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BIOACCUMULATION OF HEAVY METALS IN THE MUSCLE OF SOME COMMERCIAL FISHES FROM HLAING RIVER SEGMENT OF SHWE PYI THAR TOWNSHIP, YANGON REGION*

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Abstract

Analysis of some heavy metals in the muscle of some commercial fishes collected from Hlaing River in Shwe Pyi Thar Township, Yangon Region were conducted during April 2015 to March 2017. Suspected toxic metals from industrial sewages such as Al, Cr, Cd, As, Ni, Hg and Pb were analysed in detail. The majority of tested heavy metals were found to be high concentration in the muscles of all studied commercial fish species. The concentration of Al, Cr, Cd, As, and Hg in the fish muscles were exceeding the permissible limits of FAO/WHO (1992) standard for human consumption. Condition factors of studied fishes were negatively correlated with the concentration of heavy metals in their muscles. High bioaccumulation factor value were observed in all studied fish species, so as tested heavy metals were accumulated by fish showing long term exposure of heavy metals to fish in its surrounding. The bioaccumulation factors of demersal fishes were higher in most of the tested metals than benthopelagic fishes. Bioaccumulation factors of all tested heavy metals were found to be higher in the dry season than other seasons. Daily consumption in large amount of studied fish species captured from the Hlaing River may cause health problems if bioaccumulation continues in the same rate without taking effective management for pollution in the Hlaing River.

Key words: Heavy metals, pollution, Hlaing River, commercial fishes, bio-indicator.

Introduction

Heavy metals are metallic elements which have a high atomic weight and a density much greater at least 5 times than water. Among more than 20 heavy metals, lead (Pb), cadmium (Cd), mercury (Hg), and inorganic arsenic

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(As) are particular concern to human health. They are highly toxic and can cause damaging effects even at very low concentrations (Chang *et al*., 1996).

One of the important environmental problem is the pollution of aquatic ecosystems due to heavy metals, as heavy metals constitute some of the most hazardous substances that can bioaccumulate in various biotic systems. Bioaccumulation is a process in which a chemical pollutant enters into the body of an organism and is not excreted, but accumulated in the organism's tissues. Metals that are deposited in the aquatic environment may accumulate in the food chain and cause ecological damage, while also posing a threat to human health. Cancer and damage of the nervous system have been documented in humans as a result of metal consumption (Van den Broek *et al*., 2002).

Anthropogenic impacts including industrial discharge, domestic sewage, non-point source runoff and atmospheric precipitation are the main sources of the heavy metal pollution of aquatic ecosystems. It is often most obvious in sediments, macrophytes and aquatic animals, than in elevated concentrations in water (Linnik and Zubenko, 2000). Many aquatic organisms have been used as bioindicators, especially fish (Burger *et al*., 2002). Fishes, being major components of most aquatic habitats have also been recognized as good bio-accumulators of organic and inorganic pollutants (King and Jonathan, 2003). In addition, fish are located at the end of the aquatic food chain and may accumulate metals and pass them to human beings through food causing chronic or acute diseases (Al-Yousuf *et. al*., 2000).

Around the Yangon Region, water pollution is nearing hazardous levels as waste water and chemicals from factories and industrial zones are increasingly discharged into the rivers (Kyi Wai, 2009). **According to research from the Green Motherland Development Association (GMDA) (2015), the level of organic pollutants in waste water discharged from the Hlaing Tharyar and Shwe Pyi Thar industrial zones into Hlaing River was higher than standard specifications of WHO.**

A few research works on water pollution were carried out in Hlaing River based on the impact of industrial zones (DMR, 2013; Mya Thandar, 2014; GMDA, 2015). The academic studies on the contamination of heavy metals in fish have not been conducted yet so far in the Hlaing River.

In the study area, the small fisheries were carried out for selling in the markets of Insein, Shwe Pyi Thar, Hlaing and adjacent townships. Therefore, the present study aimed to access the pollution of Hlaing River with the following objectives:to detect the concentration of some heavy metals in the muscle of commercial fishes collected from Hlaing River Segment in Shwe Pyi Thar Township, Yangon Region, to access the possible potential human risk for consumption compared with FAO/WHO (1992) standard and, to seek the relationships of heavy metal bioaccumulation in fish muscle with size, age and feeding types, seasons and condition factor of fishes.

Materials and Methods

Study area

Study area was the Hlaing River segment situated in Shwe Pyi Thar Township, Yangon Region, and located between 16° 58' N, 96° 02' E, and 16° 55' N, 96° 04' E. Total distance of Hlaing River segment of the study area was approximately 8.19km in length (Figure 1).

Study periods

Study period lasted from April 2015 to March 2017.

Target fish species

The target fish species were the commercial fishes in the study area such as *Otolithoides pama* (Hamilton, 1822) (Pama croaker, Nga-poke-thin), *Polynemus paradiseus* Linnaeus, 1758 (Paradise threadfin, Nga-pon-nar), *Mystus spp.* (Hamilton, 1822) (Dwarf catfish, Nga-zin-yaine), *Cirrhinus cirrhosus* (**Bloch, 1795**) (Mrigal carp, Nga-gin-lone), *Illisha megaloptera* (**Swainson, 1839)** (Big eye illisha, Nga-zin-byarr), *Silonia silondia* (Hamilton, 1822) (Silond catfish, Nga-myin) and *Pangasius hypophthalmus* (Sauvage, 1878) (Striped catfish, Nga-dan). These fishes were available in all the year round in the study area and abundant enough for selling in the markets. They were found to be different habitats and food types according to Talwar and Jhingran (1991) (Table 1).

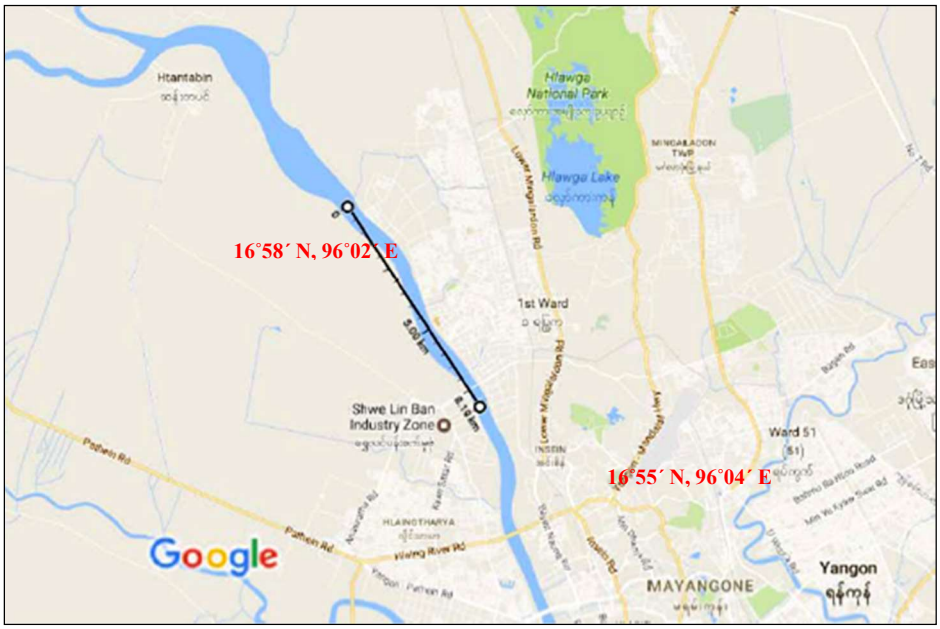

Figure 1. Location map of the study area (Source: Google Map, 2016)

Table 1. Habitats and feeding types of target commercial fishes

Scientific name	Common name	Myanmar name	Habitat	Feeding type
O. pama	Pamacroaker	Nga-poke-thin	Benthopelagic	Carnivore
P. paradiseus	Paradise threadfin	Nga-pon-nar	Demersal,	Carnivore
I. megaloptera	Bigeyeillisha	Nga-zin-byarr	Benthopelagic	Omnivore
C. cirrhosus	Mrigal carp	Nga-gin-lone	Benthopelagic	Herbivore
Mystus spp.	Dwarf catfish	Nga-zin-yaine	Demersal,	Carnivore
S. silondia	Silond catfish	Nga-myin	Demersal,	Omnivore
P. hypophthalmus	Striped catfish	Nga-dan	Benthopelagic	Omnivore

Specimen collection

Fish specimens were purchased from local fishermen in the study area. A total of 231 specimens as a ratio of three specimens for each commercial fish species per month were collected during the study period. Specimen collections were carried out from June 2015 to January 2017. Some months in

the rainy season were excluded from specimen collection due to the increasing of water level in the study area when the fishing activities were ceased temporally. Collected specimens were kept in the ice box and bring to the laboratory of Zoology Department, West Yangon University for further study.

Examination of recorded specimen

In the laboratory, collected fish specimens were identified the species according to the statement of Talwar and Jhingran (1991). Standard length and body weight were measured to calculate the condition factor of the fish. Adult and young fish were also categorized according to the development of reproductive organs. Size of fish was categorized as small size (<10cm), medium size (>10cm-<20cm) and large size (>20cm).

Preparation for heavy metal test

Collected fish specimens were skinned and approximately 50g of the axial muscles were cut out from the fish and weighted. Then, flesh of fish was cut into slices for dry rapidly. Consequently, fish slices were dried in drying oven at 60˚C overnight. Each dry specimen was weighted again and kept in separate polyethylene bag and stored in the refrigerator at 20˚C before heavy metal test. Code number of each specimen, collection date, wet weight and dry weight were labelled on the respective bag. Each specimen was homogenized by using electric blander before conducting heavy metal test.

Method of heavy metal test

The heavy metal concentration of each sample was assessed by Energy Dispersive X Ray Fluorescence spectrometer (EDXR) analysis at Department of Physics, University of Mandalay.

Bioaccumulation factor

The bioaccumulation factor (BAF) is the ratio between the accumulation of a given pollutant in any organ and dissolved concentration in water according to Authman and Abbas (2007).

$$BAF = Con_{fish} / Con_{water}$$

Con_{fish} = pollutant concentration in fish tissue (mg/kg)

Con_{water} = pollutant in water (mg/l)

The parameter is zero if the element accumulates only from the water.

If the BAF is greater than 1.0 then bioaccumulation for metals occurs by fish species (Aboul Ezz and Abdel-Razek, 1991).

Condition factor

Fulton's condition factor (K) was calculated according to Bagenal (1978) as follows: $K= 100\ W/L^3$

Where W is the total body weight in grams and L is standard length in centimeters.

Heavy metal level limit for human consumption

Standards of heavy metal safety guideline consumption for fish muscle and water were followed after FAO/WHO (1992) and WHO (1993) standards, respectively.

Statistical analysis

Recorded data were statistically analyzed using SPSS Version 16. Concentrations of heavy metals were presented as mean and standard deviation. Variation of heavy metal concentrations in fish muscle among different sizes of fish, sexes and different seasons were analyzed using ANOVA and presented with bar graph. Relation of heavy metal concentrations with size, age, condition factor, feeding habits, and also seasons were analyzed using Pearson's correlation coefficient test.

Results

Altogether 231 fish specimens were analysed by Energy Dispersive X Ray analysis (EDXR), and the results showed that 15 heavy metals including essential and toxic metals were detected in the muscles of tested fish samples.

Among detected heavy metals, Aluminium (Al), Chromium (Cr), Cadmium (Cd), Arsenic (As), Nickel (Ni), Mercury (Hg) and Lead (Pb) were analysed in detail since these metals were the suspected waste from industrial zones and highly hazardous to organisms.

Variation of heavy metal concentrations were observed among studied fish species. Some heavy metal concentrations in the studied fish muscles were exceeding the permissible limit of FAO/WHO (1992) standard as Al, Cr, As and Hg concentrations in *Polynemus paradiseus*; Al, Cr, As concentrations in *Otolithoides pama*; Al, Cr, and Hg concentrations in *Silonia silondia*; Al and Cr concentrations in *Illisha megaloptera*, *Cirrhinus cirrhosus*, *Mystus spp.* and *Pangasius hypophthalmus* (Table 2).

Table 2. Heavy metal concentrations in the muscle of some commercial fishes in the study area during the study period (ppm in wet weight)

Species name	**Heavy metal concentrations in wet weight (ppm)**						
	Al	**Cr**	**Cd**	**A**	**Ni**	**Hg**	**Pb**
Otolithoides pama	**354.09** ± 41.68	**1.43** ± 0.17	0.33 ± 0.04	**0.98** ± 0.12	0.04 ± 0.01	0.31 ± 0.04	0.20 ± 0.02
Polynemus paradiseus	**335.78** ± 40.14	**1.74** ± 0.20	0.21 ± 0.02	**0.91** ± 0.10	0.11 ± 0.01	**0.55** ± 0.06	1.14 ± 0.13
Illisha megaloptera	**522.15** ± 44.58	**1.18** ± 0.10	0.43 ± 0.04	0.47 ± 0.04	0.06 ± 0.01	0.37 ± 0.03	0.21 ± 0.02
Cirrhinus cirrhosus	**393.06** ± 92.95	**1.70** ± 0.04	0.21 ± 0.05	0.12 ± 0.03	0.12 ± 0.03	0.40 ± 0.09	0.24 ± 0.06
Mystus spp.	**421.61** ± 56.48	**1.71** ± 0.23	0.12 ± 0.02	0.34 ± 0.05	0.17 ± 0.02	0.48 ± 0.06	0.22 ± 0.03
Silonia silondia	**381.44** ± 56.35	**1.94** ± 0.27	0.49 ± 0.07	0.32 ± 0.29	0.10 ± 0.01	**0.53** ± 0.08	0.25 ± 0.03
Pangasius hypophthalmus	**306.12** ± 84.33	**1.62** ± 0.45	0.45 ± 0.12	0.38 ± 0.11	0.07 ± 0.02	0.41 ± 0.11	0.45 ± 0.12
FAO/WHO (1993)	100	0.5	0.5	0.5	0.8	0.5	2.0

Condition factor of studied fishes indicated that majority of studied fishes were in good condition (K> 1.00). Exception was observed in *I. megaloptera* in which under good condition criteria as 0.82 was observed (Figure 2).

Pearson's correlation coefficient analysis showed that all tested heavy metals were negatively correlated with condition factors of studied fishes. In *Illisha megaloptera* and *Cirrhinus cirrhosus*, concentrations of all tested heavy metals were significantly negative correlated with condition factors of those fishes. In *Otolithoides pama*, chromium and lead concentrations were significantly negative correlated with its condition factor (Table 3).

The water samples in the study area indicated that the tested heavy metals concentrations were found to be exceeding the drinking water standard of WHO (1993) (Table 4).

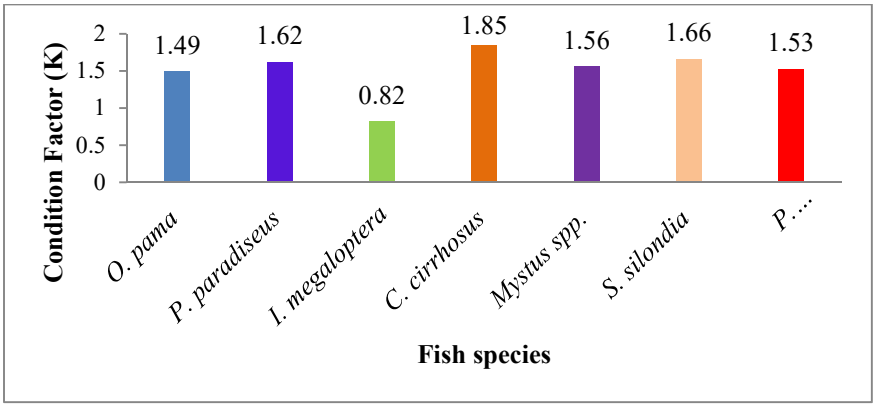

Figure 2. Condition factors of tested fish species

Table 3. Relationship between heavy metals concentrations of fish muscles and condition factors of studied fishes

Species	Coefficient of correlation (r)						
	Al	**Cr**	**Cd**	**As**	**Ni**	**Hg**	**Pb**
O. pama	- 0.740	- 0.758*	- 0.749	- 0.722	- 0.742	- 0.751	- 0.758*
P. paradiseus	- 0.380	- 0.319	- 0.283	- 0. 188	- 0.349	- 0.355	- 0.363
I. megaloptera	- 0.981*	- 0.982*	- 0.949*	- 0.977*	- 0.866*	- 0.976*	- 0.965*
C. cirrhosus	- 0.771*	- 0.767*	- 0.795*	- 0.795*	- 0.851*	- 0.790*	- 0.810*
Mystus spp.	- 0.144	- 0.256	- 0.235	- 0.095	- 0.182	- 0.091	- 0.389
S. silondia	- 0.202	- 0.385	- 0.345	- 0.321	- 0.183	- 0.404	- 0.329
P. hypothalmus	- 0.244	- 0.321	- 0.374	- 0.400	- 0.383	- 0.386	- 0.374

*Correlation is significant at the 0.05 level (2-tailed).

Table 4. Comparison of heavy metal concentration in the study site and drinking water standard of WHO (1993)

Description	Heavy metal concentrations (ppm)						
	Al	Cr	Cd	As	Ni	Hg	Pb
Drinking water standard WHO (1993)	20	0.05	0.003	0.01	0.03	0.001	0.01
Water sample in the study site	30	0.6	0.03	0.11	0.04	0.08	0.12

Among the tested heavy metals, the highest bioaccumulation factor of aluminium (17.41) was observed in the muscle of *I. megaloptera*, followed by those of *Mystus spp.*, *C. cirrhosus*, *S. silondia*, *P. paradiseus*, *O. pama*, and *P. hypophthalmus* (Table 5).

In chromium test, the highest bioaccumulation (3.23) was observed in *S. silondia*, followed by *P. paradiseus*, *Mystus spp.*, *C. cirrhosus*, *P. hypophthalmus*, *O. pama*, and *I. megaloptera* (Table 5).

Table 5. Bioaccumulation factors of studied fish species

Species	Bioaccumulation factors						
	Al	Cr	Cd	As	Ni	Hg	Pb
Otolithoides pama	11.80	2.38	11.00	**8.91**	1.33	3.88	1.67
Polynemus paradiseus	11.86	2.89	6.85	8.30	3.81	**6.85**	**9.51**
Illisha megaloptera	**17.41**	1.97	14.33	4.27	2.00	4.63	1.75
Cirrhinus cirrhosus	13.10	2.83	7.00	1.09	4.00	5.00	2.00
Mystus spp.	14.05	2.85	4.00	3.09	**5.67**	6.00	1.83
Silonia silondia	12.71	**3.23**	**16.33**	2.91	3.33	6.63	2.08
Pangasius hypophthalmus	10.20	2.70	15.00	3.45	2.33	5.13	3.75

In cadmium test, the highest bioaccumulation (16.33) was observed in *S. silondia*, followed by *P. hypophthalmus*, *I. megaloptera*, *O. pama*, *C. cirrhosus*, *P. paradiseus*, and *Mystus spp.* (Table 5).

In arsenic test, the highest bioaccumulation (8.91) was found in *O. pama*, followed by *P. paradiseus*, *I. megaloptera*, *P. hypophthalmus*, *Mystus spp.*, *S. silondia*, *C. cirrhosus* (Table 5).

In nickel test, the highest bioaccumulation (5.67) was found in *Mystus spp.*, followed by *C. cirrhosus*, *P. paradiseus*, *S. silondia*, *P. hypophthalmus*, *I. megaloptera*, and *O. pama* (Table 5).

In mercury test, the highest bioaccumulation (6.85) was found in *P. paradiseus*, followed by *S. silondia*, *Mystus spp.*, *P. hypophthalmus*, *C. cirrhosus*, *I. megaloptera*, and *O. pama* (Table 5).

In lead test, the highest bioaccumulation (9.51) was found in *P. paradiseus*, followed by *P. hypophthalmus*, *S. silondia*, *C. cirrhosus*, *Mystus spp.*, *I. megaloptera*, and *O. pama* (Table 5).

In the carnivorous fishes, bioaccumulation factors of three metals as As, Hg and Pb were found to be higher than herbivorous and omnivorous fishes. In the herbivorous fishes, bioaccumulation factors of two metals as Al and Ni were found to be higher than carnivorous and omnivorous fishes. In the omnivorous fishes, only one metal, Cd was the higher than carnivorous and herbivorous fishes (Figure 3A).

Bioaccumulation factors of demersal fishes were higher in most of the tested metals except in arsenic which showed nearly the same in both demersal and benthopelagic fishes (Figure 3B).

Among the studied fishes, higher bioaccumulation factor was observed in adult fish group than young fish group. Significant variation was found in majority of tested metals as Al, Cd, As, Hg and Pb (Figure 3C).

Variations of bioaccumulation factors of tested heavy metals among different size of fishes were observed. Bioaccumulation factors of Cd and As were found to be higher in large sized fishes, while BAF of Al and Pb were higher in medium sized fishes. Small sized fishes showed slightly higher BAF values in Cr, Ni and Hg than other sized groups (Figure 3D).

(A) Among feeding habits (B) Among different habitats

(C) Among young and adult fishes (D) Among different size

(E) Among scaled and scale-less fishes (F) Among season

Figure 3. Comparison of BAF with some descriptive account of studied fishes and seasons

Some tested heavy metals such as Cr, Cd and Hg were found to be higher bioaccumulation factors in scale-less fishes such as *Mystus spp.*, *S. silondia* and *P. hypophthalmus*. However, bioaccumulation factors of Al, As,

Ni and Pb were higher in scaled fish such as *O. pama*, *P. paradiseus*, *I. megaloptera* and *C. cirrhosus* (Figure 3E).

Bioaccumulation factors of all tested heavy metals were found to be higher in the dry season than other seasons. Significant variation was observed in cadmium as the ranking order of dry>cold>rainy seasons (Figure 3F).

Mean bioaccumulation factor of tested heavy metals was significantly negative correlated with condition factor of studied fishes ($r= -0.641$, $p<0.05$), while those bioaccumulation factor was not significantly correlated with seasons, feeding type of fish (carnivores, herbivores or omnivores), present or absent of scales, habitat types (demersal or benthopelagics) and the size of fish.

Discussion

So far, fish consumption become popular as the first choice among the people all over the world due to their nutritive and economical values which are attributed to its good and cheap, source of protein and minerals, richness in non-saturated fatty acids and Omega-3 as stated by Erkkilä *et al*. (2004). Unfortunately, the habitats of fishes became polluted in worldwide due to the progress of industries which led to increased emission of pollutants as heavy metals into ecosystems. Environmental pollution can cause poisoning, diseases and even death for fish. As xenobiotics, some of these pollutants sometimes find their way into the human system through the food chain (Gabriel *et al*., 2006). In the present study, heavy metal analysis was conducted in edible muscle tissues of 231 specimens belonging to seven commercial fish species were analysed for accessing the safety consumption on fishes in the study area.

In the present study, heavy metal concentrations in studied fish muscles and those of the surface water in the study area were significantly correlated. It indicated that the pollutant heavy metals in the water enter the Hlaing River ecosystem and bioaccumulation took place in the studied fishes. The tested heavy metals assumed to enter the study area by anthropogenic activities such as industrial wastes, chemical fertilizers and pesticides used in agricultures, sand and gravel digging in the river and storage at the river bank, fuel discharged from large vessels, etc. This finding is in agreement with the

statement of Zeitoun and Mehana (2014) that industrial wastes are potential source of heavy metal pollution in aquatic environments.

The majority of tested heavy metals were observed to be high concentration in the muscles of all studied commercial fish species, whereas aluminium, chromium, cadmium, arsenic, and mercury were exceeding the permissible limits of FAO/WHO (1992) standard for human consumption. Besides, surface water in the study area was polluted with tested heavy metals exceeding the permissible limit of the drinking water standard of WHO (1993). Therefore, people who consumed these highly contaminated fishes with heavy metals seemed to be also affected and can cause the respective health problems.

In the study area, nearly all studied fish species were in good condition showing condition factor values exceeding the critical value (K=1), although condition factors of those fishes were found to be negatively correlated with the concentration of heavy metals in their muscles. However, *Illisha megaloptera* was not in good condition in the study area due to the pollution of heavy metals as indicating significant negative correlation with the heavy metal concentrations in their muscles. This finding is in coincidence with the finding of Hashim *et al*. (2014) in the Kelantan River of Malaysia.

In all studied fish species, high bioaccumulation factor value were observed and greater than critical value (BAF=1), so as tested heavy metals were accumulated by fish showing long term exposure of heavy metals to fish in its surrounding. In addition, all tested heavy metals were significantly correlated with those in surface water of the study area. Besides, the bioaccumulation factors of demersal fishes were higher in most of the tested metals than benthopelagic fishes. This result indicated that most of the tested metals as Al, Cr, Cd, Ni, Hg and Pb were seem to be highly polluted with industrial and agricultural wastes since long been.

The seasonal variations of bioaccumulation factors were observed in studied fish species while bioaccumulation factors of all tested heavy metals were found to be higher in the dry season than other seasons. It is seemed to be fact that the heavy metal concentrations in river water were elevated by increasing temperatures and also some anthropogenic activities especially sand and gravel excavations in the river increased in the dry season. Besides,

the low heavy metal bioaccumulation during the rainy season was due to the dilution of heavy metals by heavy rain resulting increased water levels in the study area. This finding is similar to the finding of previous studies (Idodo-Umeh, 2002, and Oguzie, 2003).

In the study area, feeding habits of studied fishes were not correlated with the bioaccumulation factors of all tested heavy metals. However, the previous studies stated that the feeding habits of herbivorous, carnivorous and omnivorous fish were significantly different in heavy mental concentrations (Voigt, 2004 and Weber *et al*., 2013). The finding of the present study was contrast with the finding of previous authors. The feeding habits of studied fishes could not be the main factor of bioaccumulation of tested heavy metals in the study area. It is possible that the heavy metals in studied fish muscle came directly from water current through the gill lamellae and reached into the circulatory system and bioaccumulated in the muscles. Since bioaccumulations of tested heavy metals were higher in both demersal and benthopelagic fishes, the whole ecosystem was seem to be polluted. The same trend were expected in their food sources both plants and animals.

Among the studied fishes, adult fishes were found to be higher bioaccumulation factor than young fishes. This finding is in coincidence with the finding of the previous authors (Ahmad and Suhaimi-Othman, 2010, Hashim *et al*., 2014). They found that mature fish accumulated higher metals compared to juvenile and premature fish. It is due to the fact that adult fishes were living in continuous polluted habitats and bioaccumulation was also greater than young fishes.

In the present study, bioaccumulation of Cr, Cd and Hg were higher in scale-less fishes than scaled fishes. According to Hashim *et al*. (2014), these heavy metals were the sources from pesticides and chemical fertilizers used in agriculture. Therefore, the studied scale-less fishes probably came from the upstream of Hlaing River nearby agriculture land. In addition, bioaccumulation of Al, Ni, As and Pb were higher in scaled fish than scale-less fishes. These heavy metals were the possible sources of industrial wastes, erosion, dissolution of minerals and salts, atmospheric dust pollution and rain according to Ismailand Saleh (2012) in Malaysia. Therefore, these scaled fish

assumed to be came from the downstream of Hlaing River nearby many industries and sand and gravel excavation activities.

In Hlaing River, bioaccumulation of Cr, Ni and Hg were higher in small sized fishes, those of Al and Pb were higher in medium sized fishes and those of Cd and As were higher in large sized fishes. However, the size of the fishes was not correlated with bioaccumulation factors of tested heavy metals. Besides, bioaccumulation factors of tested heavy metals were not significantly different among the size of the fishes. Therefore, all sized of fishes were nearly the same potential of heavy metal contamination in the study area during the study period.

Conclusion

In conclusion, the high concentrations of heavy metals were observed in the muscle of all studied commercial fishes. It is noticeable that aluminium, chromium, cadmium, arsenic, and mercury were exceeding the permissible limits of FAO/WHO (1992) standard for human consumption. Like in other organisms, heavy metals are not destroyed by humans, so as they tend to accumulate within the body and threaten the health of fishes and consumers of them. Therefore, everyday consumption of studied fish species in the Hlaing River may cause health problems if bioaccumulation continues in the same rate without taking effective management for pollution in the Hlaing River. Regular monitoring of environmental parameters should be carried out as a key activity in not only managing the restoring polluted environments but also anticipating the effects of anthropogenic activities in the study area.

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SPECIES DIVERSITY OF BUTTERFLIES IN NYAUNG SHWE ENVIRONS, SOUTHERN SHAN STATE

Ei Ei Ko

Abstract

A total of 4358 butterflies representing 74 species, confined to 52 genera and five families were recorded during the study period from June 2014 to May 2015 at Nyaung Shwe environs. Among the families recorded namely, Papilionidae (10 species), Pieridae (14 species), Nymphalidae (35 species), Lycaenidae (9 species) and Hesperiidae (6 species), the highest number of species was recorded in Nymphalidae and lowest in Hesperiidae. At Nyaung Shwe environs the highest diversity indices value was found in November while the lowest indices value was occurred in June at both Site A and B. The 74 butterfly species and their host plants recorded thrive well in both the study sites. It is suggested that these butterflies should be conserved for further studies on butterflies.

Keywords: butterfly, species, Nyaung Shwe environs

Introduction

Lepidoptera means the scaly wing insects. A very large order Lepidoptera includes some of the most beautiful species and some of the economically important pests in class Insecta (Ozden, 2003).

The second largest order Lepidoptera is divided into two suborders, the butterflies (Rhopalocera) and the moths (Heterocera). The distribution of butterfly's species is subject to the availability of its preferred habitats, which are frequently determined by larval food plants, adult nectar sources and altitude or elevation above sea level (EK-Amnuay, 2012).

The degree of diversity depends upon the adaptability of a species to a particular micro habit. The dimension, population size and diversity of the species are most significant biological elements of an ecosystem (Kumar, 2013).

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Biological conservationists believe that butterflies are important as bio-indicators over all the continental land masses. Lepidopteron butterfly species are sensitive to the environmental stresses on their breeding biological situations.

Nyaung Shwe environs harbour a rich variety of butterfly species however, remained unexplored. Therefore the present study has been undertaken with the following objectives: to record the different kinds of butterfly species and to assess the diversity of the butterfly species recorded in Nyaung Shwe environs.

Materials and Methods

Study Area

Nyaung Shwe environs, the study area is located in eastern part of Nyaung Shwe Township in Southern Shan State. Two study sites were designated, Site A and B. Hta-ein-gon village (Site A), covers a land area of 0.138 square km and lies between 20º 39' 23" - 20º 39' 42" N and 96º 58' 18" - 96º 58' 48" E. The elevation is approximately 1040 m above sea level.

Kan-daw village (Site B), constitutes a land area of 0.150 square km and lies between 20º 38' 55" - 20º 39' 9" N and 96º 57' 2" - 96º 57' 25" E. The elevation is approximately 900 m above sea level (Fig. 1).

Study Period

The study period was from June 2014 to May 2015.

Collection of the Butterfly Specimen

Different habitats were selected for collection of butterflies. The foot trails in the village, the forested area and area along the bank of the stream were involved in the sites as walk transects and approximately 150 m in length. The observer travels slowly along the trails and butterflies within 5 m of both sides were counted according to Pollard (1977). Some butterflies were captured by using butterfly nets.

Butterfly collections were conducted twice a month at two study sites. Butterfly observation and collection was made between 9:00 am to 3:00 pm.

Preparation of the Specimen

The collected specimen was killed by squeezing on the thorax with the finger tip and then mounted on the setting board for a day before identification. After taking a photo, it was kept inside the insect box with the naphthalene balls to ward off ants.

For description of butterfly species and to measure the wing span five individuals were used for each species. For rare species, only one individual was used.

Identification and Classification of Collected Specimem

Identification of the butterflies was made with reference to Talbot (1939, 1947), Pinratana (1983), and classification was followed after Corbet and Pendlebury (1992). Classification of plants was followed by Hundley (1962).

Analysis of the Data

Diversity index of butterfly species were calculated by the Shannon-Wiener index (H') and Simpson index (D) and then the species richness (d) is calculated by the Margalef's index (d) as given in Ludwig and Reynolds (1988).

Shannon-Wiener's diversity index (1949) formula is given as follow:

$$H' = -\sum_{i=1}^{S}(P_i \ln P_i)$$

$$P_i = \left(\frac{n_i}{n}\right)$$

P_i = Total number of "i" species

n_i = Number of individuals in the "i^{th}" species of the sample

n = Total number of individuals of all species in the sample.

A great number of species increase species diversity, and a more even or equitable distribution among species will increase species diversity measured by Shannon-Wiener's function.

Simpson's index (1949) is given as follows;

$$D = \sum_{i=1}^{S} \frac{n_i(n_i - 1)}{n(n-1)}$$

D = Simpson's index

n_i = Number of individuals in the "i^{th}" species in the sample

n = Total number of individuals in the sample

Simpson's index is given little weight to the rare species and more weight to the common species. It value ranges from 0 to 1, where "s" is given to the number of species.

Margalef's Species Richness Index (1958) is given as follows

$$d = \frac{S-1}{\ln N}$$

d = Margalef's species richness index

S = Number of species

N = Total number of individuals

This method is incorporated to the total number of individuals and is the measure of the number of species present for a given number of individuals.

Hill's diversity numbers (1973) is given as follows:

Number 0 : $N_0 = S$

S = Total number of species

N_0 = Number of all species in the sample

Number 1 : $N_1 = e^{H'}$

H' = Shannon - Wiener's index

N_1 = Number of abundant species in the sample

Number 2 : N_2 = 1/D

D = Simpson's index

N_2 = Number of very abundant species in the sample

N_1 always intermediate between N_0 and N_2.

Effective number of species is a measure of the number of species in the sample where each species is weighted by its abundance.

Butterfly species evenness of equitability (or relative species abundance) was determined by the evenness index of modified Hill's ratio (1973), which is given as follows:

$$E = \frac{\left(1/D\right)-1}{e^{H'}-1} = \frac{N_2-1}{N_1-1}$$

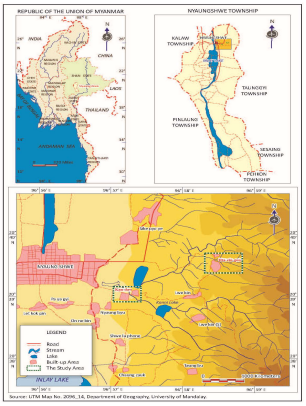
E = Hill's evenness index (which approach zero)

D = Simpson's index

H' = Shannon-Wiener's index of species diversity

N_1 = Number of abundant species in the sample

N_2 = Number of very abundant species in the sample



Result

All together 4358 butterflies accounted from 74 species and confined to 52 were recorded during the study period from June 2014 to May 2015.

The 74 butterflies species recorded during the study constitute 10 species of Papilionidae (13.51%), 14 species of Pieridae (18.92%), 35 species of Nymphalidae (43.3%), nine species of Lycaenidae (12.16%) and six species of Hesperiidae (8.11%), collected from two study sites of Hta-ein-gon village (Site A) and Kan-daw village (Site B) in Nyaung Shwe, Southern Shan State during the study period from June 2014 to May 2015 (Fig. 2).

Monthly occurrence of butterflies species and individuals were revealed to be highest in number during October and November, however only four swallowtailed butterflies *Papilio machaon* was collected in November, December and January. The genus *Pieris* was observed through the year, especially *Pieris brassicae* occurred is highest number as abundant species. Nymphalids were encountered not only in sunny places but also in shady area of trees, herbs and sometimes even on the ground. Peak number of species and individuals were significant in October, November and December at both sites (Fig. 3, 4).

Seasonal occurrence of butterfly species and individuals were observed to peak in cold season at Site A with 1268 individuals accounted from 64 species and at Site B with 1080 individuals from 61 species belonging to the families: Papilionidae, Pieridae, Nymphalidae, Lycaenidae and Hesperiidae (Fig. 5, 6).

Throughout the study period from June 2014 to May 2015 and the total number of species and individuals at study sites A and B and their corresponding species richness (d), diversity indices of Shannon-Wiener index (H') and Simpson's index (D) and evenness (E) were shown in Tables.

Comparison on the diversity indices and the seasonal occurrence of butterfly fauna revealed the highest species richness with 8.8171 at Site A in cold season. The value of Shannon’s-Wieners index, Simpson's index, N1, N2 and evenness (3.8735, 0.0241, 48.1114, 41.4552 and 0.8587) respectively were found to be highest in species richness and abundance in cold season 2014, compared to those of the rainy and hot season since the indices for d, H', D, N1 and N2 (6.3234, 3.2824, 0.0466, 26.6388 and 21.4524) respectively

were lowest in rainy season 2015 and evenness value with 0.7881 was low in hot season 2015 (Table 1).

Similarly seasonal indices of butterfly species d, H', D, N1, N2 and E with 8.5902, 3.7632, 0.0269, 43.0874, 36.1998 and 0.8601 respectively were found to be highest in species richness and abundance in the cold season 2014 at Site B. The lowest diversity indices d, H', D, N1 and N2 (6.1843, 3.277, 0.0455, 26.4956 and 21.9698) respectively appeared in rainy season and so also the evenness value with 0.7947 was lowest in hot season (Table 2).

Table 1. Comparison of diversity indices in the seasonal occurrence at Hta-ein-gon village (Site-A) in Nyaung Shwe (June 2014-May 2015)

Site-A	Rainy Season	Cold Season	Dry Season
Total no. of individuals	477	1268	549
Total no. of species	40	64	56
d	6.3234	8.8171	8.7189
H'	3.2824	3.8735	3.5592
D	0.0466	0.0241	0.0358
N1	26.6388	48.1114	34.1372
N2	21.4524	41.4552	27.9032
E	0.7977	0.8587	0.7881

Table 2. Comparison of diversity indices in the seasonal occurrence at Kan-daw village (Site-B) in Nyaung Shwe (June 2014-May 2015)

Site -B	Raining Season	Cold Season	Dry Season
Total no. of individuals	548	1080	436
Total no. of species	40	61	42
d	6.1843	8.5902	6.746
H'	3.277	3.7632	3.3562
D	0.0455	0.0269	0.0435
N1	26.4956	43.0874	28.6978
N2	21.9698	36.1998	23.0114
E	0.8225	0.8601	0.7947

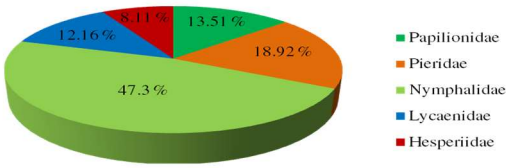


Fig. 2 Composition of butterfly species in different families at two study sites (June 2014-May 2015)

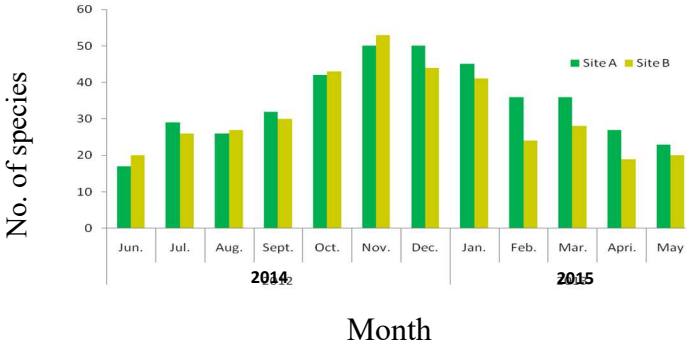


Fig. 3 Monthly occurrence of butterfly species at two study sites (June 2014-May 2015)

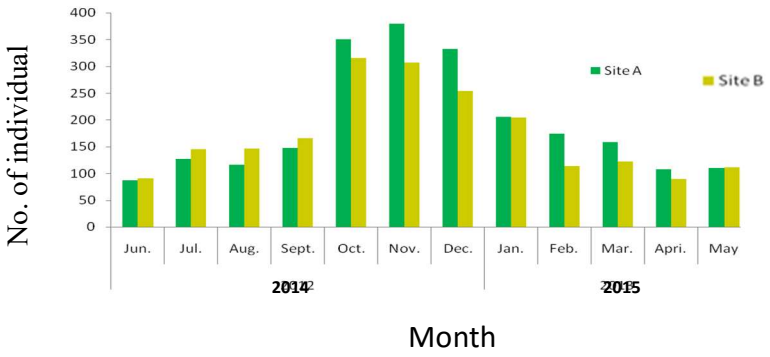


Fig. 4 Monthly occurrence of butterfly individual at two study sites (June 2014-May 2015)

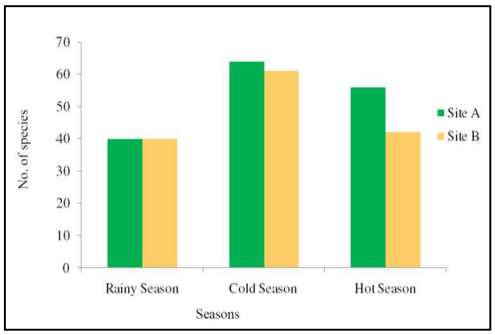


Fig. 5 Seasonal occurrence of butterfly species at two study sites of Nyaung Shwe environs (June 2014-May 2015)

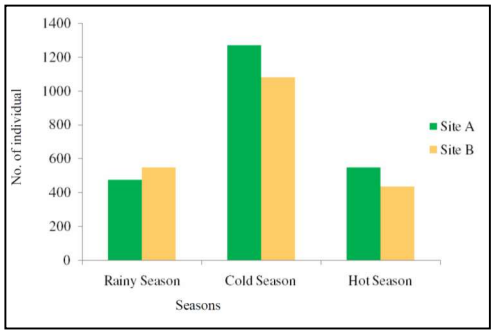


Fig. 6 Seasonal occurrence of butterfly individual at two study sites of Nyaung Shwe environs (June 2014-May 2015)

A. *Troides Helena*

B. *Pachliopta aristolochiae*

C. *Chilasa clytia*

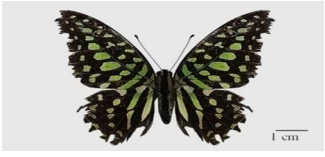
D. *Papilio demoleus*

E. *Papilio helenus*

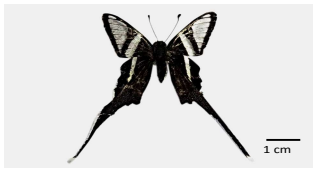
F. *Papilio*

G. *Papilio paris*

H. *Papilio machaon*



I. *Graphium agamemnon*



J. *Lamproptera curius*

K. *Delias pasithoe*

L. *Delias acalis*

M. *Delias decombesi*

N. *Pieris canidia*

O. *Pieris*

P. *Pontia daplidice*

Q. *Cepora nerissa*

R.*Hebomoia glaucippe*

Plate 1 Butterfly species in Nyaung Shwe environs

S. *Pareronia anais*

T. *Catopsilia pyranthe*

U. *Catopsilia pomona*

V. *Catopsilia scylla* W. *Eurema hecabe* X. *Eurema ada*

Y. *Danaus genutia* Z. *Tirumala limniace* AA. *Parantica*

AB. *Euploea core* AC. *Euploea klugii* AD. *Melanitis*

AE. *Elymnias casiphone* AF. *Elymnias malelas* AG. *Lethe*

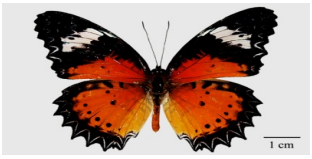
AH. *Acraea terpsicore* AI. *Acraea issoria* AJ. *Ariadne ariadne*

Plate 1 Continued

AK. *Phalanta palantha*

AL. *Vagrans egista*

AM. *Vindula erota*



AN. *Cethosia cyane*

AO. *Junonia iphita*

AP. *Junonia atlites*

AQ. *Junonia lemonias*

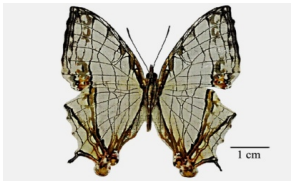
AR. *Junonia almona*

AS. *Junonia orithya*

AT. *Junonia hierta*

AU. *Hypolymnas bolina*

AV. *Kallima*



AW. *Cyrestis thyodamas*

AX. *Neptis sappho*

AY. *Neptis columella*

AW. *Cyrestis thyodamas* AX. *Neptis sappho* AY. *Neptis*

Plate 1 Continued

BC. *Tanaecia julii* BD. *Polyura athamas* BE. *Polyura*

BF. *Charaxes solon* BG. *Charaxes bernardus* BH. *Abisara*

BI. *Curetis bulis* BJ. *Castalius rosimon* BK. *Caleta elna*

BL. *Chilades pandava* BM. *Jamides elpis* BN. *Loxura atymnus*

BO. *Cheritra freju*

BP. *Talicada nyseus*

BQ. *Celaenorrhinus*

BR. *Celaenorrhinus aurivittatus*

BS. *Odontoptilum angulatum*

BT. *Onryza meiktia*

BU. *Erionota torus*

BV. *Telicota linna*

Plate 1 Continued

Discussions

Butterflies are more distributed in different localities where well favorable conditions for their larval and adult food resources in habitats. Kinyon (2004) also mentioned that total numbers of butterflies 1146 species were collected and identified in Myanmar. Nowadays, the species of butterflies have still being distributed all over Myanmar.

In the present study, a total of 4358 buterflies accounted from 74 butterfly species confined to 52 genera were recorded from Nyaung Shwe environs, Southern Shan State. Among five families recorded, Nymphalidae was the dominant family represented by (35 species, 47.3 %), followed by Pieridae (14 species, 18.92 %), Papilionidae (10 species, 13.51 %) and Lycaenidae (9 species, 12.16 %) and the last dominant family was Hesperiidae (6 species, 8.11 %) during the period from June 2014 to May 2015. Thus Nymphalids appeared as predominant butterflies in the study area

Under the genus *Papilio*, three species namely, *Papilio demoleus*, *Papilio polytes* and *Papilio paris* were recorded in large number. The abundance for these species is alluded to the abundance of host plants, including flowering plants and flowering herbaceous trees such as sein-na-ban (*Lantana aculeate*) are present among human dwellings from which nectar was collected by the butterflies. Moreover *Pieris brassicae* were also encountered abundantly as their major host plant, *Brassica* spp. appeared as major cultivated plants in the study area. *Telicada nyseus* was also recorded in large number in both the study sites but more common in Hta-ein-gon village (Site A) where their host bryophyllum plants occurred abundantly. In contrast, some species such as *Papilio machaon* appeared as rare, since only four specimens were collected from Site A during the wholes study period. Similarly, a single specimen each of *Acraea issoia* from Site B and *Modusa procris* and *Celaenorrhinus aurivittatus* from Site A respectively were recorded. The rareness of these species in the study area may rest upon the specificity in their choice for food and host plant.

During the study, highest number of species and individuals were revealed in November with 50 species and 379 individuals at Site A and 53 species and 307 individuals in Site B alluded to the presence of abundant food sources and place of shelter, following the rainy season whence vegetation thickened as new sprouting appeared followed by flowering and fruiting.

Magurran (2004) stated that biological diversity can be divided into two components: species richness and species evenness. Species richness measure that focus on the components of diversity.

Margalef's index revealed that the highest species richness was observed in cold season at Site A with 8.8171 (64 species) and at Site B with 8.5902 (61 species). Thus it appeared that the butterflies were similarly abundant at both the study sites, during the cold season.

Evenness index describes the variability in species abundance (Magurran, 2004). The result of the evenness index at Site A and Site B was approximately the same, at Site A (0.8587) and at Site B (0.8601) during the cold season. Thus it revealed abundance of species during the cold season; the environment was similarly eco-friendly to the butterflies at both the study sites.

When seasonal occurrence and diversity of butterfly species and individuals was considered the highest diversity indices value occurred in cold season because of the presence of more food sources during and after the rainy season, new sprouting appeared, the vegetation became lush green followed by flowering and fruiting providing of food sources for the butterflies to enjoy. Moreover impact of seasonal cultivation may not be ruled out, creating more chances for sustainability.

The result of present study indicated that the butterfly species appeared to thrive well in both the study areas. Thus, there is a need to maintain the environment friendlier for the butterflies to thrive even better.

Conclusions

Nyaung Shwe environ is one of the well known places in Southern Shan State, Myanmar. The result of present study indicated that Nyaung Shwe environ still harbor a variety of butterfly communities. Thus, there is a need to safeguard the sustainability of the diversity of butterflies' species by conserving their habitats in Nyaung Shwe environs.

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APPLICABILITY OF FISH SCALES NANOBIOMATERIALS IN HARD TISSUE ENGINEERING

Lei Lei Aye

Abstract

Extraction of fish scales biomaterials (from *Notopterus notopterus*) was carried out by burning the fish scales at different temperatures. These biomaterials were proved to be nano-sized hydroxyapatite by using X.R.D.(x-ray diffraction), F.T.I.R.(fourier transform infrared spectroscopy) and S.E.M.(scanning electron microscope). Purity of the sample was confirmed by T.G.A.(thermo-gravimetric analysis), protein content and Ca/P weight ratio. Porosity in the sample was calculated by image J software. Toxic heavy metal was not detected by mineral analysis. Evaluation of hardness of F.S.HAp-Glass Powder-GIC Composite was done by Mitutoyo micro-hardness testing machine. Hardness of pure Glass Ionomer (Japan) was found to be 3.5 Mohs while that of F.S.HAp-Glass Powder-GIC Composite was 4 Mohs. As the hardness is very near to teeth and skeletal tissue, it is suitable for substitution in tooth defect area.

Keywords: hydroxyapatite, *Notopterus notopterus*, Mohs, F.S.HAp-Glass Powder-GIC Composite

Introduction

Background of the Study

Nowadays, fishery by-products are subject to strict environmental regulations due to limited land and increased environmental concerns such as groundwater contamination and foul odor. Compliance with environmental standards and a better understanding of the potential values of processing by-products for a variety of applications have resulted in technological innovations for seafood wastes as nutraceuticals and functional foods. However, lack of adequate utilization of technology to convert such wastes into value-added products must be seriously addressed. Currently there is an growing interest in natural ingredients available from animal by-products to fulfill the needs of human being. Biomaterials are widely used in human body for tissue repair and substitution still expand to date. Biomaterials have recently been extracted from various calcium rich biowastes including corals, shells and vertebrate bones and the extracted biomaterials can be used in reconstruction of hard tissues.

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The aquatic biowastes, fish scale are biocomposites of highly ordered type I collagen fibers and hydroxyapatite (Fengxiang Zhang, anning wang,2011). Genetically, the same genes involved in tooth and hair development in mammals are also involved in scale development (Scale Anatomy,Fish scales, Wikipedia, the free encyclopedia).

Li Li, Haihua Pan, Jinhui Tao, Xurong Xu'(2008) stated that the hydroxyapatite is a "natural building blocks of enamel and 20nm-40nm sized hydroxyapatite particle can effectively remineralize the enamel.Their significant finding is poor adhesive ability of hydroxyapatite synthesized from Orthophosphoric acid solution. It suggests that the better biomaterials require a perfect biocompatibility to reduce the interface between inplanted biomaterials and natural materials.

Structure of Tooth

As with bone, different components of tooth (dentin, enamel, cementum) are distinguished. The periodontal ligament connects the tooth (via the cementum) to the underlying jawbone. The outer coating of the tooth as far as the gum line is enamel, a very hard material with little or no protein. Below the enamel is dentin, the major component of teeth. Separating the dentin from the surrounding jawbone is a bone-dentin composite material, cementum, and a periodontal membrane. The dentin surrounds a pulp cavity that holds the nerves and blood vessels necessary for tooth function.(Figure 1)

In tooth, collagen is the major organic constituent of dentin and cementum, but there is no collagen in enamel. Collagen is the same protein that gives flexibility to ligaments and tendons, but the addition of mineral to the collagen matrix makes it rigid and gives bones and teeth their greater load-bearing capacity. The mineral that reinforces bone and dentin matrices and the major constituent of enamel is an analogue of the mineral hydroxyapatite.

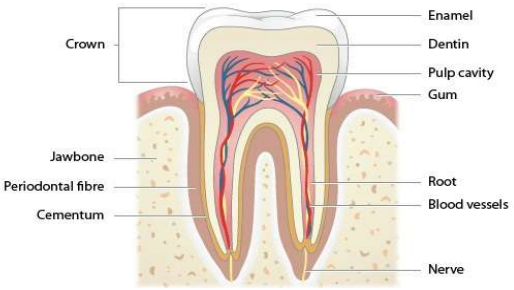


Figure1. Structure of Tooth(Source-Solanki Dental Centre)

Morphology and Component of Fish Scales

Fish scales exhibit large variations in shape, size and arrangement. Teleost scales are composed of collagen fibril type – I, and are partially mineralized with hydroxyapatite (16-59%) mineral content in weight. The outer layer of the scale is significantly more mineralized and often referred to as 'bony layer' . Whereas the inner layer 'basal' or 'collagen' layer is mineralized mostly near the bony layer, but with mineralization pockets proceeding well into the collagen layer. As hydroxyapatite of fish scales is similar to main component of teeth and bone, mineral component of fish scales can be applied in the hard tissues(teeth and skeletal tissues) engineering application of the human body.

