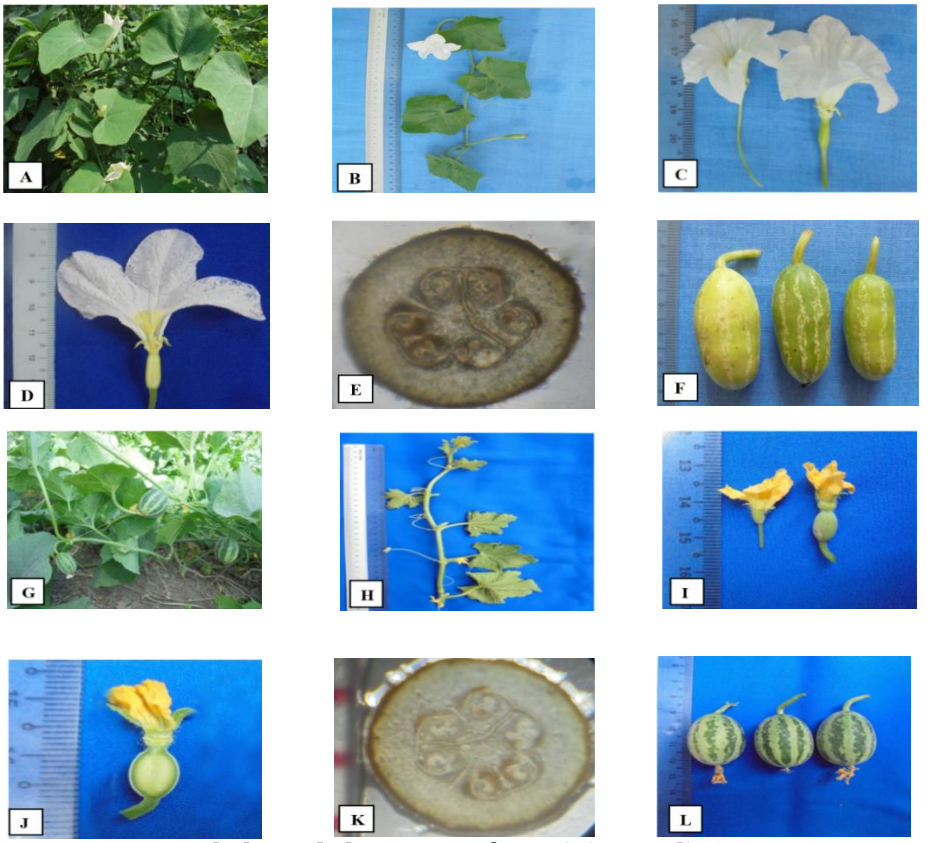


Figure 1. Morphological characters of *Coccinia grandis* (L.) Voigt. and *Cucumis trigonus* Roxb.

A. Habit, **B.** Inflorescence , **C.** Close up view of flowers,
D. L.S of pistillate flower, **E.** T.S of ovary,
F. Fruits of *Coccinia grandis* (L.) Voigt, **G.** Habit,
H. Inflorescence, **I.** Close up view of flowers,
J. L.S of pistillate flower, **K.** T.S of ovary, **L.** Fruits of *Cucumis trigonus* Roxb.

Dermal Tissue System: In surface view, the epidermal cells of both surfaces were parenchymatous, polygonal in shape, cell walls straight and thin walled. Stomata present on both surfaces, anomocytic type, oval-shaped with reniform shaped guard cells. In transverse section, both adaxial and abaxial epidermis composed of 1- layered, parenchymatous, the cells rectangular or barrel-shaped.

Ground Tissue System: Mesophyll differentiated into palisade parenchyma at upper side and spongy parenchyma at the lower side; palisade cells 1-layered, the cells elongated in shape, compactly arranged; the spongy parenchyma cells 2- to 4- layered, the cells rounded to oval in shape.

Vascular Tissue System: Vascular bundles of lateral veins were embedded in the mesophyll tissues, bicollateral type and different in size according to their position; bundle sheath distinct and composed of parenchymatous cells, rounded or oval in shape. Phloem composed of sieve tubes elements, companion cells, fibers and phloem parenchyma. Xylem composed of vessel elements, tracheids, fibers and xylem parenchyma.

Midrib

In transverse section, the midrib of *Coccinia grandis* (L.) Voigt. studied were subcircular in shape.

Dermal Tissue System: In transverse section, both upper and lower epidermis 1-layered, parenchymatous, the cells barrel or oval – shaped, anticlinal walls straight, outer and inner walls convex.

Ground Tissue System: Ground tissue composed of outer collenchymatous and inner parenchymatous cells. Outer collenchymatous cells lying internal to the epidermis, adaxial side collenchymatous cells 3- to 6- layered; abaxial side of collenchymatous cells 2- to 4-layered; parenchymatous cells lying internal to the collenchymatous cells, 3- to 7- layered, the cells oval, rounded or polygonal in shape; at the adaxial side; 4 - to 6- layered, intercellular space present.

Vascular Tissue System: Vascular bundles embedded in the ground tissues and occurred in 2 groups of fracially in crescent-shaped, collateral type, open, the middle bundle large and peripheral small, polygonal in shape; phloem present on both side of xylem separated by cambium, phloem 3- to 5- layered, the cells irregular in shape. Phloem composed of sieve tubes elements, companion cells, fibers and phloem parenchyma; xylem 4 - to 6- layered. Xylem composed of vessel elements, tracheids, fibers and xylem parenchyma.