The Properties of Glass Ionomer Cement

Glass Ionomer Cement (GIC) was invented by Wilson et. al at the Laboratory of the Government Chemist in early 1970. They are water-based cements, known as polyalkenoate cements. They possess restorative and adhesive properties- adhesion to moist tooth surface and base metals, anticariogenic propertites due to release of fluoride, thermal compatibility with tooth enamel, biocompatibility and low toxicity.(Maya Lyapina[1], Mariana Tzekova[2] *et al*., 2016). Galss ceramics are non-toxic and chemically bond to bone. Glass ceramic elicits osteoinductive property. Bioglass and glass ceramic are embedded in a biomaterial support to form prosthetics for hard tissues.(Thamaraiselvi and Rajeswari, *et al*., 2004)

The mechanical property of hydroxyapatite is very important in hard tissue engineering application. Combination of nano-sized hydroxyapatite with glass ionomer cement has been reported in 2011.The documented effects of these nano-sized particles on the chemistry of these materials include increased biocompatibility and mechanical strength(Nidhi kantharia *et al*,.2011). This study aims to highlight the efficacy of Fish scales HAp in repair of tooth defect area without regarding race and regilion.

Materials and Methods

Fish scales were collected from Mingaladon market.Characterization of fish scales HAp was done at University Research Centre and National Laboratory, Department of Research and Innovation. Extracted teeth were

collected from Dental Hospital,University of Dental Medicine, Yangon. This study was taken from 2013 to 2016.

Materials

Fish scales, 1N HCl,1N NaOH, 10% formalin, Glass Ionomer Package and extracted teeth were used in this study.(Figure 2, 3, 4, 5, 6)

Figure 2.*Notopterus notopterus* (Pallas,1769**)**

Figure 3.*Notopterus notopterus* scales

Figure 4.*Notopterus notopterus* biomaterial powders of different temperatures

Figure 5. GC Glass Ionomer

Figure 6. Extracted Tooth

Method

Extraction of Fish Scales Boimaterials

The following procedure(Figure 7) is used to extract fish scales biomaterials:

Fish(*Notopterus notopterus*) scales are washed thoroughly with tap water

↓

Cleaned fish scales are soaked in 1N HCl solution for 24 hours(Deproteinization)

↓

Washed thoroughly several times with distilled water

↓

Soaked again in 1N NaOH solution for 24 hours(Deproteinization)

↓

Filtered and washed thoroughly with distilled water

↓

Dried in oven

↓

Deproteinized, and dried fish scales are heated at different temperatures in furnace

↓

Fish scale biomaterials powders of different temperatures

Figure 7. Extraction of fish scale biomaterials

Characterization Of Fish Scale Biomaterials Powders

All powders of different temperatures were characterized by XRD and FTIR. Functional groups of all fish scales powders were proved as hydroxyapatite (F.S.HAp) by maching with light absorbant wave numbers of standard hydroxyapatite and those extracted from other biowastes. Purity of F.S.HAp. was confirmed by TGA analysis, protein content and Ca/P weight ratio. Toxic heavy metal content was examined by mineral analysis. Ca/P weight ratio of F.S.HAp. were calculated at laboratory services, AMTT company and matched with that of enamel and dentine. Particle size was observed by SEM. Porosity in the F.S.HAp. was calculated by Image J software.

Evaluation Of Hardness of Pure GC Ionomer and F.S.HAp.-Glass Powder-GIC Composite By Mitutoyo Micro-Hardness Testing Machine

GC glass ionomer (Japan) was available at University of Dental Medicine, Yangon.To make a block of pure GC glass ionomer composite, as described in direction for use, two drops of GIC was added to one scoop of glass powder.(1:2). Procedure for formation of F.S.HAp-Glass Powder-GIC Composite is stated in Figure 8.

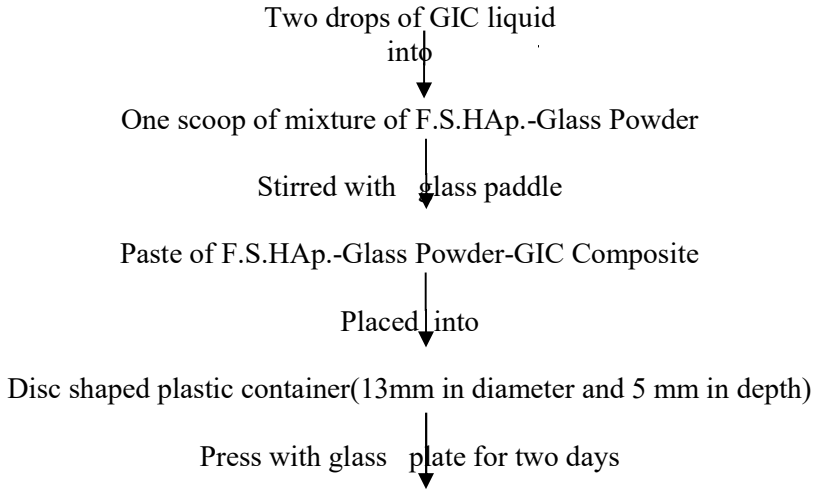


Figure 8. Formation of F.S.HAp.-Glass Powder-GIC Composite

Figure 9. Disc shaped plastic container (13mm in diameter and 5 mm in depth)

Figure 10.F.S.HAp.-Glass Powder -GIC Composite

Hardness measuring of F.S.HAp.-Glass Powder-GIC Composite in different ratios was done by Mitutoyo Micro-hardness testing machine at Meik-Hti-Lar(Figure 11). According to results, the sample composite with hardness very near to that of teeth and skeletal tissue stated in references was selected to repair the defect area in the hard tissues (Teeth and skeleton).

Figure 11 .Hardness measuring of F.S.HAp-Glass Powder-GIC Composite with Mistutoyo Micro-hardness testing machine

I have made four kinds composites in different ratios in order to know which ratio is inline with hardness of teeth(Table 1).

Table 1.The ratios of four samples

Composite	Sample no.	Ratio
Ratio of F.S.HAp.-Glass Powder-GIC composite	1	3:1:8
	2	3:2:10
	3	3:3:12
Pure GC Glass Ionomer (powder& liquid)	4	1:2(as direction for use)

Repair Of Tooth Defect Area

Extracted teeth were collected from Dental Hospital. Defect area of extracted teeth were repaired by following procedure shown in Figure 12.

Extracted tooth

↓

Treated with 10% formalin for 1 day

↓

Clean with purified water

↓

Fill up freshly prepared fish scale HAp-Glass Powder-GIC Composite (3:1:8) in tooth defect area

Figure 12.Procedure for repair of tooth defect area with F.S.HAp-Glass Powder-GIC composite

Results and Discussion

X-ray Diffraction Analysis

Presence of HAp in *Notopterus notopterus* fish scales powder (800°C) was confirmed by a strong diffraction peak at 2θ value of 31.808°(211plane) with 100% intensity (Table 2)

Table 2.Comparison of X.R.D. result datas in I%, 2θ value, hkl of F.S.HAp. and references

Datas	Milenko (2004)	X.R.D. JPCDS-09-0432	*Notopterus notopterus*(800°C)
hkl	211	211	211
I%	100	100	100
2θvalue	31.77	31.77	31.808

Thermal Analysis

The removal of the organic portion was observed at different temperatures with changes in the color of powders. The color of raw fish scale was observed as white, which gradually changed into grey, white, white and white tint with blue green respectively.No significant weight loss was observed at 800˚C,900˚C and 1000˚C(Figure 13).This indicates that fish scale powders at 800˚C is lacking of organic moieties and water.

Functional Groups Study

Functional groups of HAp (PO_4^{3-}, CO_3^{2-}, OH^- and HPO_4^{2-}) extracted from fish scale were ascertained by Fourier Transform Infrared

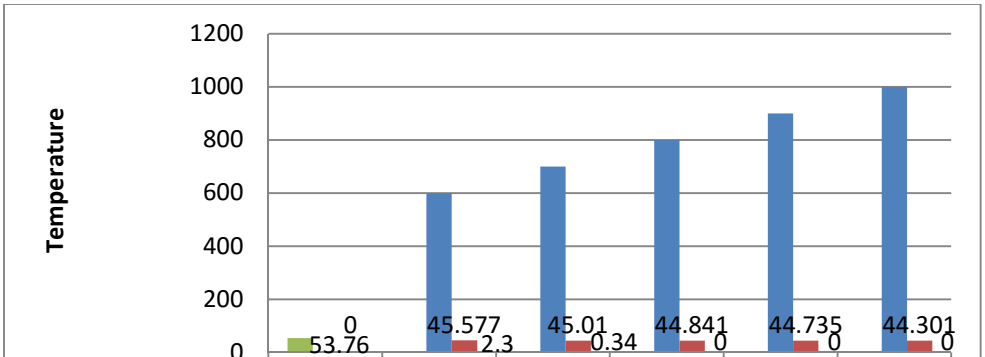
spectroscopy. Spectrums of different powders were obtained over the region of 4000 – 400 cm^{-1} . The most characteristic chemical groups in the FTIR spectrum of synthesized HAp are PO_4^{3-}, OH^-, CO_3^{2-} , as well as HPO_4^{2-} that characterize non-stoichiometric HAp. OH^- ions prove presence of HAp.(LigaBerzina-Simdina,2012). Functional groups of fish scales powder at 800°C were also found very close to those of HAp references(Table 3).

Table 3.Comparative study of F.T.I.R. wave numbers Of fish scale HAp. and HAp. references

Kinds Of HAp	Functional Groups Spectrum(cm^{-1})						
Standard Spectrum Of HAp (Lu Xiaoying, 2007)	OH^-	H_2O	CO_3^{2-}	PO_4^{3-}	HPO_4^{2-}	OH^-	PO_4^{3-}
	3570	no	no	1090, 1040,960	no	634	603, 568
Research Of Calcium Phosphate Using FTIR Spectroscopy,\ (LigaBerzina-Simdina,2012)	3420, 3500, 3540, 3570		870-880, 873, 1460-1530, 1450, 1650	1000-1100, 960, 1020-1120, 1094-1090, 1032-1046		630	460, 560-600, 603, 601 565-571
Animal Hard Tissues (LuXiaoying, 2007)	3569, 3571, 3574	1623, 1626, 1631, 1633	1400, 1456, 1460, 1470	960,962, 965,1045, 1047,1049, 1088,1089, 1091,1094	874	632, 634, 636, 642	566, 567, 568, 601, 603, 604, 609
Notopterus notopterus (800°C)	3504		1413.87, 1462.09	962.51, 1037.74, 1089.82		632.67	570.95, 601.81

Ash yield % and Protein content % of F.S.HAp

According to Figure 13 results, ash yield % was about 44%. Protein content was not observed right from at 800˚C. White color appearance of powder at 800˚C was suitable for hard tissue engineering. It is stated that fish scales are biocmposites of highly ordered type I collagen fibers and hydroxyapatite $\{ca_{10}(PO_4)_6(OH)_2\}$(Feng xiang zhang, Anning wang, 2011). As the collagen is a kind of protein, to get only hydroxyapatite from fish scale I have to choose the powder free from protein.



	1	2	3	4	5	6
Protein %	53.76	2.3	0.34	0	0	0
Temperature(°C)	0	600	700	800	900	1000
Ash yield%	0	45.577	45.01	44.841	44.735	44.301

Figure 13. Protein % and Ash yield % of *Notopterus notopterus* scales

Mineral Analysis

There is no heavy toxic metals like mercury,arsenic,cadmium and lead at ppm level shown in Table 4.The Ca/P ratio of *Notopterus notopterus* was 1.63. It is very close to the theoretical value of hydroxyapatite (1.67) and found within the range of hydroxyapatite with calcium deficient(1.5-1.67) (LigaBerzina-Simdina, 2012).(Table 4)

Table 4. Mineral analysis Of F.S.HAp

Analyte	***Notopterus notopterus* (%)**	**Ca/P**		
		Notopterus notopterus	Hydroxyapatite with Calcium deficient	Hydroxyapatite
Ca	**5.093(50930ppm)**	1.63	1.5-1.67	1.67
P	**3.062(30620ppm)**			
K	**0.098(980ppm)**			
S	**-**			
Sr	**0.016(160ppm)**			
Fe	**0.007(70ppm)**			
Zn	**0.003(30ppm)**			
Mn	**-**			
Bi	**-**			
Cu	**-**			

Ca/P Weight Ratio

Ca/P weight ratios of Fish scales were calculated at Laboratory Services , AMTT company. 1.94 Ca/P weight ratio of fish scale hydroxyapatite is identical to that of enamel, dentine and tuna bone (Table 5).

Table 5. Ca/P weight ratio of enamel, dentine, tuna bone and F.S.Hap

Ref:	Au. HwaYen liu (2013)		Jayachandran (2010)	In this study
Sample	Enamel	Dentine	Tuna bone	*Notopterus notopterus* scale
Ca/P ratio	1.94-1.83	1.91-1.78	1.94	1.94

Calculation Of Crystallite Size

For tissue engineering work, particle size is important to penetrate into the host tissue. According to X.R.D results, the biomaterial powders extracted from fish scales were proved to be HAp. Average crystallite size of *Notopterus notopterus* at 800°C was 75.8nm.The crystallite size of HAp of different powders were calculated by following equation:

$$\text{Crystallite Size} = \frac{0.9\lambda}{\beta \text{Cos}\theta}$$

SEM Analysis

Measurements of particle size and pore diameter were done by scanning electron microscope at the National Laboratory of the Department of Research and Innovation. Particle sizes are gradually increasing with the rise of temperature upto 900°C but at 1000°C some particles break up into smaller size. Average particle size of F.S.HAp at 800°C was found within the range of 151nm to 265nm (Table 6). All hexagonal shaped particles were fragile in texture.

Table 6. Comparative study of particle size of F.S.HAp at different temperatures

Temperature(°C)	*Notopterus notopterus*
600	45.45 nm to 219.7 nm
700	73.4 nm to 333.6 nm
800	**151.6 nm to265.4 nm**
900	303.3 nm to 925 nm
1000	128.9 nm to 746 nm

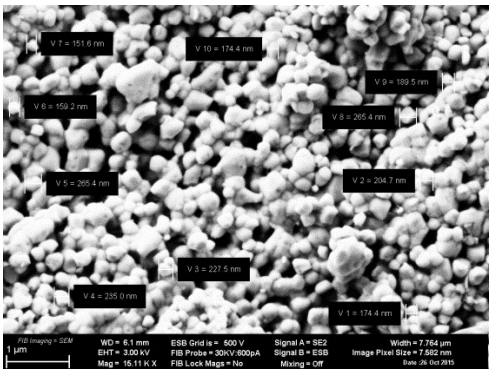


Figure 14.*Notopterus notopterus* scale (800˚C) biomaterial powder

Porosity of the F.S.HAp.

Porisity in the HAp is very important for tissue engineering work. Porosity in the HAp extracted from scales measured by image J software, was found to be 15.86% at 800˚C. (Table 7)

Table 7. Pore % of F.S.HAp.

Notopterus notopterus Scales HAp		Pore %
Total Area	41448400.44 nm^2	15.86
Pore Area	6575553 nm^2	

Toxicity Assessment of F.S.HAp.

As *Notopterus notopterus* is globally recognized as non toxic aquatic fauna, the HAp derived from it is also a non toxic biomaterial. In addition Glass powder and Glass Ionomer Cement are currently used in daily clinical practice and dental therapy. Therefore the composite applied in the present work is needless to say non toxic to human beings. Toxic heavy metals like Mercury, Arsenic, Lead and Cadmium were not detected in the fish scale powders at ppm level (Table 4). Therefore, application of fish scale HAp in hard tissue engineering,I suggest that they are not toxic to host tissue.

Evaluation of Hardness of Pure GC Glass Ionomer and F.S.HAp-Glass Powder-GIC Composite by Mitutoyo Micro-hardness Testing Machine

Hardness of all samples were measured by Mitutoyo Micro-hardness testing machine. According to results(Table.8), the hardness value of pure GC Glass Ionomer was found to be 3.5 Mohs. Ismail(2013) stated that adding of glass ionomer cement into hydroxyapatite-silica nanopowder composite makes enhancement of hardness. So many efforts were done in order to increase hardness. Incorporation of Glass Powder-GIC into F.S.HAp. gave increasement in hardness of 50HV compared to the pure GC Ionomer. Among the samples of F.S.HAp.-Glass Powder-GIC composite, according to test results(Table 8), the most hardest value of 272 (average HV) was found in the sample of 3 : 1 : 8 ratio. It was selected to use in the hard tissue engineering application.

Table 8. Hardness measurement by Mitutoyo Micro Hardness Testing Machine

Kind of Sample	**Ratio**	**Hardness(HV)**	**Hardness (Mohs)**
Pure GC Glass Ionomer (Powder : Liquid)	1 : 2	222(average)	3.5
F.S.HAp-Glass Powder-Glass Ionomer Cement Composite	3 : 1 : 8	272(average)	4
	3 : 2 : 10	261(average)	4
	3 : 3 : 12	268(average)	4
JK Chun, HH Choi and JY Lee (2014)	Enamel	274.8	
	Dentine	65.6	

SEM Analysis Of F.S.HAp– Glass Powder-GIC Composite

According to literature (Jayachandran, 2010) saying, the very important factor in the tissue engineering application is to have quite enough pore diameter in the substituted tissue for nutrient inflow and cell to cell connection. Therefore SEM analysis of the composite sample that is to be used in hard tissue engineering, was done at the Department of Research and Innovation. With Scanning Electron Microscope, pore diameter in the FS HAp-Glass Powder- GIC composite was found within the range of 26 nm – 1402 nm. Majority of pores in the composite was nano-sized.(Figure.15)

Meenakhi Mour and Debarun *et.al*.(2010) stated that minimal necessary for bone ingrowth is considered to be approximately 100 μm. The minimum pore size require to generate mineralized bone is generally considered to be 100 μm due to cell size, migration requirement and nutrient transport. Large pores(100-150 and 150-200μm) showed substantial bone growth. Small pores (75-100μm) resulted in ingrowth of unmineralized osteroid tissue. Smaller pores (10-44 and 44-75 μm) were penetrated only by fibrous tissue.

According to evidence of literature(Capillaries-Histology guide; University of Leeds), the diameter of the smallest capillary is 3 to 4 μm. I found that the significant difference in diameter of pore and capillary may lead to a barrier for nutrient supplementation and cell to cell connection into the substituted tissue.

Since enamel is mainly composed of HAp and without supplying of blood vessels and nerves, it is a structure of non living materials. So once eroded, there is no regeneration of HAp in the enamel defect area. Therefore I have done the substitution of F.S. HAp in the enamel defect area with the aid of Glass Ionomer Cement.

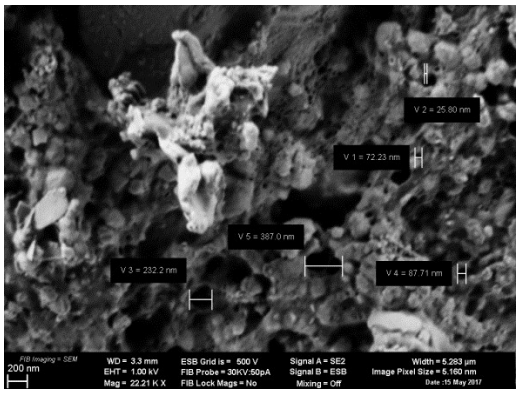


Figure 15.Pore diameter measurement of F.S.HAp-Glass Powder-GIC composite by SEM

Substitution Of F.S.HAp-Glass Powder-GIC Composite In Tooth Depfect Area(In vitro Study)

Since enamel being itself mainly composed of 20-40 nm sized HAp particles and without supplying of blood vessels and nerves, it is a structure made of non-living minerals. Once eroded the enamel, there is no regeneration of HAp in the enamel defect area. Apart from this, particle size difference between F.S.HAp and enamel is a barrier for supplementation of mineral requirement of enamel by diffusion.

In this study, the crystal size of *Notopterus notopterus* was 75.8nm. Particle size difference of HAp between tooth and fish scales was found to be a barrier for adsorption of particles to the host tissue. In order to overcome the problem of particle size difference in repair of tooth defect area, 4 drops of liquid of GC Glass Ionomer was added into the mixture of fish scale HAp-Glass Powder (1:1). Having no interference from the time of commencement of filling upto this time of presentation, the substituted HAp-Glass powder-GIC composite is still attached to the area of filling in the defect area. (Figure.16, 17, 18)

Figure 16. Before filling

Figure 17. After filling

Figure 18. Binding of vertically divided tooth

Conclusions

In this study, nano-sized hydroxyapatite biomaterial can be produced start right from *Notopterus notopterus* scales at 800°C. Significant finding is that without aid of cement, it is impossible to repair the enamel by using 75.8nm sized F.S.HAp. The application of F.S.HAp.-Glass Powder-Glass Ionomer Cement in repair of enamel defect area is possible only by substitution. It can be used in the hard tissues engineering applications for all people without regarding race and religion.

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CURRENT POPULATION STATUS, DIVERSITY AND EXPLOITATION OF TORTOISES AND FRESHWATER TURTLES IN MYEIK AREA, TANINTHARYI REGION

Kyi Thar Khaing[1] , Khin War War[2]

Abstract

Species diversity, population and exploitation of turtles and tortoises in Myeik area, Tanintharyi region were observed in the study work. The study was conducted in Myeik area, Tanintharyi Region from May 2015 to October 2016. The research designed was based on field study. The field work was conducted three times in the study period, in each study site. A total of 11 species were recorded representing two tortoise species and nine freshwater turtle species in Myeik area. Among the recorded species 18% of soft shell and 82% of hard shell were observed. The values of diversity index for three study sites indicated that the condition of these sites may be perfect habitat for the turtle species. During investigation, illegal shell trade was encountered in Tanintharyi and Palaw Township.

Keywords: Myeik area, freshwater turtles, tortoises, population, diversity, trade

Introduction

Turtles are among the most exploited and abused animals in the world. This exploitation and abuse have occurred at the hands of almost all civilizations and since ancient times. Since they are slow, docile, and easy to capture, turtles are being killed in many parts of the world. Today, the main threats are exploitation for food and commerce (Bonin *et al*.,2006). Most of turtles have been collected, illegally for food, medicinal, and pet markets in India, China and Thailand. Majority of wild caught turtles were exported to China and Thailand markets from Myanmar (Kalyar *et al*., 2014). The diversity of turtles and tortoises in the world that have existed in modern times, and currently generally recognized as distinct, consists of 334 species, of which 58 are polytypic, with 127 additional recognized subspecies, or 461 total of modern turtles and tortoises. There are about 90 species of turtles live in Southeast Asia (Sitha *et al*.,2006). Myanmar has the highest chelonian

diversity and highest chelonian endemism of any Southeast Asian country (Kuchling *et al*., 2004).

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Myanmar has 32 species of turtles and tortoises including seven endemic species (Win Maung and Win KoKo,2002). Twenty globally threatened non-marine reptile species have been recorded in Myanmar, all of which are turtles. Thus, turtles are among the most threatened of any major group of vertebrate species. The study focused on some turtles and tortoises in Myeik area, Tanintharyi region. The study area had been recognized as the hotspot of Chelonian diversity in Myanmar. Some authors reported that most endangered species of turtles and tortoises had been occurred in Tanintharyi region. According to the previous data, most of the turtle and tortoise species were reported to be extremely abundant in Tanintharyi Region. But in particular, little is known about species population and the exploitation level of the freshwater turtles and tortoises in Myeik area.

The present research is carried out with the following objectives: to record the diversity and population of freshwater turtle and tortoise species in Myeik area and to investigate the exploitation level of freshwater turtle and tortoise species in the study area.

Materials and Methods

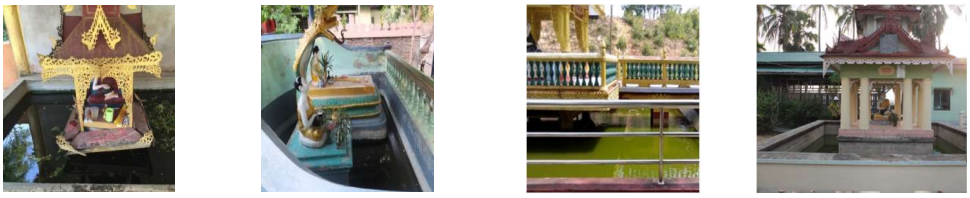
Study area and study period

Three study sites in Myeik area were selected for the study. Myeik township (12˚28'11" N, 98˚36'47" E), Tanintharyi township (12˚5'30" N, 99˚ 0'44" E), and Palaw township (12˚58'32" N, 98˚38'41" E) were chosen to study occurrence and population of freshwater turtles and tortoises in these environment. Data collections were carried out from May 2015 to October 2016.(Fig.1)

Figure 1. Location map of Myeik Area

Collection of the data

The study was carried out based on field study. Some information was first probed from some markets, villagers, natives, farmers, and fishermen lived in and around the study sites. Interviews with the local peoples and collectors using photo sheets of turtle were carried out. Visual encounter surveys were conducted at the natural habitat of the study areas.



(A) (B) (C) (D)

Plate1. Temple ponds

(A) (B) (C) (D)

Plate2. (A, B, and C) Trade and local collectors. (D) Interview survey

Identification

According to Smith (1931); CITES Identification Guide Turtles and Tortoises (1996); Win Maung and Win Ko Ko (2002) and Kalyar *et al*., (2014).

Sex differentiation

Male and female freshwater turtles usually differ in coloration, size, shell proportions and features; by enlarged base and longer tail in male than in female (Ernst and Barbour, 1989); usually differ in coloration, size, shell proportions and features (Carr, 1952).

Statistical analysis parameters

Descriptive variable and indices of species richness and diversity were utilized (Krebs et al., 1989; Solow, 1993). Shannon Diversity Index, Margalef D Index and Species richness were performed utilizing Species Richness and Diversity III Software Informer ver. 3.0 and Excel 2007.

Results

Species composition and Conservation status

Study recorded altogether 11chelonian species in Myeik area. A total of 11 species were studied representing two species of tortoises and nine species of freshwater turtles belonging to four families, Testudinidae (two species), Trionychidae (two species), Geoemydidae (six species), Emydidae (one species). There were *Indotestudo elongata* (Yellow tortoise), *Manouria emys* (Asian brown tortoise), *Siebenrockiella crassicollis* (Black marsh turtle), *Morenia ocellata* (Myanmar eyed turtle), *Cyclemys oldhamii*, (Oldham leaf turtle), *Cuora amboinensis lineata* (Myanmar box turtle), *Heosemys grandis* (Giant Asian pond turtle), *H.spinosa* (Spiny turtle), *Trachemys scripta elegan* (Red eared slider turtle), *Amyda cartilaginea* (Asiatic soft shell turtle), *Dogania subplana* (Malayan soft shell turtle) in Myeik area (Table1). As the conservation status according to the IUCN Red List (2012), three species are endangered (EN), five vulnerable (VU), one is nearly threaten (NT), and two are least concerned (LC) (Table2.Fig.2).

Population of different species

In Myeik area, a total of 361 individuals of 11 turtle species were recorded in rearing condition from three examined study sites. There were 45 individuals from Myeik, 259 individuals from Tanintharyi and 57 individuals from Palaw. The percentages population of *M.ocellata* was recorded the highest and *S.crassicollis* was the second highest population, *D.subplana* was the lowest percentage population respectively in the study area. Among the recorded species 18% of soft shell and 82% of hard shell were observed (Fig.3). Results show that overall percentage population was composed 21% juveniles, 51% females and 28% males during the study periods (Fig.4). The highest number of occurrence found for female, resulting in male to female ratio of 1:1.83 in Myeik area.

Species diversity in different study sites

Regarding the species diversity of different sites, Palaw Township was found to have the highest diversity of species with a modest H′ value 1.550 and D value 4.520. While, the second highest index value was observed in Tanintharyi Township with H′ value 1.329 and D value 2.427, which was followed by Myeik Township with H′ value 1.093 and D value 2.380 (Table3).

The species richness was found to be highest in Myeik with 1.842, second in Palaw with 1.732 and Tanintharyi had the lowest species richness value 1.429 during the study period. Although, the evenness value of Palaw with 0.383, Myeik with 0.287 and Tanintharyi with 0.239 were analyzed (Table3).

Exploitation

During the study period, most of the village in the state and potential sites with local respondents were interviewed and observed. Most turtle species were exploited for meat and trade purpose during the study period. There were about over 200 dead shell of chelonian were observed in Shaninntaw village, Tanintharyi township (Plate 3). During investigation, plastrons sale prices ranged from 1000Ks to 4000Ks per viss, while carapace could be sold for only an average of 6000Ks/viss. Moreover, live turtles were also sold for a mean of 8000Ks/viss depending on species (Plate3). Most of turtles and tortoises came from different villages around in Myeik environs. According to interview survey and several respondents, tortoises and turtles were consumed locally as food in some villages around Tanintharyi Township. The local agents collected the parts of turtles from different places and informed the main dealer (Plate3.B). Dealer visited these areas at regular intervals, collected the turtle's parts, and gave them money in return. During the study periods, most of the turtle and tortoise species were recorded by finding shells from trader. All sizes and life history stages ranging from small/juvenile, medium/subadult, and to large/adult were for sale.

Table 1. Recorded tortoises and turtles of order Testudines in Myeik area

Order	Family	Genus	Species/Subspecies	Common name
Testudines	Testudinidae	*Manouria*	*M. emys*	Asian Brown Tortoise
		Indotestudo	*I. elongata*	Yellow Tortoise
	Trionychidae	*Amyda*	*A. cartilaginea*	Asiatic Soft shell Turtle
		Dogania	*D. subplana*	Malayan Soft shell Turtle
	Geoemydidae	*Morenia*	*M. ocellata*	Myanmar Eyed Turtle (Endemic)
		Cyclemys	*C. oldhamii*	Oldham's Leaf Turtle
		Siebenrockiella	*S. crassicollis*	Black Marsh Turtle
		Cuora	*C. amboinensis lineata*	Myanmar Box Turtle (Endemic)
		Heosemys	*H. grandis*	Giant Asian Pond Turtle
		Heosemys	*H. spinosa*	Spiny Turtle
	Emydidae	*Trachemys*	*T. scripta elegan*	Red-eared Slider Turtle

Table 2. National and International protection/conservation status of recorded species

No	Species	Conservation Status			
		IUCN Red List (2012)	CITES (2010)	MWL (1994)	MFL(1993)
1	*M. emys*	Endangered	Appendix II	Protected	Not Listed
2	*I. elongata*	Endangered	Appendix II	Protected	Not Listed
3	*A. cartilaginea*	Vulnerable	Appendix II	Protected	Protected
4	*D. subplana*	Least Concerned	Not Listed	Protected	Protected
5	*M. ocellata*	Vulnerable	Appendix I	Protected	Protected
6	*C. oldhamii*	Nearly Threatened	Not Listed	Protected	Protected
7	*S. crassicollis*	Vulnerable	Appendix II	Protected	Protected
8	*C. amboinensis lineata*	Vulnerable	Appendix II	Protected	Protected
9	*H. grandis*	Vulnerable	Appendix II	Protected	Protected
10	*H. spinosa*	Endangered	Appendix II	Protected	Protected
11	*T. scripta elegan*	Least Concerned	Not Listed	Protected	Protected

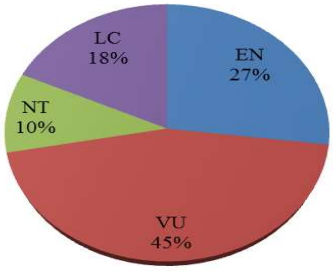


Figure 2. Conservation status of recorded turtles and tortoises in Myeik area

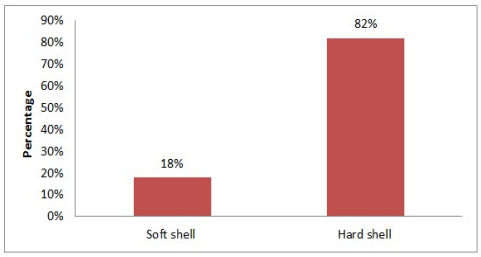


Figure 3. Percentage shell types of recorded turtles and tortoises in Myeik area

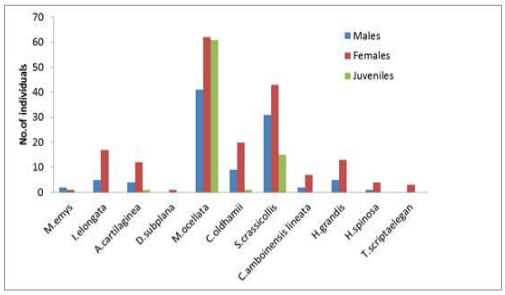


Figure 4.Comparison of recorded turtles and tortoises in Myeik area

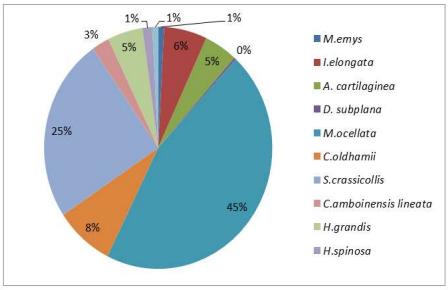


Figure 5. Percentage population of recorded turtles and tortoises in Myeik area

Table 3. Species diversity and richness in three study sites of Myeik area

Sr. No.	Particular	Myeik	Tanintharyi	Palaw
1	Species abundance (N)	45	259	57
2	Shannon Diversity Index (H)	1.093	1.329	1.550
3	Simpson's Index of Dominance (D)	2.380	2.427	4.520
4	Simpson's Index of Diversity (1 – D)	-1.380	-1.427	-3.520
5	Evenness of Shannon Index (E)	0.287	0.239	0.383
6	Margalef Index of species richness (R)	1.842	1.429	1.732

Table 4. Species number of distribution observed in captive and trade

No	Species	Captive*	Trade**	Males	Females	Juveniles
1	*M. emys*	2	1	2	1	-
2	*I. elongata*	9	13	5	17	-
3	*A. cartilaginea*	8	9	4	12	1
4	*D. subplana*	1	-	-	1	-
5	*M. ocellata*	5	159	41	62	61
6	*C. oldhamii*	2	28	9	20	1
7	*S. crassicollis*	51	38	31	43	15
8	*C. amboinen sislineata*	4	5	2	7	-
9	*H. grandis*	-	18	5	13	-
10	*H. spinosa*	-	5	1	4	-
11	*T. scripta elegan*	3	-	-	3	-

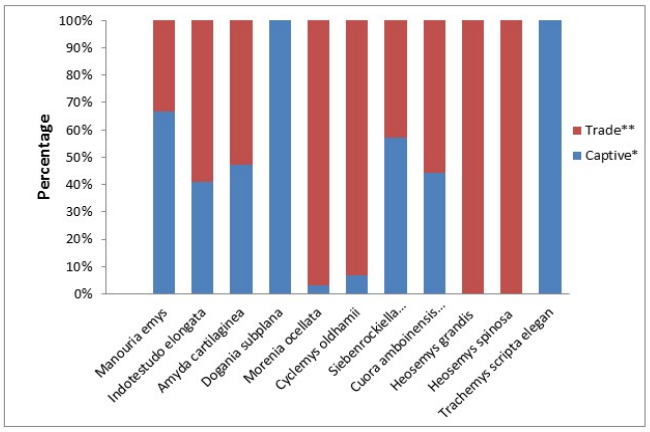
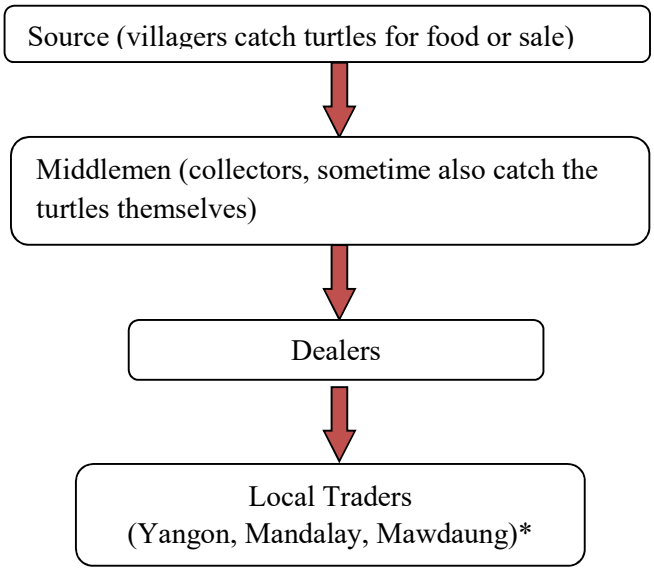


Figure 6 . Species percentage of distribution observed in captive and trade
*Specimens from captive areas (temple ponds or monastery)
**Shell trade or consumed specimens from exploited population

Trade Chain



*According to dealers, these are three main exporting regions

Discussion

Based on the species diversity of freshwater turtles and tortoises, a total of 26 species are present in Myanmar (Kalyar ,2012). Daw Mi Mi Maw (2004) reported that the total number of 17 species of some marine turtles, terrapin, tortoises, and freshwater turtles were recorded in the study area. In the present study, a total of 11 species (42%) were recorded representing two species of tortoises and nine species of turtles from Myeik area, Tanintharyi Region. Among the recorded species 18% of soft shell and 82% of hard shell were observed (Fig.3). It means that the soft shell chelonian population was less than that of hard shell species in the study environs. So, Myeik area is one of the major centers of turtle diversity in Myanmar.

According to the survey, *M.ocellata* and *S.crassicollis* were found abundant in Myeik area while other was relatively occurring with smaller numbers during the study periods. Very few individuals of *M. emys* (Asian brown tortoise), *C. amboinensis lineata* (Myanmar box turtle), *H.spinosa* (Spiny turtle), *D. subplana* (Malayan soft shell turtle) were recorded (Table.1) (Fig.5). Therefore the status of these species was rare in the study area. There are four tortoise species are present in Myanmar (Win Maung and Win Ko Ko, 2002). Among them two species of tortoises *I.elongata* and *M.emys* were recorded in the study area. Large numbers of these two tortoises are illegally exported to markets in southern China (Platt *et al.,* 2001).In the present study, these two species were found in the local shell trade. Moreover, the two species were heavily affected by local consumption, forest fires and habitat destruction. Forest fires posed a significant threat to tortoises in this area.

The present study was the first attempt concerning with species diversity and species richness of turtle fauna in Myeik area. The values of Shannon-Weiner index and Simpson's index for three study sites indicated that the condition of these sites may be perfect habitat for the turtle species. Of all the study sites, Palaw Township found to be the highest values of both indices. It may be assumed that this township provides various habitats preferable for most turtle species. Daget, 1976 stated that values greater or equal to 0.8 are usually considered as indicators of equitability in the communities. In the present study, the values of evenness are below 0.8, which suggests that the communities of turtles were not in evenness at the study area.

During investigation, illegal shell trade was encountered in Tanintharyi Township. In Shaninntaw village of turtle collector, shells of several species were obtained during the study periods. According to the owner, the price for one live individual ranged from 3000Ks to 8000Ks for a small individual, and then these were exported to Thailand. Information on trade routes was gathered through informal interviews with some collectors in Tanintharyi Township. In the present record, a total of nine species were traded species in Myeik area, all of species listed in the IUCN Red List of threatened animals. The present study found no evidence of the occurrence of *T. scripta elegan* (Red eared slider turtle) and *D. subplana* (Malayan soft shell turtle) in shell trade. The most abundant species observed during the study was the Myanmar eyed turtle, *M.ocellata*, with a total of 159 specimens observed in shell trade. Highest population size was recorded in May-June from Tanintharyi Township.

Moreover, the turtle species were more threatened by local consumption as food in the study sites (Plate.3). In some villages of Tanintharyi and Palaw Townships, turtles were caught mainly by local fishers. Large scale awareness programme could be initiated along the Tanintharyi region to protect the endangered chelonian species, because the village people have frequently been used to kill the turtle for consumption. Turtles and tortoises were protected under Myanmar Wildlife Law (1994) in Myanmar. However, local people have continuously captured the turtle species for food and for sale. The study found signs of over-exploitation or local extinction of turtle and tortoise species in Myeik area. During the study period, turtle and tortoise species were conserved to cooperate together with the local monks in some monastery of Myeik area (Plate 4). However, there was obviously a weakness in legislation regarding turtles and tortoises protection.

Conclusion

The present study highlighted the diversity and abundance of Chelonian species and its conservation threats in Myeik area, Tanintharyi region. Study revealed the existence of 11 Chelonian species under four families. By any standard, this is very high species diversity. But all of the turtle species are under threat of illegal hunting for meat and shell in this area. The conservation point of views, the population of the turtle species has been dramatically declined and conservation measures are in urgent needed in Myeik area.

Acknowledgements

I am grateful to my Professor Dr. Thida Lay Thwe, Head of Zoology Department, University of Yangon, and Professor Dr. Aye Mi San, University of Yangon, for their encouragement.

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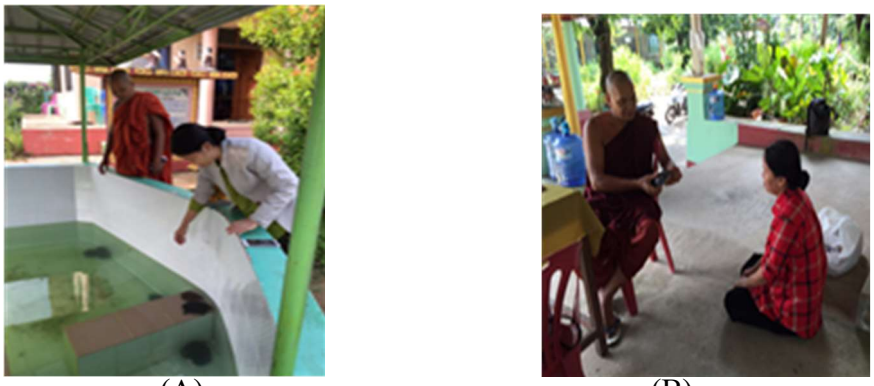
(A) (B) (C) (D)

(E) (F) (G) (H)

Plate 3.(A and B) Shell trade in Tanintharyi Township; (C) Shell trade (Carapaces) (D and E) Shell trade (Plastrons); (F, G and H) Dead of hard shell and soft shell turtles



(A) (B)

Plate 4. Conservation and cooperation with local monks (A and B)

THE MORPHOLOGICAL CHARACTERISTICS OF OTOLITHS OF SOME ESTUARINE FISHES IN THE MOUTH OF THANLWIN RIVER AND ITS ADJACENT COASTAL AREAS*

Thet Htwe Aung[1]

Abstract

Based on the external morphologies of fish, a total of 20 species of estuarine fishes selected from the mouth of Thanlwin River were identified and the sagittae otoliths were taken out from fish. The result of this study showed that otoliths of pelagic fishes compared with otoliths of demersal fishes are smaller and thinner. Furthermore otoliths of different species can have similarities in appearance but they have enough differences to be distinguished from each other. In this study, different shapes of otoliths are recorded in the fishes caught from the river mouth area of Thanlwin. These included elliptic, square, discoid, rectangle, lanceolate, triangle, pistalform and spindle shapes.

Keywords: Morphology, Thanlwin River, Otolith.

Introduction

Otoliths are structures located in the inner ear cavity of all teleost fish. Each side includes sagittae, lapilli and esterisci that are different in shape, size and location. Otoliths grow throughout a fish's life, and are formed by layers of calcium carbonate that are laid down at different rates, depending on metabolic rates during the winter form denser layers (the opaque zone), while high metabolic rates in spring and summer form less dense layers (the translucent zone). This make the otoliths look like an onion, with the opaque bands corresponding to slower growth appearing as dark rings. Because each opaque band represents a year of growth, scientists can use otoliths to estimate a fish's age. Otolith size and shape differ among species, among populations and within each species. These variations are influenced during development by both genetic and environmental factors. Due to their intra and interspecific

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*Best Paper Award Winning Paper in Marine Science (2017)

variation in shape, otoliths are useful in many studies: taxonomy, phylogeny, archeology, paleontology, species' geographical variation, stock identification, food webs and others. (Rossi-wongtschowki *et al*. 2016 and Popper *et al.* 2005)

Therefore, the identification and quantification of fish species in the diet of top predators (marine mammals, fish and seabirds, among others) usually require the analysis and identification of diagnostic hard remains found in food samples. Teleost fishes consumed by predators may be rapidly digested making them unrecognizable from external morphological features. However hard parts such as bones and otoliths are much more resilient to digestion and have regularly been used to aid in the identification of partially digested remains. Although the use of otolith increments for ageing larval and juvenile fish has become increasingly popular, characterization of the development of otolith morphology in different species is still poorly resolved. (Akkiran 1885)

In the present study, the morphological characteristics of otoliths were described for each species and then the research history on otolith morphology and the terminology used was reviewed. The present study can be used to identify fishes from otolith remains found in the digestive tracts or faeces of predators, sediment samples and fossils.

Materials and Methods

From June 2016 to January 2017, the samples of fishes and their otoliths were randomly collected along the mouth of Thanlwin River including Mawlamyine, Ahlet, Sebalar, Kadonebaw, Kyaikkhami and Setse. The samples were put in the bags and containers. The samples were brought back to the laboratory, rinsed and identified using the illustration handbooks produced by the F.A.O species identification sheets for fishery purposes Vol. I-IV. Fishbase on the website of the Academic Sinica, Taiwan was used as a supplemental guide for identification purposes. Furthermore De Bruin *et al*. 1995, Fischer 1974, Mya Than Tun 2001 and Rainboth 1996 were also used to identify the samples collected from the landing sites. Then the samples were continued removing sagittae otolith for the studies of otolith morphology. The terminology and diagonastic characters of otolith were reviewed and used in this study based on Rossi-Wongtschowiski *et al.* 2016.

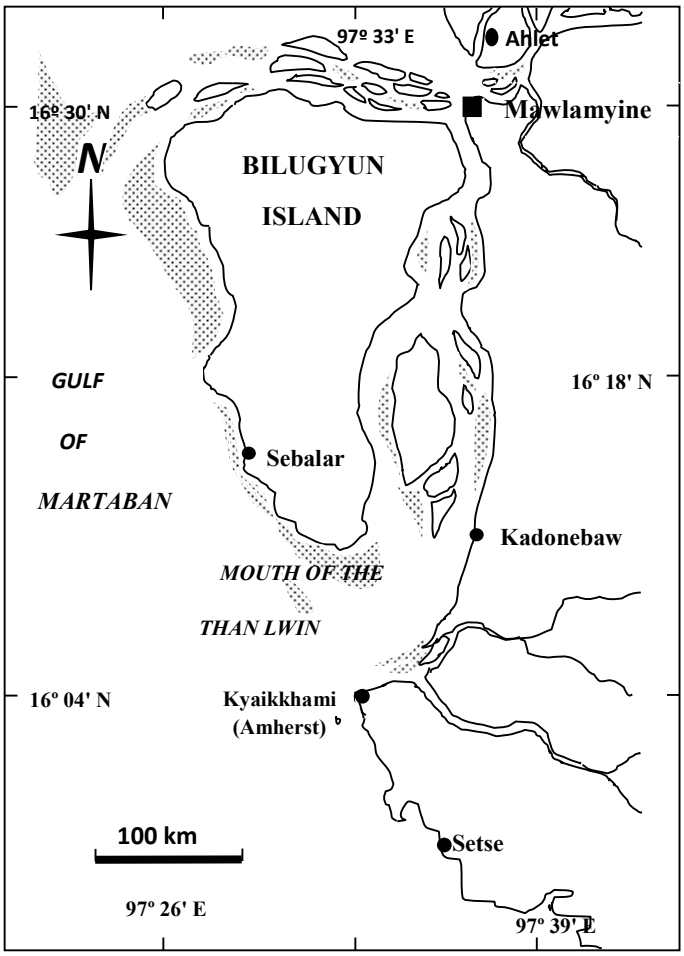

Figure 1. Map showing the specimens collection sites of fishes at the mouth of Thanlwin River.

Removing Otolith

According to the methods of Secor (1991), sagittal otoliths of fish were generally removed with a sharp fish knife and a pair of forceps or tweezers. In this way, a knife with a 15-20 cm blade and a pair of forceps or tweezers about 10 cm long were used. Firstly the head of the fish were griped by putting thumb and forefinger in its eye sockets and laid the body of the fish

on a counter with the tail pointing away. Then put the knife blade on the top of the fish's head about 1 eye diameter behind the eyes and slanted the blade away, at about a 30 angle, slice back and down about one head length and cut vertically through the top of the skull over the preopercle. After that, pushed the rear of the brain to one side, or cut it out all together. The pair of otoliths should be visible underneath the rear of the brain, still inside the skull. They may or may not be resting inside hallows in the base of the skull. Forceps were used to pull out both otoliths. They will not be attached to anything other than soft tissue. Finally the otoliths were cleaned out with water or younger fingers and soft dry in a paper envelope.

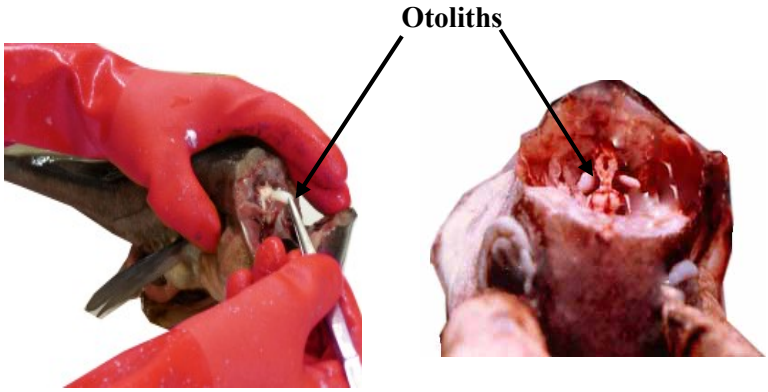

Figure 2. Removing sagittae otolith from a fish

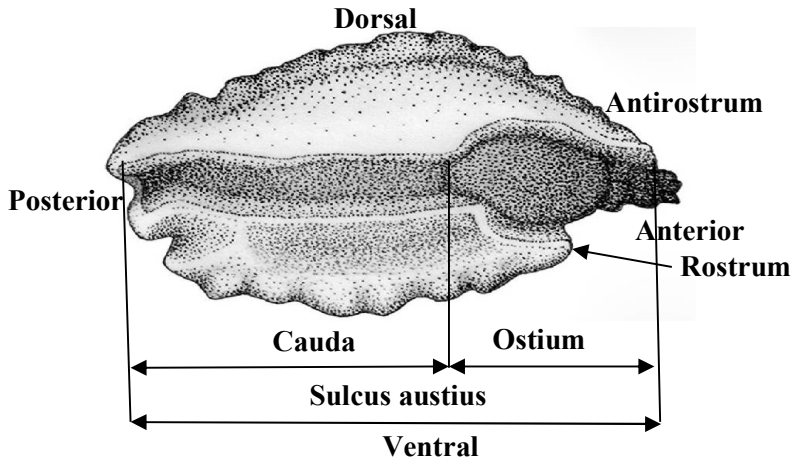

Figure 3. Illustrated glossary for sagittae otolith of fishes in the present study

Results

***Collia dussumieri* Valenciennes, 1848 (Local name- Nga-Kyan-Yat) figs. 4A, 5A, 6A**

Shape: pisiform. **Anterior region:** peaked. **Posterior region:** round. **Dorsal edge:** entire. **Ventral edge:** entire. **Rostrum:** not developed. **Antirostrum:** developed. **Sulcus acusticus: position:** median; **orientation:** horizontal; **opening:** ostial; **morphology:** homosulcoid; **colliculum:** monomorphic; **ostium**: rectangular; **cauda:** rectangular.

***Cynoglossus Bengalensis* Hamilton, 1822 (Local name- Khway- Shar) figs. 4B, 5B, 6B**

Shape: triangular. **Anterior region:** flattened or irregular. **Posterior region:** flattended. **Dorsal edge:** entire. **Ventral edge:** entire. **Rostrum and Antirostrum:** absent. **Sulcus acusticus: position:** Inframedian; **orientation:** descending; **opening:** mesial; **morphology:** homosulcoid; **colliculum:** homomorphic; **ostium**: tubular; **cauda:** tubular.