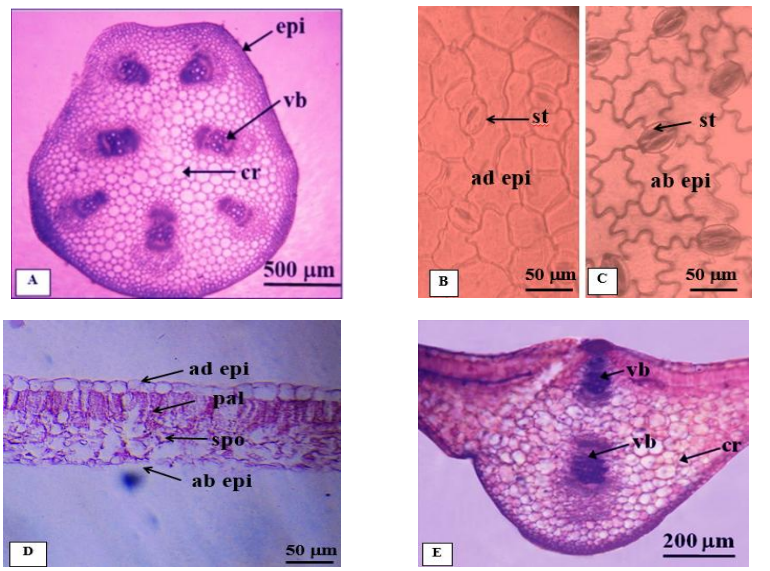


Figure 2. Internal structure of leaf *Coccinia grandis* (L.) Voigt.
A. T.S of petiole. **B.** Adaxial surface view of lamina.
C. Abaxial surface view of lamina showing stomata.
D. T.S of lamina. **E.** T.S of midrib(ab epi = abaxial epidermal cell, ad epi = adaxial epidermal cell, cr = cortex, pal = palisade parenchyma cell, ph = phloem,spo = spongy parenchyma cell, st = stoma, xy = xylem)

2.2 Internal structure of the leaves of *Cucumis trigonus* Roxb. (Figure 3)

Petiole

In transverse section, the petiole of *Cucumis trigonus* Roxb. studied were oval shape in outline.

Dermal Tissue System: In transverse section, both upper and lower epidermis 1-layered, the cells barrel or oval or rounded in shape; upper epidermal cells anticlinal walls straight, outer and inner walls convex; trichomes multiserate, 1- to 3- celled.

Ground Tissue System: Differentiated into outer collenchymatous and inner parenchymatous tissues. Outer collenchymatous tissue composed of 2- to 3-layered, the cells polygonal in shape; parenchymatous cells lying internal to collenchymatous cells, 5- to 10- layered, the cells polygonal in shape, intercellular space present.

Vascular Tissue System: Vascular bundles embedded in ground tissue, occurred in 6 to 9 groups, bicollateral type, open, 6 large bundles and 3 small bundles, each vascular bundles surrounded 5- to 7- layered of pericycle. Phloem lying on both side of xylem separated by cambium, phloem 5- to 7-layered. Xylem lying between the phloem, xylem 3- to 4-layered, the cell polygonal in shape.

Lamina

In transverse section, the lamina of *Cucumis trigonus* Roxb. studied were dorsiventral with reticulate venation, 240.0 – 300.0 μm thick.

Dermal Tissue System: In surface view, the epidermal cells of both surfaces were parenchymatous, polygonal in shape, cell walls straight and thin walled, secretory cells present. Stomata present on both surfaces, anomocytic type, oval-shaped with reniform shaped guard cells.

Ground Tissue System: Mesophyll differentiated into palisade parenchyma at the upper side and spongy parenchyma at the lower side; palisade cells 1-layered, the cells elongated in shape, compactly arranged; the spongy parenchyma 2- to 4- layered, the cells rounded to oval in shape.

Vascular Tissue System: Vascular bundles of lateral veins were embedded in the mesophyll tissues. They were bicollateral type and different in size according to their position; bundle sheath distinct and composed of parenchymatous cells, the cells rounded or oval in shape. Phloem composed

of sieve tubes elements, companion cells, fibers and phloem parenchyma. Xylem composed of vessel elements, tracheids, fibers and xylem parenchyma.

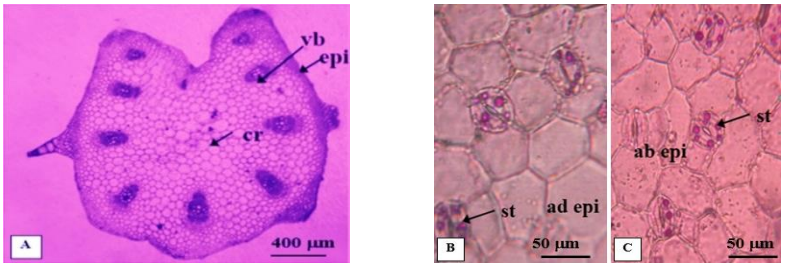
Midrib

In transverse section, the midrib of *Cucumis trigonus* Roxb. studied were subcircular in shape.

Dermal Tissue System: In transverse section, both upper and lower the epidermis 1- layered, the cells oval to barrel in shape; trichome multicellular uniseriate.

Ground Tissue System: Ground tissues composed of outer collenchymatous cells and inner parenchymatous cell. Pericycle 5- to 6- layered, the cells polygonal in shape; parenchymatous cells lying internal to the collenchymatous layers, 5- to 9-layered, the cells rounded or polygonal in shape; 5- to - 10 layered at the abaxial side; intercellular space present.

Vascular Tissue System: Vascular bundles embedded in the ground tissues, 2 bundles, collateral type, open; phloem 7- to 9- layered, the layers 60.0 – 80.0 μm thick, the cells irregular in shape. Phloem composed of sieve tubes elements, companion cells, fibers and phloem parenchyma; xylem strands arranged in 5- to 7- radial rows, 3- to 6- celled in each row, protoxylem inward and metaxylem outward.



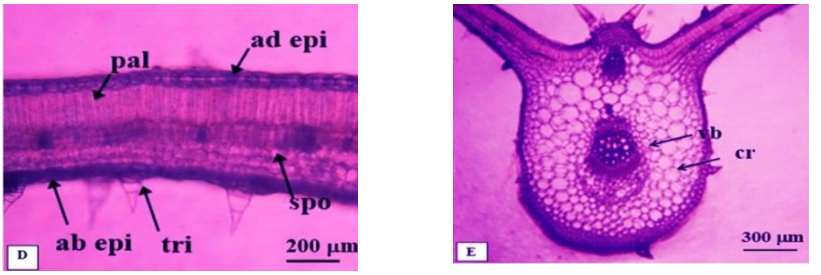


Figure 3. Internal structure of leaf *Cucumis trigonus* Roxb.
A. T.S of petiole. **B.** Adaxial surface view of lamina.
C. Abaxial surface view of lamina showing stomata. **D.** T.S of lamina.
E. T.S of midrib (ab epi = abaxial epidermal cell, ad epi = adaxial epidermal cell, cr = cortex, pal = palisade parenchyma cell, ph = phloem, spo = spongy parenchyma cell, st = stoma, tri = trichome, vb = vascular bundle, xy = xylem)

2.3 Internal structure of the stem of *Coccinia grandis* (L.) Voigt. (Figure 4)

In transverse section, the stem of *Coccinia grandis* (L.) Voigt. studied were oval – shaped in outline, distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System: In transverse section, composed of epidermis and trichomes; epidermis 1-layered, parenchymatous, the cells oval to barrel in shape, both outer and inner walls convex; trichomes present.

Ground Tissue System: Composed of cortex, endodermis, pericycle and pith. The cortex differentiated into outer collenchymatous and inner parenchymatous tissues, the outer cortex collenchymatous tissue composed of 3- to 6- layered, the cells polygonal in shape. The inner cortex parenchymatous tissue composed of 3- to 8- layered, the cells were polygonal in shape.

Endodermis layer lying inter most of cortex, 1- layered, parenchymatous, the cells barrel- shaped, thin wall. Pith present.

Vascular Tissue System: Vascular bundles embedded in the ground tissues, two circle, each with four bundles, bicollateral type, open, cambium composed of 2- to 3- layers of tangentially elongated rectangular cells; bundle sheath present and composed of parenchymatous cells, the cells round or oval in shape. Phloem 5- to 8- layered, the cells oval or irregular in shape. Xylem 3- to 5- layered, the cells round or oval in shape.

2.4 Internal structure of the stem of *Cucumis trigonus* Roxb. (Figure 4).

In transverse section, the stem of *Cucumis trigonus* Roxb. studied were cordate-shaped in outline.

Dermal Tissue System: In transverse section, composed of epidermis and trichomes, epidermis 1- layered, parenchymatous, the cells rectangular or barrel in shape; trichomes present.

Ground Tissue System: Composed of cortex, endodermis, pericycle and pith. The cortex differentiated into outer collenchymatous and inner parenchymatous tissues, the outer cortex collenchymatous tissue composed of 3- to 7- layered, the cells polygonal in shaped. The inner cortex parenchymatous tissue composed of 3- to 10- layered. Endodermis inconspicuous. Pericyclic sclerenchymatous layer forming continuous rings. Outer collenchymatous cells lying internal to the epidermis, adaxial side collenchymatous cells 3- to 6- layered, the cells polygonal in shape; abaxial side collenchymatous cells 3- to 4- layered, the cells irregular or polygonal in shape. Pith present.

Vascular Tissue System: Vascular bundles embedded in the ground tissues, two- circled, about 8- to 9-bundles, bicollateral type, phloem lying on both sides of xylem separated by cambium; cambium composed of 2- to 3- layers of tangentially elongated rectangular cells; bundle sheath present and composed of parenchymatous cells, the round or oval in shape; phloem 5- to

8-layered on both sides. Xylem 3- to 4-layered, the cells round or oval in shape.

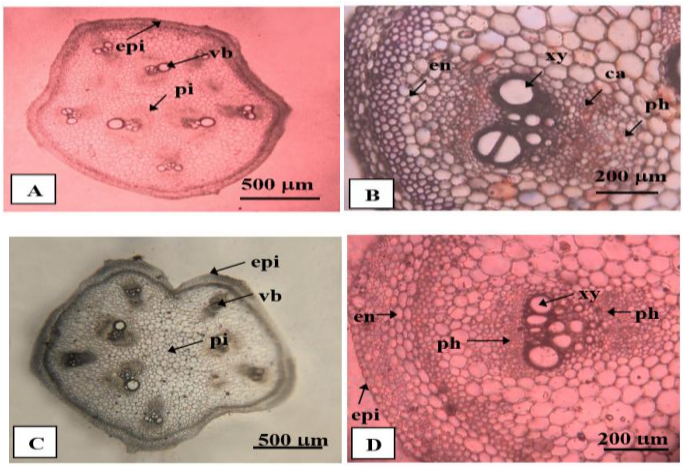


Figure 4. Internal structure of stem of *Coccinia grandis* (L.) Voigt. and *Cucumis trigonus* Roxb.

A. T.S of stem, **B.** Close up view of vascular bundle of *Coccinia grandis* (L.) Voigt.

C. T.S of stem, **D.** Close up view of vascular bundle of *Cucumis trigonus* Roxb.(ca = vascular cambium, cr = cork, en = endodermis, epi = epidermis, pi= pith, ph = phloem, vb = vascular bundle,

2.5 Internal structure of the root of *Coccinia grandis* (L.) Voigt.

(Figure 5)

In transverse section, the root of ***Coccinia grandis* (L.) Voigt**. studied were circular-shaped in outline.

Dermal Tissue System: In transverse section, epiblema 1- layered, parenchymatous, the cells oval or barrel in shape.

Ground Tissue System: Composed of cortex, endodermis, pericycle and pith. Cortex homogenous parenchymatous cells, 2- to 12- layered, the cells oval or rounded in shape. Endodermis and pericycle inconspicuous. Pith absent.