***Datnioides polota* Hamilton, 1822 (Local name- Nga- kyar- Ma) figs. 4C, 5C, 6C**

Shape: lanceolated or oval. **Anterior region:** irregular. **Posterior region:** peaked or blunt. **Dorsal edge:** crenated. **Ventral edge:** irregular. **Rostrum:**

developed. **Antirostrum:** absent. **Sulcus acusticus: position:** inframedian; **orientation:** ascending; **opening:** ostial; **morphology:** pseudo- archaesulcoid; **colliculum:** heteromorphic; **ostium**: bent; **cauda:** tubular slightly curved.

***Glossogobius giuris* Hamilton, 1822 (Local name- Kat- Tha- Poe) figs. 4D, 5D, 6D**

Shape: rectangular or irregular. **Anterior region:** oblique or flattened, sinuated. **Posterior region:** rounded or sinuated. **Dorsal edge:** entire. **Ventral edge:** dentated. **Rostrum and Antirostrum:** absent. **Sulcus acusticus: position:** median; **orientation:** horizontal; **opening:** mesial; **morphology:** homosulcoid; **colliculum:** homomorphic; **ostium**: oval; **cauda:** oval.

***Gobioides bunchanani* Day, 1878 (Local name- Nga-Yet-Ni) figs. 4E, 5E, 6E**

Shape: squared or irregular. **Anterior region:** flattened or irregular. **Posterior region:** flattended. **Dorsal edge:** entire. **Ventral edge:** entire. **Rostrum and Antirostrum:** absent. **Sulcus acusticus: position:** Inframedian; **orientation:** descending; **opening:** pseudo-ostiocaudal; **morphology:** heterosulcoid; **colliculum:** heteromorphic; **ostium**: elliptic; **cauda:** tubular.

***Hilsa ilisha* Cuvier, 1836 (Local name- Nga-Tha-Lout) figs. 4F, 5F, 6F**

Shape: lanceolated. **Anterior region:** lanceolated and peaked. **Posterior region:** oblique-round. **Dorsal edge:** dentate. **Ventral edge:** entire. **Rostrum:** developed. **Antirostrum:** developed. **Sulcus acusticus: position:** median; **orientation:** horizontal, **opening:** ostial; **morphology:** pseudo-archaesulcoid, **colliculum:** heteromorphic; **ostium**: funnel-like; **cauda:** elliptic, oval.

***Harpadon neherus* Hamilton- Buchanan, 1822 (Local name- Nga-Hnet) figs. 4G, 5G, 6G**

Shape: pisiform. **Anterior region:** peaked. **Posterior region:** round. **Dorsal edge:** entire. **Ventral edge:** entire. **Rostrum:** not developed. **Antirostrum:** developed. **Sulcus acusticus: position:** median; **orientation:** horizontal; **opening:** ostial; **morphology:** pseudo-archaesulcoid; **colliculum:** heteromorphic; **ostium**: tubular; **cauda:** funnel- like.

***Lepturacanthus savala* cuvier, 1829 (Local name- Nga-Ta-Khon) figs. 4H, 5H, 6H**

Shape: oval. **Anterior region:** flattened. **Posterior region:** lobe. **Dorsal edge:** entire. **Ventral edge:** irregular or entire. **Rostrum and Antirostrum:** absent. **Sulcus acusticus: position:** supramedian; **orientation:** horizontal; **opening:** pseudo- ostiocaudal; **morphology:** archaesulcoid; **colliculum:** monomorphic; **ostium**: funnel- like; **cauda:** tubular.

***Mugil cephalus* Linnaeus, 1758 (Local name- Kat- Bulu) figs. 4I, 5I, 6I**

Shape: lanceolated or rectangular. **Anterior region:** peaked. **Posterior region:** two peaks. **Dorsal edge:** dentate or crenate. **Ventral edge:** dentate or irregular. **Rostrum:** not developed. **Antirostrum:** developed. **Sulcus acusticus: position:** inframedian; **orientation:** ascending; **opening:** ostial; **morphology:** pseudo- archaesulcoid; **colliculum:** heteromorphic; **ostium**: bent; **cauda:** straight tubular.

***Nibea soldado* Lacepedw, 1802 (Local name- Nga-Byat) figs. 4J, 5J, 6J**

Shape: rectangular. **Anterior region:** flattened. **Posterior region:** flattenrd. **Dorsal edge:** entire. **Ventral edge:** entire. **Rostrum and Antirostrum are absent. Sulcus acusticus: position:** inframedian; **orientation:** descending; **opening:** pseudo- ostial; **morphology:** heterosulcoid; **colliculum:** heteromorphic; **ostium**: lateral; **cauda:** tubular markedly curved.

***Ompok bimaculatus* Bloch, 1794 (Local name- Nga-Nu-Than) figs. 4K, 5K, 6K**

Shape: Discoidal or seed- like. **Anterior region:** oblique. **Posterior region:** peaked. **Dorsal edge:** entire or serrated. **Ventral edge:** serrated. **Rostrum:** not developed. **Antirostrum:** not developed. **Sulcus acusticus: position:** Inframedian; **orientation:** descending; **opening:** ostial; **morphology:** pseudo-archaesulcoid; **colliculum:** heteromorphic; **ostium**: tubular; **cauda:** tubular.

***Osteobrama belangeri* Valenciennes, 1844 (Local name- Nga-Pyin-Ma) figs. 4L, 5L, 6L**

Shape: Discoidal or seed- like. **Anterior region:** irregular. **Posterior region:** round, serrated. **Dorsal edge:** serrated. **Ventral edge:** serrated. **Rostrum:** not developed. **Antirostrum:** not developed. **Sulcus acusticus: position:**

median; **orientation:** horizontal; **opening:** ostial; **morphology:** pseudo-archaesulcoid; **colliculum:** heteromorphic; **ostium**: elliptic; **cauda:** rectangular.

***Parambasis ranga* Hamilton, 1822 (Local name- Nga-zin-Set) figs. 4M, 5M, 6M**

Shape: rectangular. **Anterior region:** flattened. **Posterior region:** two peaks. **Dorsal edge:** entire. **Ventral edge:** crenated or entire. **Rostrum:** developed. **Antirostrum:** not developed. **Sulcus acusticus: position:** supramedian; **orientation:** descending; **opening:** ostial; **morphology:** pseudo-archaesulcoid; **colliculum:** heteromorphic; **ostium**: elliptic; **cauda:** tubular.

***Platycephalus indicus* Linnaeus, 1758 (Local name- Nga-Sin-Nin) figs. 4N, 5N, 6N**

Shape: oval or elongated. **Anterior region:** peaked. **Posterior region:** two peaks. **Dorsal edge:** entire. **Ventral edge:** dentated or entire. **Rostrum:** developed. **Antirostrum:** not developed. **Pseudo-rostrum and pseudo-antirostrum** present or not developed. **Sulcus acusticus: position:** supramedian; **orientation:** descending; **opening:** ostio- caudal; **morphology:** pseudo- archaesulcoid; **colliculum:** heteromorphic; **ostium**: elliptic; **cauda:** tubular.

***Puntius conchonius* Hamilton, 1822 (Local name- Nga- Khone-Ma) figs. 4O, 5O, 6O**

Shape: Discoidal or seed- like. **Anterior region:** peaked or blunt. **Posterior region:** peaked or rounded. **Dorsal edge:** serrated. **Ventral edge:** serrated. **Rostrum;** developed. **Antirostrum:** not developed. **Sulcus acusticus: position:** median; **orientation:** horizontal; **opening:** ostial; **morphology:** pseudo- archaesulcoid; **colliculum:** heteromorphic; **ostium**: elliptic; **cauda:** discoidal.

***Polynemus paradiseus* Linneus, 1758 (Local name- Nga-Pone-Nar) figs. 4P, 5P, 6P**

Shape: spindle- shape. **Anterior region:** lobed. **Posterior region:** lobed or sinuated. **Dorsal edge:** irregular. **Ventral edge:** entire. **Rostrum;** developed. **Antirostrum:** not developed. **Sulcus acusticus: position:** median;

orientation: descending; **opening:** ostial; **morphology:** pseudo-archaesulcoid; **colliculum:** heteromorphic; **ostium**: elliptic or funnel- like; **cauda:** tubular.

***Salmophasia bacalia* Hamilton, 1822 (Local name- Nga- Dar- shay) figs. 4Q, 5Q, 6Q**

Shape: Elliptic. **Anterior region:** peaked. **Posterior region:** round. **Dorsal edge:** irregular. **Ventral edge:** serrated. **Rostrum:** developed. **Antirostrum:** absent or not developed. **Sulcus acusticus: position:** median; **orientation:** horizontal; **opening:** ostial; **morphology:** pseudo- archaesulcoid; **colliculum:** heteromorphic; **ostium**: bent; **cauda:** tubular slightly curved.

***Setipinna taty* Valenciennes, 1848 (Local name- Nga-Byar) figs. 4R, 5R, 6R**

Shape: Pisiform. **Anterior region:** peaked or blunt. **Posterior region:** rounded. **Dorsal edge:** irregular. **Ventral edge:** serrated. **Rostrum;** developed. **Antirostrum:** absent or not developed. **Sulcus acusticus: position:** Inframedian; **orientation:** descending; **opening:** ostial; **morphology:** pseudo- archaesulcoid; **colliculum:** heteromorphic; **ostium**: elliptic; **cauda:** tubular.

***Taenioides gracilis* Valenciennes, 1837(Local name- Kar- att) figs. 4S, 5S, 6S**

Shape: squared or irregular. **Anterior region:** flattened. **Posterior region:** flattened. **Dorsal edge:** entire. **Ventral edge:** entire. **Rostrum and Antirostrum:** absent. **Sulcus acusticus: position:** median; **orientation:** horizontal; **opening:** mesial; **morphology:** homosulcoid; **colliculum:** homomorphic; **ostium**: oval; **cauda:** oval.

***Oreochromis aureus* (Local name- Tilapia) figs. 4T, 5T, 6T**

Shape: elliptic. **Anterior region:** peaked or blunt. **Posterior region:** rounded. **Dorsal edge:** irregular. **Ventral edge:** crenated. **Rostrum;** developed. **Antirostrum:** developed. **Sulcus acusticus: position:** supramedian; **orientation:** ascending; **opening:** ostial; **morphology:** heterosulcoid; **colliculum:** pseudo- archaesulcoid; **ostium**: rectangular; **cauda:** tubular slightly curved.

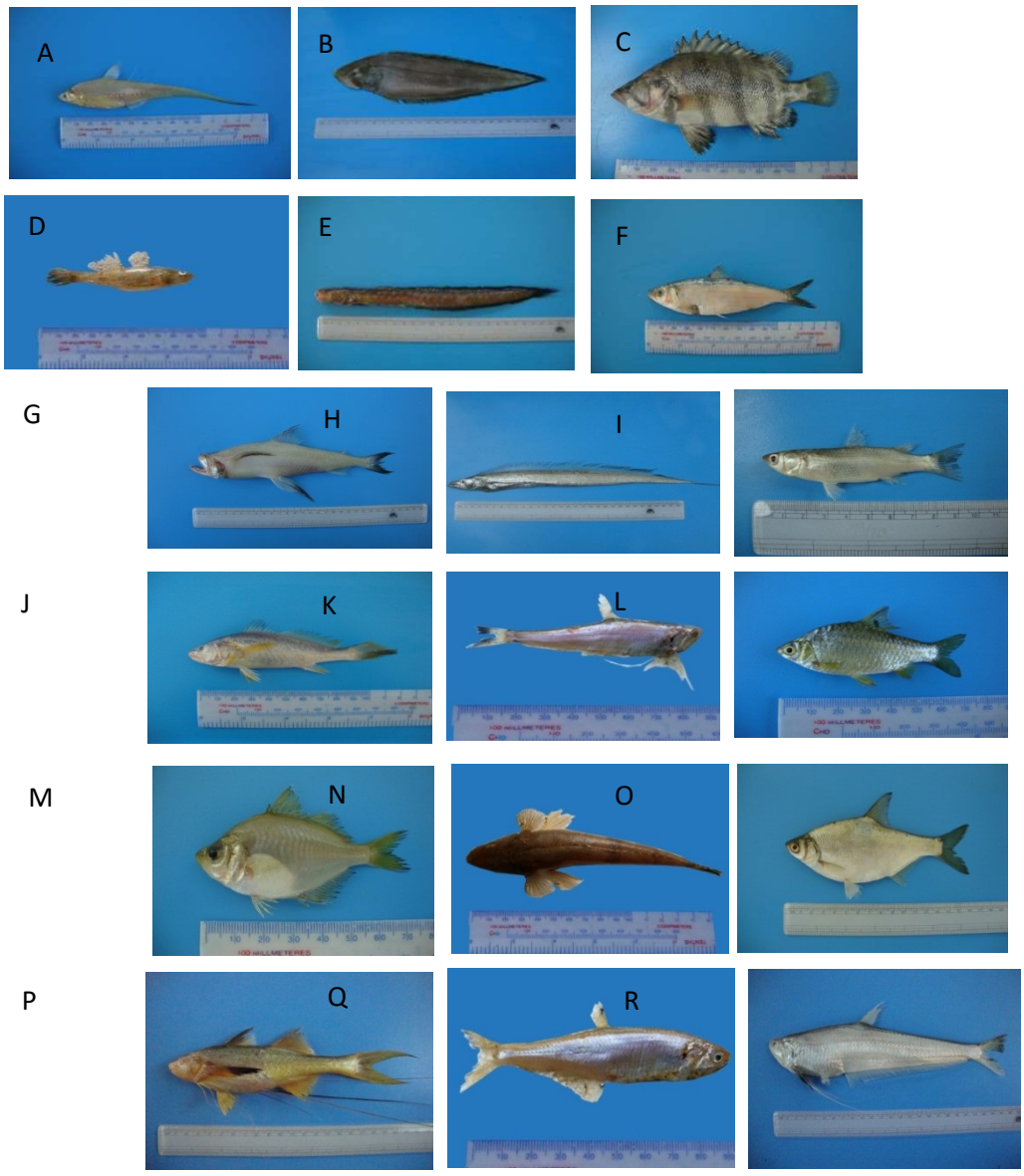

Figure 4. Habit photo of A) *Collia dussumieri,* B) *Cynoglossus Bengalensis,* C) *Datnioides polota,* D) *Glossogobius giuris,* E) *Gobioides bunchanani,* F) *Hilsa ilisha,* G) *Harpadon neherus,* H) *Lepturacanthus savala,* I) *Mugil cephalus,* J) *Nibea soldado,* K) *Ompok bimaculatus,* L) *Osteobrama belangeri*, M) *Parambasis ranga,* N) *Platycephalus indicus,* O) *Puntius conchonius,* P) *Polynemus paradiseus,* Q) *Salmophasia bacalia,*

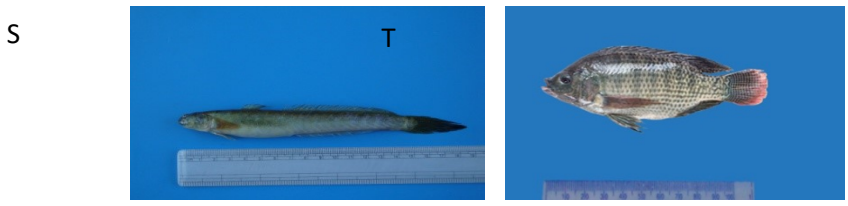

Figure 4. Habit photo of S) *Taenioides gracilis* and T) *Oreochromis aureu.*

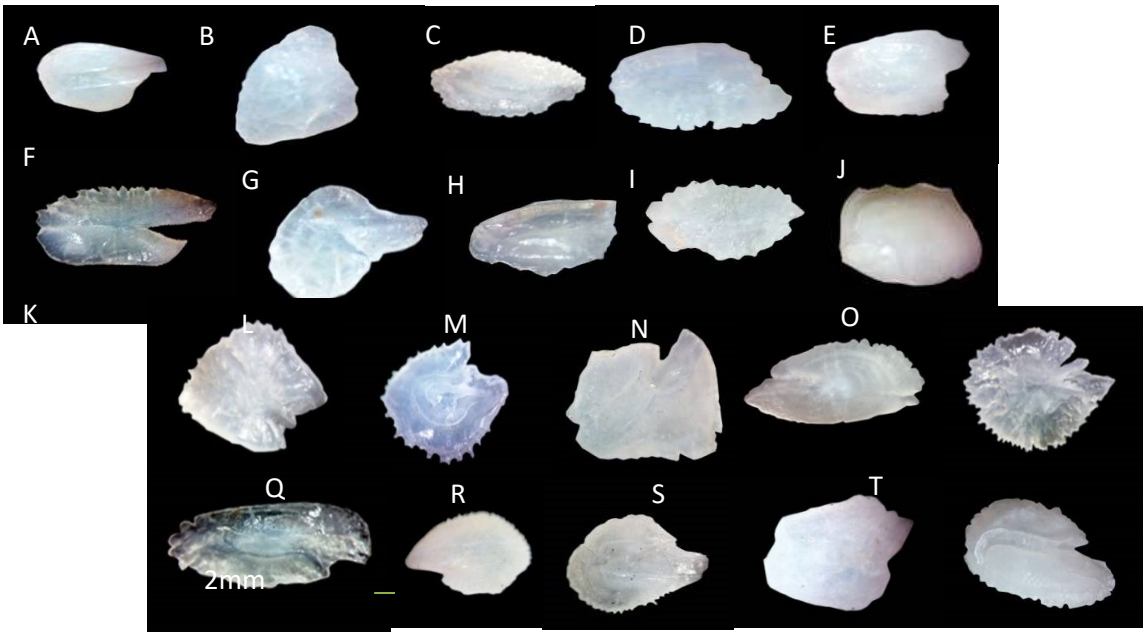

Figure 5. Otolith photo of A) *Collia dussumieri,* B) *Cynoglossus Bengalensis,* C) *Datnioides polota,* D) *Glossogobius giuris,* E) *Gobioides bunchanani,* F) *Hilsa ilisha,* G) *Harpadon neherus,* H) *Lepturacanthus savala,* I) *Mugil cephalus,* J) *Nibea soldado,* K) *Ompok bimaculatus,* L) *Osteobrama belangeri*, M) *Parambasis ranga,* N) *Platycephalus indicus,* O) *Puntius conchonius,* P) *Polynemus paradiseus,* Q) *Salmophasia bacalia,* R) *Setipinna taty,* S) *Taenioides gracilis* and T) *Oreochromis aureus* .

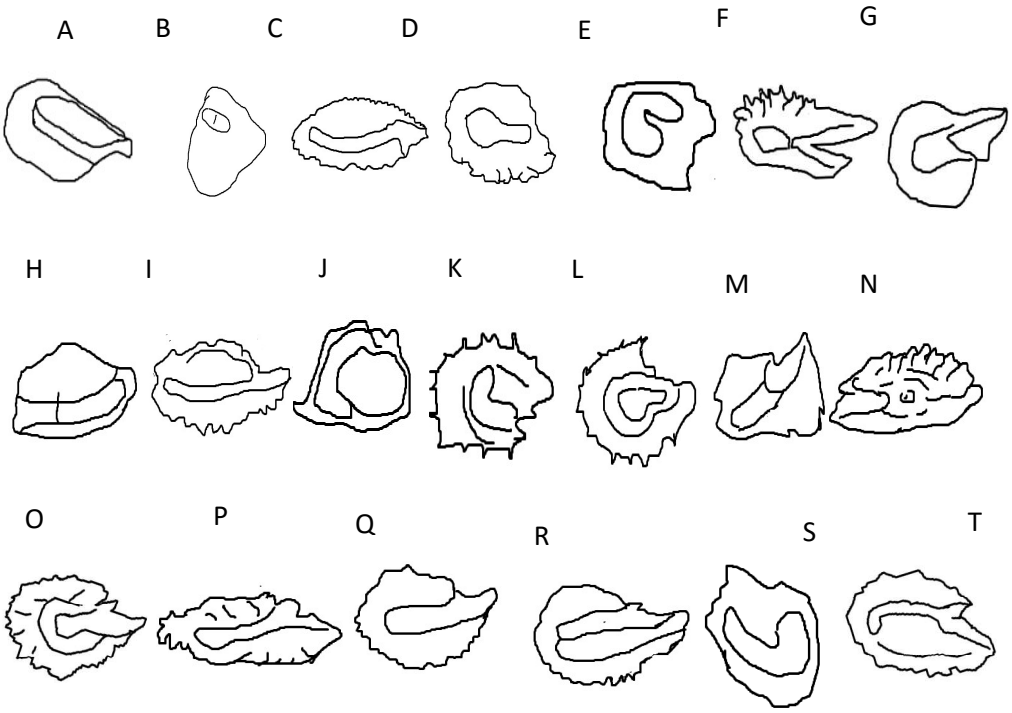

Figure 6. Otolith sketch of A) *Collia dussumieri,* B) *Cynoglossus Bengalensis,* C) *Datnioides polota,* D) *Glossogobius giuris,* E) *Gobioides bunchanani,* F) *Hilsa ilisha,* G) *Harpadon neherus,* H) *Lepturacanthus savala,* I) *Mugil cephalus,* J) *Nibea soldado,* K) *Ompok bimaculatus,* L) *Osteobrama belangeri*, M) *Parambasis ranga,* N) *Platycephalus indicus,* O) *Puntius conchonius,* P) *Polynemus paradiseus,* Q) *Salmophasia bacalia,* R) *Setipinna taty,* S) *Taenioides gracilis* and T) *Oreochromis aureus* .

Table 1. Showing the comparison of the otolith's characters among the different species

Species name	Shape	Sulcus acusticus				
		position	orientation	opening	ostium	cauda
Collia dussumieri	pisiform	median	horizontal	ostial	rectangular	rectangular
Cynoglossus Bengalensis	triangular	Inframedian	descending	mesial	tubular	tubular
Datnioides polota	lanceolated	inframedian	ascending	ostial	bent	tubular
Glossogobius giuris	rectangular	median	horizontal	mesial	oval	oval
Gobioides bunchanani	squared	Inframedian	descending	pseudo-ostiocaudal	elliptic	tubular
Hilsa ilisha	lanceolated	median	horizontal	ostial	funnel-like	oval
Harpadon neherus	pisiform	median	horizontal	ostial	tubular	funnel- like
Lepturacanthus savala	oval	supramedian	horizontal	pseudo-ostiocaudal	funnel- like	tubular
Mugil cephalus	lanceolated	inframedian	ascending	ostial	bent	straight tubular
Nibea soldado	rectangular	inframedian	descending	pseudo-ostial	lateral	tubular
Ompok bimaculatus	Discoidal	Inframedian	descending	ostial	tubular	tubular
Osteobrama belangeri	Discoidal	median	horizontal	ostial	elliptic	rectangular
Parambasis ranga	rectangular	supramedian	descending	ostial	elliptic	tubular
Platycephalus indicus	oval	supramedian	descending	ostio-caudal	elliptic	tubular
Puntius conchonius	Discoidal	median	horizontal	ostial	elliptic	discoidal
Polynemus paradiseus	spindle-shape	median	descending	ostial	funnel- like	tubular
Salmophasia bacalia	Elliptic	median	horizontal	ostial	bent	tubular
Setipinna taty	Pisiform	Inframedian	descending	ostial	elliptic	tubular
Taenioides gracilis	squared	median	horizontal	mesial	oval	oval
Oreochromis aureus	elliptic	supramedian	ascending	ostial	rectangular	tubular

Discussion

The total 20 species of fishes were selected to study their otoliths, prioring to the abundant species and different genera in Mawlamyine, Ahlet, Kadone- baw, Sebalar, kyaikkhami and Setse, along the mouth of Thanlwin River. The name of the species identified with their morphological characters are *Collia dussumieri, Cynoglossus Bengalensis, Datnioides polota, Glossogobius giuris, Gobioides bunchanani, Hilsa ilisha, Harpadon neherus, Lepturacanthus savala, Mugil cephalus, Nibea soldado, Ompok bimaculatus, Osteobrama belangeri*, *Parambasis ranga, Platycephalus indicus, Puntius conchonius, Polynemus paradiseus, Salmophasia bacalia, Setipinna taty, Taenioides gracilis* and *Oreochromis aureus*.

The result of this study showed that otoliths of pelagic fishes such as *Mugil cephalus*, *Harpadon neherus*, *Platycephalus indicus etc*. are smaller and thinner compared with otoliths of demersal fishes such as *Nibea soldado, Gobioides bunchanani, Cynoglossus Bengalensis*. According to Parmentier *et al*. 2001, fish occupying the same ecological niche show resemblances in otolith shape; pelagic fish species are known as fast swimmers and the shape of their otolirh could be an element contributing to making neurocranium lighter in order to reduce energy expenditure during swimming. On the contrary, in benthic, commensal and parasitic species, the swimming constraint is obviously weaker and does not act as a restricting factor on the otolith development. This is reinforced by their thicker otoliths. Furthermore otoliths of different species from a family can have similarities in appearance but they have enough differences to be distinguished from each other.

In the present study, the overall shape of otolith can be found pisiform shape in 3 species, triangular shape in 1 species, lanceolated shape in 3 species, rectangular shape in 3 species, squared shape in 2 species, oval or elliptic shape in 4 species, discoidal shape in 3 species and 1 species in spindle- shape. Of these 20 species studied, 15 species have rostrum and antirostrum while *Cynoglossus Bengalensis*, *Glossogobius giuris, Gobioides bunchanani*, *Lepturacanthus savala, Nibea soldado, Taenioides gracilis* are absent. Although otolith have rostrum in some fishes, antirostrum is absent or not developed. On the other hand, there is antirostrum in some fishes but rostrum is absent or undeveloped. Moreover sulcus austicus are also five types

of opening shapes in the study. They are ostial found in 13 species, ostiocaudal found in 1 species and pseudo- ostiocaudal found in 2 species, pseudo- ostial found in 1 species, mesial found in 3 species. Likewise there are six types of cauda shapes which are rectangular, elliptic or oval, bent, tubular, lateral and funnel- like and three types of ostium shapes which are tubular, elliptic and discoidal.

Conclusion

The present study was carried out by describing the detailed characters of otolith with their photos and sketchs based on the 20 species of fishes. The general otolith's shape are elliptic shape, squared shape, discoidal shape, rectangular shape, lanceolated shape, triangular shape, pisiform shape, spindle- shape. Moreover 15 species have rostrum and antirostrum while *Cynoglossus Bengalensis*, *Glossogobius giuris, Gobioides bunchanani*, *Lepturacanthus savala, Nibea soldado, Taenioides gracilis* are without rostrum and antirostrum.

Acknowledgement

I would like to express my gratitude to Dr. Aung Myat Kyaw Sein, Rector of Mawlamyine University and Dr Mie Mie Sein, Rro-rectors of Mawlamyine University for their permission to undertake this research work. I would like to express my sincere thanks to Dr.San Thar Tun, Professor and Head of Department of Marine Science in Mawlamyine University for their advices and his needful assistance.

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LEAF ARCHITECTURAL STUDY ON SOME MEMBERS OF CONVOLVULACEAE IN MANDALAY AND PYIN OO LWIN AREA*

Soe Myint Aye[1], Hnin Wai Lwin[2]

Abstract

Leaf architectural characteristics of the Family Convolvulaceae in Mandalay and Pyin oo lwin area were collected and studied during the year 2016 – 2017 . According to the identification 21 species of 7 genera were recorded in study area. The resulting species were one species of *Evolvulus*, *Jacquemontia*, *Operculina* and *Porana*, two species of *Argyreia*, five species of *Merremia* and ten species of *Ipomoea*. The leaves samples were decolorized by Method of Mishra *et al.* (2010) in Department of Botany, University of Mandalay. The qualitative features and the venation pattern of cleared leaves samples were characterized. It was found that the leaves were variously observed in shape, base, apex and margin. The margins were entire or 3-lobed. The primary veins were stout or massive and predominant tertiary vein angles were Right Right (RR), Acute Obtuse (AO), Obtuse Acute (OA), Right Obtuse (RO), Acute Right (AR), Right Acute (RA), or Acute Acute (AA). The marginal ultimate venations were looped, incomplete, or fimbriate. The primary vein categories are pinnate, actinodromous basal and actinodromous suprabasal. The secondary vein categories were brochidodromous, weak brochidodromous, cladodromous, weak cladodromous and actinodromous. Stoma types were anisocytic, anomocytic and paracytic. According to the quantitative characteristics, leaf areas were found to be variously between 40 mm^2 and 460 mm^2. The smallest leaf area was observed in *Evolvulus nummularis* (L.) L. and the largest one was in *Argyreia laxiflora* (Prain) Prain. The number of secondary veins along one side was variously observed between 4 and 13. The angle between primary and secondary vein, number of areoles per mm^2, veinlets entering areoles per mm^2, and highest vein order were different among the species. According to the different qualitative and quantitative characteristics, the leaf arichitecture provides valuable data for practical identification on members of Convolvulaceae.

Key words: Leaf architectural characters, Convolvulaceae, Myanmar

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Introduction

Convolvulaceae, known as the morning glory family, is widely distributed in tropical, subtropical and temperate regions. The Convolvulaceae are mostly twining herbs or shrubs, sometime with milky sap, comprising about 85 genera and 2,800 species in the World (Qi-ming & De-lin, 2009). In Myanmar (Burma), Hundley & Chit Ko Ko (1987) recorded 13 genera and about 90 species.

Since the time of Linnaeus the identification and reconstruction of relationships between plants have been based largely on features of the reproductive organs. Although flower and fruit characters have proved very useful in both botany and paleobotany, there are situations in which these organs are not available for study. In spite of the success of Linnaeus's sexual system and its descendants, there is a great need to be able to identify and classify dispersed leaves (Ash *et al.* 1999).

Melville (1976) stated that leaf architecture was first used and described by Hickey(1973) to represent the position and form of elements constituting the outward expression of leaf structure including venation pattern, marginal configuration, leaf shape and gland position. Leaf architecture plays an important role in ecology, plant systematics, paleobotany and conservation. Venation patterns, in particular, for identifying and classifying plants.

The arrangement of the veins in the lamina is termed venation. The veins are differentiated into number of size classes depending upon their relative size at their point of origin and behavior in relation to other veins and to the margin of the leaf. The venation is pinnate where the primary vein or midrib serves as the origin for the higher order venation. The primary (1°), secondary (2°) and tertiary (3°) veins constitute the major and those of subsequent categories (4°, 5° etc.) including the ultimate veinlets, the minor venation (Melville 1976).

The principal characteristics of the leaf venation pattern of a species are, in general, genetically fixed. This priovide the basis for using the leaf venation as taxonomic tool. Mechanical demands, transport constraints and possibly other aspects, such as ontogenetic factors, are interlinked and form a

complex pattern of factors contributing to the functional background of the evolution of leaf venation patterns (Roth-Nebelsick *et al.* 2001).

The leaves of extant terrestrial plants show highly diverse and elaborate patterns of leaf venation. One fundamental feature of many leaf venation patterns, especially in the case of angiosperm leaves, is the presence of anastomoses. Anastomosing veins distinguish a network topologically from a simple dendritic (tree-like) pattern with represents the primitive venation architecture. In palaeobotany, macrofossils showing leaf venation patterns are extensively utilized in identifying fossil taxa (Roth-Nebelsick *et al.* 2001). The high interspecific variability of leaf venation patterns indicates strong selective pressures acting on the architectural arrangement of the conducting bundles of a leaf. High variability is also demonstrated by the venation density which shows strong differences on the intra specific and individual level (Uhl & Mosbrugger 1999 as cited in Roth-Nebelsick *et al.* 2001).

Recent studies on leaf architecture of dicotyledons have much interest and led to several investigations in this field (Saibaba & Rao 1990). The study on leaf venation patterns puts forth several characteristics of leaf architecture that are diagnostics and help in the identification of species (Saibaba & Rao 1990).

Leaf architecture in some Convolvulaceae of India was studied by Inamdar & Shenoy (1980). A review of the literature revealed that no work has been done in this direction in the family Convolvulaceae of Myanmar, and therefore, the present study was undertaken to give a comprehensive account of the leaf architecture in Convolvulaceae and to evaluate its taxonomic significance. The present study was undertaken to examine the leaf architecture of the Convolvulaceae in order to provide additional information for generic circumscription and to shed new light on intrafamilial and interfamilial relationships of the family. The specific objectives of this investigation are to study leaf architecture of the Convolvulaceae, and to provide the data that will be applicable in practical identification when no reproductive organs can be found on plants.

Materials and Methods

The specimens of Convolvulaceae were collected from different localities of Mandalay area. Identification of specimen was carried out by

using literatures Dassanayake (1980) and Qi-ming & De-lin (2009). Myanmar names were referred to Hundley & Chit Ko Ko (1987) and Kress *et al*. (2003).

The leaf samples of the resulting species, a total of 21 species under 7 genera of the Convolvulaceae, were prepared to study the leaf architectural characteristics. The mature fresh leaves were cleared following the method of Mishra *et al.* (2010). Leaves were immersed in 80% ethanol for 48-72 hrs to remove chlorophyll pigments. The leaf samples were then washed and treated again with 10% NaOH for 24-36 hrs. When the leaf sample becomes cleared, it was stained in 10% safranin for 30 minutes. The pattern of veins was prominent on the cleared leaf because of the absorbing of safranin. The sample was mounted with glycerin and preserved between the wax paper.

The leaf samples showing the prominent venation pattern were putting on light box and taken the photographs. Leaf area was measured by using graph paper method. The leaf base and leaf margin were observed under microscope and taken the photomicrographs. The stoma type was studied and taken a photo. The areole size and number of vein endings were taken from different leaves. The areole and veinlet frequencies per mm² were measured by the help of square ocular micrometer after comparing to 1 mm of stage micrometer. Terminologies of Hickey (1973) and Hickey & Wolfe (1975) have been followed to describe the leaf architecture in this investigation.

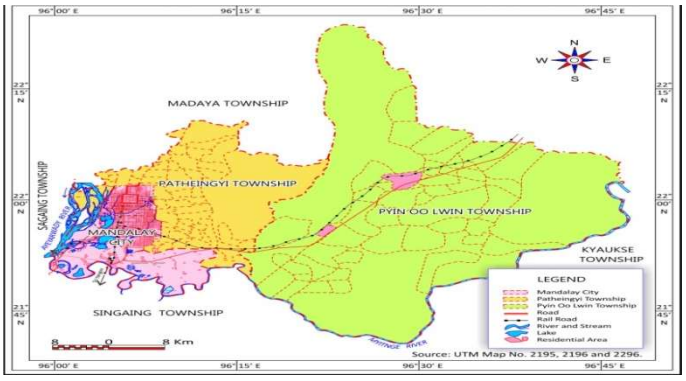

Figure 1. Location map of the specimens collected area

Results

The leaf architectural study on some members of Convolvulaceae in Mandalay area were collected, identified and studied their leaf characteristics. All together 21 species belonging to seven genera were included (Table 1). The leaf characteristics of the species were described systematically. Comparable Quantitative and Qualitative data on venation pattern of collected species was stated in Table 2 and Table 3. The cleared leaves of collected specimens represented the architectural characteristics were stated in Figure 2 and Figure 3.

Table 1 List of the Collected Species from Mandalay Area

Family	Species	Myanmar name
Convolvulaceae	1. *Argyreia hirsutissima* (Clarke) Raiz.	unknown
	2. *Argyreia laxiflora* (Prain) Prain	unknown
	3. *Evolvulus nummularis* (L.) L.	Kyauk kwe
	4. *Ipomoea aquatica* Forssk.	Ye kazun
	5. *Ipomoea biflora* (L.) Pers.	Le kazun yaine
	6. *Ipomoea cairica* (L.) Sweet	Tauk tet let wa
	7. *Ipomoea carnea* Jacq.	La thar pan
	8. *Ipomoea hederifolia* L.	Myat lay ni
	9. *Ipomoea indica* (Burm.f.) Merr.	Unknown
	10. *Ipomoea marginata* (Desr.) Verd.	Taw ka zun
	11. *Ipomoea obscura* (L.) Ker. Gawl	unknown
	12. *Ipomoea tricolor* Cav.	unknown
	13. *Ipomoea triloba* L.	Kazun yaing
	14. *Jacquemontia pentantha* (Jacq.) G. Don	unknown
	15. *Merremia aegyptia* (L.) Urban	unknown
	16. *Merremia emarginata* (Burm. f.) Hall. f.	Anya myin kwa
	17. *Merremia hederacea* (Burm. f.) Hall.f.	New shoke
	18. *Merremia umbellata* (L.) Hall. f.	Kazun war
	19. *Merremia vitifolia* (Burm. f.) Hall. f.	Sa pyit nwe
	20. *Operculina turpethum* (L.) S. Manso.	Kya hin bin
	21. *Porana volubilis* Burm. f.	Sein tha zin

Table 2. Qualitative Leaf Features of Collected Species of Convolvulaceae

Name of the taxa	Shape	Apex	Base	Margin	Texture	Primary vein category	Predominate tertiary vein angle	Marginal ultimate venation
Argyreia hirsutissima (Clarke) Raiz.	ovate	acuminate	cordate	entire	coriaceous	pinnate	RR, AO	looped
Argyreia laxiflora (Prain) Prain.	ovate	obtuse	cordate	entire	coriaceous	pinnate	OA, RO	looped
Evolvulus nummularis (L.)L.	elliptic	rounded	lobate	entire	membranaceous	pinnate	RR, AR	incomplete
Ipomoea aquatic Forssk.	ovate	acute	cordate	entire	membranaceous	pinnate	AO, RR	looped
Ipomoea biflora (L.) Pers.	broadly ovate	acuminate	cordate	entire	membranaceous	actinodromous	AA, AO	looped
Ipomoea cairica (L.) Sweet	elliptic - lanceolate	acute	acute	palmately loed entire	membranaceous	actinodromous	RR, AR	looped
Ipomoea carnea Jacq.	ovate	acuminate	cordate	entire	coriaceous	pinnate	AO, RA	looped
Ipomoea hederifolia L.	ovate	acuminate	deeply cordate	entire	membranaceous	actinodromous	AA, RO	fimbriate
Ipomoea indica (Burm.f.) Merr.	broadly ovate	acuminate	cordate	3-lobed entire	membranaceous	actinodromous	AR, RR	fimbriate
Ipomoea marginata (Desr.) Verd.	cordate	acuminate	cordate	entire	membranaceous	actinodromous	RO, OA	looped
Ipomoea obscura (L.) Ker. Gawl	cordate	acuminate	deeply cordate	entire	membranaceous	actinodromous	OA, RO	looped

Table 2 (Continued)

Name of the taxa	Shape	Apex	Base	Margin	Texture	Primary vein size	Predominate tertiary vein angle	Marginal ultimate venation
Ipomoea tricolor Cav.	ovate	acuminate	deeply cordate	entire	membranaceous	pinnate	RR, AO	fimbriate
Ipomoea triloba L.	ovate	mucronate	deeply cordate	entire	membranaceous	actinodromous	RR, AR	looped
Jacquemontia pentantha (Jacq) G. Don	ovate	mucronate	cordate	entire	membranaceous	pinnate	RA, RR	incomplete
Merremia aegyptia (L.) Urban	elliptic	acuminate	acute	palmately lobed entire	membranaceous	actinodromous	AA, AO	looped
Merremia emarginata (Burm.f.) Hall. f.	ovate to reniform	emarginate	cordate	entire	coriaceous	actinodromous	RO, AO	incomplete
Merremia hederacea (Burm. f.) Hall. f.	ovate	acuminate	Broadly cordate	entire	membranaceous	pinnate	AO, AR	incomplete
Merremia umbellata (L.) Hall. f.	ovate	rounded	cordate	entire	membranaceous	pinnate	RR, RO	looped
Merremia vitifolia (Burm. f.) Hall. f.	ovate	acuminate	cordate	palmately lobed entire	membranaceous	actinodromous	RO, RR	fimbriate
Operculina turpethum (L.) S.Manso.	ovate	mucronate	cordate	entire	membranaceous	pinnate	RO, OA	incomplete
Porana volubilis Burm. f.	ovate	acuminate	cordate	entire	membranaceous	actinodromous	OA, RA	looped

RR - Right Right, AO - Acute Obtuse, OA - Obtuse Acute, RO - Right Obtuse, AR - Acute Right, AA - Acute Acute, RA - Right Acute

Table 3. Quantitative Data on the Venation Patterns of Collected Species of Convolvulaceae (average values)

Name of the taxa	Leaf area in mm^2	Number of 2° vein along one side of midrib	Angle between 1° & 2° vein	Number of areoles per mm^2	Veinlets entering areole per mm^2	Highest vein order
Argyreia hirsutissima (Clarke) Raiz.	400	13-14	42˚-50˚	12	5	4
Argyreia laxiflora (Prain) Prain.	460	10-11	50˚-70˚	9	3	5
Evolvulus nummularis (L.) L.	40	5-6	30˚-40˚	18	6	5
Ipomoea aquatica Forssk.	270	8-9	40˚-60˚	6	2	4
Ipomoea biflora (L.) Pers.	130	7-8	45˚-50˚	15	7	5
Ipomoea cairica (L.) Sweet	150	10-11	40˚-55˚	10	5	4
Ipomoea carnea Jacq.	280	8-9	35˚-65˚	7	3	4
Ipomoea hederifolia L.	210	8-9	35˚-60˚	13	6	5
Ipomoea indica (Burm.f.) Merr.	360	5-6	40˚-60˚	8	4	5
Ipomoea marginata (Desr.) Verd.	230	6-7	50˚-70˚	18	9	5
Ipomoea obscura (L.) Ker. Gawl	240	7-8	45˚-75˚	16	7	5

Table 4.3 (Continued)

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Name of the taxa	Leaf area in mm^2	Number of 2° vein along one side of midrib	Angle between 1° & 2° vein	Number of areoles per mm^2	Veinlets entering areole per mm^2	Highest vein order
Ipomoea tricolor Cav.	120	8-9	40˚-55˚	10	4	4
Ipomoea triloba L.	190	7-8	40˚-45˚	17	10	5
Jacquemontia pentantha (Jacq) G. Don	110	6-7	55˚-75˚	12	5	5
Merremia aegyptia (L.) Urban	290	11-12	32˚-50˚	14	6	4
Merremia emarginata (Burm. f.) Hall. f.	160	5-6	35˚-55˚	9	2	5
Merremia hederacea (Burm. f.) Hall. f.	130	8-9	45˚-75˚	8	2	4
Merremia umbellata (L.) Hall. f.	240	7-8	55˚-70˚	15	8	4
Merremia vitifolia (Burm. f.) Hall. f.	360	5-6	38˚-60˚	8	3	5
Operculina turpethum (L.) S. Menso.	330	9-10	40˚-65˚	13	5	4
Porana volubilis Burm. f.	370	4-5	42˚-55˚	20	13	5

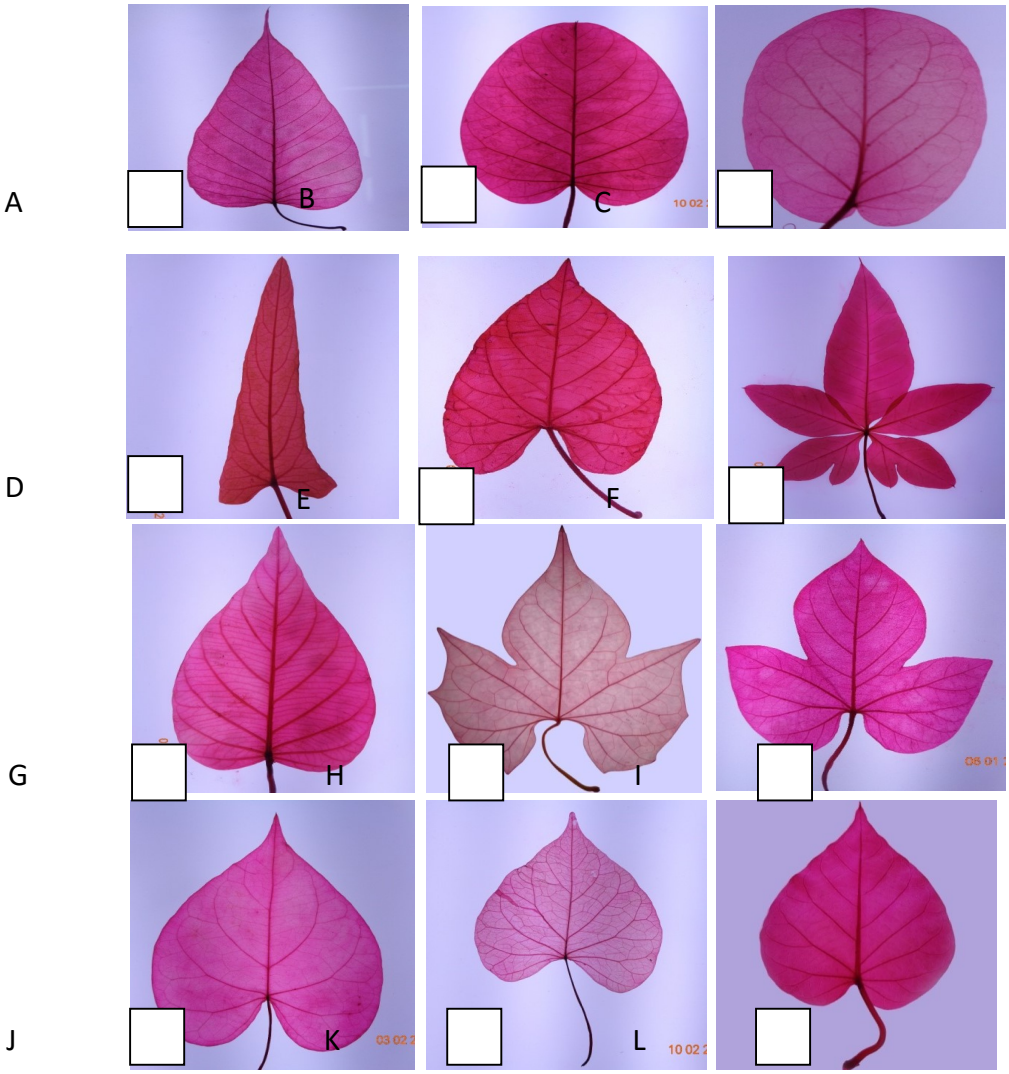

Figure 2. Cleared Leaves of **A**. *Argyreia hirsutissima* (Clarke) Raiz; **B**. *Argyreia laxiflora* (Prain) Prain.; **C**. *Evolvulus nummularis* (L.) L. ; **D**. *Ipomoea aquatica* Forssk.; **E**. *Ipomoea biflora* (L.) Pers. **F**. *Ipomoea cairica* (L.) Sweet ; **G**. *Ipomoea carnea* Jacq.; **H**. *Ipomoea hederifolia* L.; **I.** *Ipomoea indica* (Burm.f.) Merr.; **J.** *Ipomoea marginata* (Desr.) Verd. **K.** *Ipomoea obscura* (L.) Ker. Gawl **L.** *Ipomoea tricolor* Cav..

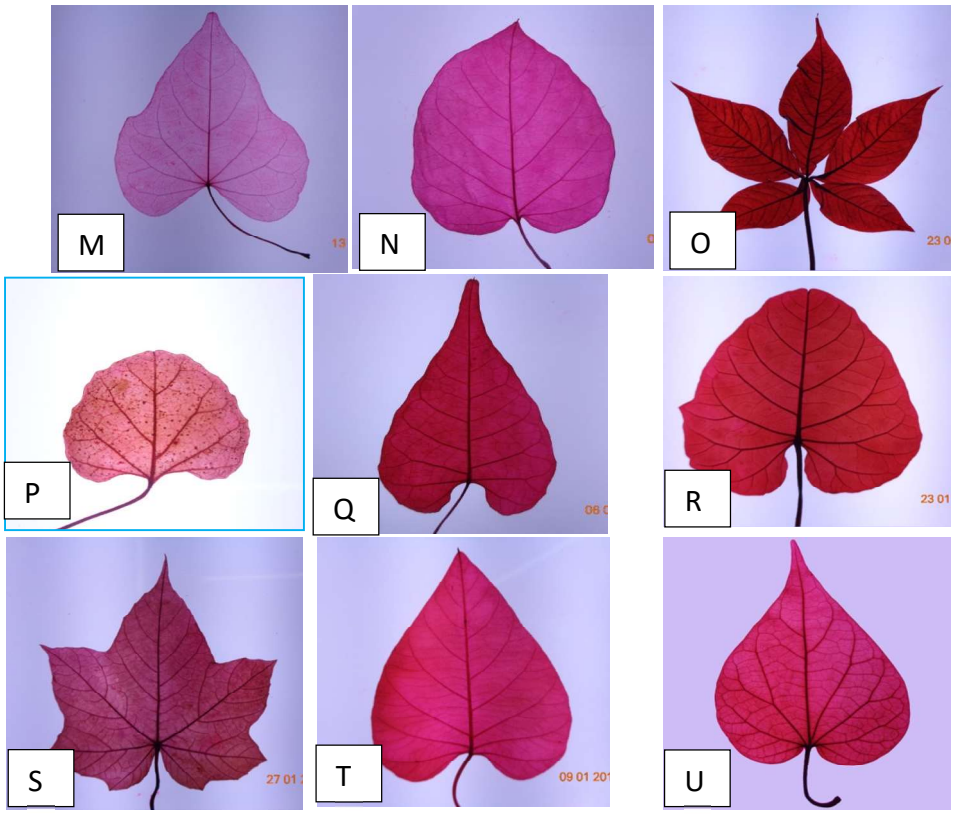

Figure 3. Cleared Leaves of **M.** *Ipomoea triloba* L.; **N.** *Jacquemontia pentantha* (Jacq) G. Don **O.** *Merremia aegyptia* (L.) Urban**.; P**. *Merremia emarginata*(Burm. f.) Hall. f. ;**Q.** *Merremia hederacea* (Burm. f.) Hall. f.; **R.** *Merremia umbellata* (L.) Hall. f. ;**S.** *Merremia vitifolia* (Burm. f.) Hall. f.; **T.** *Operculina turpethum* (L.) S. Menso.;**U.** *Porana volubilis* Burm. f.

Discussion and Conclusion

The present research work deals with the leaf architectural characteristics of Convolvulaceae found in Mandalay and Pyin oo lwin area. Altogether 21 species of 7 genera were identified based on the vegetative and reproductive characters of plant samples comparing with recent literatures and keys of Dassanayake (1980) and Qi-ming & De-lin (2009).

By the study on leaf architectural characteristics of cleared leaves, it was found that the leaves of collected species showed different characters of shape, apex, base, margin, texture. The venation patterns are also variable between the species. The leaves shapes are ovate in *Argyreia hirsutissima* (Clarke) Raiz., *Argyreia laxiflora* (Prain) Prain, *Ipomoea aquatica* Forssk, *Ipomoea carnea* Jacq., *Ipomoea hederifolia* L., *Ipomoea tricolor* Cav., *Ipomoea triloba* L., *Jacquemontia pentantha* (Jacq) G. Don, *Merremia hederacea* (Burm.f.) Hall. f., *M. umbellata* (L.) Hall. f., *Merremia vitifolia*, *Operculina turpethum* (L.) S. Manso and *Porana volubilis* Burm.f. The leaves are elliptic in *Evolvulus nummularis* (L.) L., and *Merremia aegyptia* (L.) Urban. The leaves of *Ipomoea biflora* (L.) Pers. and *Ipomoea indica* (Burm.f.) Merr. were broadly ovate. The leaves are elliptic-lanceolate in *Ipomoea cairica* (L.) Sweet. The leaves were cordate in *Ipomoea marginata* (Desr.) Verd. and *Ipomoea obscura* (L.) Ker. Gaw. The leaves were ovate to reniform in *Merremia emarginata* (Burm. f.) Hall. f.

The apex of the leaves were also different. It was acuminate in *Argyreia hirsutissima* (Clarke) Raiz., *Ipomoea biflora* (L.) Pers., *Ipomoea carnea* Jacq., *Ipomoea hederifolia* L., *Ipomoea indica* (Burm.f.) Merr., *Ipomoea marginata* (Desr.) Verd., *Ipomoea obscura* (L.) Ker. Gawl, *Ipomoea tricolor* Cav., *Merremia aegyptia* (L.) Urban., *Merremia hederacea* (Burm. f.) Hall. f., *Merremia vitifolia* (Burm. f.) Hall. f. and *Porana volubilis* Burm. f., obtuse in *Argyreia laxiflora* (Prain) Prain, rounded in *Evolvulus nummularis* (L.) L. and *Merremia umbellata* (L.) Hall. f., acute in *Ipomoea aquatica* Forssk. and *Ipomoea cairica* (L.) Sweet., mucronate in *Ipomoea triloba* L., *Jacquemontia pentantha* (Jacq.) G. Don and *Operculina turpethum* (L.) S. Manso., emarginate in *Merremia emarginata* (Burm. f.) Hall. f.