Vascular Tissue System: Vascular bundles radial type, polyarch, ray cells occur between the xylem and phloem; phloem distributed at the periphery of the xylem, 4- to 6- layered, the cells polygonal or rounded in shape; xylems trands arranged as radiated group, the cells round or polygonal in shape.

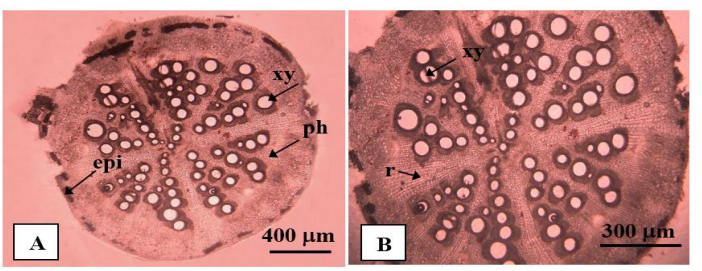
2.6 Internal structure of the root of *Cucumis trigonus* Roxb. (Figure 5).

In transverse section, the root of *Cucumis trigonus* Roxb. studied were circular-shaped in outline.

Dermal Tissue System: In transverse section, epiblema 1-layered, parenchymatous, the cells oval or barrel in shape.

Ground Tissue System: Composed of cortex, endodermis, pericycle and pith. Cortex 2- to 12-layered, parenchymatous, the cells oval or rounded in shape. Endodermis and pericycle inconspicuous. Pith absent.

Vascular Tissue System: Vascular bundles radial type, polyarch; ray cells occur between the xylem and phloem; phloem distributed at the periphery of the xylem, 3- to 5-layered. Xylem strands arranged as radiated group, 900.0 – 1320.0 μm thick, the cells polygonal or rounded in shape.



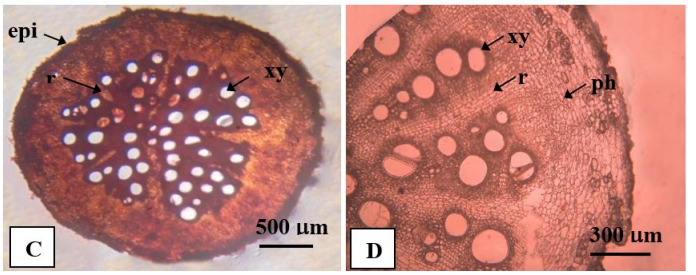


Figure 5. Internal structure of root of *Coccinia grandis* (L.) Voigt. *Cucumis trigonus* Roxb.
A. T.S of root, **B.** Close up view of vascular bundle of *Coccinia grandis* (L.) Voigt.
C. T.S of root, **D.** Close up view of vascular bundle of *Cucumis trigonus* Roxb.

Discussion and Conclusion

Morphological and anatomical characteristics of *Coccinia grandis* (L.) Voigt. and *Cucumis trigonus* Roxb. belonging to family Cucurbitaceae were studied. The leaf apices were found to be acute in *Cucumis trigonus* Roxb. and obute in *Coccinia grandis* (L.) Voigt. Tendrils of the two species were simple. The colour of flowers were white in *Coccinia grandis* (L.) Voigt. and yellow in *Cucumis trigonus* Roxb. The filaments of *Coccinia grandis* (L.) Voigt. were connate and *Cucumis trigonus* Roxb. were free. The shape of fruits was found to be oblongoid in *Coccinia grandis* (L.) Voigt. and ellipsoid in *Cucumis trigonus* Roxb. These characters were agreed with those mentioned by Dessanayake (1997) and Nian-he (2007).

In the transverse sections of petiole, the ground tissues were differentiated into collenchymatous and parenchymatous cells, collenchymatous cells located below the epidermis. The collenchymatous cells of *Cucumis trigonus* Roxb. were 2- to 3- layered and 3- to 6-layered found in *Coccinia grandis* (L.) Voigt. The parenchymatous cells were found 5- to 10- layered in *Cucumis trigonus* Roxb. and 3- to 9- layered in *Coccinia*

grandis (L.) Voigt. The vascular bundles of two species were embedded in the ground tissues and separated, arranged in a ring, bicollateral type. The number of vascular bundles were differed from one species to another, these characters were agreed with Metcalfe & Chalk (1957).

In transverse sections, the thickness of laminae was different in both species. Dermal tissues are composed of 1-layered of epidermal cells on both surfaces of the two species. Anomocytic type of stomata were found in adaxial and abaxial surface view of laminae of the two species, these characters were agreed with Metcalfe and Chalk (1957).

In transverse sections of midrib of two species were oval or shield-shaped and subcircular. The ground tissues of midribs were differentiated into two types of tissues, collenchymatous and parenchymatous cells. The vascular bundles of midribs were bicollateral type and circular in shape, these characters were agreed with Pandey & Chadha (1993).

Transverse section of stem of *Cucumis trigonus* Roxb. were found cordate-shaped and *Coccinia grandis* (L.) Voigt. were oval-shaped. The collenchymatous cells layers of *Cucumis trigonus* Roxb. were found to be 3- to 7- layered, 3- to 6- layered were found in *Coccinia grandis* (L.) Voigt. The parenchymatous cells layers of *Cucumis trigonus* Roxb. were 3- to 10-layered, 3- to 8- layered were in *Coccinia grandis* (L.) Voigt. The vascular bundles of two species were bicollateral type. The vascular bundles of stem are organized into 6 to 8 bundles in both species, these characters were agreed with Metcalfe & Chalk (1950).

In transverse section of the root of the two species were circular-shaped in outline. The vascular bundles of the root were radial type and tetrarch *Coccinia grandis* (L.) Voigt., and polyarch in *Cucumis trigonus* Roxb. These characters were agreed with Metcalfe & Chalk (1957), Pandey & Chadha (1993).