The base of the leaves was cordate in *Argyreia hirsutissima* (Clarke) Raiz., *Argyreia laxiflora* (Prain) Prain, *Ipomoea aquatica* Forssk., *Ipomoea biflora* (L.) Pers., *Ipomoea carnea* Jacq., *Ipomoea indica* (Burm.f.) Merr., *Ipomoea marginata* (Desr.) Verd., *Jacquemontia pentantha* (Jacq.) G. Don, *Merremia emarginata* (Burm. f.) Hall. f., *Merremia umbellata* (L.) Hall. f., *Merremia vitifolia* (Burm. f.) Hall. f., *Operculina turpethum* (L.) S. Manso., and *Porana volubilis* Burm. f., lobate in *Evolvulus nummularis* (L.) L., acute in *Ipomoea cairica* (L.) Sweet., and *Merremia aegyptia* (L.) Urban., deeply cordate in *Ipomoea hederifolia* L., *Ipomoea obscura* (L.) Ker. Gawl, *Ipomoea tricolor* Cav., and *Ipomoea triloba* L., broadly cordate in *Merremia hederacea* (Burm. f.) Hall.f.

The leaf margin was entire in *Argyreia hirsutissima* (Clarke) Raiz., *Argyreia laxiflora* (Prain) Prain, *Evolvulus nummularis* (L.) L., *Ipomoea*

aquatica Forssk., *Ipomoea biflora* (L.) Pers., *Ipomoea carnea* Jacq., *Ipomoea hederifolia* L., *Ipomoea marginata* (Desr.) Verd., *Ipomoea obscura* (L.) Ker. Gawl, *Ipomoea tricolor* Cav., *Ipomoea triloba* L., *Jacquemontia pentantha* (Jacq.) G. Don, *Merremia emarginata* (Burm. f.) Hall. f., *Merremia hederacea* (Burm. f.) Hall. f., *Merremia umbellata* (L.) Hall. f., *Operculina turpethum* (L.) S. Manso. and *Porana volubilis* Burm. f., 3-lobed in *Ipomoea indica* (Burm.f.) Merr., palmately lobed entire in *Ipomoea cairica* (L.) Sweet., *Merremia aegyptia* (L.) Urban., and *Merremia vitifolia* (Burm.f.) Hall. f.

The texture of leaves was membranaceous in most of the study species. The coriaceous texture was observed only in *Argyreia hirsutissima* (Clarke) Raiz., *Argyreia laxiflora* (Prain) Prain, *Ipomoea carnea* Jacq. and *Merremia emerginata* (Burm.f) Hall. f. The primary vein size was stout in most of the species. The massive size was found in *Evolvulus nummularis* (L.) L., *Ipomoea biflora* (L.) Pers., *Ipomoea cairica* (L.) Sweet., *Ipomoea hederifolia* L., *Ipomoea marginata* (Desr.) Verd., *Ipomoea obscura* (L.) Ker.-Gawl, *Ipomoea triloba* L. and *Porana volubilis* Burm. f.

The maximum leaf area of *Argyreia laxiflora* (Prain) Prain. is 460 mm². The minimum leaf area of *Evolvulus nummularis* (L.) L. in 40 mm². The maximum number of areoles was *Porana volubilis* Burm. f. in 20 per mm². The minimum number of areole was *Ipomoea aquatica* Forssk. in 6 per mm². The highest vein order of 5 in most of the species. The highest vein order of 4 in *Argyreia hirsutissima* (Clarke) Raiz., *Ipomoea aquatica* Forssk., *Ipomoea cairica* (L.) Sweet., *Ipomoea carnea* Jacq., *Ipomoea tricolor* Cav., *Merremia aegyptia* (L.) Urban., *Merremia hederacea* (Burm. f.) Hall. f., and *Operculina turpethum* (L.) S. Manso.

Stoma type was anisocytic in *Argyreia hirsutissima* (Clarke) Raiz., *Ipomoea biflora* (L.) Pers., and *Ipomoea tricolor* Cav., paracytic in *Argyreia laxiflora* (Prain) Prain., *Ipomoea cairica* (L.) Sweet., *Ipomoea carnea* Jacq., *Ipomoea hederifolia* L., *Ipomoea marginata* (Desr.) Verd., *Merremia aegyptia* (L.) Urban., *Merremia hederacea* (Burm. f.) Hall.f., and *Merremia vitifolia* (Burm. f.) Hall. f.. It was anomocytic in *Evolvulus nummularis* (L.) L., *Ipomoea aquatica* Forssk., *Ipomoea indica* (Burm.f.) Merr., *Ipomoea obscura* (L.) Ker. Gawl, *Ipomoea triloba* L., *Jacquemontia pentantha* (Jacq.) G. Don, *Merremia emarginata* (Burm. f.) Hall. f., *Merremia umbellata* (L.) Hall. f., *Operculina turpethum* (L.) S. Manso. and *Porana volubilis* Burm. f.

The first comprehensive account of venation patterns in leaves was recorded by Ettinghausen (1861 as cited in Bhat 1995). Later Kerner & Oliver (1897) and Moutan (1970) classified the venation pattern of angiosperm

leaves. They made an attempt to study the venation pattern and leaf architecture in some dicotyledons.

The present study of the leaf architecture in the family Convolvulaceae, with special reference to its taxonomic significance, is based exclusively upon the classification. The major venation pattern in all the species conformed to the typical pinnate actinodromous type. Therefore it is showing that this structure is constant criterion for taxonomic purposes in studying Convolvulaceae. Hickey (1973) stated that aeroles were well developed and usually exhibited free vein endings which were with or without terminal tracheids. Among the study species *Ipomoea hederifolia* L., *Ipomoea marginata* (Desr.) Verd., *Ipomoea obscura* (L.) Ker. Gawl., *Ipomoea tricolor* Cav., *Ipomoea triloba* L., *Jacquemontia pentantha* (Jacq.) G. Don. and *Merremia emarginata* (Burm.f.) Hall. f. possessing the terminal tracheids.

Ash *et al.* (1999) stated that the most systematically valuable features of leaves are in the venation, and quantification of vein networks. The initial sorting of a collection is usually done on the basic of toothed versus entire margins, primary and secondary vein patterns, and the presence and type of lobes. These characters are usually stable within morphotypes. According to the present study on Convolvulaceae also these characters are valuable characteristics.

Inamdar & Shenoy (1980) stated that the leaves of Convolvulaceae possess the highest vein order up to 5° or 6°. The number of second degree veins on either side of the primary vein vary from 5 to 10. In the present study the highest vein order was up to 5° and the number of second degree vein were found to be up to 14. Inamdar & Shenoy (1980) observed that eventually both the branches of secondary vein fuse to form common strand it was peculiarly bifurcated at the point of origin from the primary in *Evolvulus nummularis*. According to the present study these characteristics was also found in *Ipomoea hederifolia* L., *Ipomoea indica* (Burm.f.) Merr., *Ipomoea obscura* (L.) Ker. Gawl.

During the present study, simple or branched vein endings were observed. The marginal ultimate venation was incomplete and this remained more or less consistent within the species of one genus. The major leaf architectural characters, therefore, can be used for taxonomic consideration.

Bhat (1995) stated that because of the minor characters, such as aeroles and vein endings differ even within the species, they cannot be used as taxonomic criteria among the genus *Hibiscus*. Zhang *et al*. (2015) stated that characters from leaf venation were once considered difficult to use for taxonomic purposes, owing to problems with their description. The more

detailed investigations of minor characters of venation of a wide range of species will be helpful for a better understanding of leaf architecture. In the present study, the leaf architectural characters are found as valuable characters for members of Convolvulaceae. Todzia & Keating (1991) stated that although leaf architecture of extant angiosperms has been shown to be useful to identify fossil remains and elucidate to intra- and interfamilial relationships, leaf architecture has been studied in only a relatively minute fraction of angiosperms. Therefore, it is needed to extend to research works on various members and groups of flowering plants.

It is sincerely concluded that the architectural characteristics of present study among the species under Convolvulaceae are the valuable evidence for taxonomic identification and systematic study on that family in the future.

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STUDY ON ANTIFUNGAL ACTIVITY OF ENDOPHYTIC FUNGI ON PATHOGENIC FUNGI FROM SUNFLOWER SEEDS AND IDENTIFICATION OF SELECTED ENDOPHYTIC AND PATHOGENIC FUNGI

Taik Paing*

Abstract

In the isolation of endophytic fungi, 5 different plant sources were collected in Pathein Area and10 kinds of endophytic fungi (TP-01,02,03,04,05,06,07,08,09,10) were isolated. For the antifungal activities, the pathogenic fungi were isolated from the seeds of sunflower grown in Hinthada Area. Three kinds of pathogenic fungi (PF-01,PF-02,PF-03) were isolated by serial dilution methods. Ten kinds of isolated endophytic fungi were tested for antifungal activities. Among them, 4 kinds of endophytic fungi (TP-01,08,09,10) showed the antifungal activities (15 mm, 14 mm, 17 mm and 21 mm of inhibitory zones) on pathogenic fungus (PF-03) of sunflower seeds. According to these inhibitory zones, the biggest zone (21 mm) of endophytic fungus from *Polygonum barbatum* L. (suzat-pan) belonging to the family of Polygonaceae was selected for further investigation. Selected endophytic fungus (TP-10) and pathogenic fungus (PF-03) were identified by using reference keys. These selected fungus were important for all farmers because the seed-borne fungi were deterioration in sunflower oil and lost yield.

.**Key words**: isolation of fungi, antifungal activities, identification of fungi

Introduction

Soil, plant parts (living and fallen leaves, leaf litters), dung, insect, fresh water and marine water are the typical materials for microbial sources to isolate the microorganisms (Harayama and Isono, 2002). Plants can be considered as a new isolation source of microorganisms. This means that there is much possibility of findings new microorganisms (Scott and Lori, 1996). The medicinal plants in Myanmar are useful for therapeutic and microorganisms which living inside these healthy plant parts are also useful to produce the metabolites (Saisamon and Nipawam, 2007).

The deterioration in sunflower oil due to seed-borne fungi is of a great importance. Sunflower (*Helianthus annuus* L.), considered as commercial oil crop all over the world, the crop is widely cultivated in Egypt and in many countries all over the world. Sunflower is particularly used for production of

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edible oils as well as for seed consumption. The crop is attacked by numerous seed mycoflora and these pathogens may affect the crop resulting in a reduction of the seed quantity and quality. The direct impact of storage fungi on the economical part of the plant (seed) need further studies to find out the different effects of storage fungi on sunflower oil in order to increase oil yield and crop quality for human consumption and food industries (El-Wakil, 2014).

In continuation of screening programme for biological activities of the higher plants, studied *Polygonum barbatum* L. *Polygonum barbatum* L. is called joint weed, smartweed and knotgrass in Philippine. Its young leaves and shoots are cooked as vegetables. In medicinal properties, the sap of pounded leaves applied to wounds is considered an effective cicatrizant. Seeds are used to relieve colic pains. Roots are used as astringent. In China, leaves and stems are used to wash wounds and ulcers. The sap is applied to wounds as antiseptic. The paste of roots is used for treatment of scabies (Mao, 2012). The leaves of *Polygonum barbatum* L. were used for isolation of endophytic fungi for the antifungal activity.

Aims and objectives of present work are (i) to isolate the endophytic fungi from different plant sources;(ii) to study the isolation of pathogenic fungi from the infected seeds that cause the diseases;(iii) to investigate the antifungal activities of endophytic fungi on pathogenic fungi and (iv) to identify selected endophytic and pathogenic fungi.

Materials And Methods

Isolation of endophytic fungi

Five different plants were utilized in the isolation and they were collected from Pathein Area (Table-1). In the isolation procedure of endophytic fungi (Figure-1), the leaves were washed in running tap water for 15 minutes and sterilized by soaking in 95% alcohol for 15 seconds. Then, the leaves were cut into small pieces and dried on the sterilized tissue paper. After that, cut pieces were incubated on nutrient agar plate (LCA medium) for 3 days to 1 week at room temperature.

Table-1 Plant samples used for screening of endophytic fung

No.	Scientific Name	Myanmar name	Family
1	*Premna corymbosa* Rottle & Willd	Pyae sone	Lamiaceae
2	*Tadehagi triquetrum* (L) H. Ohashi	Lauk thay	Fabaceae
3	*Cissus discolor* Blumi, Bijd.	Tabin-taing- mya-nan	Vitaceae
4	*Gynura procumbens* (Lour.) Merr.	Pya-me-swae	Asteraceae
5	*Polygonum barbatum* L.	Suzat pan	Polygonaceae

The procedure of isolation method for endophytes (Tomita, 1998)

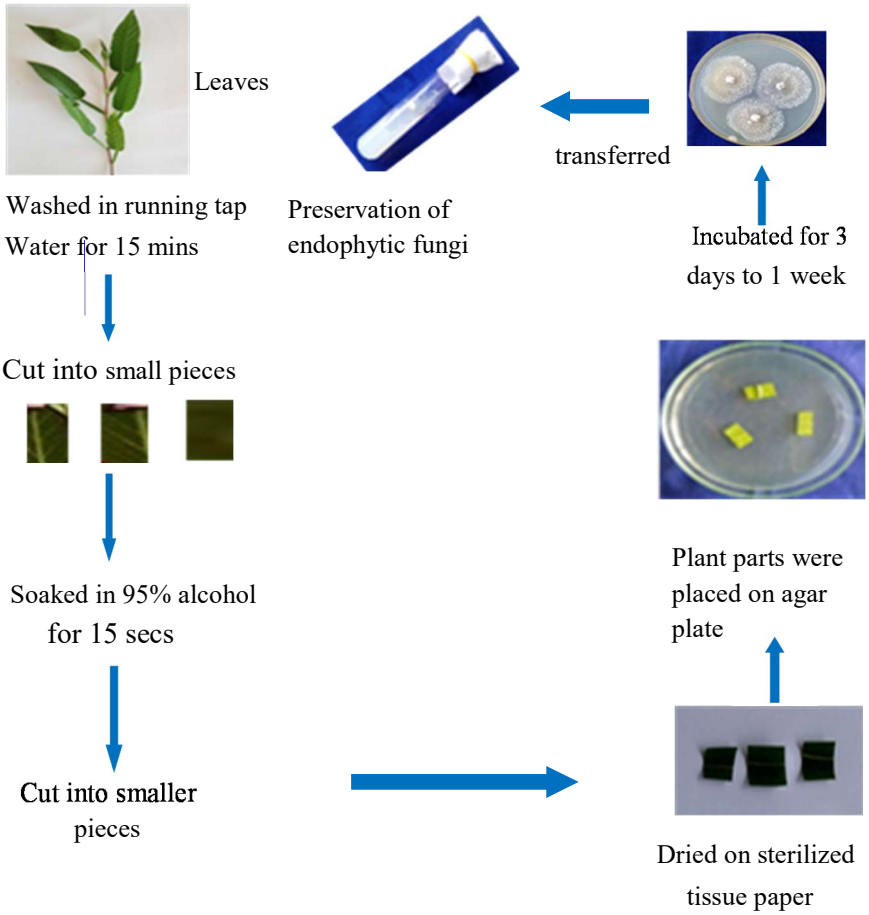


Figure 1. Isolation procedure of endophytes from plant parts

Isolation of pathogenic fungi from dried seeds of *Helianthus annuus* L

Seeds may carry spores of some pathogenic fungi. In such cases, a sedimentation or seed-washing test is useful for detecting spores. Seeds with spores samples were placed in test tube containing 10 ml of 0.85% saline. The aqueous suspension (0.5 ml) was transferred into other test tube containing 4.5 ml of distilled water and then 0.5 ml suspension into 4.5 ml distilled water tube after that 1 ml suspension into 4 ml distilled water. After serial dilution for spores suspension, 1 ml suspension was inoculated onto the nutrient agar plates (glucose 1%, peptone 0.3%, agar1.8%) incubated for 3-7 days at room temperature (Omura, 1985).

Serial dilution method, in biotechnology, Japan (Omura, 1985)

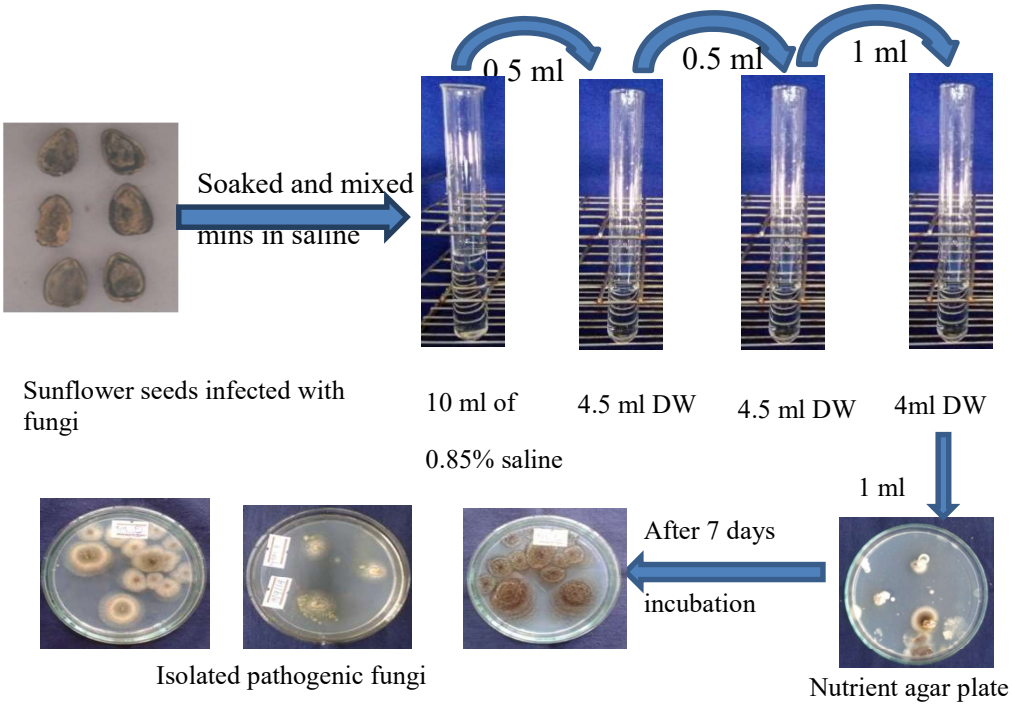


Figure 2. Isolation procedure of pathogenic fungi

Medium used of the isolation of fungi

LCA medium (Ando, 2004)

Glucose	0.1 g
K_2HPO_4	0.1 g
$MgSO_4.7H_2O$	0.02 g
$NaNO_3$	0.2 g
KCl	0.02 g
Yeast Extract	0.02 g
Agar	1.8 g
DW	100 mL

PGA medium (Ando, 2004)

Potato	20.0 g
Glucose	2.0 g
Agar	1.8 g
DW	100 mL

(After autoclaving chloramphenicol were added to the medium)

Preliminary study of the activities of isolated fungi

The isolated fungi were inoculated into the preculture medium (glucose 1%, potato dextrose broth 10%, at pH 7.0) for 3 days at room temperature. After three days, the preculture (1%) was transferred into the fermentation medium (glucose 1%, yeast extract 0.3%, peptone 0.3%, at pH 7.0) and carried out for 10 days by static culture. Then, the fermented broth was used to check the antifungal activity by paper disc diffusion assay method (Suto, 1999). Paper disc having 8 mm in diameter (Advance, Tokyo Roshi Kaisha Co., Ltd., Japan) were utilized for antifungal activity. The paper discs were soaked in fermented broth each and allowed to dry.

Paper disc diffusion assay method (Suto, 1999)

This method was used for the antifungal activity by the pathogenic fungi. The assay medium (glucose 1%, peptone 0.3%, agar 1.8% at pH 7.0) was utilized for these fungi. The pathogenic fungi were inoculated in assay broth for 3 days at room temperature. One percent of pathogenic fungi was added to assay medium and then poured into petridishes. After solidification, the paper discs impregnated with fermented broth samples were applied on the

agar plates and the plates were incubated for 24-36 hrs. Clear zones (inhibitory zones) surrounding the paper discs indicate the presence of bioactive compounds which inhibit the growth of the pathogenic fungi.

Preliminary identification of selected endophytic fungus and pathogenic fungus

The morphological and microscopical characters were observed by the methods of Ando and Inaba (2004) and Barnett (1956): Fungi Imperfecti.

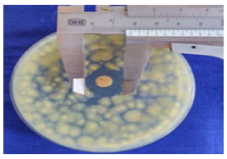
RESULTS

Table -2. Isolated endophytic fungi from plant sources

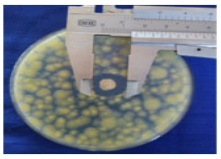
No.	Scientific Name	Myanmar name	No. of isolates	Isolated endophytic fungi
1	*Premna corymbosa* Rottle &Willd	Pyait sone	1	TP-01
2	*Tadehagi triquetrum* (L.) H. Ohashi	Lauk thay	2	TP-02, 03
3	*Cissus discolor* Blumi, Bijd.	Tabin-taing-mya-nan	3	TP-04,05,06
4	*Gynura procumbens* (Lour.) Merr.	Pya-me-swae	1	TP-07
5	*Polygonum barbatum* L.	Suzat pan	3	TP-08,09,10
	Total isolates		10	

Table-3. Antifungal Activities of Isolated Endophytic Fungi

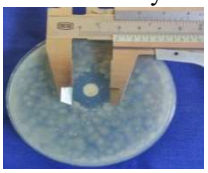
Isolated endophytic fungi	Activities on three pathogenic fungi		
	PF-01	PF-02	PF-03
TP-01	-	-	+(15 mm clear zone)
TP-02	-	-	-
TP-03	-	-	-
TP-04	-	-	-
TP-05	-	-	-
TP-06	-	-	-
TP-07	-	-	-
TP-08	-	-	+(14 mm clear zone)
TP-09	-	-	+(17 mm clear zone)
TP-10	-	-	+(21 mm clear zone)



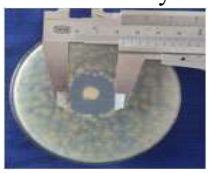
TP-01
(15 mm inhibitory zone)



TP-08
(14 mm inhibitory zone)



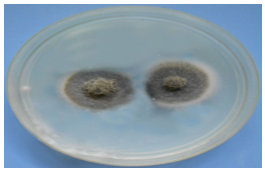
TP-09
(17 mm inhibitory zone)



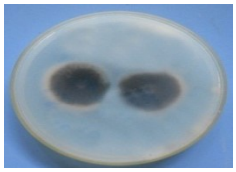
TP-10
(21mm inhibitory zone)

Figure 3. Antifungal activities of endophytic fungi on pathogenic fungi (PF-03)

Morphological and microscopical characters of selected endophytic fungus (TP-10)



Front colour



Back colour

Figure-3 Morphological Character of Selected Endophytic Fungus (TP-10)

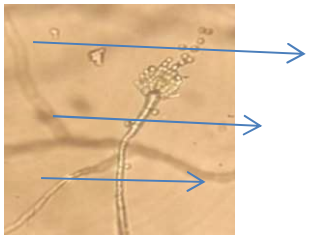
After 3 days of cultivation, it was observed that dark brown colonies were reached 2.5cm diameter at room temperature on PGA medium.

Distinctive character of selected endophytic fungus (TP-10)

conidia

conidiophore

hyphae



Photomicrograph X 100

Distinctive character of selected endophytic fungus (TP-10) was found that hyphae are hyaline, septate and branched; conidiophore brown, unbranched, hyaline at the base, bearing clusters of multi-phialides; conidia hyaline, lacking septum, one-celled, ameroconidium, globose, conidial chain long with many conidia(6-10), borned at the apex of the short phailide.

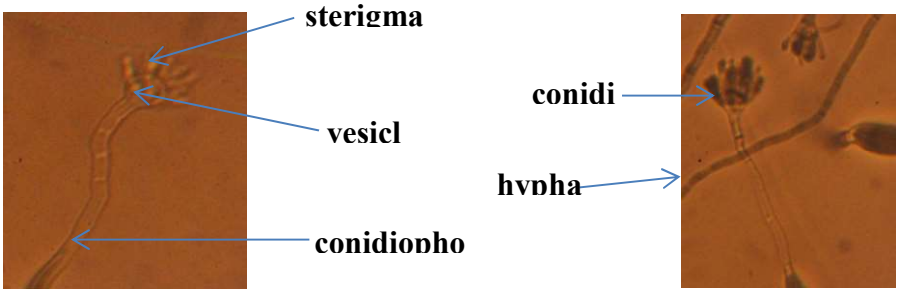
According to the morphological and microscopical characteristics features and based on the references keys of Ando and Inaba (2004), Barnett,(1956),this endophytic fungus was grouped as the fungi imperfecti may be *Aspergillus* sp.

Morphological and microscopical characters of pathogenic fungus (PF-03)

Front colour Back colour

Figure-4 Morphological Character of Pathogenic Fungus (PF-03)

Distinctive character of selected pathogenic fungus (PF-03)



Distinctive character of pathogenic fungus (PF-03) was found that hyphae are septate and hyaline;conidiophore with septum and hyaline, simple, long and ends in a dome–shaped multinucleate head called the vesicle, subglobose, club-shaped; sterigmata are tubular outgrowths from the vesicle, bearing cluster of 4-6 phialides; conidia hyaline, one celled, amerospore, globose.

According to the morphological and microscopical characteristics features and based on the references keys of Ando and Inaba (2004), Barnett, H.L., (1956), this pathogenic fungus was grouped as the fungi imperfecti may be *Aspergillus* sp.

Disscussion and Conclusion

In the course of the isolation for antifungal metabolites producing microorganisms, ten fungi were isolated from five different kinds of plant leaves collected at Pathein Township, Ayeyarwady Region. One fungi (TP-01) was isolated from the leaf of *Premna corymbosa* Rottle & Willd.; two

fungi (TP-02, TP-03) were isolated from the leaf of *Tadehagi triquetrum* (L.) H. Ohashi.; three fungi (TP-04, TP-05, TP-06) were isolated from the leaf of *Cissus discolor* Blumi, Bijid..; one fungi (TP-07) was isolated from the leaf of *Gynura procumbens* (Lour.)Merr. and three fungi (TP-08, TP-09, TP-10) were isolated from the leaf of *Polygonum barbatum* L. by surface sterilization method. For the antifungal activities, the pathogenic fungi were isolated from the seeds of sunflower (*Helianthus annuus* L. belonging to Asteraceae) grown in Hinthada Area. Three kinds of pathogenic fungi (PF-01, PF-02 and PF-03)were isolated by serial dilution method.

In order to find new antimicrobial metabolites, it is very important to set up an effective screening which has a unique target and deals with unique microorganism (Phay, 1997). During the study of antifungal activities, isolated endophytic fungi, TP-01 showed the antifungal activities (15 mm of inhibitory zone) on pathogenic fungus (PF-03) of sunflower seeds; TP-08 against 14 mm, TP-09 showed 17 mm and TP-10 showed 21 mm clear zones of antifungal activities on pathogenic fungus (PF-03). Among them, endophytic fungus TP-10 showed more highly antifungal activities (21 mm clear zone) against pathogenic fungus (PF-03) than other isolated fungi, these endophytic fungus (TP-10) from *Polygonum barbatum* L. (suzat-pan) belonging to the family of Polygonaceae. Therefore this strain TP-10 was selected for further investigation of identification.

In the identification of selected endophytic and pathogenic fungi, antifungal metabolite producing selected endophytic fungus TP-10 was grouped and keyed out fungi imperfecti. Based on the macroscopical - microscopical characters and the reference keys, this fungus TP-10 was grouped as the fungi imperfecti may be *Aspergillus* sp. However, identification of pathogenic fungus PF-03, according to the morphology and microscopical charasteristics features and based on the references keys, this pathogenic fungus (PF-03) was grouped as the fungi imperfecti may be *Aspergillus* sp. These selected fungi benefit for the against of infected seed-borne fungi. These investigations clearly indicate that the selected endophytic fungus, the genus *Aspergillus* sp. is useful for the production of antifungal substances. The type of antifungal agents produced by these research will be investigated as well.

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SCREENING OF SOIL FUNGI FROM MAGWAY TOWNSHIP AND IDENTIFICATION OF SELECTED SOIL FUNGUS ESPECIALLY AGAINST *STAPHYLOCOCCUS AUREUS*

Phyo Phyo Soe San

Abstract

In the study on the isolation of soil fungi, 43 fungi were isolated from five different soil samples collected at Magway Township, Magway Region. In the investigation of antimicrobial activities of 43 soil fungi, *Agrobacterium tumefaciens, Bacillus pumalis, Candida albicans, Escherichia coli* and *Staphylococcus aureus* were used for the test throughout the research studies. Among them, since PS-13, PS-14, PS-15, PS-16, PS-17 and PS-18 showed the antimicrobial activities against *Staphylococcus aureus and E. coli.* PS-13 showed most highly selective antimicrobial activity against *Staphylococcus aureus* than the other fungi. The characters of selected strain PS-13 are similar to those of *Cephalosporium* sp. (Ando and Inaba (2004), Barnett (1956)). So that strain PS-13 was determined as *Cephalosporium* sp.

Key words: antimicrobial activity, identification

Introduction

Microorganisms live in all parts of the biosphere where there is liquid water, including soil, hot springs, on ocean floor, high in the atmosphere and deep inside rocks within the earth's crust. The typical materials for microbial sources are soil living and fallen leaves, leaf litters, dung, insect, fresh water and marine water. The soil sample is the most effective and popular materials for the isolation of fungi and actinomycetes (Harayama and Isono, 2002). Microorganisms are a virtually unlimited source of novel chemical structures with many potential therapeutic applications (Behal, 2000).

Fungi are well known as prolific producers of biologically active natural products (Hara *et al.*, 2007). Most of the naturally occurring antibiotics have been isolated from soil microorganisms. Isolating microorganisms from the environment is the first step in screening for natural products such as secondary metabolites and enzymes (Hunter-Cevera and Belt, 1999).

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Aim and objectives of present work are to know the isolation programs and varieties of fungi from different soil samples, to study the morphology of isolated soil fungi and their antimicrobial potentials.

Materials And Methods

Study area

The fourty-three soil fungi were isolated from five different soil sampls Magway Township, Magway Region (Figure 1). The study site five places(1) Agricultural oil-crops research centre, located between 20˚9'23.29"N and 94˚56'0.24"E, (2)Local Audit Organization, located between 20˚9'19.81" N and 94˚57'10.76" E, (3) Kanther Lake, located between 20˚ 8'43.37" N and 94˚ 56'20.40" E, (4) Magway University, located between 20˚ 7'54.58" N and 94˚56'17.76" E, and (5) Magway Airport, located between 20˚9'29.08"N and 94˚ 58' 22.62" E (Figure 1).

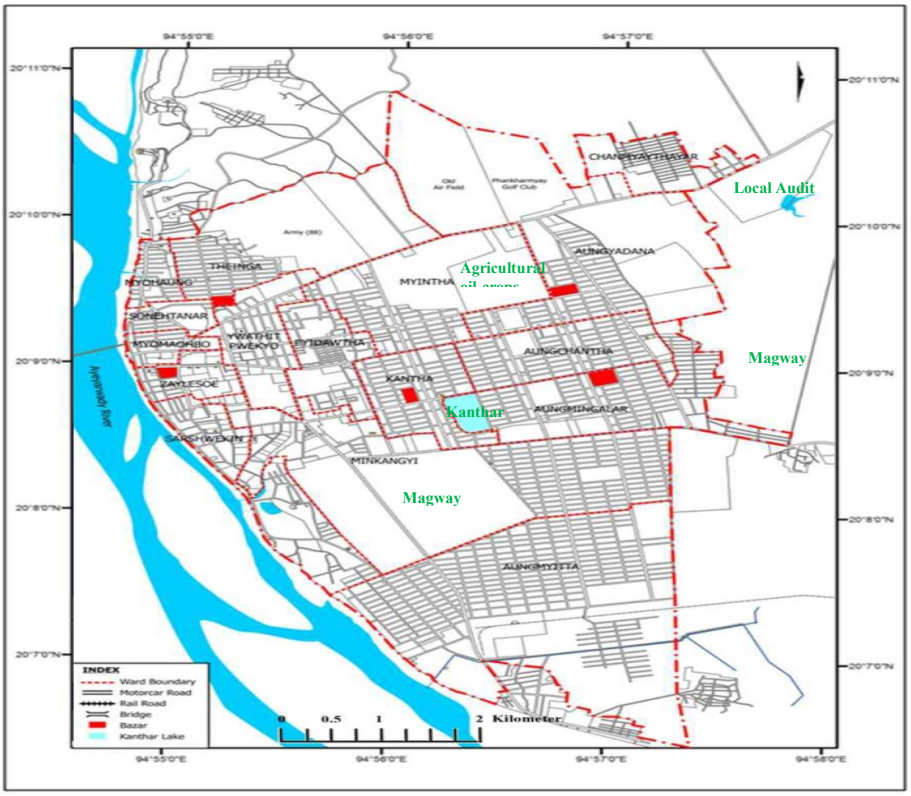


Figure 1. Location map of Magway Township

Study method

1. Physical dilution method (Phay & Amachi,2005)
2. Physical treatment serial dilution method (PBCC, 2004)
3. Physical and chemical treatment dilution method (Phay & Amachi, 2005)
4. Feeding method (PBCC, 2004)

Screening or Preliminary Study for Antimicrobial Activities by Paper Disc Diffusion Assay (Tomita, 1998)

The isolated fungi were grown at 25°C for 7 days on PGA medium. The isolated fungi were inoculated into seed medium and incubated at 25°C for 3 days. 25 ml of seed culture was transferred into the fermentation medium. The fermentation was carried out for 7 days. After the end of fermentation, the fermented broth (20 µl) was used to check the antimicrobial activity against test organisms by paper disc diffusion assay (Figure 4). Paper disc having eight millimeter diameter (Advantec, Toyo Roshi Kaisha Co., Ltd., Japan) was utilized for antimicrobial assays.

The assay medium (Glucose -1%, Polypepton- 0.3%, KNO_3- 0.1 %, Agar-1.8%, Distilled water-100 ml, pH-6.5) was used for the antimicrobial activity test. One percent of test organism was added to assay medium, then poured into plates. After solidification, paper discs impregnated with samples (fermented both) were applied on the agar plates and the plates were incubated for 24-36 hours at 28 to 30°C. Clear zones (inhibitory zones) surrounding the test discs indicate the presence of bioactive metabolites which inhibits the growth of test organisms.

The test organisms used in paper disc diffusion assay were *Agrobacterium tumefaciens, Bacillus pumalis* NITE 47239, *Candida albicans* NITE 83297, *E.coli AHU* 5436, and *Staphylococcus aureus* AHU 8465. The test organisms were supported by NITE (National Institute of Technology and Evaluation, Japan) and Faculty of Agriculture, Hokkaido University, Japan (Table 1).

Table 1. Test organisms used in antimicrobial activities (NITE)

No.	Test organisms	Infections
1.	Agrobacterium tumefaciens (IFO5431)	Crown gall diseases
2.	Bacillus pumalis (NITE47239)	Fever and food poisoning
3.	Candida albicans (NITE09542)	Candidosis
4.	Escherichia coli (AHU5436)	Diarrhoea
5.	Staphylococcus aureus (AHU8465)	Skin disease and Food poisoning

Results

Isolation from Soil Samples

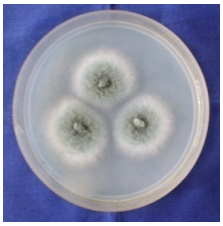
In the course of the isolation for antimicrobial metabolite producing microorganisms, 43 fungi were isolated from five different kinds of soil samples (Table-2 and Figure-2 and 3).

Table 2. Isolated soil fungi from two different soil samples by using four methods (PS-01 to PS-18)

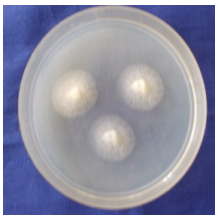
Soil No.	**Isolation method**	**Isolated fungi**
S-1	Physical dilution method	PS-01,02,03
	Physical Treatment serial dilution method	PS-04,05
	Physical and chemical treatment dilution method	PS-06,07
	Feeding method	PS-08,09
S-2	Physical dilution method	PS-10,11,12
	Physical treatment serial dilution method	PS-13,14
	Physical and chemical treatment dilution method	PS-15,16
	Feeding method	PS-17,18

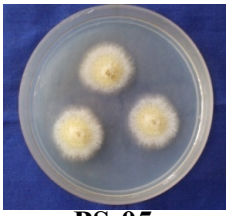
Soil No.	Isolation method	Isolated fungi
S-3	Physical dilution method	PS-19,20
	Physical treatment serial dilution method	PS-21,22
	Physical and chemical treatment dilution method	PS-23,24
	Feeding method	PS-25,26
S-4	Physical dilution method	PS-27,28
	Physical treatment serial dilution method	PS-29,30,31
	Physical and chemical treatment dilution method	PS-32,33
	Feeding method	PS-34,35
S-5	Physical dilution method	PS-36,37
	Physical treatment serial dilution method	PS-38,39
	Physical and chemical treatment dilution method	PS-40,41
	Feeding method	PS-42,43

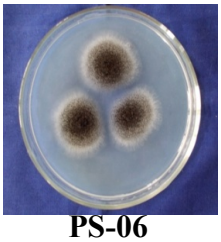

PS-01

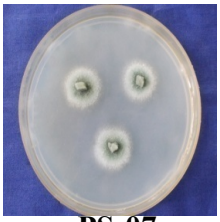

PS-02


PS-03


PS-04


PS-05


PS-06


PS-07


PS-08

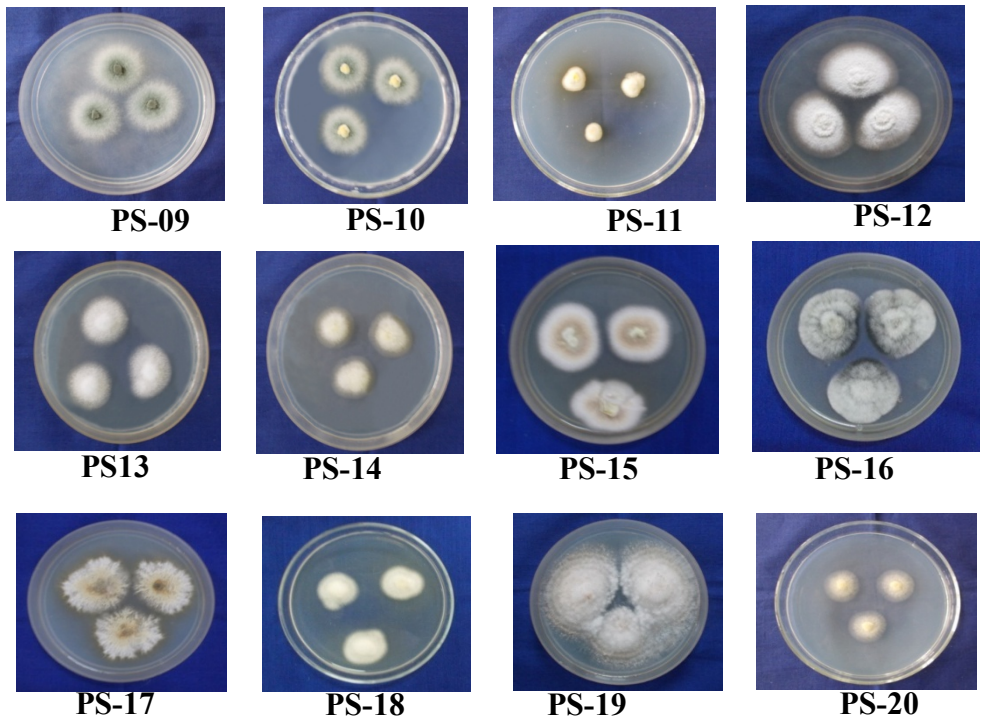
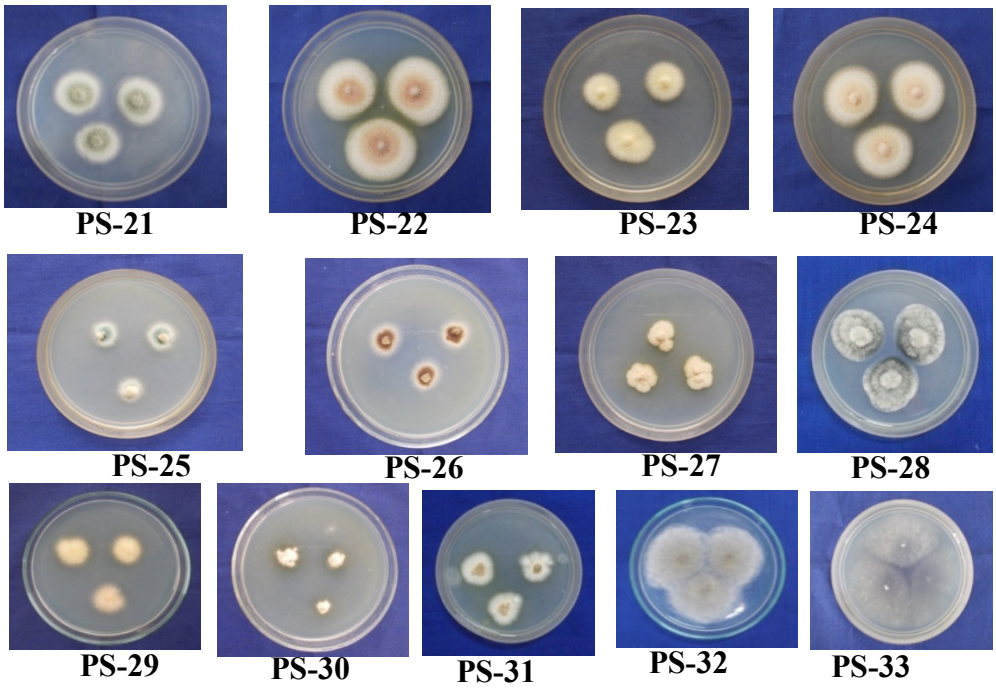


Figure 2. Morphology of isolated fungi on PGA medium (5 days old culture)



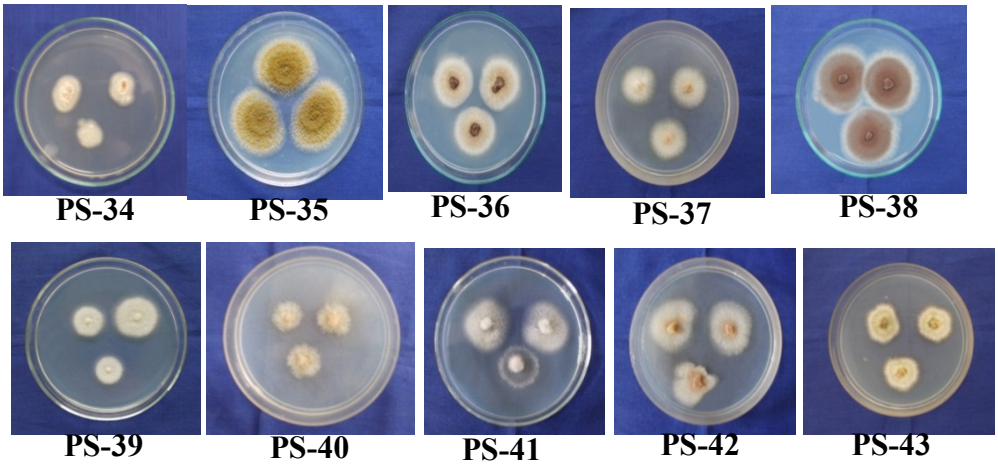


Figure 3. Morphology of isolated fungi on PGA medium (5 days old culture)

Table 3. Antimicrobial activities and starch hdrolyzing activities of isolated soil fungi

Stain No.	*Bacillus pumalis*	*Candida albicans*	*E. coil*	*S. aureus*	*Agro. tumefaciens*
PS-01	-	-	-	-	-
PS-02	-	-	-	-	-
PS-03	-	-	-	-	-
PS-04	-	-	-	-	-
PS-05	-	-	-	-	-
PS-06	-	-	-	-	-
PS-07	-	-	-	-	-
PS-08	-	-	-	-	-
PS-10	-	-	-	-	-
PS-11	-	-	-	-	-
PS-12	-	-	-	-	-
PS-13	-	-	27mm	37mm	-
PS-14	-	-	26mm	32mm	-
PS-15	-	-	23mm	29mm	-
PS-16	-	-	25mm	30mm	-

Stain No.	*Bacillus pumalis*	*Candida albicans*	*E. coil*	*S. aureus*	*Agro. tumefaciens*
PS-17	+	-	27mm	28mm	-
PS-18	-	-	29mm	33mm	-
PS-19	-	-	-	-	-
PS-20	-	-	+	-	-
PS-21	-	-	-	-	-
PS-22	-	-	-	-	-
PS-23	-	-	-	-	-
PS-24	-	-	-	-	-
PS-25	-	-	-	-	-
PS-26	-	-	-	-	-
PS-27	-	-	-	-	-
PS-28	-	-	-	-	-
PS-29	+	-	-	-	-
PS-30	-	+	-	-	-
PS-31	-	-	-	-	-
PS-32	-	-	-	-	-
PS-33	-	-	-	-	-
PS-34	-	-	-	-	-
PS-35	-	-	-	-	-
PS-36	-	+	-	-	+
PS-37	-	-	-	-	-
PS-38	-	-	-	-	-
PS-39	-	-	-	-	-
PS-40	-	-	-	-	-
PS-41	-	-	-	+	-
PS-42	-	-	-	-	-
PS-43	-	-	-	-	-

Screening of Effective Microorganisms Isolated from Soil by Paper Disc Diffusion Assay

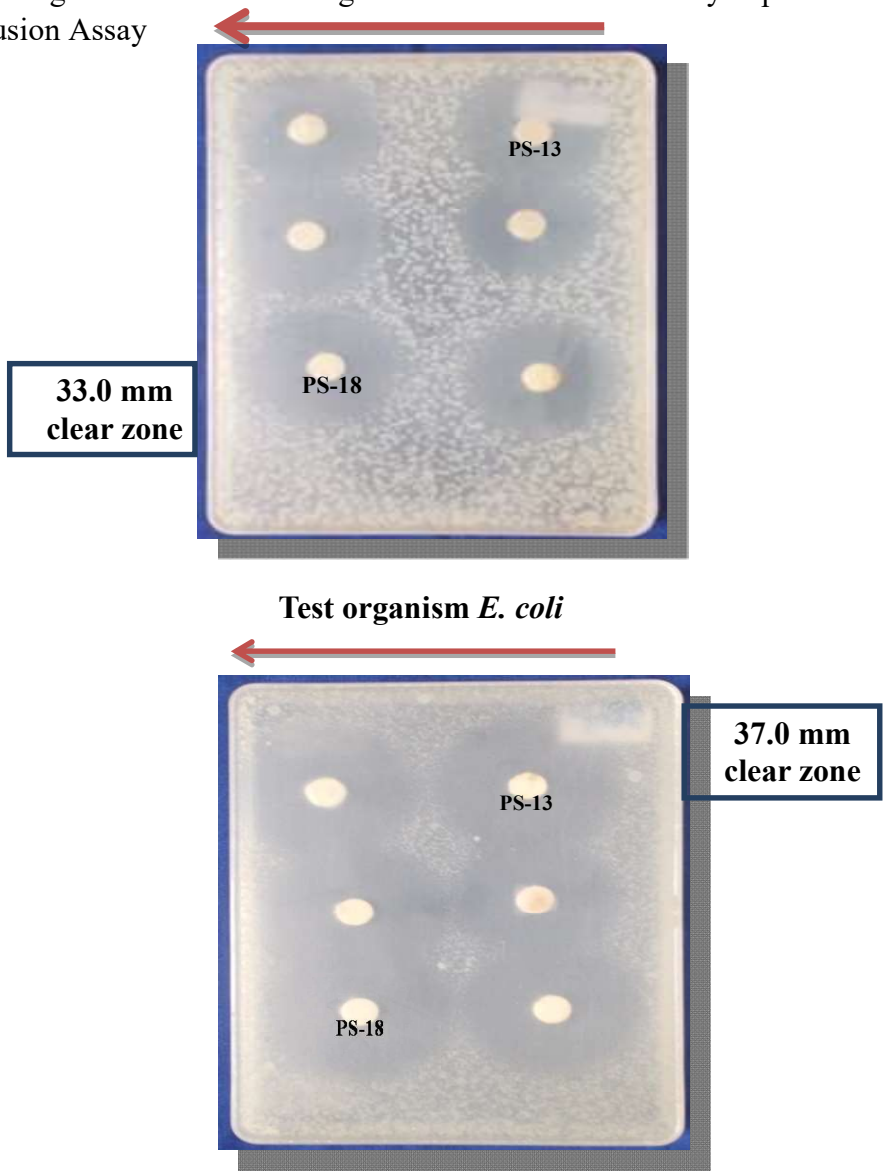
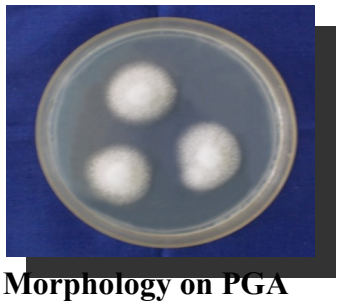
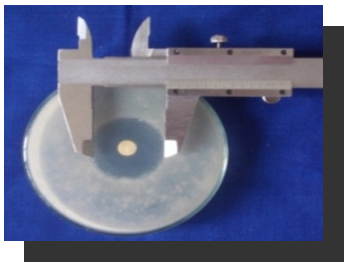


Figure 4. Antibacterial activity of isolated soil fungi



Morphology on PGA medium (5 days old culture)

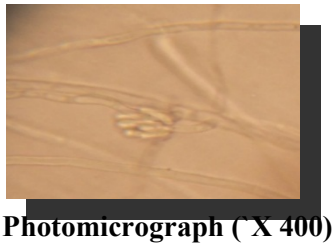


This fungus showed the antibacterial activity against *Staphylococcus aureus*(37.0 mm, clear zone)

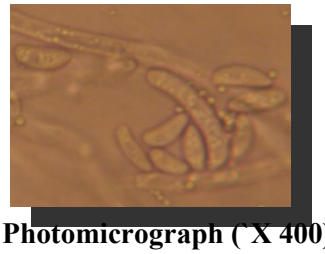
Figure 5. Morphology and antimicrobial activity of selected soil fungus PS-13.

Morphology of soil fungus PS-13

- Characteristically present hyphae with septa regularly
- Conidiophore upright, hyaline, unbranch, elongated with conidial production
- Conidia hyaline, lacking septum, amerospore, elliptical to elliptic fusiform, produce successively at the tip and collecting in a slime drop.



Photomicrograph (`X 400)



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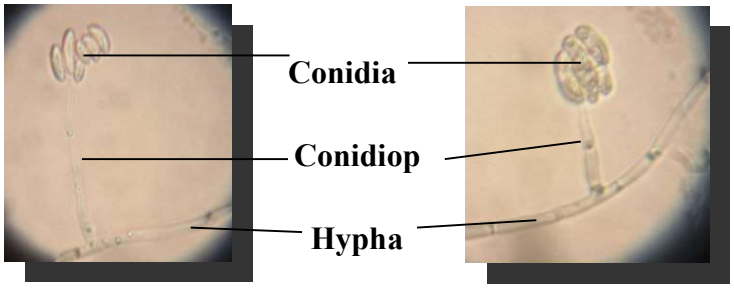

Photomicrograph (`X 400)** **Photomicrograph (`X 400)

Figure 6. Photomicrograph of *Cephalosporium*

Kingdom - Fungi
Division - Ascomycota
Class - Ascomycetes
Order - Moniliales
Family - Moniliaceae
Genus – *Cephalosporium*

Discussion And Conclusion

In the course of the screening of antimicrobial metabolite producing soil fungi from five different places collected at Magway township were utilized in order to find a new and effective antimicrobial activities with a specific target of fungi.In the precent study, 43 soil fungi were isolated from five different soil samples. During the studies of antimicrobial activities of 43 soil fungi, *Agrobacterium tumefaciens, Bacillus pumalis, Candida albicans, Escherichia coli* and *Staphylococcus aureus* were used for the test throughout the research studies. Among them, PS-13, PS-14, PS-15, PS-16, PS-17 and PS-18 showed the antimicrobial activities against *Staphylococcus aureus* and *Escherichia coli.* Fungus PS-13(37.0 mm clear zone) showed the highest activity against *Staphylococcus aureus* than the other fungi. Selected fungus PS-13 was isolated from the soil collected at Local Audit Organization. In the investigation of identification, fungus PS-13 possessing antibacterial activity was identified as *Cephalosporium* sp. on the basis of morphological-microscopial characters and reference keys (Ando and Inaba (2004), Barnett (1956)). In conclusion these selected soil fungus *Cephalosporium* sp. obtained from Local Audit Organization, Magway township, Magway region was observed that inhibit harmful diseases causing agents *Staphylococcus aureus.*So, these selected soil fungus can be regarded as a good source of antibiotic for human and animals.

Acknowledgements

I wish to express my sincere gratitude to Dr Nyunt Phay, Rector, Pathein University for his permission to do this research. I am greatefully indebted to Dr Wai Wai Nyunt and Dr Mi Mi Gyi, Pro-Rectors, Pathein University, for their encouragement. My special thanks go to Professor Dr Kay Thi Mya, Head of Botany Department Pathein University, for her invaluable support and suggestions. I extend my thanks to Dr Wah Wah Lwin, Professor, Department of Botany Pathein University, for her advice and kind help. I would like to express my heartfelt gratitude to my supervisor Dr Mya Htet Htet Aung, Lecturer, Department of Botany, Pathein University, for her invaluable overall guidance, suggestions and co-operation during my study.

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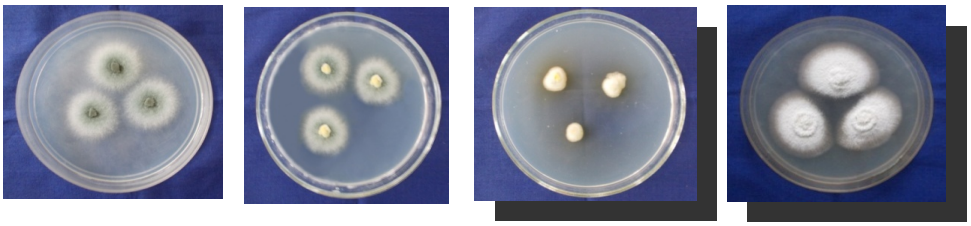

FERMENTATION STUDIES OF BIOACTIVE FUNGAL STRAIN *ASPERGILLUS* SP. SS 7 ISOLATED FROM *ZINGIBER CASSUMUNAR* ROXB

Soe Soe Yu Hnin[1], Yee Yee Thu[2], Aye Pe[3]

Abstract

Endophytic fungal strain *Aspergillus* sp. SS 7 isolated from the rhizome of *Zingiber cassumunar* Roxb. was used for the investigation of optimal fermentation conditions such as various carbon and nitrogen sources, different culture media, age of inoculum, size of inloculum and pH utilization. In utilization of carbon sources, starch and glycerol were the best whereas yeast extract and soybean were the best nitrogen sources. In antimicrobial activity of various carbon sources, glucose medium showed very high activity against *Candida albicans* whereas various nitrogen sources, oat meal medium indicated very high activity against *Malassezia furfur*. The investigation of the morphological characters on different media, medium 1, 3, 7, 9 and 10 were good media. As a result of antimicrobial activity on different media, medium 7 and medium 9 were the best for fermentation medium. In the study of inoculum optimization, two days old (age of inoculum) and 1.5% of seed culture at fifth day fermentation were suitable for the production of bioactive metabolites. In the study of pH utilization, pH 6 was the best for extraction of the bioactive compounds.

Key words: antimicrobial activity, *Aspergillus* sp. SS 7, *Zingiber cassumunar* Roxb.

Introduction

Fermentation is a metabolic process that converts sugar to acids, gases or alcohol. From thousands of year's mankind has used natural product, chemicals produced by plants, fungi, bacteria and other living organism in a variety of application: drugs and food. During the last two decades endophytes have been targeted as valuable sources of new bioactive compounds (Tadych and White, 2009). In the past two decades, many valuable bioactive compounds with antimicrobial, insecticidal and anticancer activities have been successfully discovered from the endophytic fungi. Methods to obtain

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bioactive compounds include the extraction from a natural source and the microbial production via fermentation (Parton and Willis, 1989). In the present study, optimal fermentation conditions and different media of endophytic fungal strain *Aspergillus* sp. SS 7 were conducted for extraction of the bioactive compounds.

Materials and Methods

Collection and outstanding characters of plant sample

The plant sample was collected from the Mhawe-Bi Township. Outstanding characters of plant sample was identified by Backer and Bakhuizen, 1968.

Morphological and microscopical characters of isolated fungal strain SS 7

Isolated fungal strain SS 7 grown on slant culture was transferred onto the plate containing sucrose, yeast extract medium. Then this plate was incubated at room temperature for 3-7 days. Colony forms, surface and reverse pigments of isolated strain and microscopical characters were studied according to Barnett, 1998.

Utilization of carbon and nitrogen sources

In this study the morphological characters of strain SS 7 were studied by using various carbon and nitrogen sources. Carbon sources are sucrose, glucose, starch, mannitol and glycerol whereas nitrogen sources are yeast extract, meat extract, malt extract, oat meal and soybean. Basal media for finding out suitable carbon sources are yeast extract 0.3%, K_2HPO_4 0.01%, $MgSO_4$ 0.01%,and $CaCO_3$ 0.01% while basal media for finding out suitable nitrogen sources are glycerol 1.0%, K_2HPO_4 0.01%, $MgSO_4$ 0.01% and $CaCO_3$ 0.01% (Monaghan *et al*., 1999).

Antimicrobial activity of strain SS 7 by using various carbon and nitrogen sources

Fungal strain SS 7 grown on slant culture was transferred into 50 ml flasks containing 25 ml of various carbon and nitrogen sources and incubated for ten days. The fermented broth was used to check antimicrobial activity by paper disc diffusion assay (Phay, 1997).

Morphological characters of strain SS 7 on different media

In this study, different media were used for media optimization. A piece of fungus from plate culture of strain SS 7 was inoculated on each of different media plates and incubated for 3-5 days (Monaghan *et al*., 1999). Various media were **medium 1 (**Polypeptone,Yeast medium), **medium 2** (Meat, Polypeptone, NaCl medium), **medium 3** (Yeast, Malt, Glucose medium), **medium 4** (Glycerol, K_2HPO_4,$MgSO_4$, NaCl medium), **medium 5** (Oat meal medium), **medium 6** (Glycerol, K_2HPO_4 medium), **medium 7** (Soybean, Mannitol medium), **medium 8** (K_2HPO_4,$MgSO_4$, NaCl medium), **medium 9** (Sucrose, Yeast extract medium), **medium 10** (Malt, Meat extract medium) and **medium 11** (Sucrose, Malt extract, Soluble starch medium)

Antimicrobial activity of strain SS 7 by using different media

A piece of fungus from plate culture of strain SS 7 was inoculated into each of eleven (50 ml) conical flasks containing 20 ml of different fermentation medium. These flasks were incubated at room temperature for two days. After two days these fermented broths were checked for their inhibitory activity by paper disc diffusion assay (Phay, 1997) as shown in Figure 1.

Figure 1. Different media for media optimization

Age of inoculum for strain SS 7

Two days old and three days old of seed cultures were transferred into 50 ml fermentation flasks containing 25 ml of medium 9 (SY: sucrose, yeast extract medium). They were incubated for seven days at room temperature. Then, these fermented broths were checked for their inhibitory activity by paper disc diffusion assay (Strobel and Sullivan, 1999).

Size of inoculum of strain SS 7

The proper cultivation and amount of inoculums are essential for the optimal production of bioactive metabolites. A piece from fungal plate culture of strain SS 7 was inoculated into 300 ml conical flasks containing 100 ml of medium 9 (SY; sucrose, yeast extract medium) seed medium. The flasks were incubated at room temperature for two days. After two days, the seed cultures (0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0% and 3.5%) were transferred into seven conical flasks containing 100 ml of fermentation medium as shown in Figure 2. The fermentation was carried out for ten days.

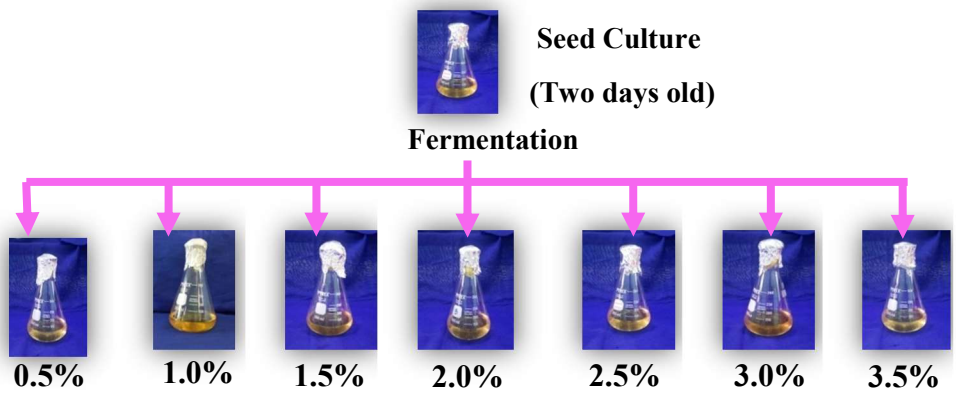


Figure 2. Seed culture and fermentation for size of inoculum

pH utilization of strain SS 7 (Monaghan *et al*., 1999)

For the seed culture, a piece from fungal plate culture of strain SS 7 was inoculated into 300 ml of conical flask containing 100 ml of medium 9 (SY; sucrose, yeast extract medium) and then flasks were incubated at room temperature for two days. Seven 300 ml conical flasks containing 100 ml fermentation medium were adjusted at pH 4, 5, 6, 7, 8, 9, 10 and autoclaved. After two days, the seed culture (1.5%) was transferred to each fermentation flask with pH 4 to 10 and fermentation was carried out for 3 days. After three days, seven fermentation flasks were checked their antimicrobial activity.

Ferm

Result

Outstanding characters of plant sample

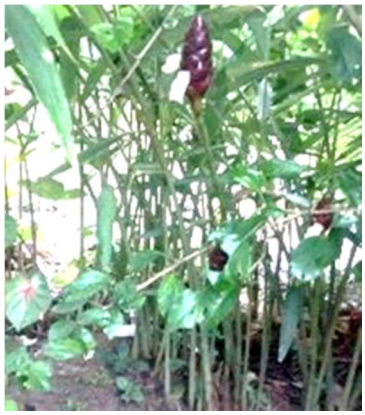


Figure 3. Habit of *Zingiber cassumunar* Roxb.

Scientific name - *Zingiber cassumunar* Roxb.
English Name - Bengal ginger
Myanmar name - Meik-Tha-Lin
Family - Zingiberaceae

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 $1+(2)^{st} + 2^{st}$, epipetalous; Filaments exserted, anthers dithecous, dorsifixed, longitudinal dehiscence; Pistil 1, tricarpellary, syncarpous, axile placentation, style long and slender, stigma capitates inferior; Fruits and seeds not seen.

Morphological and microscopical characters of isolated fungal strain SS 7

Surface and reverse colour of strain SS 7 was dark green and yellow. Conidiophores upright, simple, terminating in a globose swelling, bearing phialides at the apex, conidia 1 celled, globose, often variously colored in mass. Therefore, strain SS 7 was identified as *Aspergillus* sp. (Figure 4)

Figure 4. Morphological and Microscopical character of strain SS 7 (X 400)

Carbon utilization

Among carbon sources, starch and glycerol media were the best carbon sources whereas sucrose, glucose and mannitol media were also suitable for fermentation as shown in and Figure 5.

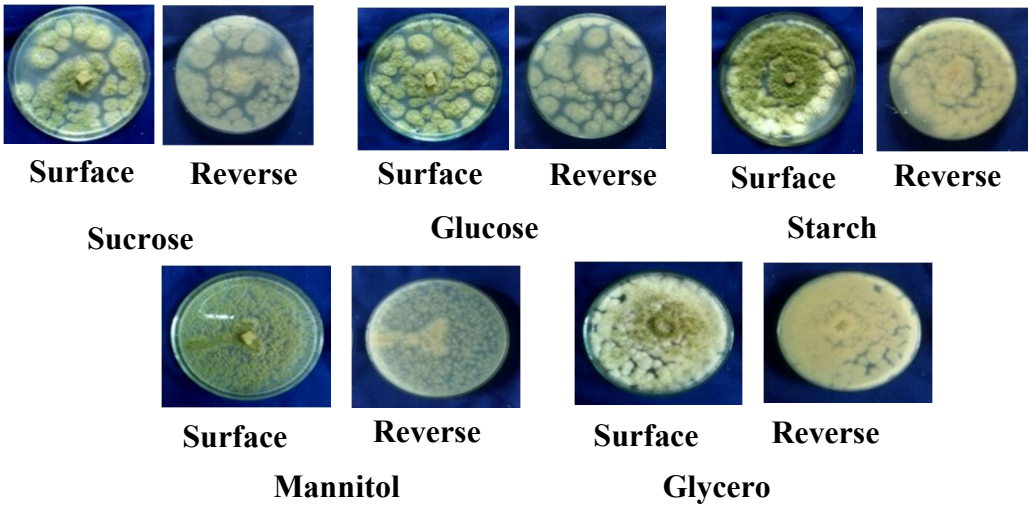


Figure 5. Strain SS 7 grown on the plates of various carbon source

Nitrogen utilization

In nitrogen sources, yeast extract and soybean media were the best nitrogen sources. Meat extract, malt extract and oat meal media were suitable for fermentation as shown in Figure 6.

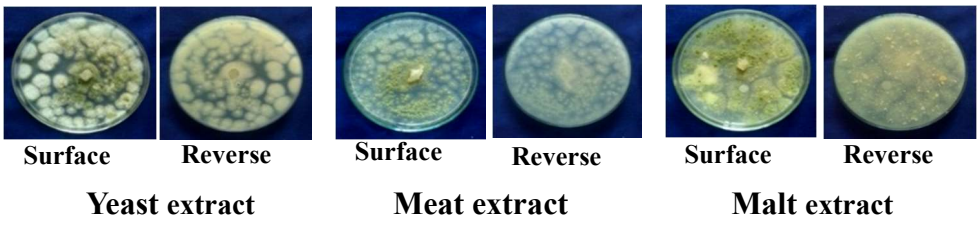


Figure 6. Strain SS 7 grown on the plates of various nitrogen sources

Antimicrobial activity of strain SS 7 by using various carbon sources

Antimicrobial activity of strain SS 7 in glucose medium showed very high activity against *Candida albicans*, starch medium indicated high activity against *Candida albicans*, *Salmonella typhi* and *Staphylococcus aureus* as shown in Figure 7.

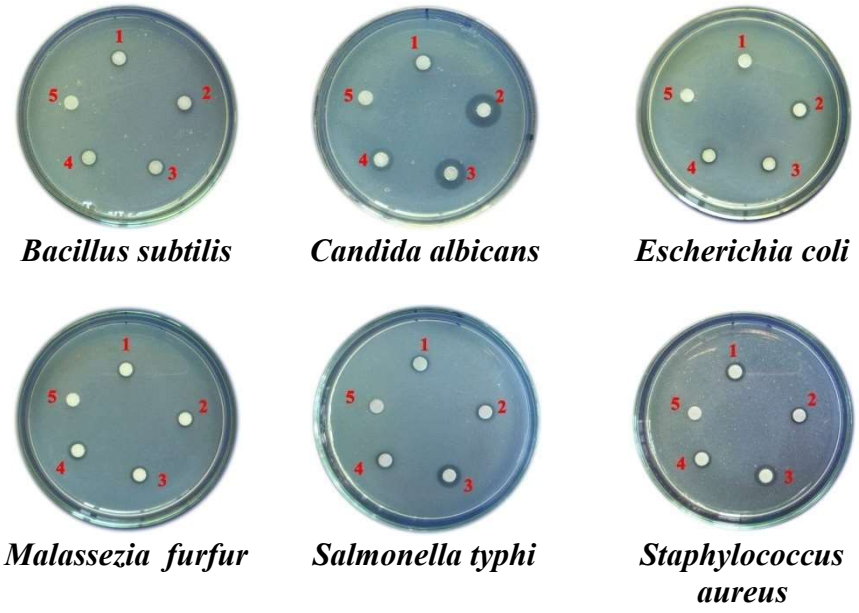


Figure7. Inhibitory zones of strain SS 7 on various carbon sources against six test organisms

Antimicrobial activity of strain SS 7 by using various nitrogen sources

Antimicrobial activity of strain SS 7 in yeast extract medium showed high activity against *Bacillus subtilis*, *Candida albicans*, *Salmonella typhi* and *Staphylococcus aureus*. Meat extract medium indicated high activity against *Candida albicans*. Malt extract medium indicated high activity against *Candida albicans* and *Staphylococcus aureus.* Oat meal medium indicated very high and high activity against *Bacillus subtilis*, *Candida albicans*, *Escherichia coli* and *Malassezia*. Soybean medium indicated high activity against *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur* and *Staphylococcus aureus* respectively as shown in Figure 8.

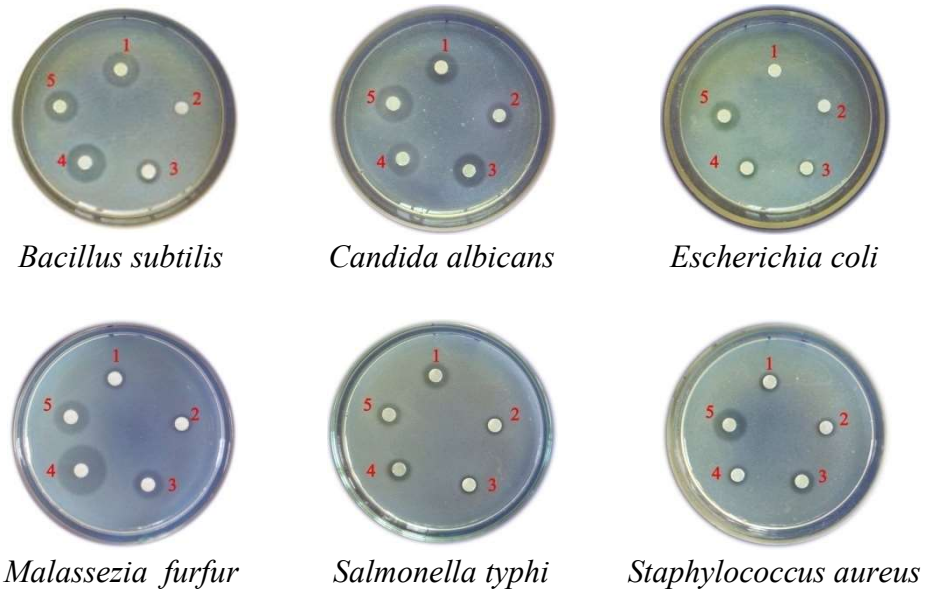


Figure 8. Inhibitory zones of strain SS-7 on various nitrogen sources against six test organisms

Morphological characters of strain SS 7 on different media

In the investigation of morphological characters of strain SS 7 on different media, medium 1, 3, 7, 9 and 10 were good. In these media, the surface and reverse colour of medium 1 was white to light green and cream colour, another media such as medium 3, 7, 9 and 10 were green and cream colour. Medium 2 and 5 were moderate for fermentation. These surface and reverse colour were white to light green and white for medium 2 and green and white colour for medium 5. However, medium 4, 6, 8 and 11 were not suitable for fermentation to produce antimicrobial metabolites from strain SS 7. The surface and reverse colour of medium 4, 6 and 8 were white colour respectively whereas medium 11 was light green and white colour as shown in Figure 9.

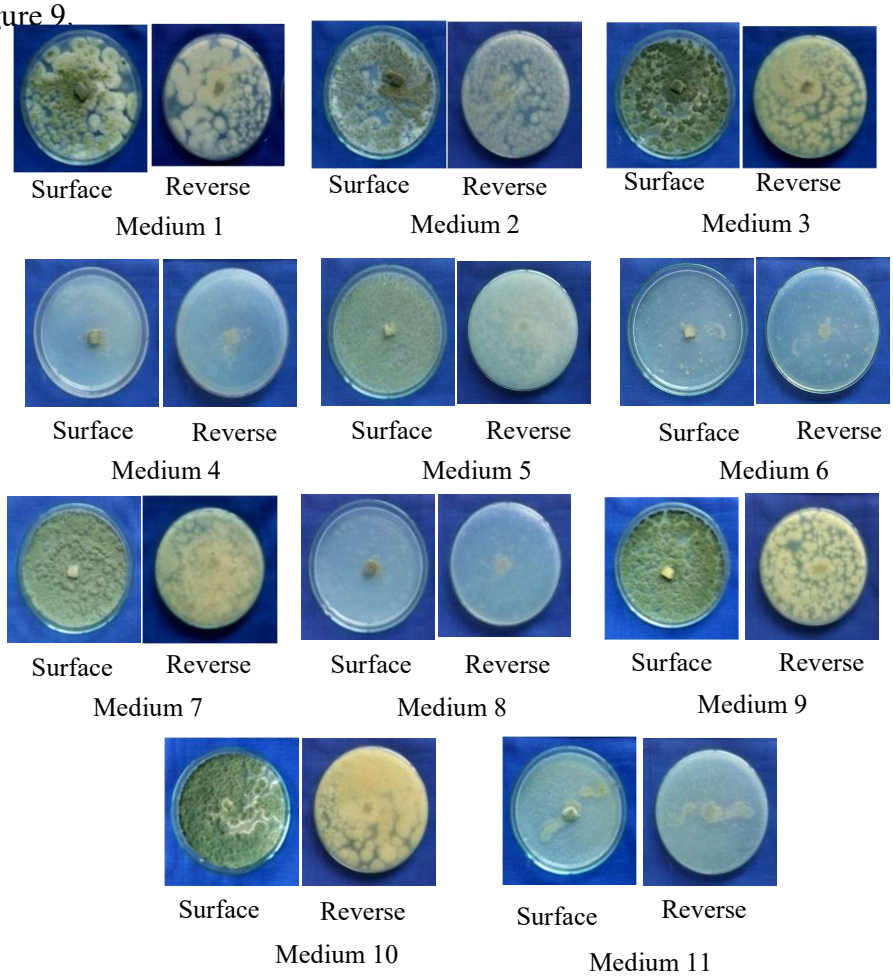
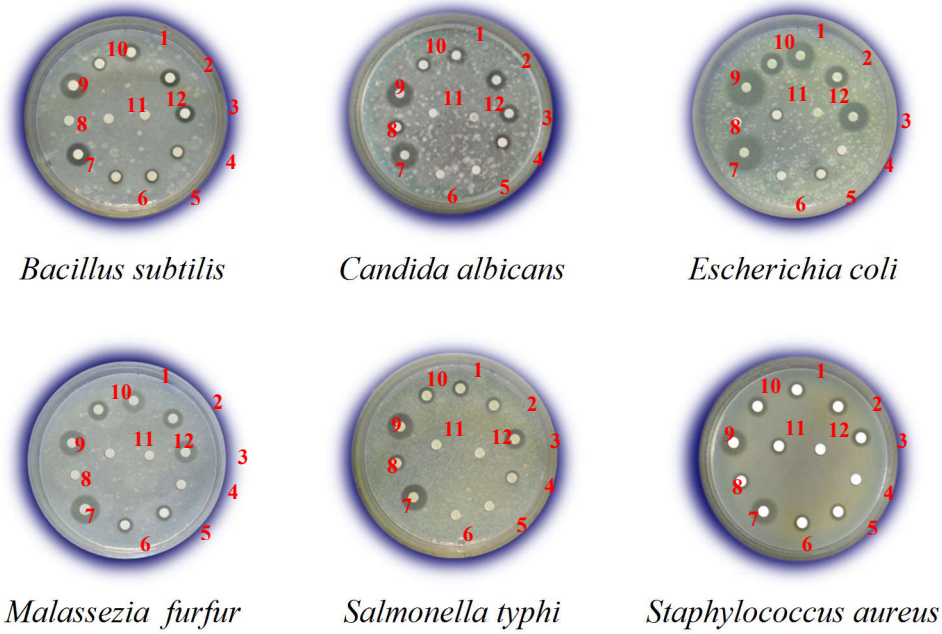


Figure 9. Surface and reverse color of strain SS 7 grown on different media

Antimicrobial activity of strain SS 7 by using different media

Antimicrobial activity of strain SS 7 on different media, soybean, mannitol medium (M-7) showed highest activity against *Malassezia furfur* and *Escherichia coli*. Sucrose, Yeast extract medium (M-9) also showed highest activity against *Escherichia coli* and *Salmonella typhi* as shown in Figure 10.



1. Medium 1	2. Medium 2	3. Medium 3	4. Medium 4
5. Medium 5	6. Medium 6	7. Medium 7	8. Medium 8
9. Medium 9	10. Medium 10	11. Medium 11	12. Control

Figure 10. Inhibitory zones of strain SS 7 on different media against six test organisms

Fermentation studies of strains SS 7

Age of inoculum

In the study of age of inoculum, fermentation 1 (Two days old) showed the highest activity against *Bacillus subtilis*, *Candida albicans, Malassezia furfur* and *Escherichia coli* respectively as shown in Table 1 and Figure 11. Fermentation 2 (Three days old) showed weak activity against four test organisms as shown in Table 2 and Figure 11.

Table 1. Inhibitory zones (mm) of strain SS 7 for Fermentation 1 (Two days old)

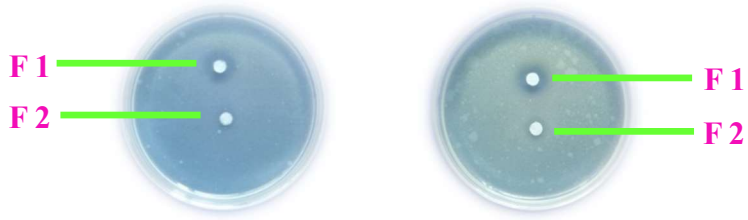
Day / Test organism	1	2	3	4	5	6	7
Bacillus subtilis	-	12	14	**16**	14	12	11
Candida albicans	-	15	15	**17**	12	11	10
Escherichia coli	-	12	13	15	**17**	14	12
Malassezia furfur	-	12	14	17	14	12	10

10-12 mm = weak activity, 13-17 = high activity, >18 mm =very high activity (Disc size =6mm

Table 2. Inhibitory zones (mm) of strain SS-7 for Fermentation 2 (Three days old)

Day / Test organism	1	2	3	4	5	6	7
Bacillus subtilis	-	11	10	10	10	10	-
Candida albicans	-	10	10	10	10	10	-
Escherichia coli	-	10	10	10	10	11	-
Malassezia furfur	-	10	11	10	10	10	-

10-12 mm = weak activity, 13-17 = high activity, >18 mm =very high activity (Disc size =6mm)



Bacillus subtilis *Candida albicans*

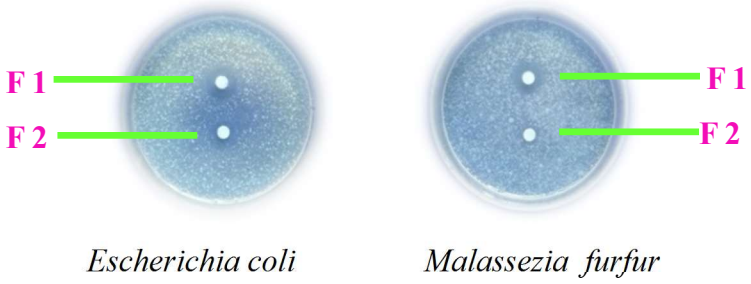


Figure 11. Inhibitory zones of strain SS 7 for Fermentation 1 and fermentation 2 against four test organisms

Size of inoculum

In the study of size of inoculum optimization, among the seed culture (0.5%, 1.0%, 1.5%, 2%, 2.5%, 3.0% and 3.5%) 1.5% of seed culture at fifth day fermentation was suitable for the production of the bioactive compound as shown in Figure 12.

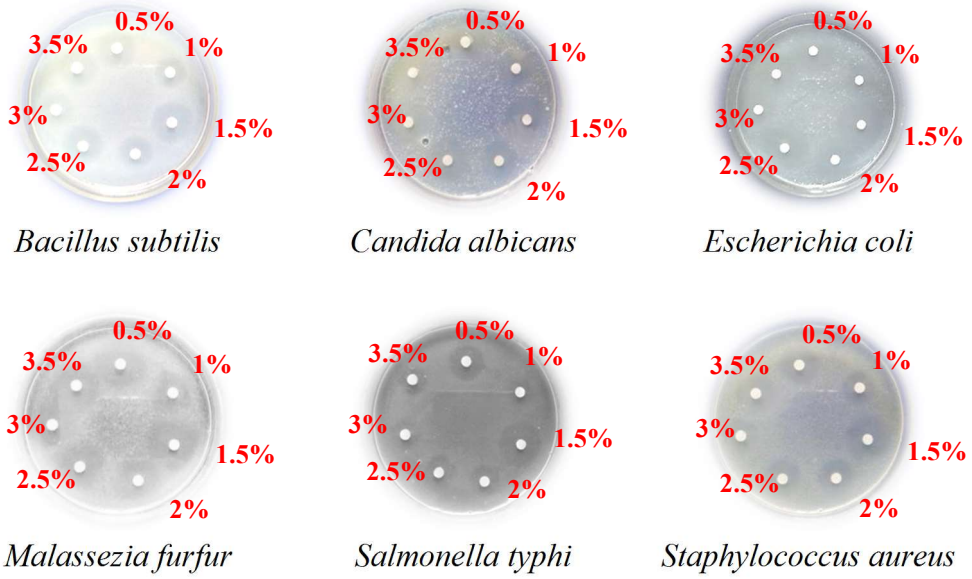


Figure 12. Inhibitory zones (mm) of size of inoculum for strain SS 7

Effect of different pH of strain SS 7

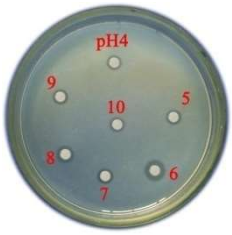
Among pH 4, 5, 6, 7, 8, 9 and 10 of fermented broth of strain SS 7, pH 6 was the best for extraction of the bioactive compounds from fermented broth according to the result of inhibitory zones against six test organisms as shown in Table 3 and Figure 13.

Table 3. Inhibitory zones (mm) of different pH of strain SS 7 on antimicrobial activity

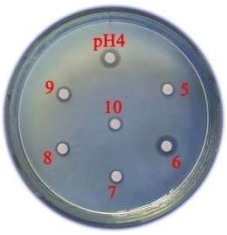
pH	***Bacillus subtilis***	***Candida albicans***	***Escherichia coli***	***Malassezia furfur***	***Salmonella typhi***	***Staphylococcus aureus***
pH 4	10	11	12	13	11	13
pH 5	10	10	10	10	10	11
pH 6	10	**13**	**14**	**13**	10	**15**
pH 7	10	10	14	11	10	10
pH 8	10	11	10	10	10	11
pH 9	10	10	10	10	10	12
pH 10	10	10	10	11	10	10

10 -12 mm = weak activity, 13 - 17 mm= high activity, >18 mm = very high activity. (disc size = 6 mm)

Bacillus subtilis



Candida albicans



Escherichia coli

Malassezia furfur *Salmonella typhi* *Staphylococcus aureus*

Figure 13. Inhibitory zones (mm) of pH utilization for strain SS 7

Discussion and Conclusion

Endophytic fungal strain *Aspergillus* sp. SS 7 isolated from the rhizome of *Zingiber cassumunar* Roxb. was used for the investigation of optimal fermentation conditions in order to produced its bioactive secondary metabolites. In utilization of carbon sources, starch and glycerol were the best fermentation whereas in nitrogen sources, yeast extract and soybean were the best fermentation for strain SS 7. Kyawt Kyawt Aung (2014) also reported that in utilization of carbon sources, starch was the best fermentation whereas in nitrogen sources, yeast extract and soybean were the best fermentation for endophytic fungal strain.

In antimicrobial activity of various carbon sources, glucose medium showed very high activity against *Candida albicans* whereas starch medium showed high activity against *Salmonella typhi* and *Staphylococcus aureus*. In various nitrogen sources oat meal medium indicated very high and high activity against *Malassezia furfur* and *Bacillus subtilis* whereas yeast extract media showed high activity against *Salmonella typhi* and soybean media indicated high activity against *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*. Yee Yee Thu (2006) reported that glucose and yeast extract media indicated high activity against *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*. Kyawt Kyawt Aung (2014) reported that starch and soybean medium showed high activity against *Escherichia coli*. In the study of morphological characters of different media, 1, 3, 7, 9 and 10 were good for fermentation to produce antimicrobial metabolites from strain SS 7. As a result of antimicrobial activity on different media, medium 7 and medium 9 were the best for fermentation medium.

In the study of size of inoculum optimization 1.5% of seed culture at fifth day fermentation was suitable for the production of bioactive metabolites. Yee Yee Soe (2014) observed the highest activity against *Bacillus subtilis* at 1.5% of seed culture for fermentation of bioactive strain. The large numbers of known bioactive compounds of microbial origin are currently produced by fermentation (Parkinsan, 1994). Optimal fermentation conditions such as proper age and size of inoculum are very important for the production of metabolites (Omura 1984). In the screening of optimal pH for fermentation, pH 6 was the best for extraction of the bioactive compounds from fermented broth of strain SS 7 according to the results of inhibitory zones on six test organisms. Yee Yee Thu (2006) has reported that endophytic fungus isolated from *Mimusops elengi* L. showed high activity at pH 6. Kyawt Kyawt Aung (2014) reported that endophytic fungus isolated from *Coccinia indica* Wight and ARN indicated high activity at pH 7. In conclusion, the best fermentation medium for strain SS-7 should consist of starch, mannitol and glycerol for carbon sources, yeast extract and soybean for nitrogen sources. The best fermentation condition was 1.5 % of two days old seed culture and pH 6 to produce bioactive metabolites from strain SS 7.

Acknowledgements

I would like to express my thank to Dr. San San Aye, Professor, Department of Botany, University of Yangon, for her kind help for my research.

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ANTIMICROBIAL ACTIVITIES OF *PIPER BETLE* L. LEAVES

Tin Zar Aye[1] , Nwet Nwet Aung[2]

Abstract

Piper betle L. (Kun) is a well-known medicinal plant and widely distributed in Myanmar. In this study the leaves of *Piper betle* L. were collected from Yamethin Township, Mandalay Region, in the month of June to July, 2016. The extraction were done with 95%, 70%, 50% ethanol, aqueous and fresh juice. Endophytic microorganisms were also isolated from the leaves of *Piper betle*. Six endophytic bacterial strains and three endophytic fungal strains were obtained. Antimicrobial activities of leaf extract and endophytic microorganisms were tested on the seven pathogenic organisms by paper disc diffusion method. The ethanolic leaves extracts of 95% showed significant effect against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* Endophytic bacterial strains TZ-2 and TZ-5 showed activities against *Candida albicans* but endophytic fungal strains did not show the effect against on seven pathogenic organisms.

Introduction

Piper betle L. (Kun) belongs to the Piperaceae family. The betel plant is an evergreen and perennial creeper (Houghton, 2001). The betel plant is indigenous to South and South East Asia (Mukherjee, 2000). Betel leaf is used as paan by Asian emigrants, with or without tobacco. The betel leaf is a heart shaped with different size (Dixit *et al*., 1995).

Betel leaf is traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, conjunctivitis, constipation, headache, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, swelling of gum, rheumatism, cuts and ayurvedic preparations (Sharma, 1991). Leaves were considered useful in treating bronchitis and dyspnea. The leaves were chewed by singers to improve their voice. The fruit of *Piper betle* employed with honey as a remedy for cough (Usmanghani *et al*., 1997).

Myanmar Traditional Medicine is the national heritage and have been existing since time immemorial. Eighty percent of Myanmar population live

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in countryside and there have many difficulties in gaining access to modern medicine. Thus, herbal medicines have become valuable and readily available resources for primary health care. Meanwhile, it has been realized that medicinal plants are invaluable resources for new pharmaceutical products and potential sources of new drugs as well as for economic development. A growing interest in the usage of 2 medicinal plants has created the need for scientific investigation. To enhance the quality and promote the systematic development of traditional herbal medicine assurance of the safety, quality and efficacy of medicinal plant has now become a key issue. *Piper betle* L. is an evergreen perennial creeper. Betel leaves can be used in many ways; for example as masticatory in betel quid, as spice, as poultice and as an applicant to the chest and abdomen. Myanmar people have the custom of offering the betel quid in traditional ceremonies (Kay Thwe Aung, 2008).

The fresh betel leaves possess antimicrobial, antifungal, antiseptic and antihelminthic effects (Chandra *et al*., 1987).The leaf has a significant antimicrobial activity against broad spectrum of microorganisms (Sarkar *et al*., 2013).The betel shows the antimicrobial activity against *Streptococcus pyrogen, Staphylococcus aureus, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa* etc., and beside this, the leaf extract also possess the bactericidal activity against the urinary tract pathogenic bacteria (Agarwal and Singh, 2012).

In this work, the comparative study was done on antimicrobial activities of leaf extract and endophytic microorganisms were tested on seven pathogenic organisms.

Materials and Methods

Sample collection of *Piper betle* L.

Plants samples of *Piper betle* L. were collected from Yamethin Township, Mandalay Region, in the month of June to July, 2016.

Extraction of *Piper betle* L. leaves

Hundred grams of powdered leaves were soaked in 500 ml of aqueous and (95%, 70% and 50% ethanol), shaking 200rpm for seven days respectively. Hundred grams of fresh leaves were ground for fresh juice. Then the infusion was filtered by using filter paper and the residue was discarded. The solvent extracts were concentrated by using water bath at 70°C to

evaporate the solvent. After complete solvent evaporation, the crude extracts were weighed and recorded.

Preparation of *Piper betle* L. leaves extract for antimicrobial activity

Leaves extracts 0.1g, 0.5g and 1g were dissolved in 1 ml of aqueous, 95%, 70% and 50% ethanol and these were used for antimicrobial test.

The morphological characters of endophytic microorganisms

The morphological characters of endophytic microorganisms were studied under light microscope. According to the morphological characters, the isolated microorganisms were named TZ-1, TZ-2, TZ-3, TZ-4, TZ-5, TZ-6, TZ-7, TZ-8, and TZ-9.

Test organism for antimicrobial activities

Seven strains of clinical pathogen and one strain of phytopathogenic bacteria were obtained from Department of Medical Research (PyinOoLwin branch). These test organisms were sub cultured on nutrient agar slant for 24 hours. Then, these were inoculated into 5 ml of nutrient broth and incubated for 4-5 hour on shaker of 200rpm at room temperature.

Antimicrobial activity of leaves extract

The paper disc diffusion method (Atlas, 1993) was used to determine antibacterial activities of *Piper betle* L. leaves extract. Twenty-five milliliter of sterilized nutrient agar cool to 45°C and mixed with 20µl of test organisms. The mixture was poured onto each petri dishes, spread anticlockwise and allowed to stand for 15 minutes to solidify. The petri plates were placed in inverted position. Each disc with 20µl extract were gently pressed down on nutrient agar medium by using sterilized forceps. The plates were incubated at 37°C, overnight in incubator. Twenty microliter of 95% ethanol were applied on paper disc as control. Antibacterial activity showed as the zone of inhibition produced by plant extract. Zone of inhibition were assessed to evaluate by ruler and recorded in milliliter. This experiment was done in the Department of Medical Research (PyinOoLwin Branch) and it was carried out duplicate.

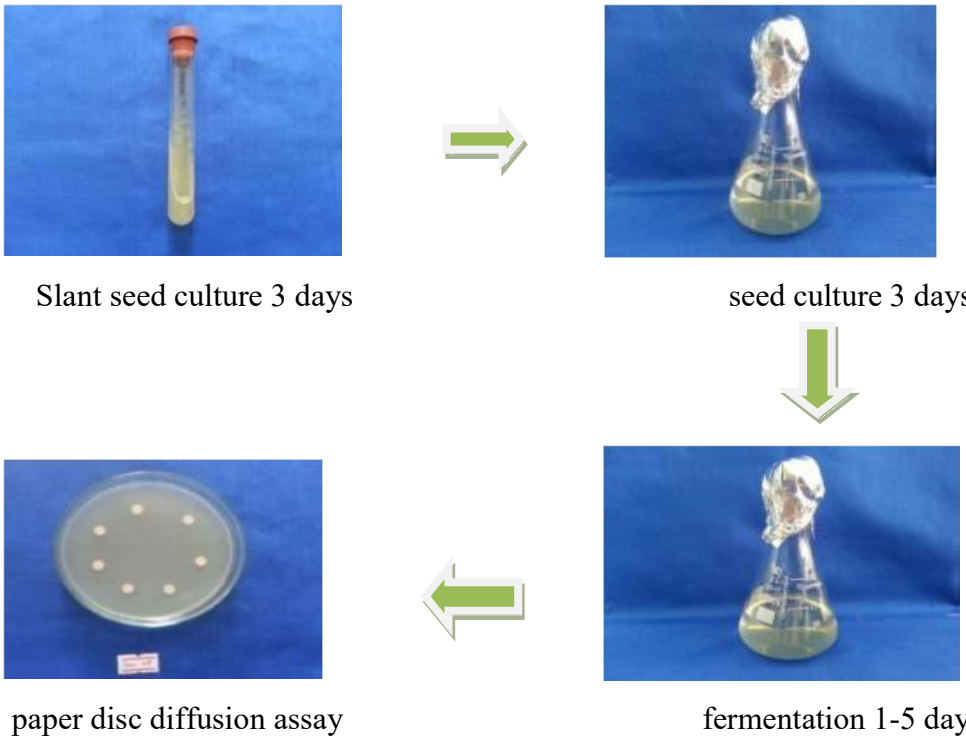


Figure 1. The antimicrobial activities by paper disc diffusion assay (Atlas, 1993)

Antimicrobial activity of endophytic microorganisms by paper disc diffusion assay

The isolated microorganism strains were grown on nutrient agar medium then one loop of isolated microorganisms was transferred into 50ml of seed medium and incubated at 30ºC for 3days. Five milliliter seed culture was transferred into 50ml fermentation medium and incubated at 30ºC for 5days on reciprocal shaker at 200 strokes/min. The fermentation was carried out for 5days. During the fermentation, the fermented broth between 1-5 days was applied on the paper disc (6mm in diameter) and allowed to dry in the air. For the antimicrobial activity, test organisms were separately inoculated in the nutrient broth for 4-5 hours, and 20µl of each test organisms were added to each medium, and then poured into plates. After solidification, paper disc impregnated with fermented broth were applied on agar plates and the plates were incubated for 24-36 hours and the inhibitory zones were recorded.

Medium used Antimicrobial activities test (Atlas, 1993, Phay N.1997)

Seed medium		**Fermentation medium**	
Composition per liter		Composition per liter	
Glucose	20.0g	Glucose	20.0g
Peptone	3.0g	Peptone	3.0g
KNO_3	1.0g	K_2HPO_4	0.1g
K_2HPO_4	0.1g	$MgSo_4$	0.1g
pH	6.5	$CaCo_3$	1.0 g
		pH	6.5

Results

The ethanolic leave extracts

The results of crude extract of *Piper betle* L. leaves by various ethanolic concentrations, aqueous and fresh juice. The highest amount of extracts 15.23g were obtained from aqueous, followed by 13.11g from 50% ethanol, 10.32g from 70% ethanol and lowest amount of extracts 10.21g from 95% ethanol.

Table(1) The crude extract of *Pipper betle* (L) leaves by various ethanol concentration

	Ethanol concentration	Crude extract (g)
1	95%ethanol	10.21
2	70%ethanol	10.32
3	50%ethanol	13.11
4	Aqueous	15.23

The morphological characters of isolated endophytic bacteria from leaf of *Piper betle* L.

The morphological characters of isolated endophytic bacteria from leaf of *Piper betle* L. were shown in table 2.

Table 2. Colony characters of isolated bacteria Morphology

	TZ – 1	TZ - 2	TZ - 3	TZ - 4	TZ - 5	TZ - 6
Color of colony	Cream	Pale yellow	Cream	Pale yellow	Pale yellow	Cream
Nature of colony	Irregular, Mucous present	Circular, Mucous present	Irregular, Mucous present	Circular, Mucous present	Irregular, Mucous present	Circular, Mucous present
Edges	Undulate	Entire	Undulate	Entire	Undulate	Entire
Size	2-3mm	3mm	1-2mm	0.5mm	1.5mm	2mm
Cell Shape	Chain	Short rod	Chain	Short rod	Short rod	Chain
Gram-staining	Positive	Negative	Positive	Negative	Negative	Negative
Oxygen requirements	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic
Translucency	Opaque	Translucent	Opaque	Translucent	Translucent	Opaque

Antimicrobial activities of ethanolic extracts *Piper betle* L.

Antimicrobial activities of various ethanol concentrations were carried out by paper disc diffusion method. Only 95% ethanol leaves extracts showed significant effect against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus.* The tested leave extracts 0.1g dissolved in 1 ml of 95% of ethanolic extracts showed antimicrobial activity against on *Bacillus subtilis* (8mm), *Escherichia coli* (7mm), *Staphylococcus aureus* (8mm). The tested leaves extracts 0.5g dissolved in 1 ml of 95% of ethanolic extracts was showed effect on *Bacillus subtilis* (8mm), *Escherichia coli* (7mm), *Staphylococcus aureus* (9 mm), *Pseudomonas aeruginosa* (20mm). The tested leaves extracts 1gdissolved in 1 ml of 95% of ethanolic extracts

was showed significant effect against *Bacillus subtilis* (9mm), *Escherichia coli* (8mm), *Staphylococcus aureus* (9mm), *Pseudomonas aeruginosa* (22mm). The highest inhibitory zone size was recorded from 1g of 95% ethanolic extracts that against *Pseudomonas aeruginosa* (22mm). The fresh juice and aqueous extracts were not effect on test organisms. The diameters of inhibition zones that appeared were shown in Table 3, Figure 2.

Table3. Antimicrobial activity of different concentrations of leaves by 95% ethanol extract against clinical and pathogenic bacteria

No	Test organisms	Size of clear zone given by different concentration in 95% ethanol extract		
		0.1 g	0.5 g	1.0 g
1	*Aspergillus brasiliensis*	-	-	-
2	*Bacillus subtilis*	8 mm	8 mm	9 mm
3	*Candida albicans*	-	-	-
4	*Escherichia coli*	7 mm	7 mm	8 mm
5	*Pseudomonas aeruginosa*	-	20 mm	22 mm
6	*Staphylococcus aureus*	8 mm	9 mm	9 mm
7	*Salmonella enterica*	-	-	-

Size of paper disc = 6mm

Antimicrobial activities of isolated endophytic bacteria from leaf of *Piper betle*L.

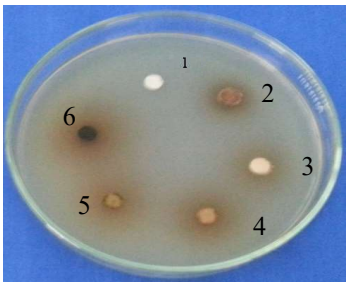
Antimicrobial activities of endophytic bacterial strains were tested on the seven pathogenic organisms by paper discs diffusion method. Endophytic bacterial strains TZ-2 and TZ-5 showed against *Candida albicans*. Days 2 fermentation of endophytic bacterial strain TZ-2 was showed antimicrobial activities against *Candida albicans* (15mm). Days 3 fermentation of endophytic bacterial strain TZ-2 was showed antimicrobial activities against *Candida albicans* (15mm). Days 4 fermentation of endophytic bacterial strain

TZ-2 was showed antimicrobial activities against *Candida albicans* (10mm). Days 5 fermentation of endophytic bacterial strain TZ-2 was showed antimicrobial activities against *Candida albicans* (10mm). Only days 2 fermentation of endophytic bacterial strain TZ-5 was showed antimicrobial activities against *Candida albicans* (10mm). The diameters of inhibition zones that appeared were shown in Table 4 and Figure 3.

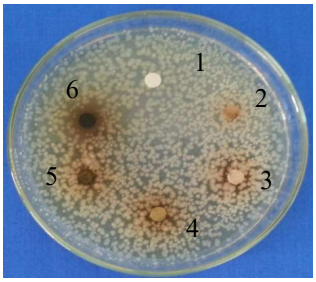
Table 4. Antimicrobial activities of TZ- 2

Test organisms	Antimicrobial activity in millimeter (mm)				
	Day1	Day2	Day3	Day4	Day5
Aspergillus brasiliensis	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-
Candida albicans	-	15	15	10	10
Escherichia coli	-		-	-	-
Pseudomonas aeruginosa	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-
Salmonella enterica	-	-	-	-	-

Size of paper disc = 6mm



Zone of inhibition on *A. brasiliensis*



Zone of inhibition on *B. subt*

1=control

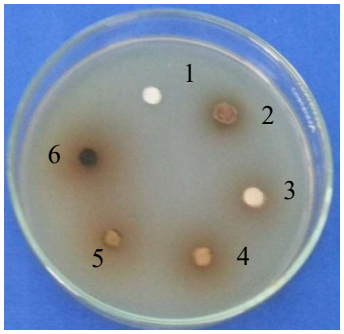
2=fresh juice

3=aqueous

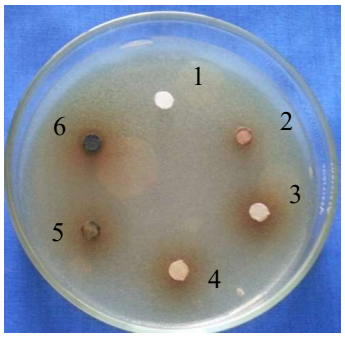
4=50%EtOH

5=70%EtOH

6=95%EtOH



Zone of inhibition on *C. albicans*



Zone of inhibition on *E. coli*

1=control

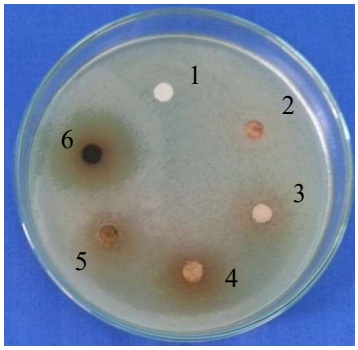
2=fresh juice

3=aqueous

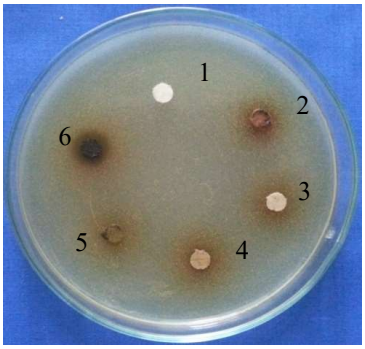
4=50%EtOH

5=70%EtOH

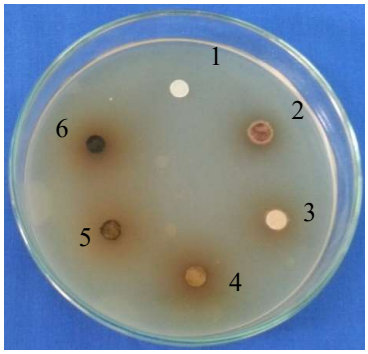
6=95%EtOH



Zone of inhibition on *P. aeruginosa*

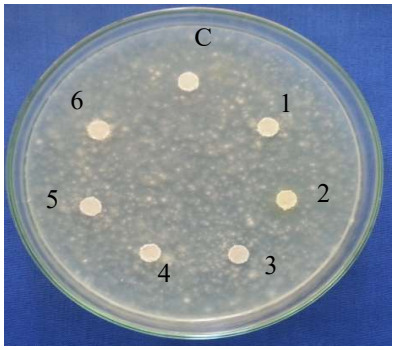


Zone of inhibition on *S. aureus*

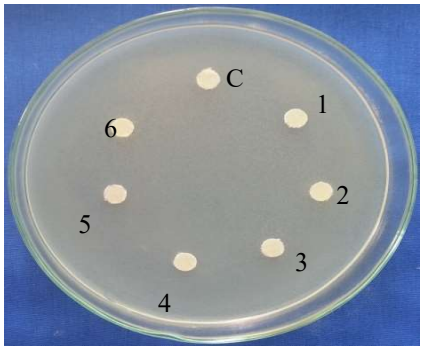


Zone of inhibition on *S. enterica*

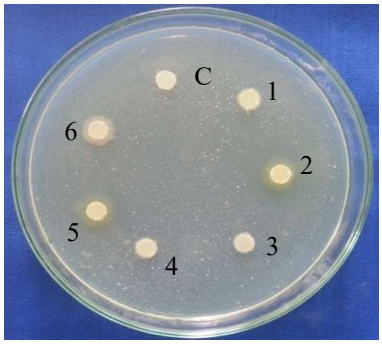
Figure 2. Antimicrobial activity provided by leaf extract 1.0 g dissolve in 95% ethanol



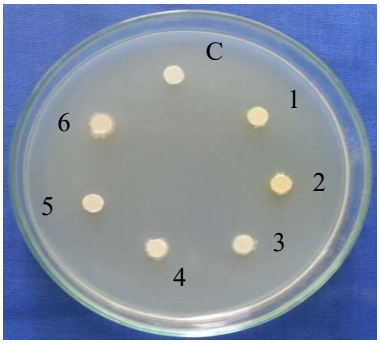
Zone of inhibition on *A. brasiliensis*



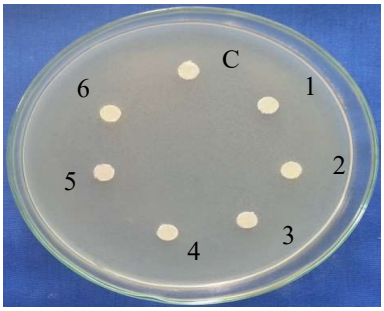
Zone of inhibition on *B. subtilis*



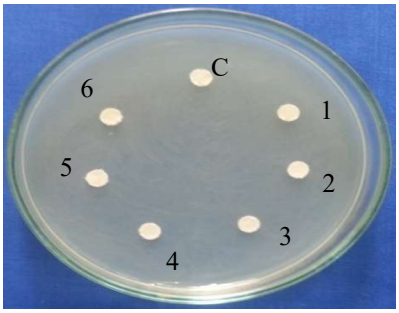
Zone of inhibition on *C.albicans*



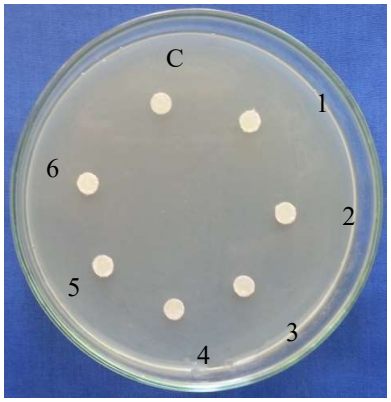
Zone of inhibition on *E.coli*



Zone of inhibition on *P.aeruginosa*



Zone of inhibition on *S.aureus*



Zone of inhibition on *S.enterica*

Figure 3. Antibacterial activity of isolated bacteria

Antimicrobial activities of isolated endophytic fungi from leaf of *Piper betle* L.