In conclusion, the present research can provide the information of similarities and differences of morphological and anatomical characteristics of the two species. It is hoped that the results of the present work will be useful in identification of family Cucurbitaceae.

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We would like to express our heartfelt thanks to Dr Nu Nu Yee, Professor and Head, Department of Botany, University of Mandalay for permitting me to undertaken this research, for her supervision, for her invaluable advice and for providing all departmental facilities. We are also thankful to Dr Soe Soe Aung, Professor, Department of Botany, University of Mandalay for her suggestion.

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POLLINARIAL MORPHOLOGY OF TEN SPECIES OF FAMILY APOCYNACEAE FOUND IN SOUTHERN SHAN STATE

Hnin Hnin Yu[1], Nwè Nwè Yi[2]

Abstract

Pollinarial morphology of 10 species belonging to eight genera of Apocynaceae were studied. All specimens were collected from Southern Shan State from July 2016 to September 2017. The flowering plants were collected, classified, identified and preserved. The pollen grains were found as tetrads and pollinia. Pollen tetrads were observed in two species *Hemidesmus indicus* (L.) R. Br. and *Streptocaulon tomentosum* Wight. And the eight species were pollinia. The small size of pollen tetrad was found in *Hemidesmus indicus* (L.) R. Br. and the large size of pollen tetrad was occurred in *Streptocaulon tomentosum* Wight. Pollinial morphology of 8 species was recorded with their size, shape, colour, orientation and translator attachment to the pollinia. The pollinia of each species were presented with photomicrographs.

Key words: Apocynaceae, Pollinarium, Southern Shan State

Introduction

Apocynaceae is one of the largest families of angiosperms, with 375 genera and over 5000 species. The notable morphological variation in reproductive traits in the family has resulted in distinct interpretations about the appropriate choice of characters for taxonomic classifications. Of the five subfamilies currently recognized in Apocynaceae, four (Apocynoideae, Asclepiadoideae, Periplocoideae, Secamonoideae) have some of the most elaborate and complicated flowers of all the angiosperms (Simoes *et al*. 2010).

Pollen unit refers to the number of pollen grains united together at the time of release. Most commonly the four microspores formed after microsporogenes is separate prior to pollen release such single, unfused pollen grains are called monads, found in the great majority of angiosperms. Pollen

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grains that are connate in precise units of more than four are called polyads. Fusion of pollen grains in large, often irregular number, but less than an entire theca is called massulae (Singular massula). Finally, the fusion of all pollen grains of an entire theca is called a pollinium (plural pollinia), found in the families Apocynaceae and Orchidaceae (Simpson 2006).

Pollen morphology of Asclepiadoideae is different from the pollen morphology of other families, due to the presence of pollen grains that form hard sac-like definite structure called pollinium. Asclepiadoideae is composed of 2 or more pollinia, in which all the pollen grains of a single anther locule are embedded in a hard structure and a translator attachment, which develops from a stigmatic secretion and mechanically attach the pollinia to the pollinator (Corry 1883, Schill & Jakel 1978, Kunze 1993, and Swarupannandan *et al*. 1996 cited in Rapini 2012). Pollinial wall is made of amorphous sporopollenin enclosing the pollen mass each with a lamellate exine (Sinha &Mondal 2011).

Taxonomic study of family Apocynaceae had been reported in the floristic studies on various regions of Myanmar. However, pollinarial morphology study of Apocynaceaeis still lacking. Therefore, a research on the pollinarial morphology of this family was selected and studied.

The aim and objectives of present study were to investigate the differences between the morphological characteristics of pollen and pollinarium of Apocynaceae, to provide the valuable pollinarium morphological characteristics that can be used in plant classification and identification of Apocynaceae.

Materials and Methods

Collection of Plant Materials

The specimens of the Family Apocynaceae were collected from Southern Shan State from July 2016 to September 2017. Morphology of pollen and pollinarium from mature flowers of 10 species belonging to 8

genera were examined at Department of Botany, University of Mandalay.

Identification of specimens were carried out by referring to the literature such as Hooker (1882), Backer & Bakhuizen (1965), Dassanayake (1983) and Middleton *et al*. (1999), Myanmar names were referred to Hundley & Chit KoKo (1961).

Collection of Pollen Samples

All the fresh pollen and pollinarium were collected from the anthers of open flowers. The collected flowers of each species were stored in glass vial with glacial acetic acid and labeled. For the isolation of pollinarium, pollinarium were manually picked under a dissecting microscope using forceps and sharp needles.

Results

List of the collected plants

Ten species belong to eight genera were studied. The list of collected species were presented in Table 1.

Table 1. List of the collected plants

Family	Subfamily	No.	Scientific Name	Myanmar Name
Apocynaceae	Periplocoideae	1	*Hemidesmus indicus* (L.) R. Br.	Than hlat pin
		2	*Streptocaulon tomentosum* Wight.	Myinsagoni
	Secamonoideae	3	*Toxocarpus wangianus* Tsiang	Unknown
	Asclepiadoideae	4	*Calotropis gigantea* (L.) R. Br.	Ma yogyi
		5	*Cynanchum dalhousiae* Wight.	Unknown
		6	*Hoya revoluta* Hook.	Unknown
		7	*Hoya thailandica* Thaithong	Unknown
		8	*Pentasachme caudatum* Wall. ex Wight.	Kyauk pan
		9	*Pergularia minor* Andr.	Daung da late
		10	*Pergularia pallida* Wight & Arn.	Taw daung da late

Subfamily Periplocoideae

1. *Hemidesmus indicus* (L.) R. Br. in Men. wern. Soc. 1. 1809. (Figure 1 A)

Periploca indica L. Sp. Pl. 211. 1753.

Hemidesmus wallichii Mig. f. Fl. Brit. Ind. 4. 5. 1883.