Antimicrobial activities of isolated endophytic fungal strains were tested on the seven pathogenic organisms by paper discs diffusion method. Endophytic fungal strains showed no effect on seven pathogenic organisms.

Discussion and Conclusion

The fresh leaves of betel was collected from Yamethin Township, Mandalay Region. The total of 3520g of fresh leaves were dried at 25°C for 20days for dry weight. The leaves dry weight of 438g were constant. The constant leaves dry weight 438g were powdered to use for extraction. The dry weight of leaves was significantly reduced 3520g to 438g. Each 100g of leaves powdered were mixed with 95%, 70%, 50% of ethyl alcohol and also aqueous. The results of crude extract of *Piper betle* L. leaves by various ethanolic concentration, aqueous and fresh juice were shown in Table (2). The highest amount of extracts 15.23g were obtained from aqueous, followed by 13.11g from 50% ethanol, 10.32g from 70% ethanol and lowest amount 10.21g from 95% ethanol respectively. The highest amount of extracts was recorded from aqueous. The aqueous extract of *Piper betle* L. leaf is higher than other ethanolic extracts. It may be due to the leaves constituents are more soluble in distilled water than ethanol.

Antimicrobial activities of various ethanol concentration were carried out by paper disc diffusion method. Only 95% ethanol leaves extracts showed significant effect against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*. The tested leaves extracts (0.1g) 95% of ethanolic extracts was showed against on *Bacillus subtilis* (8mm), *Escherichia coli* (7mm), *Staphylococcus aureus* (8mm). The tested leaves extracts (0.5g) 95% of ethanolic extracts was showed effect on *Bacillus subtilis* (8mm), *Escherichia coli* (7mm), *Staphylococcus aureus* (9 mm), *Pseudomonas aeruginosa* (20mm). The tested leaves extracts (1g) 95% of ethanolic extracts was showed significant effect against *Bacillus subtilis* (9mm), *Escherichia coli* (8mm), *Staphylococcus aureus* (9mm), *Pseudomonas aeruginosa* (22mm). The highest inhibitory zone size was recorded from 1g of 95% ethanolic extracts that against *Pseudomonas aeruginosa* (22mm).The fresh juice and aqueous extracts were not effect on test organisms. Fresh juice did not show the inhibition zone on Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (Aye Thida Htun, 2015), but not agree with Ratnasooriya and Premakumara, 1997, they reported that the fresh betel leaves possess antimicrobial, antifungal, antiseptic and antihelminthic effects. The diameters of inhibition zones that appeared were shown in table 5, 6 and 7. According to the table control disc prepared solely with 95%, 70% and 50%ethanol showed no antimicrobial activity. Thus, it can be assumed that, the zone of inhibition could not be due to the ethanol solvent. Among the different extracts, only 95% ethanol leaves extracts showed significant effect against B*acillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*. Agarwal and Singh (2012) reported that the betel shows the antimicrobial activity against *Streptococcus pyrogen*, *Staphylococcus aureus, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa*. The betel leaf has a significant antimicrobial activity against broad spectrum of microorganisms (Jesonbabu *et al*., 2012).

Six endophytic bacterial strains and three endophyti7[c fungal strains were also isolated from the leaves of *Piper betle*. Antimicrobial activities of endophytic bacterial strains were tested on the seven pathogenic organisms by paper discs diffusion method. Endophytic bacterial strains TZ-2 and TZ-5 showed against *Candida albicans*. Days 2 fermentation of endophytic bacterial strain TZ-2 showed antimicrobial activities against *Candida albicans* (15mm). Days 3 fermentation of endophytic bacterial strain TZ-2 was showed

antimicrobial activities against *Candida albicans* (15mm). Days 4 fermentation of endophytic bacterial strain TZ-2 was showed antimicrobial activities against *Candida albicans* (10mm). Days 5 fermentation of endophytic bacterial strain TZ-2 was showed antimicrobial activities against *Candida albicans* (10mm). Antimicrobial activities showed 15 mm in diameter against *Candida albicans* on days 2 and days 3 fermentation, but the activities reduced 10mm in diameter on days 4 and days 5 fermentation were observed. Only days 2 fermentation of endophytic bacterial strain TZ-5 was showed antimicrobial activities against *Candida albicans* (10mm). TZ-5 strain inhibited the growth of *Candida albicans* only on days 2 fermentation, but the activities did not show on days 3, days 4 and days 5 fermentation. In this study, seven test organisms were used and one out of seven was inhibited by endophytic bacterial strains TZ-2 and TZ-5 were observed. Endophytic fungal strains did not effect on seven pathogenic organisms. It is not agreed with Ali *et al*., 2010, stated that the leaf of *Piper betle* possess the antifungal activity against many fungal infections.

It is conclude that among the different ethanolic extracts, aqueous and fresh juice of *Piper betle* L. leaf, the antimicrobial activity of only 95% extract was effective against the test organisms such as Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus. Comparing with the antimicrobial activities of leaf extracts and endophytic microorganisms, it is observed that, the activity against the test organisms were not the same. The ethanolic leaf extract is more active than that of the endophytes. It is agreed with Jesonbabu *et al*., 2012 who stated that the leaf has a significant antimicrobial activity against broad spectrum of microorganisms. It could be suggested that the leaves of *Piper betle* L. can be useful as a medicine for some local diseases.

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ANTIBACTERIAL COMPOUND PRODUCED BY *STACHYBOTRYS* SP. AGAINST *AGROBACTERIUM TUMEFACIENS*

Tin Tin Hla

Abstract

A total of 15 fungi were isolated from four different soil samples collected from Pathein Area. All 15 fungi showed no antimicrobial activity against eight test organisms except *Agrobacterium tumefaciens*. Among them, fungus TH-15 exhibited more activities than other fungi. After examining the biological activities, TH-15 was found to be similar with those of genus *Stachybotrys* sp. In the fermentation studies, the maximum activity reached at 72 hrs of fermentation for the production of antibacterial compound. After fermentation with optimal parameter, the broth was adjusted pH 7.0 and studied by paper chromatography. Thin layer chromatography (TLC) by using the crude extract to obtain the basal data. The purification of antibacterial compound was undertaken by silica gel column chromatographies with various elution solvents collected by Preparative Thin Layer Chromatography (PTLC).

Introduction

Various microbial isolates may produce a wide variety of new or unusual compounds. Soil samples are good substrata for isolating microbes. Although microorganisms are harmful to human, they can be beneficial in medicines, industrials and various environment issues by means of secondary metabolite produced from these microorganisms (Hunter *et al*., 1999). *Agrobacterium tumefaciens* is the causal agent of crown gall disease (the formation of tumours) in over 140 species of eudicots (Young, *et al*., 2001).

The genus *Stachybotrys* is an asexually reproducing, belonging to the mitosporic Hypocreales group. *Stachybotrys chartarum* is a known producer of trichothecene mycotoxins and stachylysin (a hemolysin). The best

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characterized trichothecenes include satratoxins F, G, and H, roriden E, verrucarin J, and trichoverrols A and B (Dongyou and Paterson, 2011).

Verrucarin belongs in the class of tricothecenes, a group of sesquiterpene toxins produced by several fungi. Satratoxin-H, a trichothecene mycotoxin, is a naturally occurring mold byproduct of *Stachybotrys chartarum* which is toxic to humans and animals (Croft *et al.,* 1986). The aim and objectives are to collect soil samples in nature for the isolation of useful soil fungi and to identify soil fungi.

Materials and Methods

Collection and Preparation of soil samples

Four different soil samples were collected from Pathein Area, and were dried at room temperature in the laboratory.

Isolation of microorganisms from different soil samples

The isolation of fungi was undertaken by the methods of chemical treatment dilution method (Phay and Yamamura, 2005) and feeding method (NITE, 2002) as shown in Figure 1.

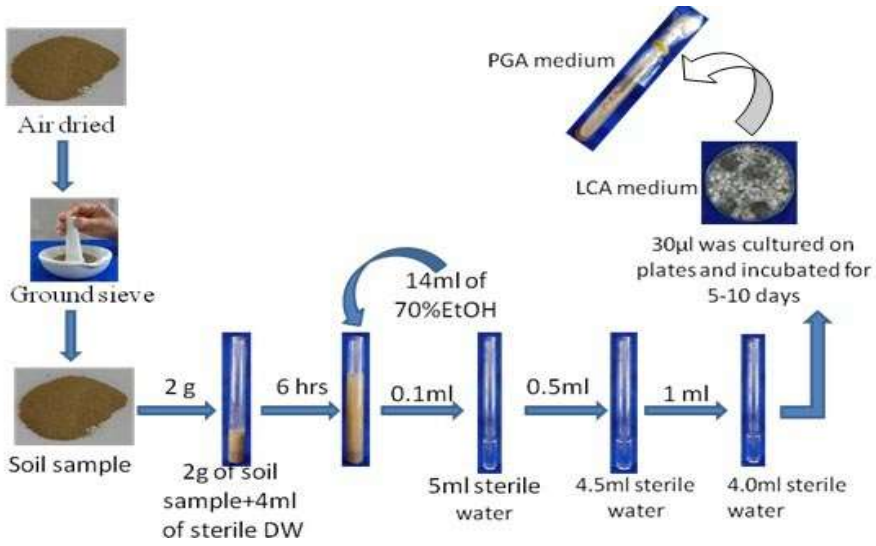
Chemical treatment dilution method (Phay and Yamamura, 2005)

Soil sample was air dried at room temperature and was grounded and sieved. Two grams of the sieved soil was then put into 4 mL of sterilized distilled water and settle for 6 hours to germinate early-germinating soil fungi. Fourteen ml of 70% ethanol solution was then added into the tube containing soil suspension, and shaken for 1 minute and diluted with sterile water. After serial dilution, 30 µL of soil suspension was placed on low carbon agar medium (LCA medium) and then incubated for 5-7 days as shown in Figure 1.

Feeding method (NITE, 2002)

Soil sample (1.0 g) was poured onto 100 mL LCA liquid culture medium and it was incubated overnight and 20 mL of 70% methanol was added. After shaking for 2 minutes, 30 µL sample was cultured on low carbon agar medium (LCA medium) plates and incubated for 5-10 days as shown in Figure 1.

Method 1. Chemical treatment dilution method (Phay and Yamamura , 2005)



Method 2. Feeding method (NITE, 2002)

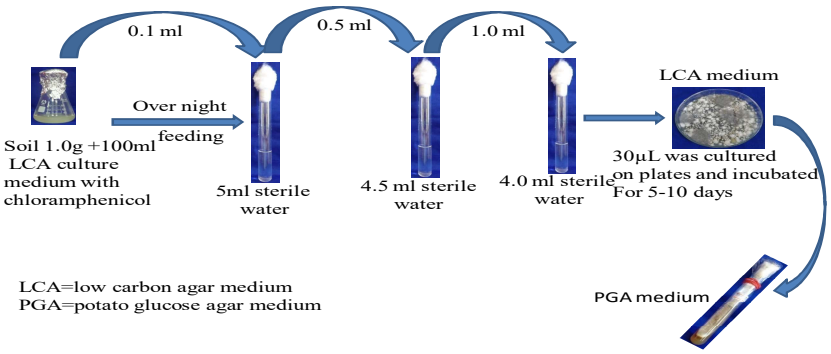


Figure 1. Procedure for chemical treatment dilution method and feeding method

Preliminary study for antimicrobial activities by paper disc diffusion assay (Ando *et al*., 2004)

The isolated fungi were inoculated on seed medium and incubated at room temperature for 3 days. Twenty mL of seed culture was transferred into the fermentation medium and incubated at room temperature for 7 days. Twenty µL of fermented broth was put on paper disc (size - 8mm) and placed

on assay plate containing test organisms and the plates were incubated for 24 hrs at 28˚C.

Identification of the antibacterial metabolite producing fungus TH-15

Morphological and microscopical characters of antibacterial metabolite producing fungus TH-15 was observed by the methods of identification of mitosporic fungi by Kasuhiko Ando (Biology Research Centre, 2015) and Barnett (1956).

Studies on microbial growth kinetics on fungus TH-15 (Omura, 1985, Crueger, 1989)

The strain TH-15 was inoculated into medium and cultured for 120 hrs at 100 rpm rotary shaker. The samples were taken out at 12 hrs intervals and PCV% (Packed cell volume) was calculated.

Study on the effects of ages and sizes of seed culture on the fermentation

The strain TH-15 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.

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 reported by Ando, 2002.

The effects of carbon and nitrogen sources on fermentation

In the investigation of carbon sources, the 1.5 % of each carbon source used in this study such as 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 of antibacterial activity against *Agrobacterium tumefaciens* respectively.

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 (Patrick, 1998; Simon and Gray, 1998)

The *n-butanol* layer was tested the antibacterial activity compared with original fermented broth.

Thin layer chromatography and bioautography overlay assay (Touchstone, 1992, and Aszalo, 1980)

The TLC plates were developed in the solvents and these TLC plates were calculated by the following equation.

$$\text{This is defined as: } R_f \text{ value} = \frac{\text{Distance of compound from origin}}{\text{Solvent front from origin}}$$

Investigation of silica gel column chromatography (Jarvis *et al*., 1986 and Marcelo *et al.*, 2006)

Based on the TLC-bioautography's result, silica gel column chromatography was carried out and checked by TLC and tested with *Agrobacterium tumefaciens.* The column size and flow rate were the following.

Column size 14.5cm x50cm

Flow rate 0.75 mL/min

Eluting solvent $CHCl_3$, $CHCl_3$: MeOH (100:5 v/v) and $CHCl_3$: MeOH (100:10 v/v)

Fraction size 3mL / test tube

Study on silica gel column re-chromatography

Fraction two of silica gel column chromatography were combined and concentrated *in vacuo*. The UV spectrum and FTIR of isolated compound have been obtained. The UV spectrum of isolated compound ethanol is firstly dissolved in and subjected in the estimation of UV spectrum have been obtained. By using Amtt Company, Bago University and West Yangon

University of Department of Chemistry (UV - 1800 Spectrophotometer, Shimadzu, Japan). In FTIR were recorded by using FTIR-8400, Shimadzu, Japan at Universities′ Research Centre (URC), University of Yangon.

Results

Collection and Isolation of microorganisms from soil samples

In this study, 15 kinds of soil fungi were isolated from four different soil samples from Pathein Area, Ayeyarwady Region as shown in Table 1 and 2.

Table 1. Seven different soil samples collected at seven different places

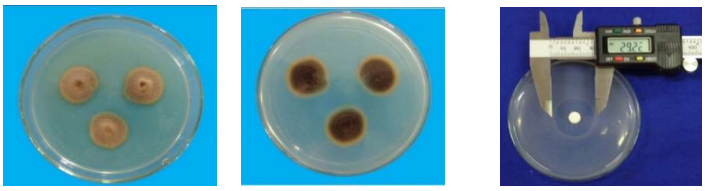
Soil sample No.	pH	Soil type	Collect date	Collected place	Location
S-1	6.22	Sandy Loam	23.6.2013	Shwe-wet-lue	N 16º 46.665″ E 094º 43.705″
S-2	4.83	Loam	7.7.2013	Thu-taw-gone	N 16º 48.978″ E 094º 42.540″
S-3	5.28	Sandy Clay Loam	7.7.2013	Tar-kaing	N 16º 45.392″ E 094º 42.757″
S-4	4.86	Silty Clay Loam	28.7.2013	Yae-oe-sin	N 16º 41.172″ E 094º 42.654″

Table 2. Numbers of fungi isolated from seven different soil samples by chemical dilution method and feeding method

Soil No.	Collected places	Total isolated fungi			Fungi No.
		Chemical treatment dilution method	Feeding method	Total fungi	
S-1	Shwe-wet-lu	2	1	3	TH-01,02,03
S-2	Thu-taw-gone	2	2	4	TH-04,05,06,07
S-3	Tar-kaing	2	2	4	TH-08,09,10,11
S-4	Yae-o-sin	2	2	5	TH-12,13,14,15
Total isolated fungi		8	7	15	

Screening of effective fungi isolated from soil by paper disc diffusion assay

The isolated 15 fungi were tested in nine kinds of test organism. Among them, TH-15 showed the best antibacterial activity (29.2mm of inhibitory zone) especially against *Agrobacterium tumefaciens.* According to the result, fungus TH-15 was selected for further investigation as shown in Figure 2.



Fungus TH-15 (Front and Reverse side) Antibacterial activity

Figure 2. Morphologies and activity of TH-15 agains *Agrobacterium tumefacie*ns

Distinct characters of fungus TH-15

In this study, the fungus TH-15 was cultured on PGA medium for seven days. It was found that, morphology of front color is brownish and reverse is black. The microscopical characters are conidial production - chain, type of conidial production – phialo, type of conidial ontogeny – enteroblastic, conidiophores - typical conidiophores but not branch, conidiophore elongates or not - conidiophores elongate along with conidial production, arrangement of conidiogenous cells - longitudinal (parallel), development of conidiogenous cells – stable, conidial production loci of conidiogenous cells – multi, conidia shape - simple spore, conidia septa – amerospore and hyphae - with septa regularly were observed.

According to the morphological and microscopical characters and based on the reference keys, fungus TH-15 was similar to genus *Stachybotrys* (Corda, 1837 & Jarvis *et al*.,1986) as shown in Figure 3 and morphology of TH-15 was mentioned above in Figure 2.

Kingdom	Fungi
Division	Ascomycota
Class	Sordariomycetes
Order	Hypocreales
Family	Stachybotryaceae
Genus	*Stachybotrys*

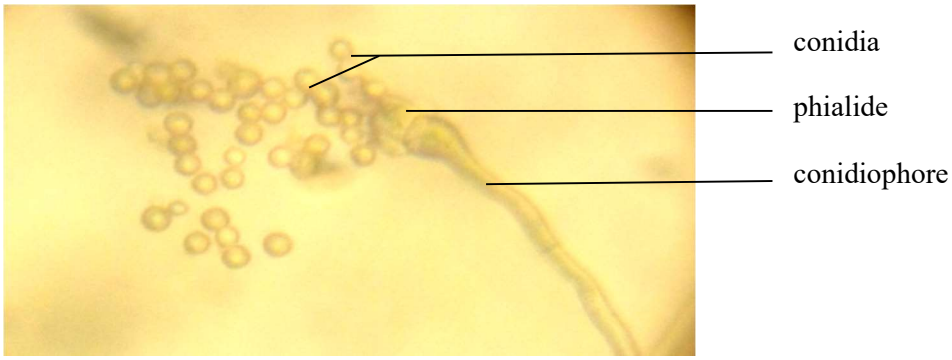


Figure 3. Photomicrograph of fungus TH-15 (x400)

Studies on microbial growth kinetics of fungus TH-15

In the studies of microbial growth kinetics, it was observed that growth phase of fungus TH-35 between 48hrs and 108hrs. According to Crueger and Crueger (1989), ages of inoculum 48hr, 60hr, 72hr, 84hr, 96hr and 108hr were utilized for the optimization of fermentation as shown in Figure 4.

Studies on the ages and sizes of fungus TH-15 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. In this investigation, 72hrs seed culture was the best for fermentation in mention Table 3 and Figure 5. Based on the result of age of inoculum in Table 4 and Figure 5, 20% sizes of inoculums were optimized for the fermentation to produce the antibacterial metabolites.

Studies on the effects of carbon and nitrogen sources of fungus TH-15 on the antimicroobial activity against on *Agrobacterium tumefaciens*

The best carbon source is glucose and yeast extract gave the best activity of nitrogen source were optimized in Figure 4.

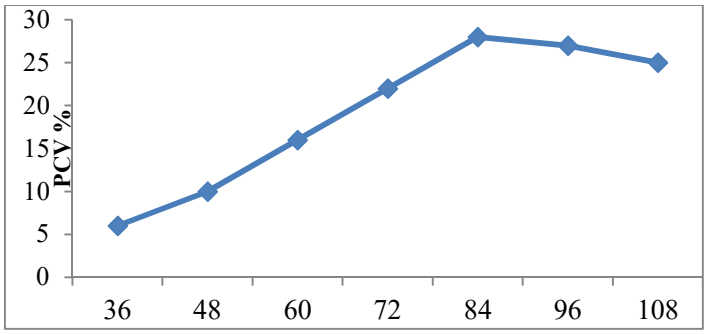


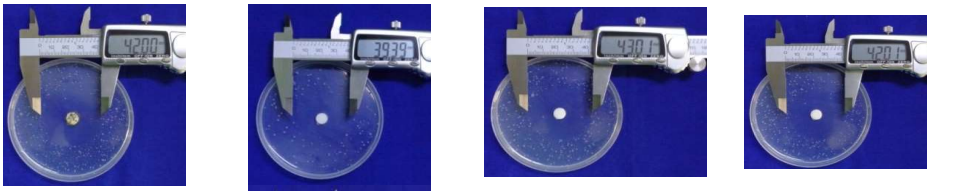
Figure 4. Microbial growth kinetics of fungus TH-15

Table 3. The effects of ages of culture on fermentation

Culture time (Ages of culture, hrs)	Activity (clear zone, mm)
48	38.45
60	39.67
72	**42.00**
84	41.17
96	40.54
108	39.04

Table 4.The effects of sizes of inoculum on the fermentation

Sizes of inoculum at 72hrs (%)	Activity (clear zone, mm)
5%	42.65
10%	40.54
15%	39.39
20%	**43.18**
25%	40.30

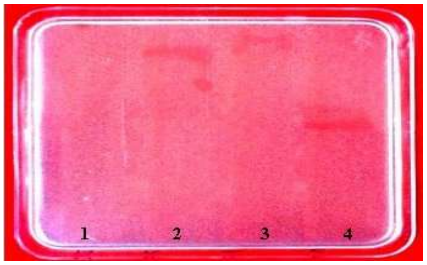


72hr (42.00mm) 20% (39.39mm) glucose (43.01 mm) Yeast extract (42.01 mm)

Figure 5. The best inhibitory zone of age, size, carbon source and nitrogen source against *Agrobacterium tumefaciens*

Determination of solvent for the extraction of antibacterial metabolite by bio-assay of Paper chromatography

According to the R_f value, solvent 2 n-butanol more suitable for the extraction of antibacterial metabolite than solvent 4 ethyl acetate as shown in Figure 6.



1- 20%NH_4Cl

2 - n- butanol saturated with water

3- ethyl acetate-acetic acid-water(3:1:1)

4- ethyl acetate saturated with water

Figure 6. Paper chromatography Bioautographic overlay-assay

Thin layer chromatography and bioautography assay

Based on the TLC results (R_f values), it was found that chloroform-acetone (9:1) solvent system was suitable for the separation of compound by silica gel column chromatography.

Extraction of antibacterial metabolite adjusted pH with organic solvent

It was found that antibacterial metabolite could be extracted with *n-butanol* at pH 7.0 and *n-butanol* was concentrated *in vacuo*.

Silica gel column chromatography

The presence of antibacterial compound produced by TH- 15 was detected by silica gel column chromatography, it was found that three main fractions (F-1, F-2 and F-3) were collected and checked by TLC and tested on *Agrobacterium tumefaciens*. In antibacterial activities, fraction two was showed the best inhibitory zone (33.58 mm) more than fraction one (30.02 mm of inhibitory zone) and fraction three (31.78 mm of inhibitory zone) respectively in Figure 7.

Silica gel re-chromatography

In the experiment of silica gel column re-chromatography, four fractions (F-A, F-B, F-C and F-D) were collected and then checked by TLC. By using PTLC method, compound 1and 2 were isolated from fraction B and

C, fraction A and D were showed tailing characters as shown in Figure 7 – 11and Table 5 and 6.

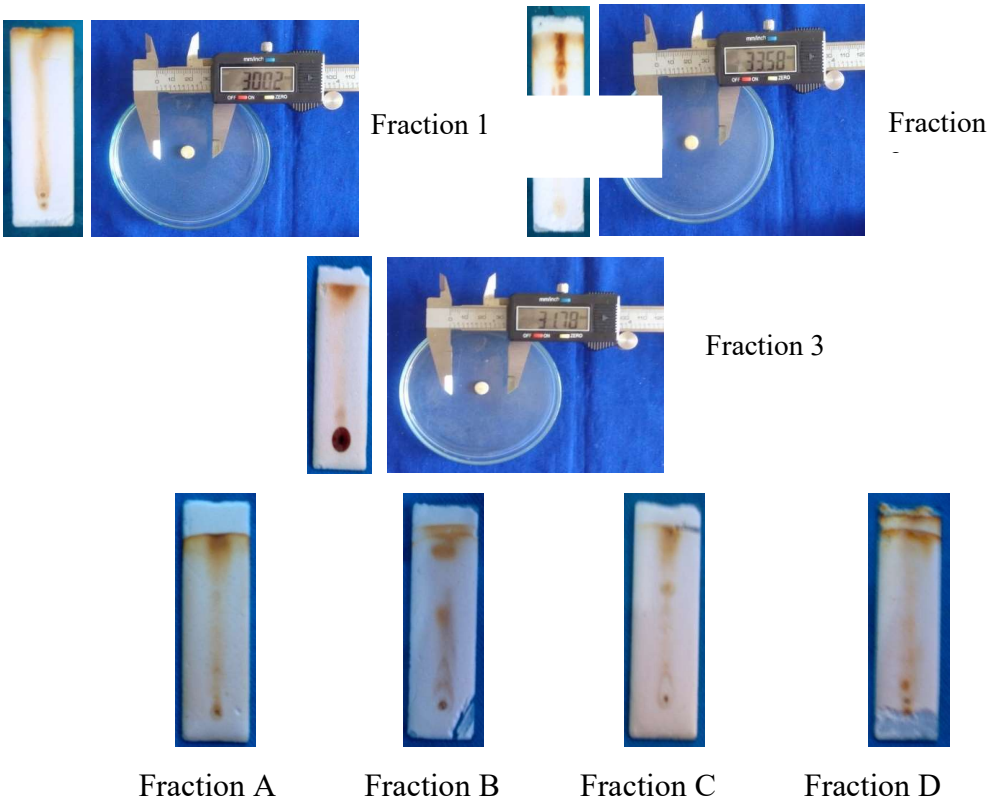
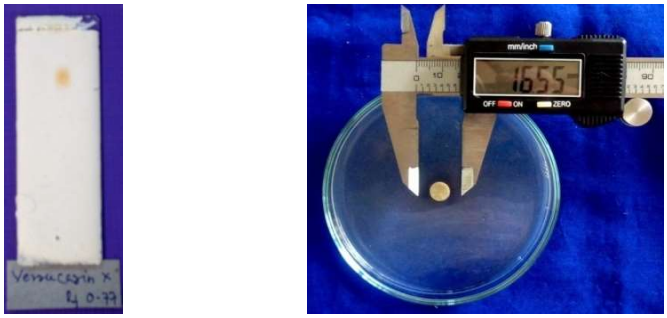


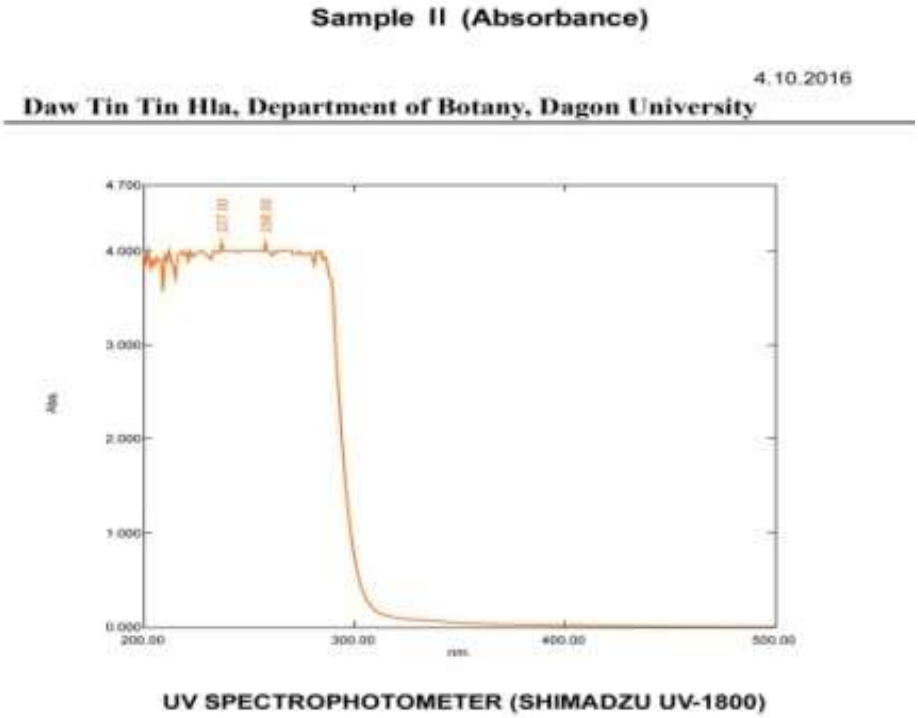
Figure 7. Checked by TLC and tested with *Agrobacterium tumefaciens*

Solvent system	ethylacetate : hexane 7 : 3 v/v
Spray reagent	conc: H_2SO_4 (Δ)
R_f value	0.77
UV	258 nm observed (literature 259 nm)
Melting point	>300°C



Inhibitory zone(16.55mm)

Figure 8.TLC plate and antibacterial activity against on *A tumefaciens*



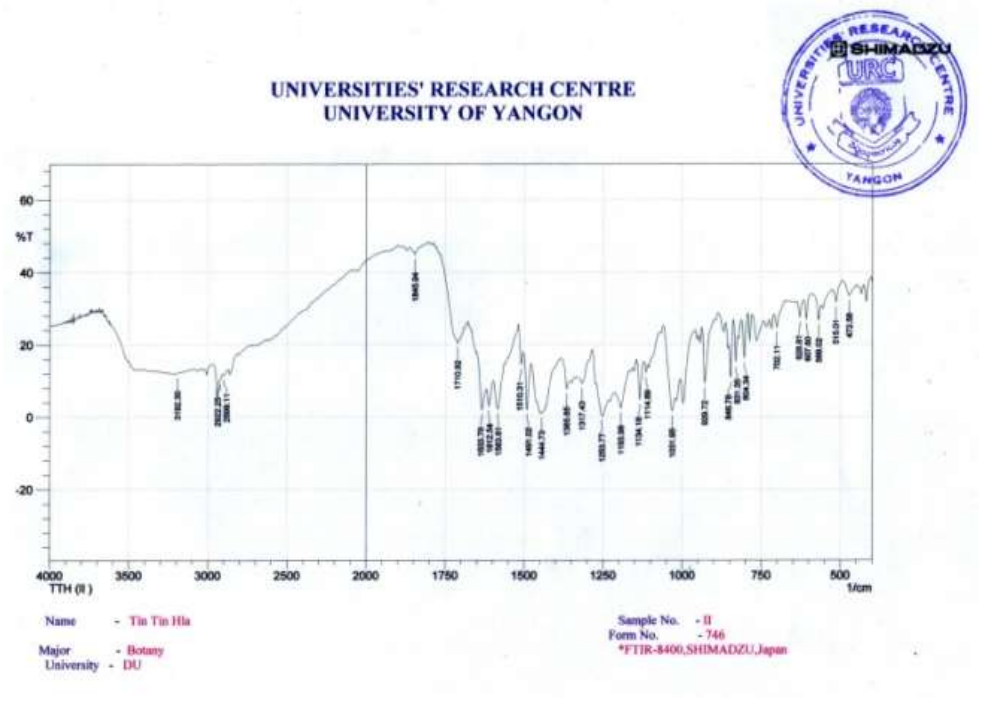


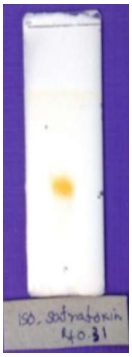
Figure 9.UV and FTIR spectrum of isolated compound 1 (in Amtt Company and URC, Yangon)

Table 5. FTIR spectrum of isolated compound 1 and comparison with literature value

No.	Wave no. (cm^{-1})		Functional group
	isolated	literature	
1	3445	3440	OH stretching hydroxy group
2	2922	2970	CH stretching for CH =CH group
3	1710	1715	C=O stretching
4	1630	1635	C=C stretching for aromatic ring
5	1444	1410	CH banding
6	1263, 1193	1270, 1190	C-O- C stretching
7	1031	1030	C-O stretching in alcohol
8	970, 625	973, 615	CH out of plane banding

S

Solvent system	ethylacetate : hexane 7 : 3 v/v
Spray reagent	conc: H_2SO_4 (Δ)
R_f value	0.31 (literature 0.33)
UV	225.10nm observed (literature 226nm)
Melting point	165-166°C (literature 168-171)

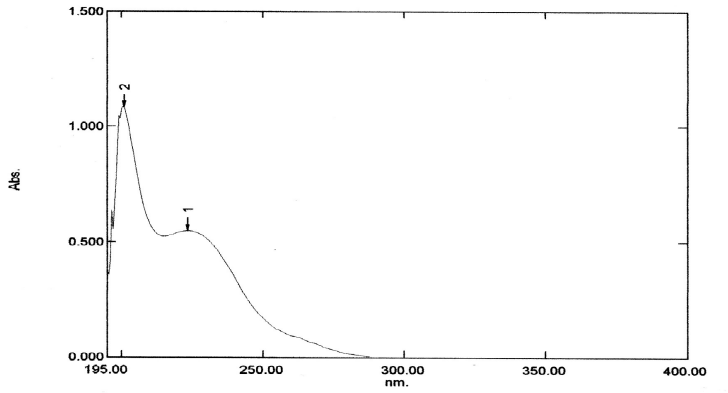


Inhibitory zone (18.88mm

Figure 10. TLC plate and antibacterial activity against o n *A. tumefaciens*

WEST YANGON UNIVERSITY
DEPARTMENT OF CHEMISTRY
UV- 1800 SPECTROPHOTOMETER, SHIMADZU, JAPAN

Sample Name : III
Operator : Kyaw Khaing



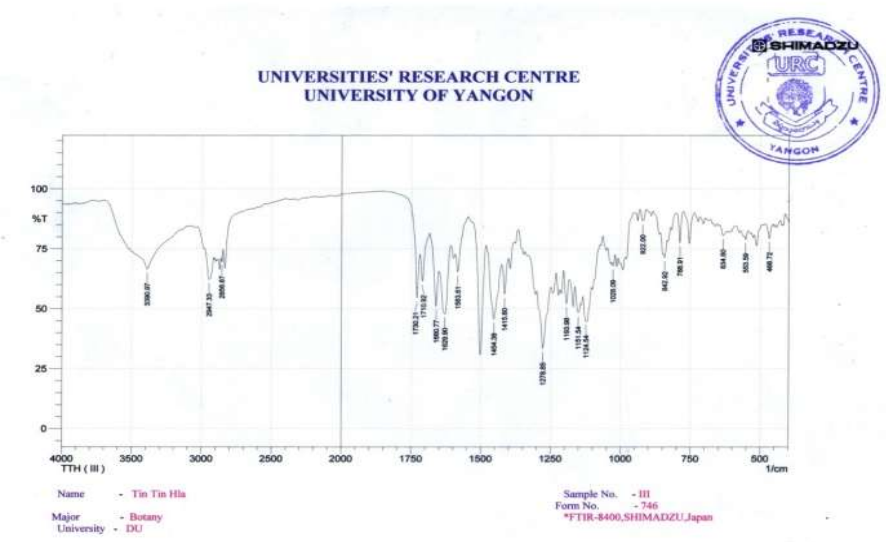


Figure 11.UV and FTIR spectrum of isolated compound 2

(in West Yangon University, Chemistry Department and URC, University of Yangon)

Table 6. FTIR spectrum of isolated compound 1 and comparison with literature value

No.	Wave no. (cm^{-1})		Functional group
	isolated	literature	
1	3550,3400	3560,3440	OH stretching for hydroxy group
2	2947, 2868	2975, 2855	CH stretching for CH_2=CH_3 group
3	1730, 1660	1730, 1660	C=O stretching
4	1583	1595	C=C stretching for aromatic ring
5	1278, 1193	1270, 1200	C-O.C stretching (asymmetric)
6	1151, 1124	1165, 1145	C-O stretching (symmetric)
7	1028	1080	C-OH stretching
8	970	975	CH out of plane banding

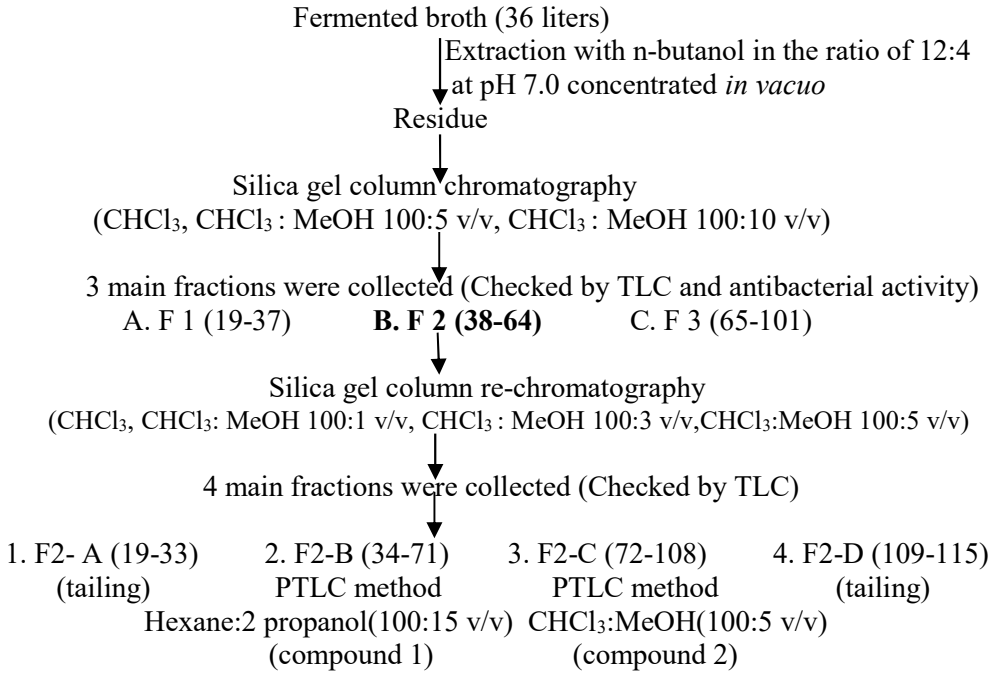


Fig. 11. Flow diagram of separation and isolation of antibacterial compound from extract TH-15

Discussion and Conclusion

A total of 15 fungi were isolated from four different soil samples from Pathein Area. The isolated 15 fungi were tested in *Aspergillus paracitius, Bacillus subtilis, Candida albicans, Escherichia coli, Micrococcus luteus, Pseudomonas fluroscens, Saccharomyces cerevisiae* and *Staphylococcus aureus*, these isolated fungi showed no antimicrobial activity except for *Agrobacterium tumefaciens.* Among them, it was found that strain TH-15 distinctly showed the antimicrobial activity (29.2mm of inhibitory zone). Therefore, this fungus TH-15 was selected for the production of the antibacterial metabolite.

Stachybotrys is characterized by macronematous, mononematous, unbranched or branched conidiophores, with discrete terminal and phialidic conidiogenous cells, and aseptate, reniform, ellipsoidal to spherical, smooth or verrucose conidia (Brazilian Journal of Botany, 2010), in the investigation of fungus TH-15 is similar genus *Stachybotrys.*

The optimal parameter such as growth phase between 48 hrs and 108 hrs; 20% sizes and 72 hrs age of inoculum were observed. In the studies of

carbon and nitrogen sources utilization, glucose (43.01mm, inhibitory zone) and yeast extract (42.01mm, inhibitory zone) were the best for the production of the antibacterial metabolites. According to the result, the extraction of antibacterial metabolite was found in 5 days period.

Preliminary studies of paper chromatography are required to extract the compound from the fermented broth (Kyowa Hakko Co. Ltd., Japan, 1980). In the study of paper chromatography, it was found that n-butanol is suitable for the extraction of the antibacterial metabolite. In TLC, it was found that chloroform-methanol (9:1) solvent system was suitable. Base on the result, the active fractionated compounds, purified by various chromatographies and identification of pure compound had been studied.

In the study of isolation of the antibacterial compound from TH-15, two compounds were isolated from fraction two. Trichothecenes consists of verrucarin A, E and X, piperidine and satratoxins F, G, and H. Trichothecenes are mycotoxins, mycotoxins were used as antibiotics (Jarvis *et al*., 1986 and Marcelo *et al*., 2006). The metabolite extracted from fermented broth of the fungus TH-15, when the analysis of fraction two were isolated verrucarin X and satratoxin H. These isolated two compounds (compound 1 and compound 2) were re-checked by melting point, UV, FTIR, TLC and tested with *Agrobacterium tumefaciens*.

In compound 1, melting point is >300° C, in TLC of R_f value is 0.77, UV 258 nm, tested with *Agrobacterium tumefaciens* (16.55 mm) inhibitory zone were observed compared as literature UV 259 nm (Marcelo *et al*., 2006). Therefore, isolated compound 1 suggested as verrucarin X. In compound 2, melting point is 165 - 166˚C, in TLC of R_f value is 0.31, UV 225.10 nm and 18.88 mm inhibitory zone observed. In literature, melting point is 168 - 171°C, TLC of R_f value 0.33, UV 226 nm (Rusell *et al.*, 1984). Therefore, the isolated compound 2 may be satratoxin H. These collected two compounds were mentioned that Figure flow chart.

The antibiotic trichothecene can be used for its antimicrobial in the form of pharmaceutical compositions containing verrucarin X and satratoxin H. The compositions may also contain other active antibacterial and/or antitumor agents and these made up in any pharmaceutical form appropriate (Rusell *et al.*, 1984). Thus, it is necessary to clarify how these isolated two compounds can be applied as novel pharmaceuticals will be conducted in the near future.

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ISOLATION OF EIGHT BACTERIAL STRAINS FROM RHIZOSPHERIC SOIL OF PEANUT FIELDS

May Zin Moe[1], Zar Zar Yin[2] and Soe Soe Aung[3]

Abstract

The bacterial strains were isolated from the rhizospheric soil of peanut fields. The samples were collected from Shan Ka Lay Kyun village, Amarapura Township, Mandalay Region during August 2016. This experiment was carried out at the Microbiology Laboratory, Department of Botany, University of Mandalay from August 2016 to February 2017. The bacterial strains were isolated by using the King's B medium. The bacterial strains MZ 1, MZ 2, MZ 3, MZ 4, MZ 5, MZ 6, MZ 7, MZ 8 were isolated. These bacterial strains were identified based on their colony morphology and biochemical activities such as catalase test, oxidase test, sugar fermentation test (sucrose, lactose), methyl red (MR) test, voges-proskauer (VP) test, lysine decarboxylase test, citrate utilization test, nitrate reduction test, urease test, triple sugar iron test, starch hydrolysis test, oxygen requirement and motility test. According to the results, the isolated bacterial strains, MZ 1- MZ 8 were confirmed as *Pseudomonas* sp. The colony and cell morphology of eight bacterial strains were described and presented with photomicrographs.

Keywords: rhizospheric soil of peanut, isolation, identification of bacterial strains, *Pseudomonas* sp.

Introduction

Soil microorganisms, such as bacteria and fungi, control ecosystem functioning through decomposition and nutrient cycling and may serve as indicators of land-use change and ecosystem health (Balser *et al*., 2010). The rhizosphere area includes the soil connected to the plant roots and often extends a few millimeters of the root surface, being an important environment for the plant and microorganism interactions (Lynch, 1990 and Gray and Smith, 2005 as cited in Souza *et al*., 2015), because plant roots release a wide range of compounds involved in attracting organisms which may be beneficial, neutral or detrimental to plants (Lynch, 1990 and Badri and Vivanco, 2009 as cited in Souza *et al*., 2015).

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[3.] Professor, Department of Botany, University of Mandalay

The plant growth-promoting bacteria (PGPB) belong to a beneficial and heterogeneous group of microorganisms that can be found in the rhizosphere, on the root surface or associated to it, and are capable of enhancing the growth of plants and protecting them from diseases and abiotic stresses (Dimkpa *et al.,* 2009, Grover *et al.,* 2011 and Glick, 2012 as cited in Souza *et al*., 2015). Certain plant growth promoting microorganisms could enhance defensive activity and stimulate plant resistance against soil borne pathogens (Whipps *et al*., 2001 as cited in Ardebili *et al.,* 2011). Both rhizosphere and rhizoplane comprised *Bacillus, Enterobacter* and *Pseudomonas* can be applied as PGPB to improve and enhance growth in arid soils. Isolated bacteria from soils were found to exhibit capabilities in fix atmospheric nitrogen, produce ammonia, indoleacetic acid (IAA), siderophores, solubilize phosphate and zinc (El-Sayed and El-Naggar, 2014).

Beneficial microorganisms that improve plant health through the enhancement of plant resistance and tolerance against biotic stresses include bacteria, such as *Pseudomonas* sp. or *Bacillus* sp. and fungi such as *Trichoderma* sp., *Gliocladium* sp. or mycorrhizal fungi (Pozo *et al.,* 2002 as cited in Ardebili *et al.,* 2011). Many rhizosphere colonizing bacteria, including *Azotobacter*, *Azospirillum*, *Bacillus*, *Clostridium*, and *Pseudomonas*, typically produce substances that stimulate plant growth or inhibit root pathogens (Vázquez *et al*., 2000 as cited in Ardebili *et al.,* 2011).

Therefore, the present study was carried out the isolation and identification of bacterial strains from the rhizospheric soil of peanut growing areas. The aim and objectives of this study were to isolate the bacterial strains from rhizospheric soil of peanut and to study their colony morphology characters, gram straining, microscopical characters and biochemical activities.

Materials and Methods

The soil samples were randomly collected from rhizospheric soil of peanut fields in Shan Ka Lay Kyun Village, Amarapura Township, Mandalay Region during August 2016. The soil samples were collected from these regions (Figure 1). The study was carried out at the Microbiology Laboratory, at the Department of Botany, University of Mandalay from August, 2016 to February, 2017.

Serial dilutions of soil samples, plating and streaking techniques were used to isolate the microorganisms from soil according to Salle (1948); Collins (1964) and Pelezer and Chan (1972).

The identification of isolated bacterial strains were carried out using their colony morphology, gram staining methods (Dubey and Maheshwari, 2002), and biochemical activities which include Catalase test (Dickey and Kelman, 1988), Oxidase test (Dickey and Kelman, 1988), Sugar fermentation test (sucrose, lactose) (Atlas, 1993), Methyl red test (Aneja, 1996), Voges-Proskauer-VP test (Cruickshank, 1963), Lysine decarboxylase test (Downes, and/to 2001), Citrate utilization test (Atlas, 1993), Nitrate reduction test (Dickey and Kelman, 1988), Urease test (Woodland, 2004), Triple sugar iron test (Woodland, 2004), Starch hydrolysis test (Aneja, 1996), Oxygen requirement (aerobic/anaerobic) (Prescott, 2002)and motility test (Tittsler and Sandholzer, 1936).

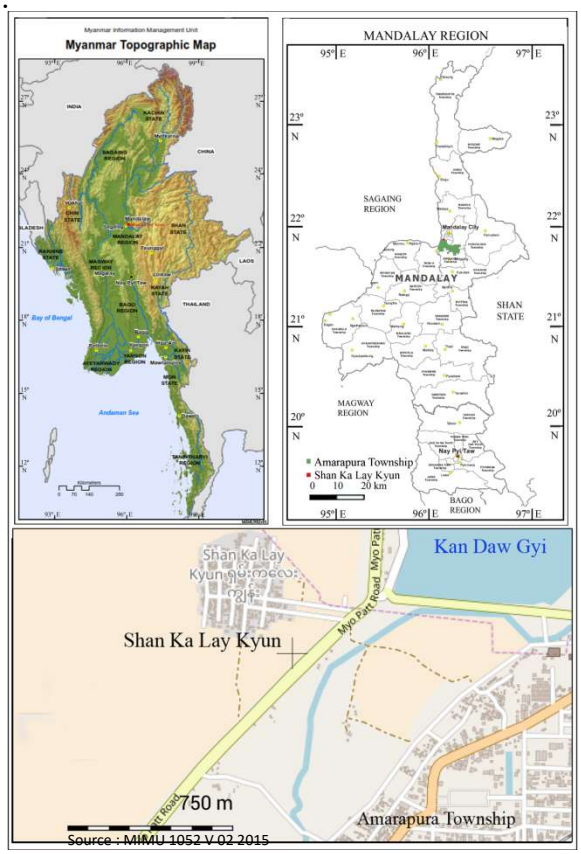

Figure. 1 Location Map of Specimen Collection Site in Shan Ka Lay Kyun village, Amarapura Township

Results

The total of 8 bacterial strains such as MZ 1, MZ 2, MZ 3, MZ 4, MZ 5, MZ 6, MZ 7 and MZ 8, were isolated from the rhizospheric peanut soil. The colonies morphology of those isolated strains MZ 1-MZ 8 were small, moderate, large in sizes; circular, irregular, entire in margins; cream, white, pale green and pale yellow in color; raised and flat in elevation and form; shiny and dull in pigments on agar. Those bacterial strains were short-rot and rod in their cell morphologies, facultative and aerobic, positive in motality. The results of their colony morphology, cell morphology and biochemical activities were shown in Table 1-3 and Figure 2-22.