Myanmar name : Than hlat pin

English name : Unknown

Flowering period : August to November

Pollinarial Morphology (Figure 1B, C)

Pollen translator 150 – 450 × 90 – 310 μm in length and breadth, spatulate, mimosa yellow, orientation of pollen translator erect; pollen tetrad, rhomboidal in shape, 31.5 – 47.5 × 42.5 – 50.0 μm in length and breadth; single grain small, 20.0 – 21.3 μm in diameter; exine about 2.5μm thick; sculpturing psilate.

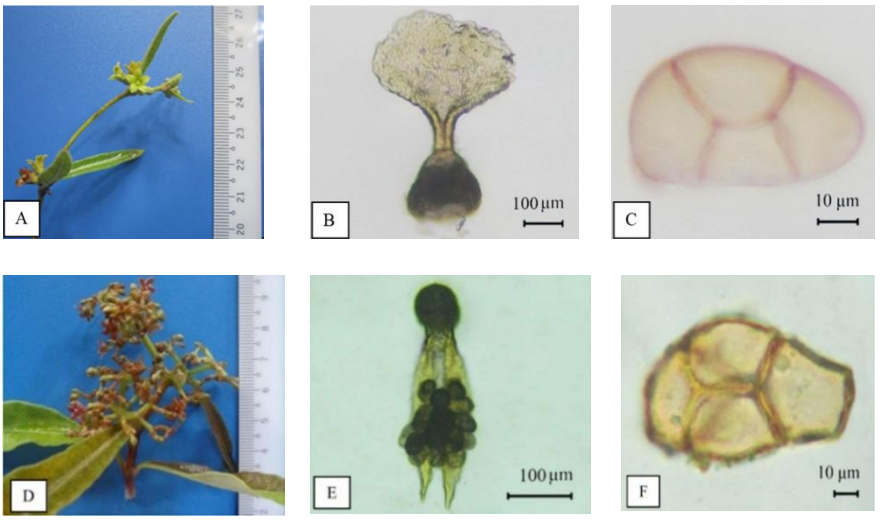

Figure 1 A. Inflorescences of *Hemidesmus indicus* (L.) R. Br.
B. Pollinial translator of *H. indicus* (L.) R. Br.
C. Tetrad of *H. indicus* (L.) R. Br.
D. Inflorescences of *Streptocaulon tomentosum* Wight.
E. Pollinial translator of *S. tomentosum* Wight.
F. Tetrad of *S. tomentosum* Wight.

Subfamily Secamonoideae

3. *Toxocarpus wangianus* Tsiang, Sunyatsenia. 4: 100. 1939. (Figure 2 A)

Myanmar name : Unknown
English name : Unknown
Flowering period : May to August

Pollinarial Morphology (Figure 2 B, C)

Pollinia 4, pollinial sac 250-325 × 200-250 μm in length and breadth, oblong in shape, lemon yellow, orientation of pollinium erect; corpusculum 200-250 × 225-275 μm in length and breadth, rounded in head of shape, brown; pollen tetrad, rhomboidal in shape, 62.5-75.5 × 37.5-40.5 μm in length and breadth; single grain 12.5-25.0 × 18.5-20.0 μm in length and breadth; exine about 1.3 μm thick, sexine thicker than nexine; sculpturing psilate.

Subfamily Asclepiadoideae

4. ***Calotropis gigantea*** (L.) R. Br. in Ait. Hort. Kew. ed 2, 2:78. 1811. (Figure 2D)

Asclepias gigantea L., Sp. Pl. 214. 1753.

Myanmar name : Ma yogyi
English name : Giant swallow word
Flowering period : Throughout the year

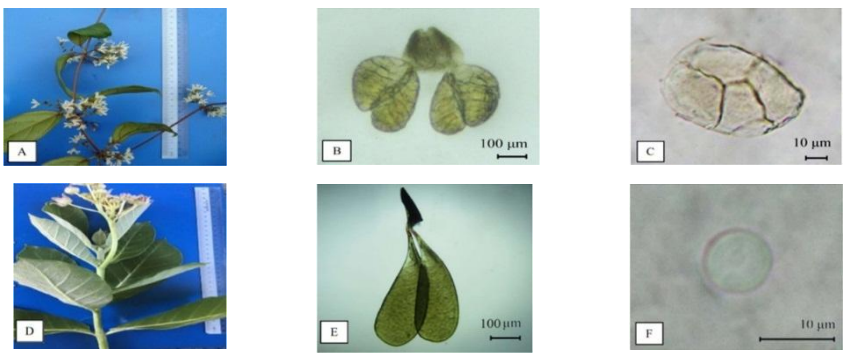

Figure 2. A. Inflorescences of *Taxocarpus wangianus* Tsaing
B. Pollinarium of *T. wangianus* Tsiang
C. Single grain of *T. wangianus* Tsiang
D. Inflorescences of *Calotropisgi gantea* (L.) R. Br.
E. Pollinarium of *C. gigantea* (L.) R. Br.
F. Single grain of *C. gigantea* (L.) R. Br.

Pollinarial Morphology (Figure 2E, F)

Pollinia 2, pollinial sac 1313 – 1375 × 500 – 538 μm in length and breadth; oblong in shape, sulphur yellow, orientation of pollinium pendulous; corpusculum 413 – 525 × 125 – 188 μm in length and breadth, angular in shape of head, reddish brown; translator arm 250 – 313 × 38 – 50 μm in length and breadth, cylindrical in shape, black yellow; translator attachment to the pollinia basal; single grain small, spherical, 1.3 – 10.0μm in diameter.

5. ***Cynanchum dalhousiae*** Wight., Contrib. Bot. Ind. 55. 1834.(Figure 3 A)

Myanmar name : Unknown

English name : Unknown

Flowering period : September to December

Pollinarial Morphology (Figure 3 B, C)

Pollinia 2, pollinial sac 487.5-562.5 × 212.5-237.5 μm in length and breadth, oblong in shape, lemon yellow, orientation of pollinium pendulous; corpusculum 362-412 × 175-180 μm in length and breadth, rounded in shape, reddish brown; translator arm 125-188 × 175-180 μm in length and breadth, triangular in shape, pale yellow; translator attachment to the pollinia basal; single grain small, spherical, 3.8 -17.5 μm in diameter.

6. ***Hoya revolute*** Hook. f. Fl. Brit. Ind. 4. 1883. (Figure 3D)
Hoya ovalifolia Wall., Cat. 8160 b. 1847.

Myanmar name : Unknown

English name : Unknown

Flowering period : June to September

Pollinarial Morphology (Figure 3 E, F)

Pollinia 2, pollinial sac 750-938×250-388 μm in length and breadth; ovate-oblong in shape, lemon yellow, orientation of pollinium horizontal; corpusculum 500-650 × 250-287 μm in length and breadth, angular in shape of head, reddish; translator arm about 50-63 μm in length and breadth, triangular in shape, yellow; translator attachment to the pollinia terminal; single grain small, spherical, 1.3-13.0 μm in diameter.

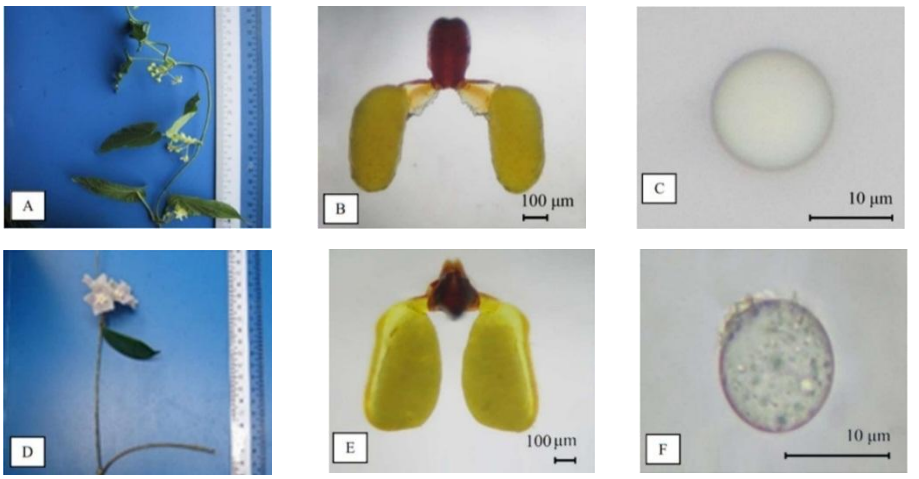

Figure 3. A. Inflorescences of *Cynanchum dalhousiae* Wight.
B. Pollinarium of *C. dalhousiae* Wight.
C. Single grain of *C. dalhousiae* Wight.
D. Inflorescences of *Hoya revoluta* Hook.
E. Pollinarium of *H. revolute* Hook.
F. Single grain of *H. revolute* Hook.

7. *Hoya thailandica* Thaithong. Nordic J. 21(2): 143. 2001.
(Figure 4 A)

Myanmar name : Unknown

English name : Unknown

Flowering period : June to August

Pollinarial Morphology (Figure 4 B, C)

Pollinia 2, pollinial sac 600-760 × 225-275 μm in length and breadth; ovate-oblong in shape, lemon yellow, orientation of pollinium horizontal; corpusculum 375-400 × 95-150 μm in length and breadth, angular in shape of head, reddish; translator arm absent; single grain small, spherical, 5-40 μm in diameter.

8. ***Pentasachme caudatum*** Wall. ex. Wight, Contr. Bot. India 60. 1834 (Figure 4 D)
Pentasachme championii Benth. Hooker's J. Bot. Kew Gard. Misc. 5: 54-55.

Myanmar name : Kyukpan
English name : Unknown
Flowering period : April to October

Pollinarial Morphology (Figure 4 E, F)

Pollinia 2, pollinial sac 275-363 × 313-363 μm in length and breadth; ovoid in shape, lemon yellow, orientation of pollinium erect; corpusculum 100-163× about 62.5 μm in length and breadth, rounded in shape of head, orange; translator arm 10.0-12.5 × 5.0-7.2 μm in length and breadth, triangular in shape, white; translator attachment to the pollinia basal; single grain small, spherical, 1.3-6.3 μm in diameter.

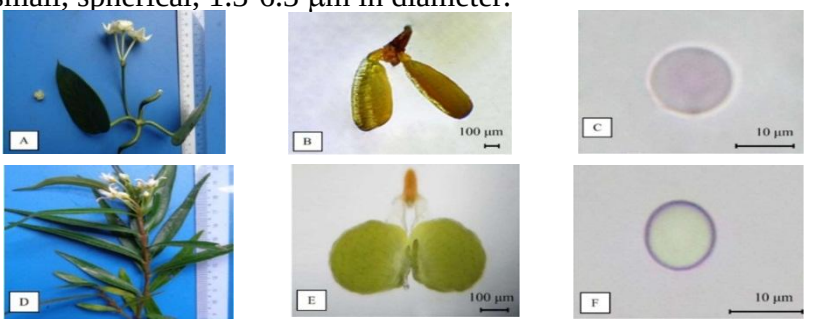

Figure 4. A. Inflorescences of *Hoya thailandica* Thaithong
B. Pollinarium of *H. thailandica* Thaithong
C. Single grain of *H. thailandica* Thaithong
D. Inflorescences of *Pentasachme caudatum* Wall. ex Wight.
E. Pollinarium of *P. caudatum* Wall. ex Wight.
F. Single grain of *P. caudatum* Wall. ex Wight.