Table 1. Colony characteristics of eight strains

Strains	Size of Colony	Margin	Color	Elevation and Form	Apperance
MZ 1	small	circular	cream	raised	shiny
MZ 2	moderate	circular	white	raised	shiny
MZ 3	small	circular	white	flat	shiny
MZ 4	large	irregular	pale green	raised	dull
MZ 5	small	entire	pale green	raised	shiny
MZ 6	small	irregular	cream	raised	shiny
MZ 7	small	entire	white	raised	shiny
MZ 8	moderate	entire	pale yellow	raised	shiny

Table 2. Cell morphology and physical characteristics of eight strains

Strains	Gram Staining	Cell Shape	Physical Characteristics
MZ 1	–	rod	aerobic
MZ 2	–	short rod	aerobic
MZ 3	–	rod	aerobic
MZ 4	–	rod	aerobic
MZ 5	–	short rod	aerobic
MZ 6	–	rod	aerobic
MZ 7	–	rod	aerobic
MZ 8	–	short rod	facultative

negative reaction = –

Table 3. Biochemical activities of eight strains

No.	Biochemical activities	Strains							
		MZ 1	MZ 2	MZ 3	MZ 4	MZ 5	MZ 6	MZ 7	MZ 8
1	Catalase	+	+	+	+	+	+	+	+
2	Oxidase	+	+	+	+	+	+	+	+
3	Sugar Fermentation (lactose)	+	+	+	+	–	–	–	+
4	Sugar Fermentation (sucrose)	+	+	+	+	+	+	–	+
5	Methyl Red (MR)	–	–	–	–	–	–	–	–
6	Voges-Proskauer (VP-Test)	–	–	–	–	–	–	–	–
7	Lysine	+	–	+	–	–	+	+	–
8	Citrate	+	+	+	+	+	+	+	+
9	Nitrate	+	–	+	–	–	+	+	+
10	Urease	+	+	–	+	+	–	–	–
11	Triple sugar								
	(i) Fermentation	+	+	+	+	+	+	+	+
	(ii) H_2S	–	–	–	–	+	–	–	–
	(iii) Gas	–	+	–	–	–	–	–	+
12	Starch Hydrolysis	+	–	+	–	–	–	–	–
13	Motality	+	+	+	+	+	+	+	+

positive reaction = +, negative reaction = –

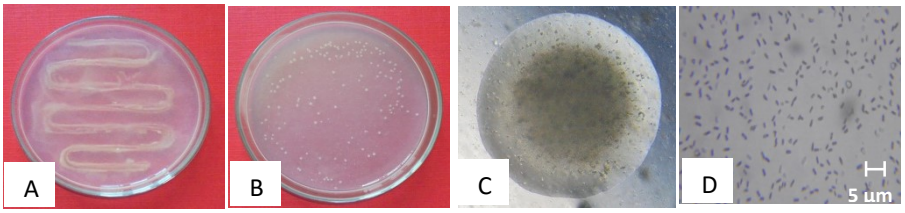

Figure 2. Colony characteristics and cell morphology of MZ 1

A. Colonies on streaks plate, B. Colonies, C. Single colony,

D. Photomicrograph of cells

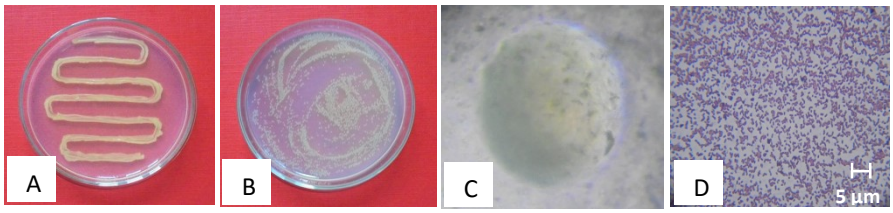

Figure 3. Colony characteristics and cell morphology of MZ 2
A. Colonies on streaks plate, B. Colonies, C. Single colony,
D. Photomicrograph of cells

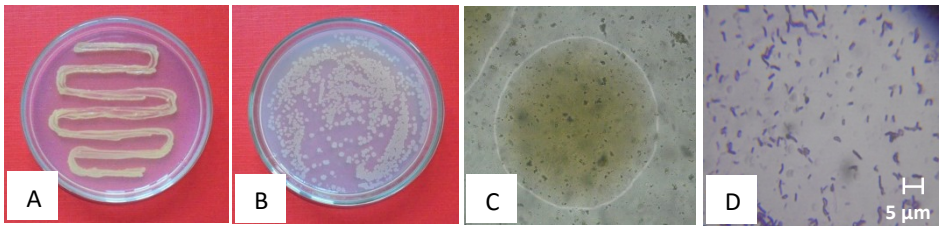

Figure 4. Colony characteristics and cell morphology of MZ 3
A. Colonies on streaks plate, B. Colonies, C. Single colony,
D. Photomicrograph of cells

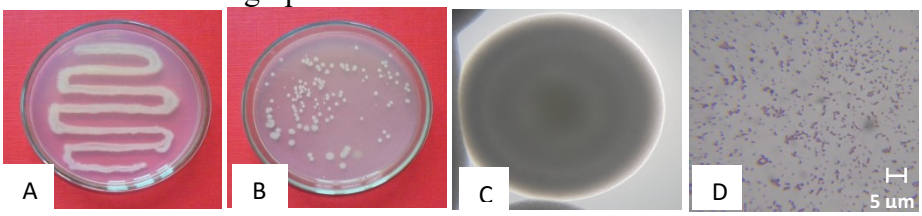

Figure 5. Colony characteristics and cell morphology of MZ 4
A. Colonies on streaks plate, B. Colonies, C. Single colony,
D. Photomicrograph of cells

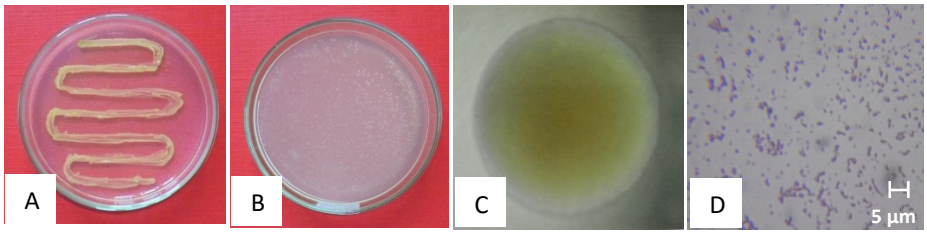

Figure 6. Colony characteristics and cell morphology of MZ 5
A. Colonies on streaks plate, B. Colonies, C. Single colony,
D. Photomicrograph of cells

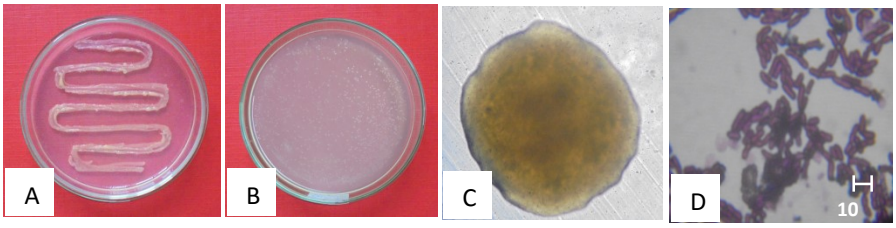

Figure 7. Colony characteristics and cell morphology of MZ 6
A. Colonies on streaks plate, B. Colonies, C. Single colony,
D. Photomicrograph of cells

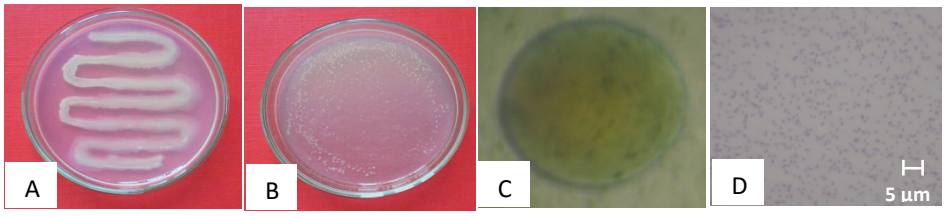

Figure 8. Colony characteristics and cell morphology of MZ 7
A. Colonies on streaks plate, B. Colonies, C. Single colony,
D. Photomicrograph of cells

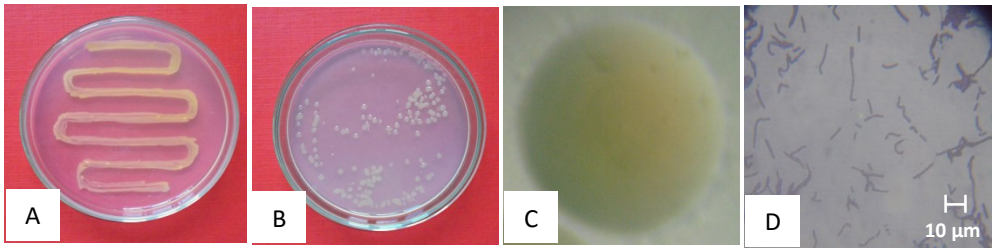

Figure 9. Colony characteristics and cell morphology of MZ 8
A. Colonies on streaks plate, B. Colonies, C. Single colony,
D. Photomicrograph of cells

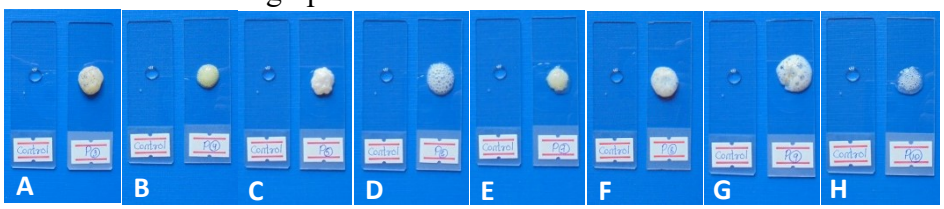

Figure 10. Catalase test A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive).

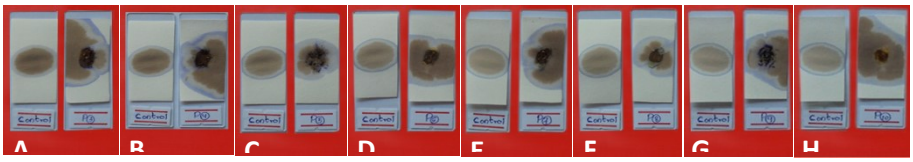

Figure 11. Oxidase test A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive)

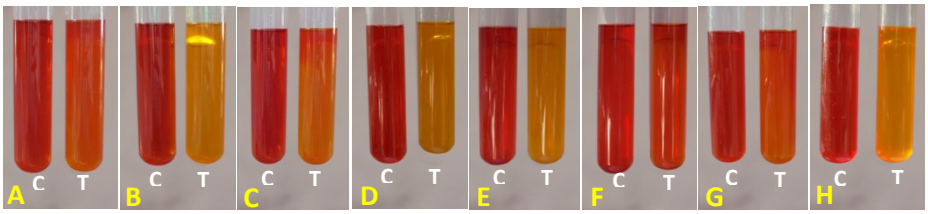

Figure 12. Sugar fermentation test (lactose) A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Negative), F. MZ 6 (Negative), G. MZ 7 (Negative), H. MZ 8 (Positive). C = control, T = treatment.

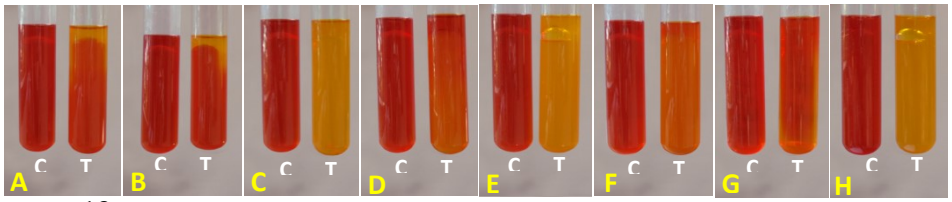

Figure 13. Sugar fermentation test (sucrose) A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Negative), H. MZ 8 (Positive). C = control, T = treatment.

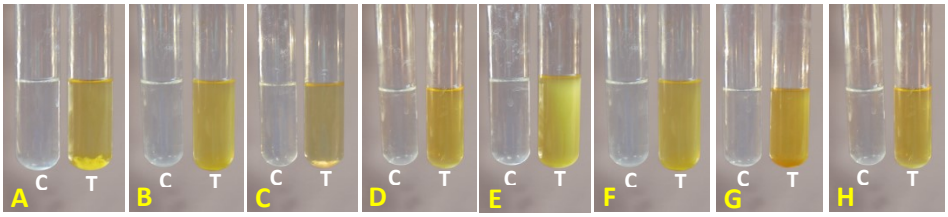

Figure 14. Methyl red (MR) test A. MZ 1 (Negative), B. MZ 2 (Negative), C. MZ 3 (Negative), D. MZ 4 (Negative), E. MZ 5 (Negative), F. MZ 6 (Negative), G. MZ 7 (Negative), H. MZ 8 (Negative).C = control, T = treatment.

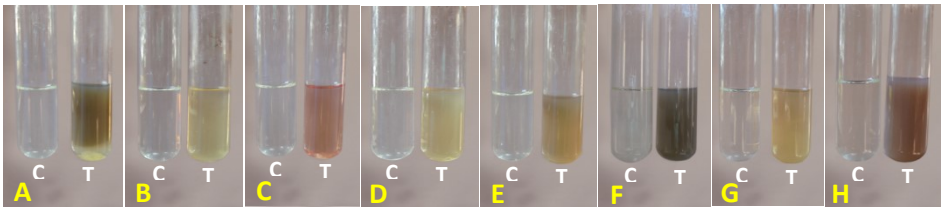

Figure 15. Voges-Proskauer (VP) test A. MZ 1 (Negative), B. MZ 2 (Negative), C. MZ 3 (Negative), D. MZ 4 (Negative), E. MZ 5 (Negative), F. MZ 6 (Negative), G. MZ 7 (Negative), H. MZ 8 (Negative). C = control, T = treatment.

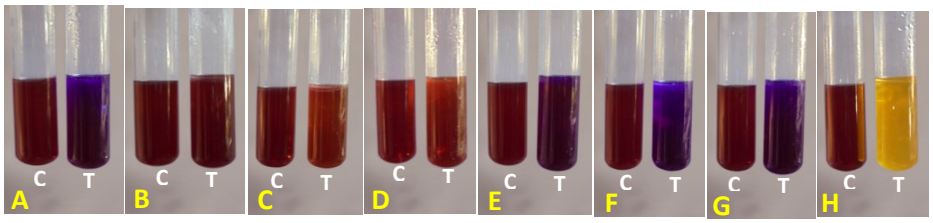

Figure 16. Lysine test A. MZ 1 (Positive), B. MZ 2 (Negative), C. MZ 3 (Positive), D. MZ 4 (Negative), E. MZ 5 (Negative), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Negative). C = control, T = treatment.

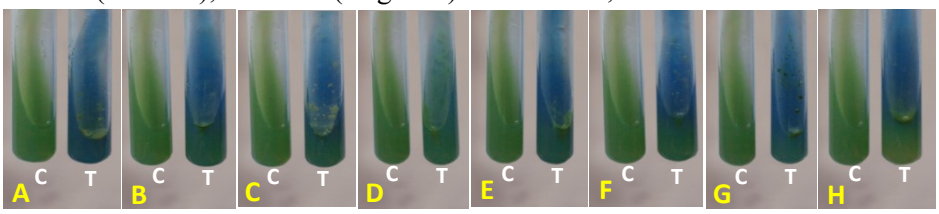

Figure 17. Citrate test A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive). C = control, T = treatment.

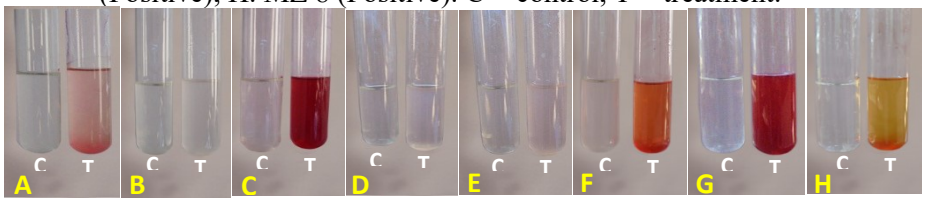

Figure 18. Nitrate test A. MZ 1 (Positive), B. MZ 2 (Negative), C. MZ 3 (Positive), D. MZ 4 (Negative), E. MZ 5 (Negative), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive). C = control, T = treatment.

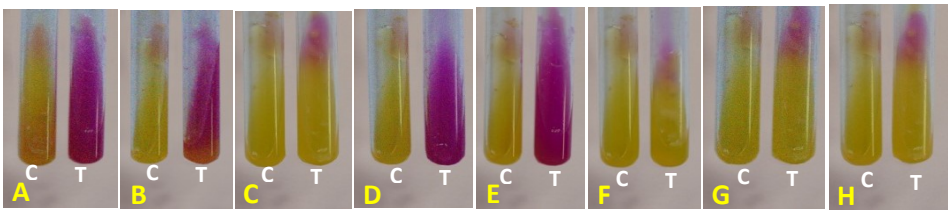

Figure 19. Urease test A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Negative), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Negative), G. MZ 7 (Negative), H. MZ 8 (Negative). C = control, T = treatment.

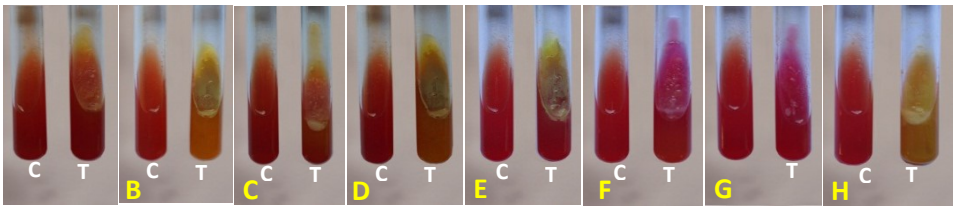

Figure 20. Triple sugar test A. MZ 1 (Negative), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ6 (Positive), G. MZ 7 (Negative), H. MZ 8 (Positive). C = control, T = treatment.

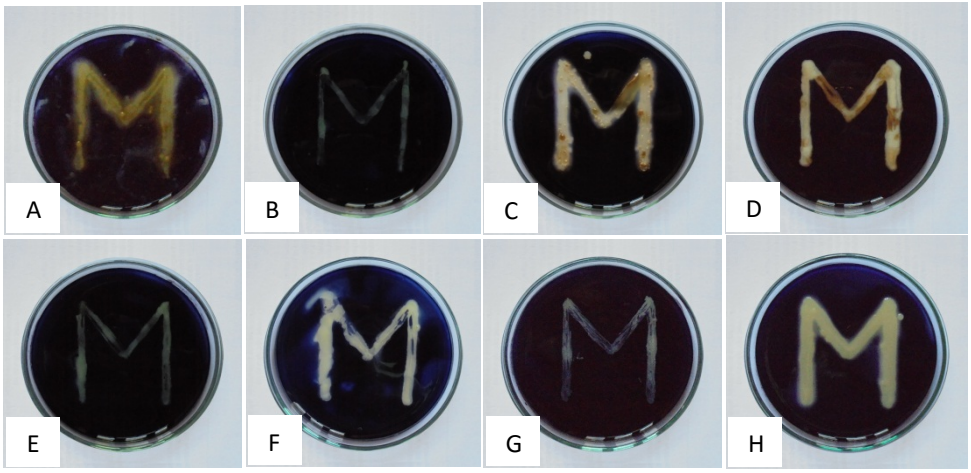

Figure 21. Starch hydrolysis test [A. MZ 1 (Positive), B. MZ 2 (Negative), C. MZ 3 (Positive), D. MZ 4 (Negative), E. MZ 5 (Negative), F. MZ 6 (Negative), G. MZ 7 (Negative), H. MZ 8 (Negative)].

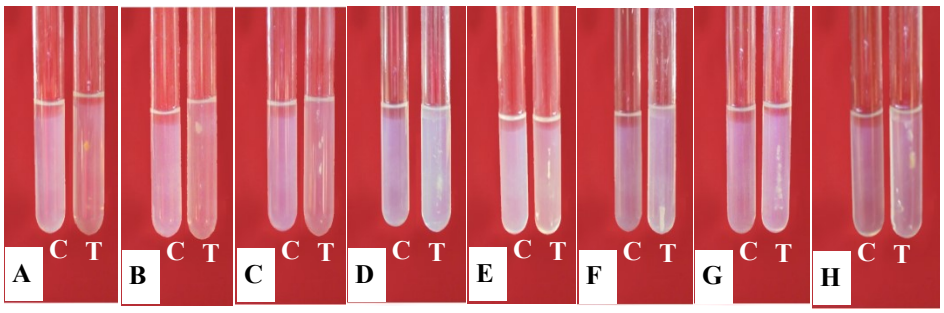

Figure 22. Motality tests. A. MZ 1 (Positive), B. MZ 2 (Positive), C. MZ 3 (Positive), D. MZ 4 (Positive), E. MZ 5 (Positive), F. MZ 6 (Positive), G. MZ 7 (Positive), H. MZ 8 (Positive). C = control, T = treatment.

Discussion and Conclusion

Microorganisms play an important role on the nutritional chains that are an important part of the biological balance in the life in planet. Microorganisms can be used to determine the bioavailability of a given chemical compounds in soil (Bating *et al*., 2008).

The present study, the rhizospheric soil samples were randomly collected from the peanut fields of Shan Ka Lay Kyun village, Amarapura Township, Mandalay Region during August 2016. The study was carried out at Microbiology Laboratory of Botany Department, University of Mandalay from August 2016 to February 2017.

The present findings showed that total of eight bacterial strains were isolated and identified from the rhizospheric peanut soils. The isolated strains were designated as MZ 1, MZ 2, MZ 3, MZ 4, MZ 5, MZ 6, MZ 7, MZ 8. In order to identify the isolated strains, the colony morphology, gram strains, microscopical characters and biochemical tests were carried out during the study. As the results, the colony sizes of MZ 1 - MZ 8 were small, moderate and large. Their margins were circular, irregular and entire. Their colony color were cream, white, pale-green and pale yellow. The elevation of these strains was raised and flatted. The colony's pigment was shiny and dull on agar medium. All of the isolated strains MZ 1 to MZ 8 in their cell morphology were gram-negative, rod and short-rod, aerobic, motile, catalase positive; oxidase positive. In addition, lactic acids were produced from sucrose and lactose, negative results in methyl red (MR) and (VP) tests, positive in the

citrate and nitrate reactions. However, H_2S gas was not produced except in MZ 5. All the isolated strains showed negative in starch hydrolysis except in MZ 1 and MZ 3.Those results were based on the identification of colony morphology, microscopical characters and biochemical reactions from the isolated strains which were more or less the same characters of *Pseudomonas* spp. These findings were also in agreement with Soesantoetal. (2011) who reported that *Pseudomonas* was greenish yellow colony, smooth edges, convex surfaces and fluorescent under UV light on King's B agar medium. These results were the same with the descriptions of Buchanan and Gibbons (1974). Therefore, these isolated strains MZ 1 - MZ 8 were identified as the genus, *Pseudomonas.*

As the results, it would be concluded that the present findings of those isolated bacterial strains MZ 1 - MZ 8 can be noted as the *Pseudomonas* bacterial strains. Those bacterial strains would be isolated from the rhizopheric soil of peanut fields and confirmed as *Pseudomonas* spp. However, further study should be undertaken for the antimicrobial activities and biocontrol agents by using the effective bacterial strains which can be isolated from different rhizospheric soils.

Acknowledgements

I would like to acknowledge to the following persons who have supported for this research work: Dr. Nu Nu Yee, Professor and Head of Department of Botany, University of Mandalay for her invaluable advice and encouragement, Dr. Soe Myint Aye, Professor from Department of Botany, University of Mandalay for his critical reviews and suggestions and Dr. Win Win Khaing, Lecturer, Department of Botany, University of Mandalay for great helps and suggestions.

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SCREENING OF AMYLOLYTIC FUNGI FROM DIFFERENT SOURCES

Kyaw Soe Khaing

Abstract

In this study, amylolytic fungi were screened from various sources such as bread, *Daucus carota* L. (carrot), *Musa* sp. (banana), *Sechium edule* Sw. (chayote), *Vitis vinifera* L. (grape) and *Cucurbita maxima* Duch. (pumpkin). and soil. Isolated fungi were cultured on the glucose yeast peptone agar medium (GYP medium) for 5 to 11 days. Amylolytic activity was screened as sharp and distinct clear zone using with Gram's iodine solution. Three different amylolytic fungi were directly taken from bread (*Amblyosporium* sp.(1) , *Aspergillus* sp. (1), *Amblyosporium* sp. (2)), four different strains from carrot (*Aspergillus* sp. (2), *Aspergillus* sp. (3), *Aspergillus* sp. (4), *Aspergillus* sp. (5)), 1 strain from banana (*Botrytis* sp.), 1 strain from chayote (*Aspergillus* sp. (6)), 2 strains from grape (*Aspergillus* sp. (7), *Aspergillus* sp. (8)), 1 strain from pumpkin (*Penicillium* sp.) and 4 strains from soil (*Aspergillus* sp. (9), *Aspergillus* sp. (10), *Aspergillus* sp. (11), *Paecilomyces* sp.), respectively. The total number of (16) amylolytic fungi were obtained.

Keywords: amylolytic fungi

Introduction

Enzymes that hydrolyze starch are known as amylases. α- Amylase catalyzes the first step in the digestion of starch, a main source of carbohydrate in the human diet. Without the enzymes in our digestive tract for example, it would take us about 50 years to digest a single meal. The first industrially amylase enzyme was produced from a fungal source in 1894, which was used for the treatment of digestive disorder (Crueger and Crueger, 1984). *Aspergillus oryzae* has been largely used in the production of food such as soy sauce, organic acid such as citric and acetic acids and commercial enzymes including α-amylase (Kammoun et. al., 2008). Fungal Amylase - α-amylases with a slightly different action pattern yield mostly maltose and some oligomers. They are an alternative to β-amylases for making maltose syrup. Fungal amylases are more heat labile than those from bacterial and plant sources (U Win, 2004).

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In this research, amylolytic fungi were isolated from different sources and screened their amylolytic activity with Gram's iodine method and they were identified and classified into genus level. The following aims and objectives of present work are:

(a) To isolate the amylolytic fungi from various sources

(b) To identify the amylolytic fungi from different sources into genus level

Meterials and Methods

Collecting samples from different sources

The soil samples were collected from University of Yangon campus. The diluted soil were cultured on starch agar medium (GYP) for isolation of amylolytic fungi. Bread, carrot, banana, chayote, grapes, pumpkin were bought from different markets. The samples are kept in room temperature for several days. After one week, fungi colonized on samples. These fungi are directly taken for isolation of amylolytic fungi on starch agar medium (GYP) and inoculated in room temperature for 5- 11days. The resulting fungi were routinely subcultures to get pure isolates and screened with Gram's iodine stain (Kasana, et al., 2008). Glass wares that used in this experiment were sterilized at 121° C in 15lbs/sq inch for 15 minutes.

Soil Dilution Method

Soil dilution method was used according to method of Johnson (1957). One gram of soil sample was placed in a 300 ml conical flask. Water was added to the soil so that a total volume of 100 ml was reached; the suspension was stirred and shaken for 30 minutes. One ml of soil sample was transferred immediately through successive 9 ml sterile water in test tubes until the desired final dilution was reached.

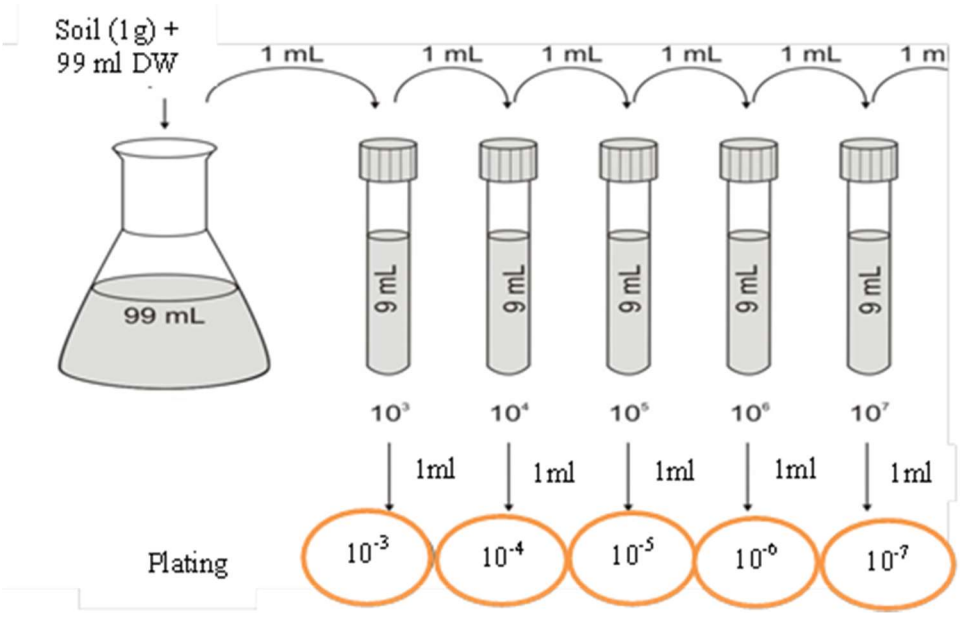


Figure.1 Soil Dilution Method (Johnson, 1957)

Preparation of GYP medium for preliminary test (GYP medium)

Glucose yeast peptone agar medium was used for amylolytic enzyme activity according to the method of Elsa and Bhimba (2012). GYP media was prepared by mixing soluble starch 2.00 g, glucose 0.1 g, yeast extract 0.01g, peptone 0.05 g, Agar 1.60 g, distilled water100.0 ml in a 250-ml conical flask. The pH of the solution was adjusted to 6.0.

Grams Iodine Stain

Grams Iodine Stain was used for screening amylolytic fungi by Kasana, et al. (2008).

Potassium iodide (KI)	- 2.0 g
Iodine	- 1.0 g
Distilled water	- 300 ml

Identification of fungi

Fungal isolates were identified on the basis of routine cultural, morphological and microscopic characters according to Watanabe (1937), Barnett (1969) and Ingold (1967).

Czapekdox agar medium

Czapekdox agar medium was used as stock culture medium or sub-culture medium for maintenance the fungus according to the method of Raper and Thom (1945). This medium were contain Sucrose 30.00 g, $NaNO_3$ 2.00 g, K_2HPO_4 1.00 g, $MgSO_4$ 0.50 g, KCL 0.50 g, $FeSO_4$ 0.10 g, Agar 20.00 g, Demineralized water 1000.0 ml. The pH of the solution is 7.0.

Results

Soil sample was collected from University of Yangon campus and starch rich sources such as bread, *Daucus carota* L. (carrot), *Musa* sp. (banana), *Sechium edule* Sw. (chayote), *Vitis vinifera* L. (grape) and *Cucurbita maxima* Duch. (pumpkin) were bought. Three different amylolytic fungi were directly taken from bread (*Amblyosporium* sp. (1), *Aspergillus* sp. (1), *Amblyosporium* sp. (2)), were shown in figure-(3), (4), (5). Four different strains were directly taken from carrot (*Aspergillus* sp. (2), *Aspergillus* sp. (3), *Aspergillus* sp. (4), *Aspergillus* sp. (5)), were shown in figure-(7), (8), (9), (10). One strain was isolated from banana (*Botrytis* sp.), was shown in figure – (12). One strain was isolated from chayote (*Aspergillus* sp. (6)), was shown in figure-(14). Two strains were isolated from grape (*Aspergillus* sp. (7), *Aspergillus* sp. (8)) were shown in figure-(16), (17). One strain was isolated from pumpkin (*Penicillium* sp.), was shown in figure – (19) and four strains were isolated from soil (*Aspergillus* sp. (9), *Aspergillus* sp. (10), *Aspergillus* sp. (11), *Paecilomyces* sp.), were shown in figure- (21), (22), (23), (24) respectively. The total number of (16) amylolytic fungi were obtained and showed in Table 1 and 2.

Table 1. Indentification of amylolytic fungi from different source

Sr. No.	Sampling sources	No. of amylolytic fungi	Genus
1	Bread	3	*Amblyosporium* sp. (1), *Aspergillus* sp.(1), *Amblyosporium* sp. (2)
2	Carrot	4	*Aspergillus* sp.(2), *Aspergillus* sp.(3), *Aspergillus* sp.(4), *Aspergillus* sp.(5)
3	Banana	1	*Botrytis* sp.
4	Chayote	1	*Aspergillus* sp.(6)
5	Grape	2	*Aspergillus* sp.(7), *Aspergillus* sp.(8)
6	Pumpkin	1	*Penicillium* sp.

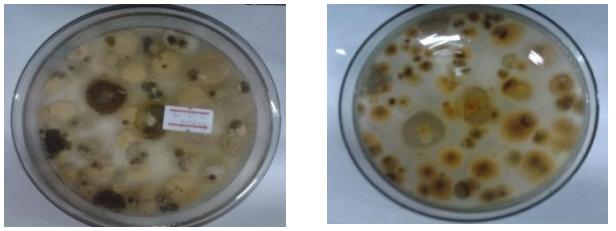
Table 2. Identification of amylolytic fungi isolated from soil

Sr.No.	Soil concentration	Genus
1	10^{-3} SS-10 (1)	*Aspergillus* sp.(9)
2	10^{-3} SS-10 (2)	*Aspergillus* sp.(10)
3	10^{-3} SS-10 (3)	*Aspergillus* sp.(11)
4	10^{-6} SS-10 (1)	*Paecilomyces* sp.

Formation of fungal colonies on bread

Three different amylolytic fungi were directly taken from bread; they were *Amblyosporium* sp. (1), *Aspergillus* sp. (1), *Amblyosporium* sp.(2).

Various fungal colonies grow on bread.

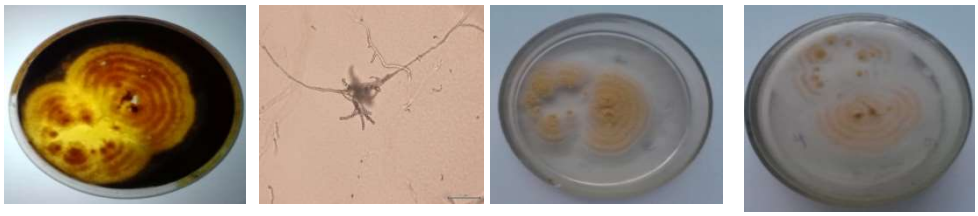


Various fungal colonies grown on starch agar medium from bread

Figure 2- Various fungal colonies grow on bread and starch agar medium.

Characters of mycelium and spore formation of *Amblyosporium* sp. (1)

Mycelium pale to yellow-orange; conidiophores erect, septate, lower portion unbranched, bearing a number of irregular branches near or at the apex, from which conidial chains are formed by segmentation; conidia (arthrospores) 1-celled, hyaline or yellow-orange in mass, barrel-shaped, catenulate; saprophytic in soil or often growing on fleshy or woody basidiomycetes. The micrograph of *Amblyosporium* sp. (1) was shown in figure – (3).



Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Amblyosporium* sp. (1) (X 400)

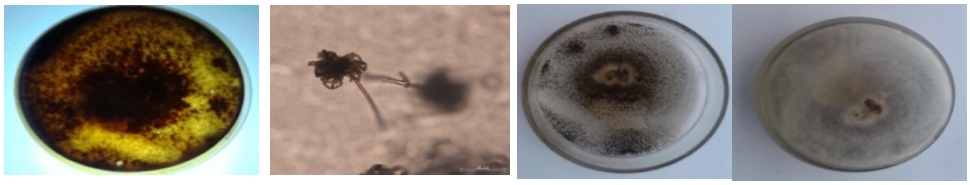
Pure fungal colony (pale yellow) 8 days old

Reverse view

Figure 3 - *Amblyosporium* sp. (1) isolated from bread

Characters of mycelium and spore formation of *Aspergillus* sp. (1)

Conidiophores upright, simple, terminating in a globose swelling, bearing phialides at the apex or radiating from the apex or the entire surface; conidia (phialospores) 1-celled, globose, black colour in mass, in dry basipetal chains. The micrograph of *Aspergillus* sp. (1) was shown in figure – (4).



Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp. (1) (X 400)

Pure fungal colony (black), 8 days old

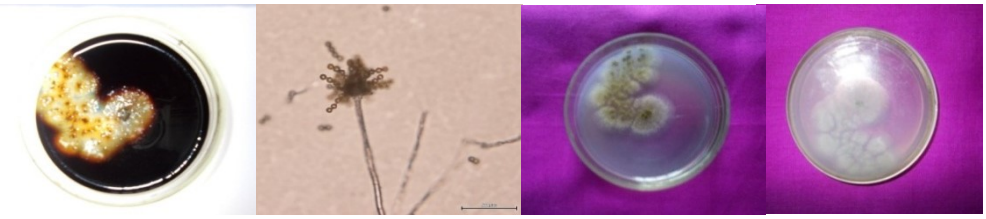
Reverse view

Figure 4 - *Aspergillus* sp. (1) isolated from bread

Characters of mycelium and spore formation of *Amblyosporium* sp. (2)

Mycelium pale to yellow-orange; conidiophores erect, septate, lower portion unbranched, bearing a number of irregular branches near or at the apex, from which conidial chains are formed by segmentation; conidia (arthrospores) 1-celled, hyaline or yellow-orange in mass, barrel-shaped,

catenulate; saprophytic in soil or often growing on fleshy or woody basidiomycetes. The micrograph of *Amblyosporium* sp. (2) was shown in figure – (5).



Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Pure fungal colony (yellow), 8 days old

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; sp. (1)
0)

Reverse view

Figure 5 - *Amblyosporium* sp. (2) isolated from bread

Formation of fungal colonies on carrot

Four different strains were isolated from carrot, which are *Aspergillus* sp. (2), *Aspergillus* sp. (3), *Aspergillus* sp. (4), *Aspergillus* sp. (5).

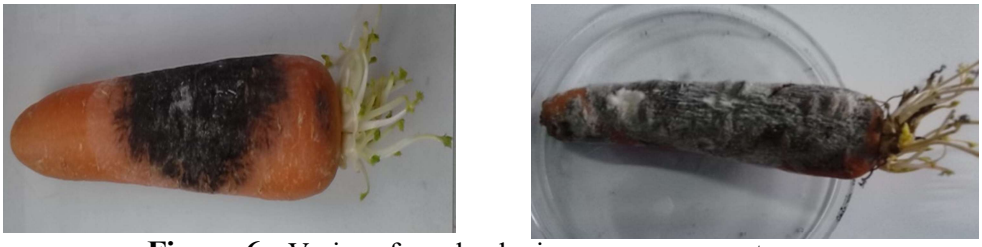
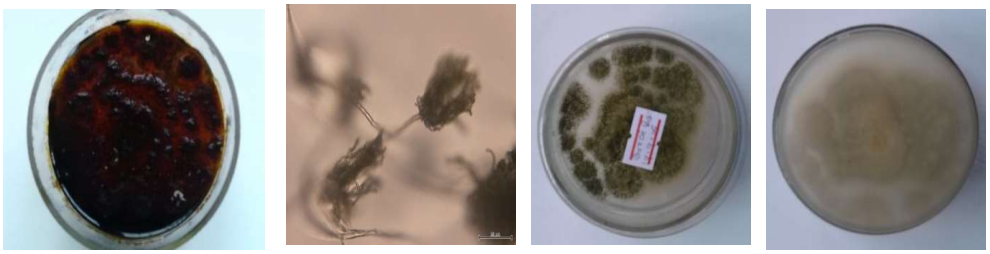


Figure 6 - Various fungal colonies grown on carrot.

Characters of mycelium and spore formation of *Aspergillus* sp. (2)

Conidiophores hyaline, simple, occasionally thick-walled, inflated globosely or ellipsoidally at the apex (called vesicles), bearing spore heads composed of catenulate conidia borne on uniseriate phialides on vesicles: conidial heads dark green, loosely columnar. Conidia phialosporous, pale brown to yellowish brown, globose, delicately rough at the surface. The micrograph of *Aspergillus* sp. (2) was shown in figure – (7).



Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp. (2) (X 400)

Pure fungal colony (yellowish brown), 5days old

Reverse view

Figure 7 - *Aspergillus* sp. (2) isolated from

Characters of mycelium and spore formation of *Aspergillus* sp. (3)

Conidiophores upright, simple, terminating in a globose swelling, bearing phialides at the entire surface; conidia (phialospores) 1-celled, globose, black colour in mass, in dry basipetal chains. The micrograph of *Aspergillus* sp. (3) was shown in figure – (8).

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Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp. (3) (X 400)

Pure fungal colony (black), 5 days old

Reverse view

Figure 8 - *Aspergillus* sp. (3) isolated from carrot.

Characters of mycelium and spore formation of *Aspergillus* sp. (4)

In the conidial (*Aspergillus*) stage the conidiophore is erect and unbranched, and its apex swells into a globular head, from this there bud out a number of projections (phialides) each of which produces a growing chain of

conidia with the youngest at the bottom, spores are dry, easily detached by air currents, culture of this species is bright yellow because of the pigmentation of the conidia, saprophytes in the soil and on decaying vegetable matter. The micrograph of *Aspergillus* sp. (4) was shown in figure – (9).

Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp. (4) (X400)

Pure fungal colony (yellow), 5days old

Reverse view

Figure 9 - *Aspergillus* sp. (4) isolated from carrot.

Characters of mycelium and spore formation of *Aspergillus* sp. (5)

Conidiophores upright, simple, terminating in a globose swelling, bearing phialides at the apex or radiating from the apex; conidia (phialospores) 1-celled, globose, often variously colored in mass, in dry basipetal chains. The micrograph of *Aspergillus* sp. (5) was shown in figure – (10).

Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp. (5) (X400)

Pure fungal colony (bright green), 5 days old

Figure 10- *Aspergillus* sp. (5) isolated from carrot.

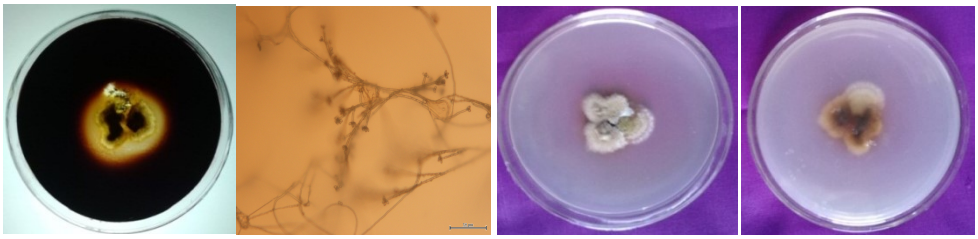
Formation of fungal colonies on banana

One amylolytic fungi strain was isolated from banana, it was *Botrytis* sp.

Figure 11 - Fungi grown on banana

Characters of mycelium and spore formation of *Botrytis* sp.

Conidiophores tall, slender, determinate, pigmented, branched irregularly in upper portion, apical cells enlarge or rounded, bearing clusters of conidia; conidia (botryoblastospores) hyaline or gray in mass, ovoid; black irregular sclerotia often present; causing "gray mold" on many plants or saprophytic. The micrograph of *Botrytis* sp. was shown in figure – (12).



Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *botrytis* sp. (X400)

Pure fungal colony (white), 6 days old

Reverse view

Figure 12 - *Botrytis* sp. isolated from banana.

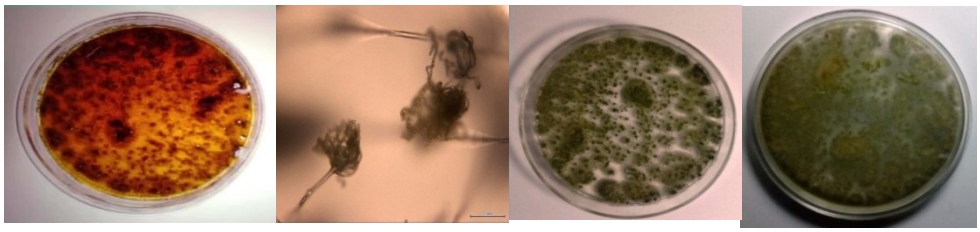
Formation of fungal colonies on chayote

One amylolytic fungi strain was isolated from chayote; it is *Aspergillus* sp. (6).

Figure 13 - Fungi grown on chayote

Characters of mycelium and spore formation of *Aspergillus* sp. (6)

Conidiophores erect, simple, and rough in the surface, with foot cells basally, inflated at the apex forming globose vesicles, bearing radiate conidial heads composed of catenulate conidia borne on uniseriate or rarely biseriate phialides: conidial heads yellowish green, radiate, columnar. Conidia phialosporous, pale green, globose, echinulate. The micrograph of *Aspergillus* sp. (6) was shown in figure – (14).



Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp. (6) (X400)

Pure fungal colony (green) 7 days old

Reverse view

Figure 14 - *Aspergillus* sp. (6) isolated from chayote.

Formation of fungal colonies on grape

Two amylolytic fungi strains were isolated from grapes which are *Aspergillus* sp. (7) and *Aspergillus* sp. (8).

Figure 15 - Fungi grow on grape

Characters of mycelium and spore formation of *Aspergillus* sp. (7)

Conidiophores hyaline or pale brown, erect, simple, thick-walled, with foot cells basally, inflated at the apex forming globose vesicles, bearing conidial heads split into over 4 loose conidial columns with over 4 fragments apically, composed of catenulate conidia (over 15 conidia/chain) borne on

uniseriate or biseriate phialides on pale brown, globose vesicles and phialides acutely tapered at apex. Conidia phialosporous, brown, black in mass, globose, minutely echinulate. The micrograph of *Aspergillus* sp. (7) was shown in figure- (16).

Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp. (7) (X400)

Pure fungal colony (black color), 11 days old

Reverse view

Figure 16 - *Aspergillus* sp. (7) isolated from grape.

Characters of mycelium and spore formation of *Aspergillus* sp. (8)

Conidiophores hyaline or pale brown, erect, simple, thick-walled, with foot cells basally, inflated at the apex forming globose vesicles, bearing conidial heads split into over 4 loose conidial columns with over 4 fragments apically, composed of catenulate conidia (over 15 conidia/chain) borne on uniseriate or biseriate phialides on pale brown, globose vesicles and phialides acutely tapered at apex. Conidia phialosporous, brown, black in mass, globose, minutely echinulate. The micrograph of *Aspergillus* sp. (8) was shown in figure- (17).

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Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp. (8) X400

Pure fungal colony (black color), 11 days old

Reverse view

Figure 17 - *Aspergillus* sp. (8) isolated from grape

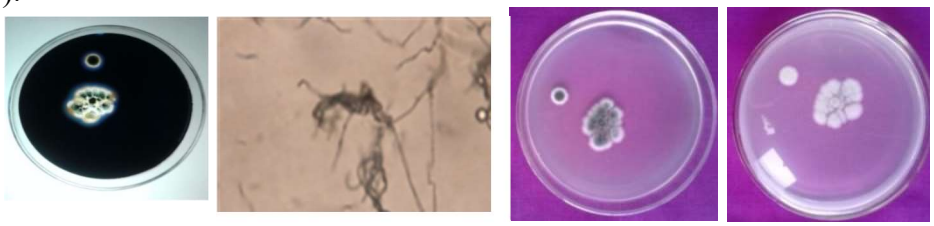
Formation of fungal colonies on pumpkin

One amylolytic fungi strain was isolated from pumpkin, it was *Penicillium sp.*.

Figure 18 - Fungal colony grown on pumpkin

Characters of mycelium and spore formation of *Penicillium* sp.

Conidiophores hyaline, branched penicillately at the apexes with verticillate metula, terminal phialides and catenulate conidia on each phialide, forming rather divergent conidial heads: Conidia phialosporous, subglobose, 1-celled, and smooth. The micrograph of *Penicillium* sp. was shown in figure-(19).



Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Penicillium* sp. (X400)

Pure fungal colony (green inside and white periphery), 7 days old

Reverse view

Figure 19 - *Penicillium* sp. isolated from pumpkin

Isolation of fungi from soil

There are six different kinds of fungi were isolated from soil serial dilution (10^{-2} to 10^{-7}). Some amylolytic fungi can be seen in every dilution plates and pure four different fungi were isolated from soil dilution 10^{-3} and 10^{-6}.

.

Figure 20 - Various fungal colonies grown on starch agar medium from soil dilution 10^{-3}

Character of mycelium and spore formation of *Aspergillus* sp. (9)

Conidiophores upright, simple, terminating in a globose swelling, bearing phialides radiating from the apex or the entire surface; conidia (phialospores) 1-celled, globose, often variously colored in mass, in dry basipetal chains. The micrograph of *Aspergillus* sp. (9) was shown in figure- (21).

Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp. (9) (X400)

Pure fungal colony from soil dilution 10^{-3} (dark green) 5 days old

Reverse view

Figure 21 - *Aspergillus* sp. (9) isolated from soil dilution 10^{-3}

Character of mycelium and spore formation of *Aspergillus* sp. (10)

Conidiophores hyaline, simple, occasionally thick-walled, inflated globosely or ellipsoidally at the apex (called vesicles), bearing spore heads composed of catenulate conidia borne on uniseriate phialides on vesicles: conidial heads dark green, loosely columnar. Conidia phialosporous, pale brown to yellowish brown, globose, delicately rough at the surface. The micrograph of *Aspergillus* sp. (10) was shown in figure- (22).

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Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp. (10) (X400)

Pure fungal colony from soil dilution 10^{-3} (Yellowish brown), 5 days old

Reverse view

Figure 22 - *Aspergillus* sp. (10) isolated from soil dilution 10^{-3}

Character of mycelium and spore formation of *Aspergillus* sp. (11)

Conidiophores upright, simple, terminating in a globose swelling, bearing phialides radiating from the apex or the entire surface; conidia (phialospores) 1-celled, globose, often variously colored in mass, in dry basipetal chains. The micrograph of *Aspergillus* sp. (8) was shown in figure-(23).

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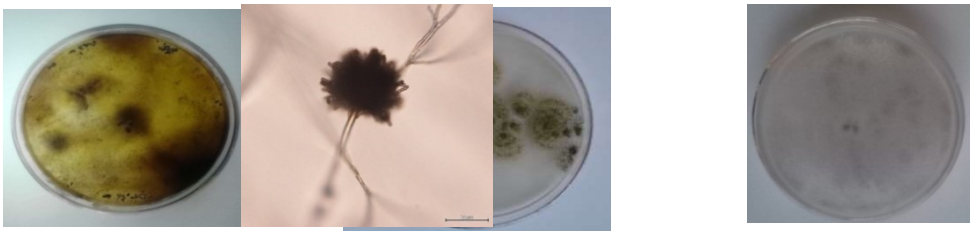

Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp (11) (X400)

Pure fungal colony (black), 5 days old

Reverse view

Figure 23 - *Aspergillus* sp. (11) isolated from soil dilution 10^{-3}.

Characters of mycelium and spore formation of *Paecilomyces* sp.

Conidiophores (phialides) simple or rarely branched, erect, hyaline, 1-septate basally, tapering from base toward apex, bearing over 10 catenulate conidia apically. Conidia phialosporous, hyaline, ovate, 1-celled, slightly

apiculate at one end. The micrograph of *Paecilomyces* sp. was shown in figure- (24).

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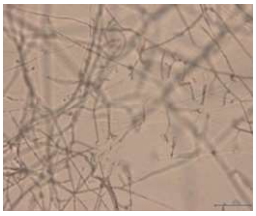
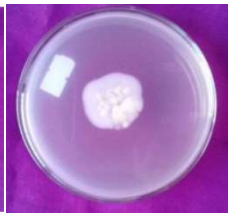
Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution



Micrograph of *Paecilomyces* sp. (X400)

Pure fungal colony (white), 7 days old



Reverse view

Figure 24 - *Paecilomyces* sp. isolated from soil dilution 10^{-6}.

Discussion and Conclusion

In this study, amylolytic fungi were screened from different sources. Fungi which possess amylolytic activity were selected based upon the clear zone around the fungal colony. Twelve different amylolytic fungi were directly collected from bread, *Daucus carota* L. (carrot), *Musa* sp. (banana), *Sechium edule* Sw. (chayote), *Vitis vinifera* L. (grape) and *Cucurbita maxima* Duch. (pumpkin) The appearance of clear zone can be seen after the flooding with Gram's iodine.This is the evidence that the fungi showed amylolytic activity due to hydrolysis of starch by α-amylase enzyme.