9. *Pergularia minor* Andr. Bot. Rep. t. 1:2.1899. (Figure 5A)

Myanmar Name : Unknown
English Name : Unknown
Flowering period : May to August

Pollinarial Morphology (Figure 5B, C)

Pollinia 2, pollinial sac 450-575 × 250-275 μm in length and breadth; globosely obovoid in shape, canary yellow, orientation of pollinium erect; corpusculum 262.5-312.5 × 175.0-212.5 μm in length and breadth, rounded in shape of head, reddish; translator arm 75.0-137.5 × 62.5-87.5 μm in length and breadth, triangular in shape, pale yellow; translator attachment to the pollinia subbasal; single grain small, spherical, 12.5-50.0 μm in diameter.

10. ***Pergularia pallida*** Wight &Arn, Contrib. 42.2:76.1879. (Figure 5 D)

Myanmar name : Taw daung da late
English name : Unknown
Flowering period : June to September

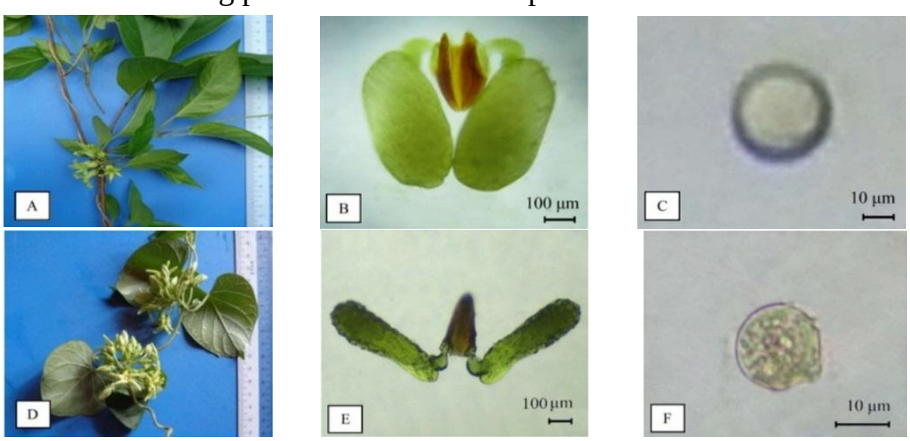

Figure 5. A. Inflorescences of *Pergularia minor* Andr.
B. Pollinarium of *P. minor* Andr.
C. Single grain of *P. minor* Andr.
D. Inflorescences of *Pergularia pallida* Wight &Arn.
E. Pollinarium of *P. pallida* Wight & Arn.
F. Single grain of *P. pallida* Wight & Arn.

Pollinarial Morphology (Figure 5 E, F)

Pollinia 2, pollinial sac 562.5–587.5×162.5–187.5 μm in length and breadth; obovate in shape, canary yellow, orientation of pollinium erect; corpusculum 300–313× 150–163 μm in length and breadth, rounded in shape of head, reddish brown; translator arm 112.5–125.0 μm in length and breadth, angular in shape, pale yellow; translator attachment to the pollinia basal; single grain small, spherical, 1.3–18.8 μm in diameter.

Discussion and Conclusion

The present study deal with pollen morphology of family Apocynaceae found in Southern Shan State. Apocynaceae is a large, widespread family of woody and herbaceous plants.

In the present study, 2 species of Periplocoideae, 1 species of Secamonoideae and 7 species of Asclepiadoideae were studied. *Hemidesmus indicus* (L.) R. Br. and *Streptocaulon tomentosum* Wight. were belong to the subfamily Periplocoideae. *Toxocarpus wangianus* Tsiang. was belong to the Secamonoideae. *Calotropis gigantea* (L.) R., *Cynanchum dalhousiae* Wight., *Hoya revoluta* Hook., *Hoya thailandica* Thaithong., *Pentasachme caudatum* Wall. ex. Wight., *Pergularia minor* Andr. and *Pergularia pallida* Wight &Arn. were belong to the subfamily Asclepiadoideae.

According to the collected data, 6 species are climbing herbs and shrubs and 3 species are woody lianas. Only one epiphytic species, *Hoya thailandica* Thaithong. can be found in the study area.

The pollen grains of 2 species were tetrads and the remaining 8 species were polyads or pollinia. The pollen grains of angiosperms display variation in many of their morphological features.

The size and shape of pollinial sac, colour of pollinia, nature of corpuscular, position of pollinia, structure of caudicle or translator were differed from one species to another. *Hemidesmus indicus* (L.) R. Br. possess

spatulate-shaped translator; *Streptocaulon tomentosum* Wight. hadboat-shaped translator. *Hemidesmus indicus* (L.) R. Br. and *Streptocaulon tomentosum* Wight. had the rhomboidal shape of pollen tetrads. These characters agreed with Arekal & Ramakrishna (1980).

The pollen tetrad of *Toxocarpus wangianus* Tsiang. had the orientation of pollinia erect and pollen tetrads are agglutinated to four per pollinia anther. These characters were agreed with Ramarkrishna *et. al.* (2012).

The pollinia showed a great variation in form, varying from ovate to oblong. In the present study, different shapes of pollinia were observed in 7 species of subfamily Asclepiadoideae. Pollinia were ovate-oblong in *Hoya revolute* Hook. f. Fl. and *Hoya thailandica* Thaithong. and the remaining species were ovoid, obovate, globosely obovoid and obovate. These characters were in agreement with those reported by Sinha & Mondal (2011).

In this study, the horizontal orientation of pollinia was found in *Hoya revoluta* Hook. f. Fl., and *Hoya thailandica* Thaithong.; pendulous orientation of pollinia was occurred in *Calotropis gigantea* (L.) R. Br. and *Cynanchum dalhousiae* Wight. and the erect orientation of pollinia was observed in the remaining species. These characters were agreed with those stated by Sinha & Mondal (2011).

According to result, pollen and pollinarium characters are now being used as important taxonomical tool for reassessing the different types of plant groups. It is hoped that these differences of palynological characters will support the classification and identification of Apocynaceae.

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