Four amylolytic strains were obtained from soil sample which was collected from Botanical garden at University of Yangon campus by soil dilution method. According to the results, Gram iodine is the best plate assay method for determining amylolytic activity and gives the best result with prominent and distinct zone within 3-5 minutes. Amylolytic fungi in the plate break down the polysaccharide which surrounded by fungal colony were exhausted with polysaccharides so monosaccharide and disaccharides were remained. Florencio *et al*., (2012) reported that mono and disaccharide cannot bind with dyes efficiently, so clear zone around the colony can be seen. In the present investigation, the total number of amylolytic fungi were (16) strains which were (11) strains of *Aspergillus,* (2) strains of *Amblyosporium*, (1) strain of *Penicillium,* (1) strain of *Botrytis*, and (1) strain of *Paecilomyces*

were isolated from different sources. Among them, the best amylolytic activity of fungi will be used for the future investigation.

Acknowledgements

I wish to express my deep gratitude to Dr. Thidar Oo, Professor and Head of the Botany Department, West Yangon University, for her encouragement and suggestion in the preparation of this paper. I owe much thanks and appreciation to my supervisor, Dr. Bay Dar, Professor, Botany department, Myeik University for her precious advices in this research work.

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FERMENTATION STUDIES OF BIOACTIVE FUNGAL STRAIN MM4 ISOLATED FROM THE LEAVES OF *PSIDIUM GUAJAVA* L.

Hnin Wit Mhon[1], Yee Yee Thu[2]

Abstract

In this study, bioactive strain MM4 isolated from the leaves of *Psidium guajava*L. was utilized to investigate optimal fermentation conditions such as carbon and nitrogen sources, various culture media, age of inoculum, size of inoculum and pH utilization. In carbon sources, sucrose, starch and glycerol were the best whereas yeast, meat, malt and soybean were good nitrogen sources. In antimicrobial activity, sucrose, glucose, starch and mannitol in carbon sources showed high activity against *Candida albicans, Escherichia coli* and *Malassezia furfur* while in nitrogen sources, yeast extract, meat extract, and soybean indicated very high activity against *Candida albicans*and *Escherichia coli.* In the investigation of morphological characters on eleven media, fermentation media 9, 7 and 10 were good to produce antimicrobial metabolites from strain MM4. Antimicrobial activity of eleven media, fermentation medium 9 showed highest activity against test organisms. Two days old age of inoculum showed highest activity against test organisms. According to the results, size of inoculum (1.5%) and pH 4 with two days old age of inoculum were the best for extraction of the bioactive compounds from fermented broth.

Keywords*: Bioactive strain,optimal fermentation, bioactive compounds*

Introduction

Endophytes are microorganisms that include bacteria and fungi living within plant tissues without causing any immediate negative effects (Kumar and Sagar, 2007). Fungi are considered as a good natural source for a production of bioactive secondary metabolites that contain different bioactive agents including antibiotics, antitumors, and antioxidants (Elaasser *et al*., 2011). Microbial fermentation is the basis for the production of a wide range of pharmaceutical products, targeting practically any medical indication. Optimization of the fermentation conditions of the endophytic fungus may

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lead to the development of an economically process for the production of bioactive compounds (Puri *et al*., 2005). The large numbers of known bioactive compounds of microbial origin are currently produced by fermentation (Parkinsan, 1994). Parton and Willis (1989) studied strain preservation, inoculum preparation and development for fermentation of active strains. In this research work, optimal fermentation conditions and various media of endophytic fungal strain MM4 were carried out for extraction of the bioactive compounds.

Materials and Methods

Collection of plant samples

The plant samples were collected from Nyaung-Hna-Pin area, Hmawbi Township and studied their outstanding characters by Backer and Bakhuizen, 1968 and Hooker, 1885.

Fermentation Studies

Utilization of carbon and nitrogen sources of strain (MM 4) (Monaghan *et al*., 1999)

In this research, morphological characters of strain MM 4 were studied by using various carbon and nitrogen sources. Carbon sources are sucrose, glucose, starch, mannitol and glycerol whereas nitrogen sources are yeast extract, meat extract, malt extract, oat meal and soy bean. Basal media for finding out suitable carbon sources contained yeast extract 0.3%, K_2HPO_4 0.01%, $MgSO_4$ 0.01%, $CaCO_3$ 0.01% and for nitrogen sources the basal medium consisted glycerol 1.0%, K_2HPO_4 0.01%, $MgSO_4$ 0.01%, $CaCO_3$ 0.01%.

Antimicrobial activity of strain MM 4 by using various carbon and nitrogen sources (Monaghan *et al*., 1999)

Fungal strain MM 4 grown on slant culture was transferred into 50ml flasks containing 25 mL of various carbon and nitrogen sources and incubated for ten days. The fermented broth was used to check antimicrobial activity by paper disc diffusion assay.

Morphological characters of strain MM 4 on eleven different media (Monaghan *et al*., 1999)

In this study, various media were employed for media optimization. A piece from fungal plate culture of strain MM 4 was inoculated on each of various media plates and incubated for 3-10 days.

Antimicrobial activity of strain MM 4 on eleven different media (Monaghan *et al*., 1999)

Fungal strain MM4 grown on slant culture was transferred into 50 mL flasks containing 25 mL of eleven different media. The fermented broth was used to check antimicrobial activity by paper disc diffusion assay.

Eleven different media

Eleven media were Polypeptone, Yeast medium (FM-1)/ Meat, Polypeptone, NaCL medium (FM-2)/Yeast, Malt, Glucose medium (FM-3)/ Glycerol, K_2HPO_4, $MgSO_4$, NaCL medium (FM-4)/ Oat meal medium (FM-5)/ Glycerol, K_2HPO_4 medium (FM-6)/ Soybean, Mannitol medium (FM-7) / K_2HPO_4, $MgSO_4$, NaCL medium(FM-8) Sucrose, Yeast extract medium (FM-9)/ Malt, Meat extract medium (FM-10) and Sucrose, Malt extract, Soluble strach medium (FM-11) as shown in Figure 1.

Figure 1. Eleven different media for media optimization

Age of inoculum of strain MM 4 (Strobel and Sullivan, 1999)

First fermentation (two days old) and second fermentation (three days old) seed cultures were transferred into 50 ml fermentation flasks containing 25 mL of sucrose/yeast extract medium. They were incubated for ten days. Then, these fermented broths were checked for their inhibitory activity by paper disc diffusion assay.

Size of inoculum of strain MM 4 (Monaghan *et al*., 1999)

The proper cultivation and transfer (size of inoculum) are essential for the production of bioactive metabolites. A piece from fungal plate culture of strain MM 4 was inoculated into 300 mL conical flask containing 100 mL of sucrose/yeast extract seed medium. These flasks were incubated at room temperature for 2 days. After two days, the seed cultures (0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0% and 3.5%) were transferred into seven conical flasks (300 mL) containing 100 ml of fermentation medium. The fermentation was carried out for 10 days.

pH utilization of strain MM 4 (Monaghan *et al*., 1999)

For the seed culture, a piece from fungal plate culture of strain MM 4 was inoculated into 300 mL of conical flask containing 100 mL of sucrose/yeast extract medium and then flasks were incubated at room temperature for two days. Seven 300 mL conical flasks containing 100 mL fermentation medium were adjusted at pH 4, 5, 6, 7, 8, 9 10 and autoclaved. After two days, the seed culture (1.5%) was transferred to each fermentation flask with pH 4 to 10 and fermentation was carried out for 3 days. After three days, seven fermentation flasks were checked their antimicrobial activity.

Table1. Test organisms and diseases

Test organisms	Code	Diseases
Bacillus subtilis	JAP- 0225025	It can cause dysentery, but at the first sign of diarrhea
Candida albicans	IFO- 1060	Skin infection, vaginal candidasis, alimentary tract infection urogenital infection.
Escherichia coli	ATCC- 25922	Cholera, diarrhea and vomiting, urinary tract infections
Salmonella typhi	ST-3/JEP-69	Typhoid, strong fever.
Staphylococcus aureus	ATCC- 12877	Skin disease, food poison, wound infection, burns, abscesses, blood stream infection, staphylococcal pneumonia
Malassezia furfur	AUW- 0255	Dandruff

Results

Figure 2. Habit of *Psidium guajava* L.

Scientific Name - *Psidium guajara* L.

English Name - Guava

Myanmar Name - Malaka

Family - Myrtaceae

Outstanding characters

Mostly shrubs to small tree; Leaves opposite and distichous, simple, petiolate, exstipulate;Inflorescence axillary, cymose; Flowers ebracteate, pedicellate, bisexual, actinomorphic, epigynous; Sepals 5, valvate, persistent; Petals 5, imbricate; Stamens numerous, polyandrous, filaments long and filiform, anthers dithecous, dorsifixed, introrse; Pistil 1, pentacrpellary, syncarpous, pentalocules, placentation axile, style long and simple, stigma capitates, ovary inferior; Fruits fleshy berry; Seed exalbuminous, embryo curved as shown in Figure 2.

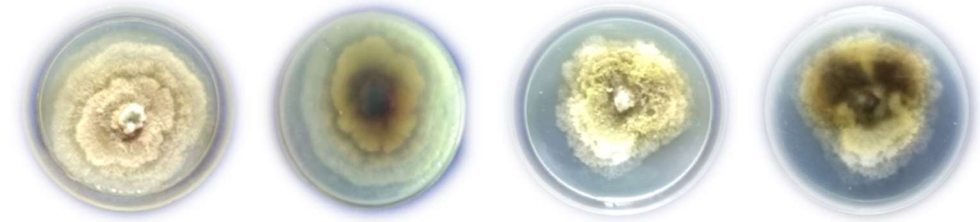
Utilization of carbon and nitrogen sources of strain MM4

Carbon utilization

Among carbon sources, sucrose, starch and glycerol were the best carbon sources whereas glucose and mannitol were also suitable for fermentation as shown in Table 2 and Figures 3 & 4.

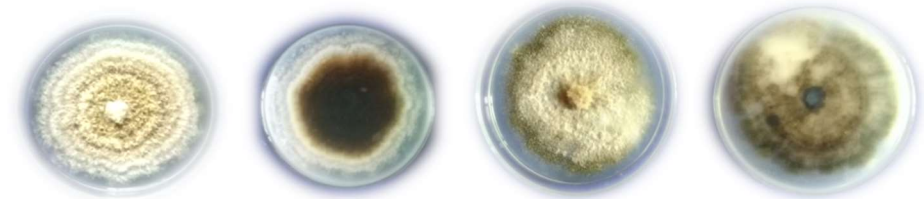
Table 2. Morphological characters of strain MM4 on various carbon sources

No.	Carbon source	Growth	Surface colour	Reverse colour
1	Sucrose	Good	White	Pale yellow
2	Glucose	Moderate	Cream	Light brown
3	Starch	Good	White	Dark brown
4	Mannitol	Moderate	Light gray	White
5	Glycerol	Good	White	Red



Surface view Sucrose Reverse view Surface view Glucose Reverse view

Figure 3. Strain MM4 grown on the plates of various carbon sources



Surface view Starch Reverse view Surface view Mannitol Reverse view

Surface view Starch Reverse view

Figure 4. Strain MM4 grown on the plates of various carbon

Nitrogen utilization

Among nitrogen sources, yeast extract, meat extract, malt extract and soy bean were the best nitrogen sources while oat meal was poor for fermentation as shown in Table 3 and Figures 5 & 6.

Table 3. Morphological characters of strain MM4 on various nitrogen sources

No.	Nitrogen source	Growth	Surface colour	Reverse colour
1	Yeast extract	Good	White	White
2	Meat extract	Good	Cream	Cream
3	Malt extract	Good	Pale yellow	Light yellow
4	Oat meal	Poor	Dark cream	Dark cream
5	Soybean	Good	Cream	Dark

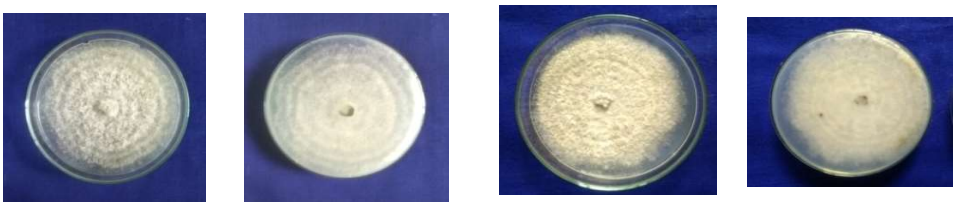


Figure 5. Strain MM4 grown on the plates of various nitrogen sources

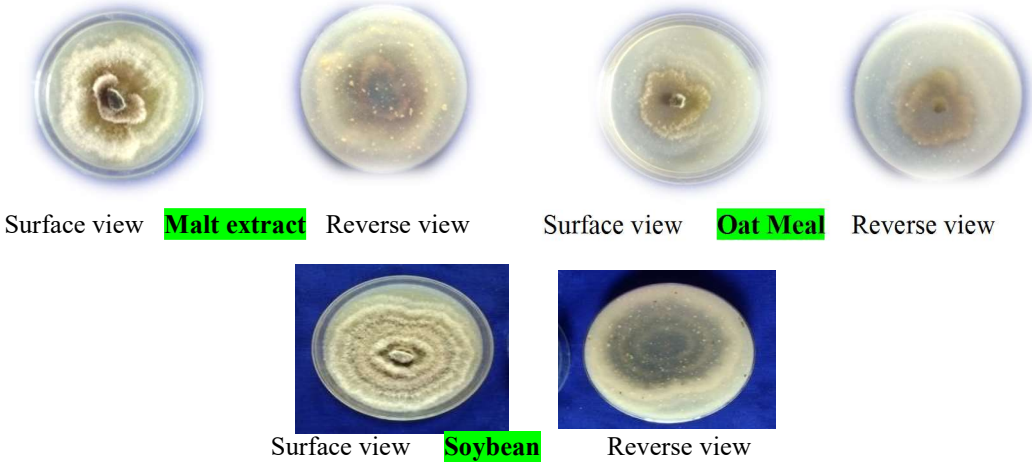


Figure 6. Strain MM4 grown on the plates of various nitrogen sources

Antimicrobial activity of strain MM 4 by using various carbon sources

Antimicrobial activity of strain MM 4 by using various carbon sources. Fermented broth of strain MM4 in sucrose medium showed high activity against *Candida albicans* seventh day (15cm).Strain MM 4 in glucose medium exhibited high activity against *Escherichia coli* at fourth day and *Candida albicans* seventh day (15cm).Strain MM4 in starch indicated high activity against *Escherichia coli* at fourth day(15cm).Strain MM 4 in mannitol showed high activity against *Escherichia coli, Malassezia furfur* at fourth day (15cm) as shown in Figure 7.

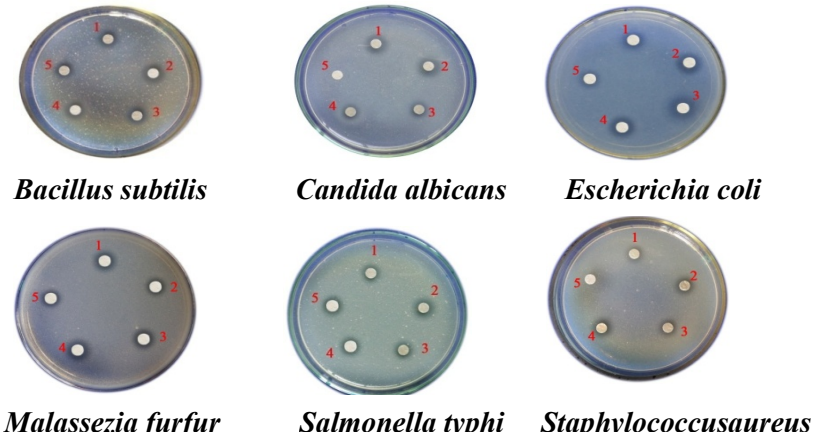
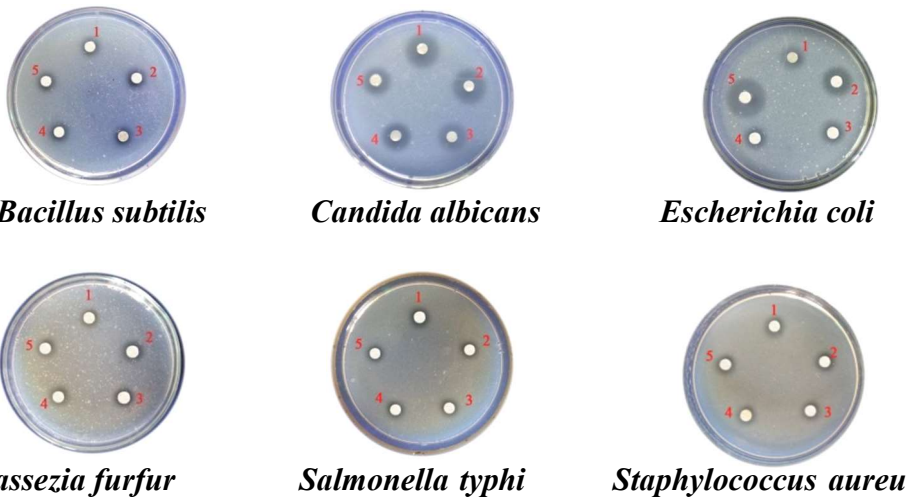


Figure 7. Inhibitory zones of fermented broths on carbon sources

Antimicrobial activity of strain MM 4 by using various nitrogen sources

Fermented broth of strain MM 4 in yeast extract showed very high activity against *Candida albicans* fourth day and *Escherichia coli* at eighth day (20cm). Strain MM 4 in meat extract indicated very high activity against *Candida albicans* at fourth day (20cm). Strain MM 4 in malt extract and oat meal exhibited high activity against *Candida albicans* at fourth day (18cm).Strain MM 4 in soybean indicated very high activity against *Escherichia coli* at fourth day (20cm) as shown in Figure 8.



1.Yeast extract 2. Meat extract 3. Malt extract 4.Oat meal 5. Soybean

Figure 8. Inhibitory zones of fermented broths on nitrogen source

Morphological characters of strain MM 4 on eleven different media

In the investigation of morphological characters of strain MM 4 on eleven media, fermentation medium 9, 7 and 10 media were good whereas fermentation medium 1, 2, 3 and 11 were moderate for fermentation. However, fermentation medium 6, 4, 8 and 5 were not good for fermentation to produce antimicrobial metabolites from strain MM 4 as shown in Table 4and Figures 9-11.

Table 4. Cultural characters of strain MM 4 on eleven different media

Medium	Growth	Surface view	Reverse view
1	Moderate	Light brown	Dark brown
2	Moderate	Brown	Dark brown
3	Moderate	White	Cream
4	Poor	Gray	Gray
5	Poor	Dark gray	Dark gray
6	Poor	White	White
7	Good	Cream	Yellowish red

Medium	Growth	Surface view	Reverse view
8	Poor	Light gray	Light gray
9	Good	Dark cream	Red
10	Good	Cream	Light red
11	Moderate	Light brown	Light brown

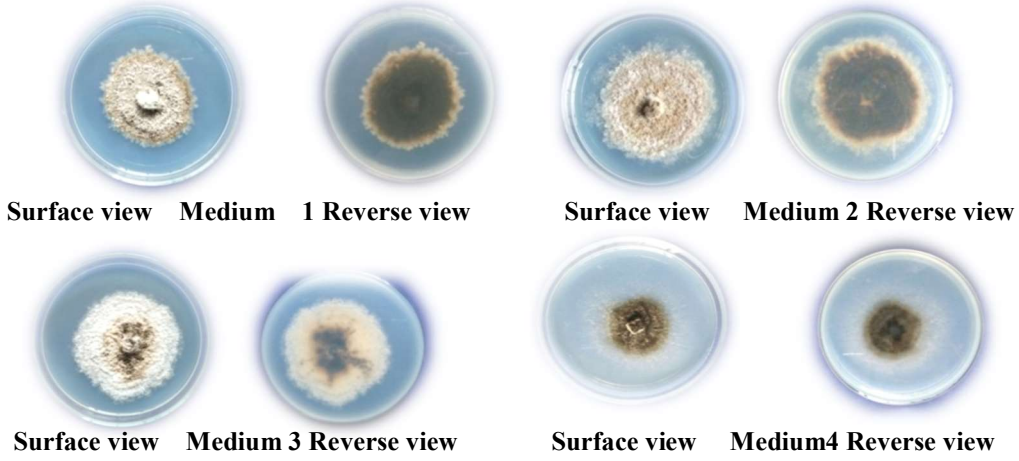


Figure 9. Strain MM 4 grown on the plates of media 1-4

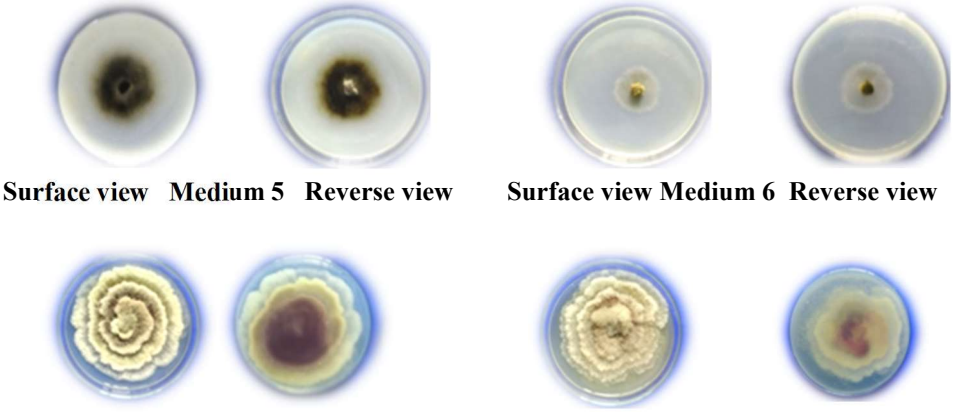


Figure 10. Strain MM 4 grown on the plates of media 5-8

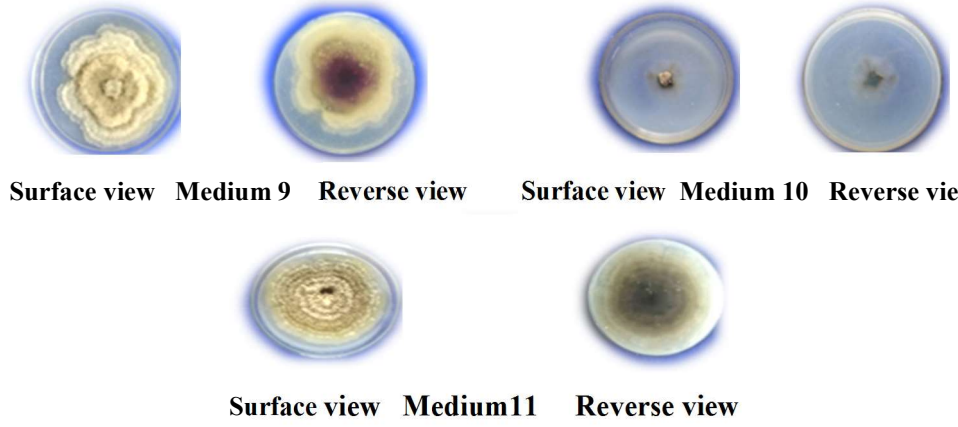


Figure 11. Strain MM4 grown on the plates of media 9-11

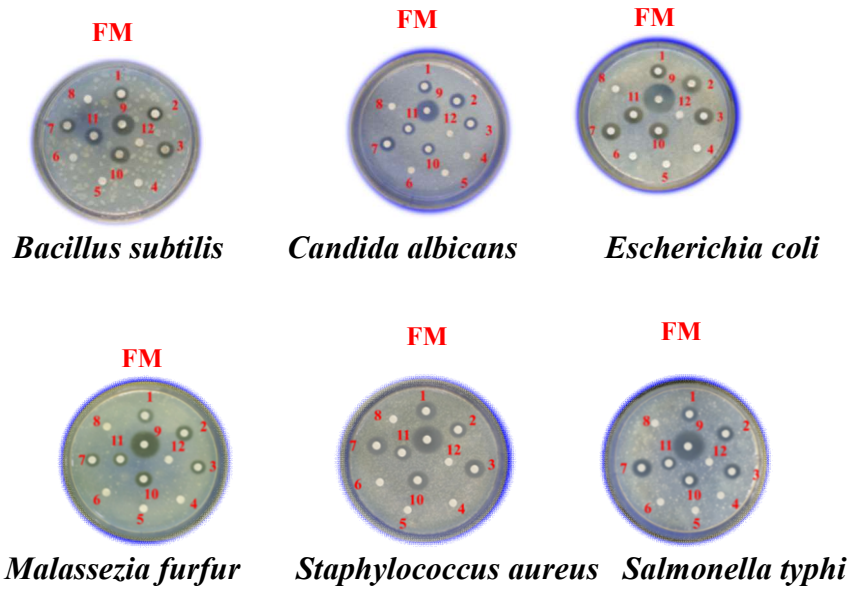
Antimicrobial activity of endophytic fungal strain (MM 4) by using eleven media

In this study, fermented broth of strain MM 4 in sucrose/ yeast (SY) fermentation medium (9) showed very high activity against *Bacillus subtilis, Candida albicans, Escherichia coli, Malassezia furfur, Salmonella typhi*, *Staphylococcus aureus.* Strain MM4 in fermentation medium 4, 5, 6 and 8 media indicated no activity and other media exhibited high activity against six test organisms. Therefore, among eleven media, sucrose/yeast (FM-9) medium was suitable for large fermentation as shown in Table 5 and Figure 12.

Table 5. Inhibitory zones (mm) of strain MM 4 on eleven different media

Media / T.O	FM 1	FM 2	FM 3	FM 4	FM 5	FM 6	FM 7	FM 8	FM 9	FM 10	FM 11
Bacillus subtilis	12	1	15	-	-	-	12	-	18	15	15
Candida albicans	13	15	12	-	-	-	15	-	20	12	12
Escherichia coli	13	15	15	-	-	-	15	-	22	15	15
Malassezia furfur	12	15	12	-	-	-	12	-	25	15	12
Salmonella typhi	15	12	15	-	-	-	15	-	20	15	12
Staphylococcus aureus	12	13	12	-	-	-	15	-	22	12	10

10 -12 mm = weak activity, 13 - 18 mm = high activity, >18 mm = very high activity (disc size = 6 mm), T.O = Test Organisms



FM – 1-11= Eleven fermentation medium 12- Control

Figure 12. Inhibitory zones of fermented broths on eleven different media

Fermentation studies of strain MM4

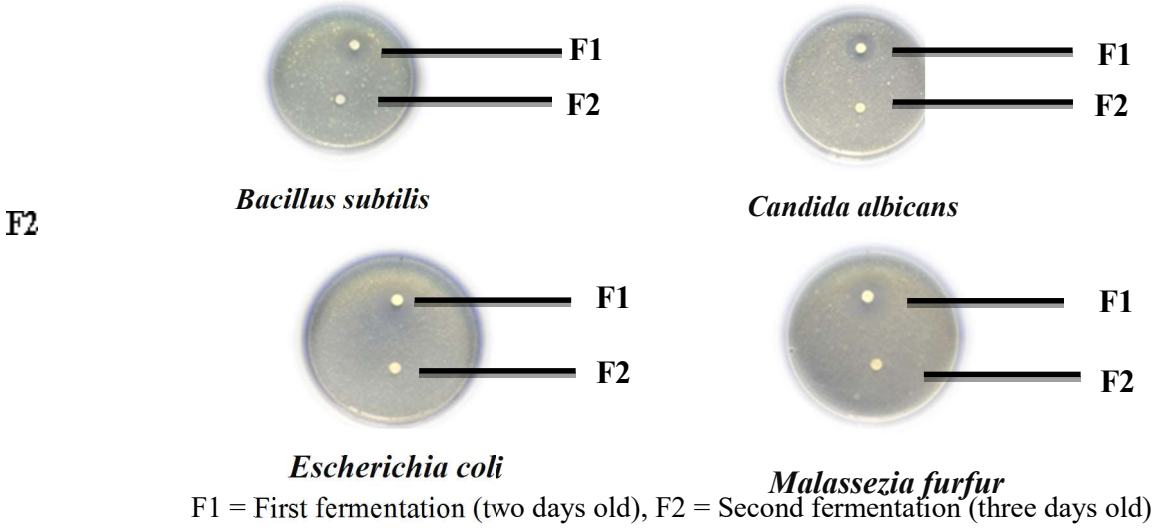
Age of inoculum

In this study, first fermentation (two days old) showed very high activity at seventh day of fermentation on *Baillus subtilis, Candida albicans, Escherichia coli* and *Malassezia furfur* and second fermentation (three days old) indicated weak activity. Therefore, the study of first and second fermentation, first fermentation showed the higher activity than second fermentation on *Bacillus subtilis, Candida albicans, Escherichia coli* and *Malassezia furfur* as shown in Table 6 and Figure 13.

Table.6. Inhibitory zones (mm) of strain MM4 for first fermentation

Days / T.O	1	2	3	4	5	6	7	8	9	10
Bacillus subtilis	-	16	14	22	15	20	**20**	12	18	18
Candida albicans	-	12	10	15	17	15	**18**	15	12	15
Escherichia coli	-	10	18	13	16	13	**20**	15	18	18
Malassezia furfur	-	14	16	15	15	13	**20**	18	13	18

10 -12 mm = weak activity, 13 - 18 mm = high activity, >18 mm = very high activity (disc size = 6 mm), T. O = Test Organisms



F1 = First fermentation (two days old), F2 = Second fermentation (three days old)

Figure 13. Inhibitory zones of age of inoculum

Size of inoculum

In the study of size of inoculum, among the seed culture (0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0% and 3.5%) **1.5** % of seed culture within five-seven days fermentation was suitable for the production of the bioactive compounds. Strain MM4 inhibited bioactivity against six test organisms but they indicated very high antimicrobial activity on *Bacillus subtilis, Salmonella typhi* and *Staphylococcus aureus* at seventh days (25 cm, 30 cm, 30 cm), *Candida albicans, Escherichia coli, Malassezia furfur* at fifth day (28 cm, 30cm, 30cm) as shown in Figure 14.

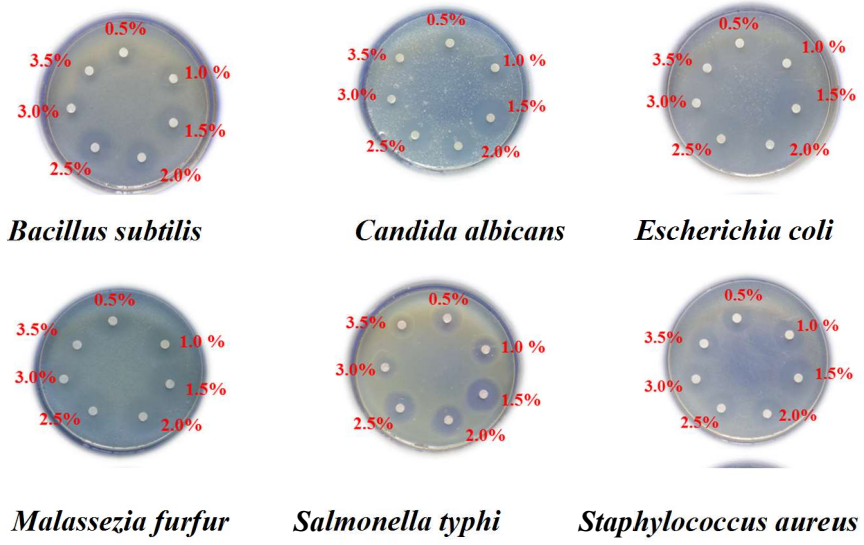


Figure 14. Inhibitory zones of size of inoculums

Effect of different pH on antimicrobial activity of strain MM 4

Among pH 4, 5, 6, 7, 8, 9 and 10 of fermented broths of strain MM4, pH 4 was the best for the extraction of the bioactive compounds from fermented broth.According to their results of inhibitory zones, strain MM4 showed high activity on *Bacillus subtilis, Escherichia coli, Malassezia furfur, Staphylococcus aureus* at pH 4 as shown in Table 7 and Figure 15.

Table 7. Inhibitory zones (mm) of different pH of strain MM 4

T.O \ pH	4	5	6	7	8	9	10
Bacillus subtilis	**15**	12	12	12	12	10	10
Candida albicans	10	10	12	12	12	10	10
Escherichia coli	**18**	15	12	12	15	10	10
Malassezia furfur	**18**	10	10	10	10	12	10
Salmonella typhi	12	15	10	10	10	10	10
Staphylococcus aureus	**18**	15	10	15	15	12	10

10 -12 mm = weak activity, 13 - 18 mm = high activity,>18 mm = very high activity (disc size = 6 mm)

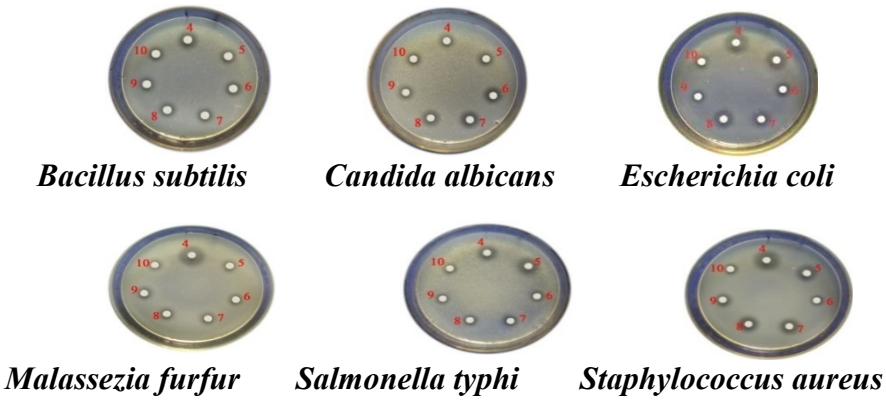


Figure 15. Inhibitory zones of pH utilization

Discussion and Conclusion

In this study, endophytic fungal strainMM4 was isolated from the leaves of *Psidium guajava* L. Fermentation studies of strain MM4 were investigated in order to produce its bioactive secondary metabolites. The large numbers of known bioactive compounds of microbial origin are currently produced by fermentation (Parkinsan, 1994). Among carbon sources, sucrose,

starch and glycerol were the best whereas glucose and mannitol were also suitable for fermentation.

Ritchie *etal.,* (2009) reported that sucrose was the most suitable carbon source for the growth of some isolates of ***Rhizoctoniasolani.*** Yeast extract, meat extract, malt extract and soybean were the best nitrogen sources for fermentation.

KyawtKyawtAung (2014) stated that sucrose and starch in carbon sources and yeast extract, meat extract and soybean in nitrogen sources were the best media for fermentation.The antimicrobial activity of strain MM4 in carbon sources, sucrose, glucose, starch and mannitol showed high activity against *Candida albicans, Escherichia coli* and *Malassezia furfur* whereas in nitrogen sources, yeast extract, meat extract and soybean indicated very high activity against *Candida albicans* and *Escherichia coli.*

The production of antimicrobial metabolites by fungi is also influenced by nutrients mainly carbon and nitrogen sources (EL-Banna , 2006).In morphological characters on various media, 9, 7 and 10 were good whereas media 1, 2, 3 and 11 were moderate for fermentation. In the present study, two days old of age of inoculum was the best for fermentation. It is in agreement with the statement of Yee Yee Thu (2006). In inoculum optimization, among the seed culture (0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0% and 3.5%) 1.5% of seed culture within five-seven days fermentation was suitable according to the results of their antimicrobial activity.

Yee YeeSoe (2014) reported that 1.5% size of inoculum for fermentation of bioactive strain showed highest activity against *Bacillus subtilis* .Optimal fermentation conditions such as proper age and size of inoculum are very important for the production of metabolites (Omura, 1985).

Festus *et al.,* (2015) isolated endophytic fungal strain from *Psidium guajava* L. leaves and reported that rice medium was the best for fermentation. In the screening of optimal pH for fermentation, pH 4 was the best for extration of bioactive compound according to the result of inhibitory zones of against six test organisms. Yee Yee Thu (2006) has reported that endophytic fungus isolated from *Chaetomium* sp. indicated high activity at pH 4.5.

In conclusion, the best fermentation medium for strain MM4 should consists of sucrose, glucose or starch for carbon sources, yeast extract or meat extract for nitrogen sources. The best fermentation conditions were 1.5% of two days old seed culture and pH 4 to produce bioactive metabolites from strain MM4.

Acknowledgements

I wish to thank especially my Head of Professor Dr Aye Pe, Professor and Head,Department of Botany, University of Yangon, for his scholarly advises and encourage during the study and this period.

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MORPHOLOGICAL AND HISTOLOGICAL STUDIES OF *MIRABILIS JALAPA* L. GROWN IN YANGON UNIVERSITY CAMPUS

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

Abstract

Mirabilis jalapa L. belongs to the family Nyctaginaceae. In this paper, identification of plant is carried out by using available literatures. Morphological and histological characters of leaves, stems, rhizomes and roots were also carried out by using microscope. In morphological study, the plant is perennial herbs. Leaves opposite. Inflorescences terminal cymes. Flower bracteolate (5-sepaloid) and hypogynous. Perianth fused. Stamen shortly connate around the ovary. Placentation is basal. In microscopical study, the anticlinal wall of lower surface of lamina is wavier than upper. Anomocytic stomata present only in lower surface of lamina. Vascular bundles of lamina, midrib, and petiole are collateral, medullary bundles also present in middle of midrib and petiole. Raphides are present in mesophyll layer of lamina and also in cortical region of midrib and petiole. In stem, vascular bundles are collateral and medullary bundles are scattered in the ground tissue. In rhizome, vascular bundles are collateral. Rhaphides and starch grains are present. In root, vascular bundle is diarch. Raphides found within the pericycle layer in circular manner. The powered sample has been investigated and presented as diagnostic characters for the standardization of powdered drugs.

Keywords: *Mirabilis jalapa* L., Morphological and Histological Studies

Introduction

Medicinal plants are rich in Myanmar. Among them, *Mirabilis jalapa* L. belonging to the family Nyctaginaceae is included. This family is mainly distributed in subtropical, particularly America; temperate North America to temperate South America, Africa, South Asia, Australia, New Zealand. The Nyctaginaceae consist of 30 genera and 290 species (Goldberg, 1986).

According to the Hutchinson (1967), this family consists of about 33 genera and 560 species, distributed in tropics and subtropics, but mainly tropical and temperate America. The native of this plant is tropical America (Cooke, 1958). It is generally found cultivated in gardens (Duke, 2002).

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The flowers of *Mirabilis jalapa* L. open in the evening, which give rise to one of the common names "Four O'clock" flowers. Another common name, Marvel of Peru, relates to the polychromic flowers, which are white, yellow, or red (Heywood *et al*., 2007). Myanmar name of this species is Mye-su, Lay-nar-yi-pan and common name is Marvel of Peru or Four o'clock flower (Hundley and Chit Ko Ko, 1961 and Kress *et al*., 2003).

The plant is used in the treatment of carminative, diuretic, laxative, fungicide, stomachic, tonic, vermifuge, cancer, diabetes, diarrhoea, dysentery, inflammation, leucorrhea, syphilis, wound, and whitlow (Duke, 2002). It is used in urinary tract infection (Nagathein, 1972). The leaves are applied to boils, phlegmons and whitlow, as a maturant. The root is used as a purgative in La Reunion and the Philippine Islands (Chopra *et al*., 1956 and Kartikar and Basu, 1975).Tuber is used as a poultice on carbuncles. Root is a mild purgative (Nadkarni, 1954).

The aims and objectives are to verify the plant *Mirabilis jalapa* L. based on morphological character and to examine the histological characters of leaves, stems, rhizomes and roots and to identify the diagnostic characters of powdered samples for the standardization of traditional medicine.

Materials and Methods

Collection and Identification of *Mirabilis jalapa* L.

The plants *Mirabilis jalapa* L. were collected from Kamaryut Township, Yangon University campus, from July to October (2014). The morphological study of plant was undertaken with the help of available literatures (Hooker, 1885; Cooke, 1958; Wealth of India, 1962; Backer *et al*., 1963; Lawrence, 1964; Hutchison, 1967; Kirtikar and Basu, 1975; Dassanayake, 1996; Subrahmanyam, 1999 and Qi-ming, 2007).

Histological study of *Mirabilis jalapa* L.

In histological studies, free hand sections of laminas, midribs, petioles, stems, roots and rhizomes from the fresh specimens were prepared by using chloral hydrate solution for clearing reagents, phloroglucinol solution followed by conc: HCl for testing lignin and I_2 B.P solution for starch. These characters were determined according to the literature of Metcalfe and Chalk, (1950), Wallis, (1967), Pandey, (1993) and Pandey and Chadha, (1996).

Preparation of powdered samples of *Mirabilis jalapa* L.

The collected samples (leaves, stems, rhizomes and roots) were washed with water to remove impurities. After washing the samples were cut into small pieces then air dried at room temperature and weighed. When constant weight was obtained, different plant parts were grounded to get powder and stored in air tight containers to prevent moisture changes and contamination. Powders were examined of different plant parts identify to get standardization for traditional medicine.

Results

Morphological characters of *Mirabilis jalapa* L.

Scientific Name - *Mirabilis jalapa* L.
Myanmar Name - Mye-su
Common Name - Marvel of Peru or Four o'clock
Family - Nyctaginaceae

Perennial herbs, about 2ft-4ft high, the stems herbaceous, cylindrical, angular, erect, much branched, often reddish, with tumid nodes, pubescent. Leaves opposite, simple, leaf blade ovate to ovate-triangular, deep green, about 10.9- 6.9 x 5.1- 2.8 cm, the tips acuminate, the margins entire, the margins entire, the base cordate, both the surface glabrous; petioles about 3.5- 1.3 cm long and 0.3- 0.2mm wide, pubescent, exstipullate. Inflorescence terminal, cymose, simple-corymb, about 5.6- 3.5 cm, persistent, ebracteate, bracteolate, pedicellate (very short). Flowers funnel-shaped, yellow, purple, white, or variegated and opening in the evening, about each flower subtended by an involucre, complete, actinomorphic, pentamerous, hypogynous. Perianth tepal (5), syntepalous, funnel shaped, various coloured (yellow, purple, white or variegated), inferior. Stamen 5, usually exserted, shortly connate around the ovary, filament unequal, filiform, anther dithecous, longitudinal dehiscent. Ovary 1-celled, monocarpellary, one ovule, basal placentation, superior, styles filiform, exserted, stigma capitellate with stipitate papillae, a nectariferous disc surround the ovary. Fruit globose, about 0.8- 0.7 cmblack, ribbed, persistent perianth. Seed-testa adherent to the pericarp, about 0.5- 0.4 cm, embryo hooked. The results are shown in Figure.1-8.

Flowering and fruiting time – August to December

Morphological characters of *Mirabilis jalap* L.

Figure.1 Habit

Figure.2 Tuber

Figure.3 Arrangements of leaves and inflorescence

Figure.4 L.S of flower

Figure.5 T.S of ovary

Figure.6 L.S of ovary

Figure.7 Mature fruits

Figure.8 Seeds

Histological characters of *Mirabilis jalapa* L. leaves

Lamina

In surface view, the cuticle is smooth, the epidermal cells both surfaces are parenchymatous and thin wall the anticlinal wall of the lower surfaces are waiver than the upper. Anomocyctic stomata are present only on

the lower surface. The stomata are oval in outline with two-reniform shaped guard cells and contain abundant chloroplasts. Bundles of raphides are present on both surfaces.

In transverse section of lamina, cuticle layer is thin and smooth on both surfaces. Both upper and lower epidermal cells are barrel shaped, thin walled and parenchymatous. Palisade parenchyma found beneath the upper epidermis is only one-layered. These cells are vertically elongated and compact, with abundant chloroplast. The spongy mesophyll cells are 5-6 layers thick, loosely arranged, irregular in shape, with many intercellular spaces. Vascular bundles are embedded in the mesophyll cells. They are collateral type. Each bundle is surrounded by a parenchymatous bundle sheath. The phloem tissue composed of sieve tube elements, companion cells, phloem fibre and phloem parenchyma. The xylem composed of vessels, tracheids, fibres and xylem parenchyma. Bundles of raphides are abundantly present among the mesophyll cells. The results were shown in Figures 9-11.

Midrib

In surface view, the epidermal cells are rectangular in shaped and axillary elongated. Uniseriate, multicellular trichomes with rounded tips are present.

In transverse section, the cuticle layer is thin. Both upper and lower epidermal cells are more or less barrel shaped parenchymatous cells. Below the epidermis, 2-3 layers of lamellar type collenchymatous cells are present towards the upper surface and 1-2 layers embedded in the parenchymatous layers. Inner to the upper and lower collenchymatous layers consists of parenchymatous cells which are rounded to polygonal in shape. Vascular bundles are collateral types, embedded in the parenchymatous layers. The numbers varies from 3-5 and arrange in semicircular shape with 1-2 medullary bundles in the middle. Bundles of raphides are occasionally present between the vascular bundles. Xylem composed of vessels, tracheids, xylem fibres and xylem parenchyma. The phloem tissue composed of sieve tube elements, companion cells, phloem fibre and phloem parenchyma. Uniseriate, multicellular trichomes are present on the upper epidermis. The results were shown in Figure 12.

Petioles

In surface view, the epidermal cells are thin walled, polygonal in shaped and axillary elongated.

In transverse section, the cuticle layer is thin. The epidermal cells of both surfaces are more or less barrel shaped, with thin walled parenchymatous cells. The lamellar type collenchymatous cells present below both epidermises are 2-3 layers and thickened, rounded to polygonal shaped in both surfaces. The parenchymatous cells between two collenchymatous layers are 5-7 layers are thickened and are polygonal to isodiametric in shape. Vascular bundles are collateral types, embedded in the parenchymatous layers. The numbers varies from 3-5 and arrange in semicircular shape with 1-2 medullary bundles in the middle. Bundles of raphides are occasionally present between the vascular bundles. Xylem composed of vessels, tracheids, fibres and xylem parenchyma. The phloem tissue composed of sieve tube elements, companion cells, phloem fibre and phloem parenchyma. Uniseriate, multicellular trichomes are present on the upper epidermis. The results were shown in Figure 13.

Histological characters of *Mirabilis jalapa* L. stems

Stems

In surface view, the epidermal cells are thin walled parenchymatous cells, polygonal in shaped and axillary elongated.

In transverse section, the stem has two groves lying opposite to each other. The cuticles layer is thin. Epidermal cells are barrel shaped, parenchymatous cells. 2-3 layered of lamellar type collenchymatous cells, 1-3 layered chlorenchymatous cells and 1-3 layered of parenchymatous cells with large intercellular spaces. The endodermal layer is composed of barrel-shaped parenchymatous cells. Pericycle layer lies below this layer and are polygonal shaped. Collateral type vascular bundles and medullary vascular bundles are scattered in the ground tissue. Bundles of raphides are present in cortical region. Uniseriate, multicellular trichomes are present. The results were shown in Figure 14.

Histological characters of *Mirabilis jalapa* L. rhizome

Rhizome

In surface view, the cork cells are polygonal in shaped; anticlinal walls are straight.

In transverse section, periderm consists of 35-40 layers, thin walled parenchymatous cells, rectangular to irregular in shaped. Periderm composed of phellem or cork, phellogen or cork cambium and phelloderm or secondary cortex. Cortex 11-13 layers, thin walled parenchymatous cells, irregular in

shaped. As secondary growth has taken place vascular elements are all scattered throughout the cortical region. Xylem bundles composed of vessels, tracheids, xylem fibre and xylem parenchyma. Phloem composed of sieve tube, companion cells, phloem fibres and phoem parenchyma. Bundles of raphides and starch grains are present. The results were shown in Figure 15.

Histological characters of *Mirabilis jalapa* L. roots

Roots (Young root)

In surface view, epidermal cells are thin walled parenchymatous cells.

In transverse section, the young root is circular in outline. The epiblemal layer is made of barrel shaped, one layer, and thin walled parenchymatous cells. The roots hairs are present. Cortex layer composed of 6-7 layers of parenchymatous cells with are polygonal shaped with large intercellular spaces are present. The endodermis is made up of barrel-shaped parenchymatous cells. Pericycle layer lie below this endodermal layers are made up of barrel shaped. Raphides are present within the pericycle layer in a circular manner. Vascular bundle is diarch. Bundles of the xylem are exarch i.e. the metaxylem towards the central and protoxylem towards the periphery. Xylem bundles composed of sieve tube, companion cells, xylem fibre and xylem parenchyma. Phloem composed of sieve tube, companion cells, phloem fibre and phloem parenchyma. The results were shown in Figure 16.

In mature root

In transverse section, the mature root is circular in outline. Periderm consists of 30-32 layers thin walled parenchymatous cells, rectangular to polygonal in shaped. Endodermis layer is not distinct in mature root. As secondary growth has taken place, all tissues outside the stele become irregular and crushed between secondary phloem and pericycle. The diarch vascular bundles changed into anomalous structure. Patches of raphides are left around the stele. The results were shown in Figure 17.

Sensory characters the whole plant powders of *Mirabilis jalapa* L.

The whole plant powdered of *Mirabilis jalapa* L. was pale green coloured and odourless. It was tasteless and fibrous in texture as shown in Figure 18.

Microscopical characters the whole plant powders of *Mirabilis jalapa* L.

It consists of multicellular trichome, fragment of upper surface, stomata, spiral vessel, pitted vessel, annular vessels, fibres, tracheids and raphides as shown in Figures 19-29.

Histological characters of leaves, stems, rhizomes and roots of *Mirabilis jalapa* L.

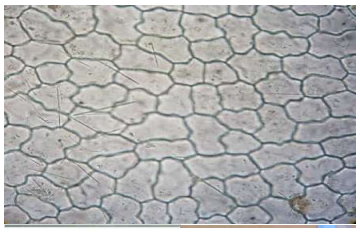

Figure.9 Surface view of upper epidermis (X400)

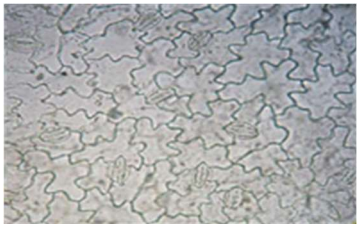

Figure.10 Surface view of lower epidermis (X 400)

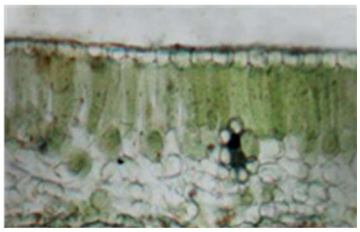

Figure.11 T.S of lamina showing vascular bundle (X 400)

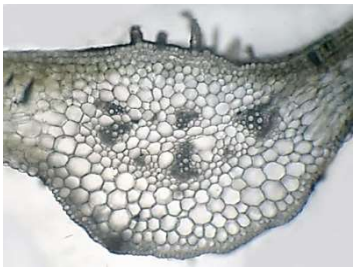

Figure.12 T.S of midrib (X 100)

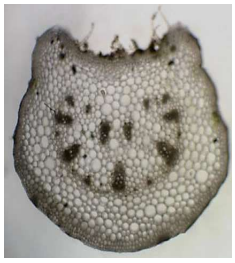

Figure.13 T.S of petiole (X 100)

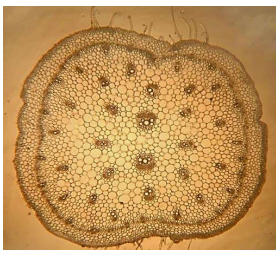

Figure.14 T.S of stem (X 40)

Figure.15 T.S of rhizome showing vascular bundles and raphides (X 100)

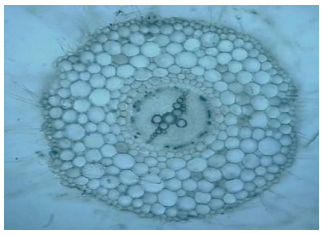

Figure.16 T.S of root in outline (X 100)

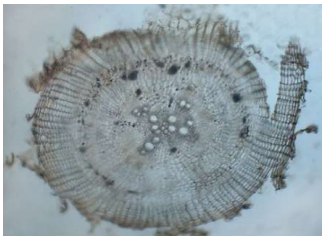

Figure.17 T.S of mature root (X 100)

Figure.18 Powderplant of *Mirabilis jalapa*L.Diagnostic characters the whole plant powders of *Mirabilis jalapa* L.

Sensory characters of the whole plant powders of *Mirabilis jalapa* L.

Figure.19 Multicellular trichome (X 400)

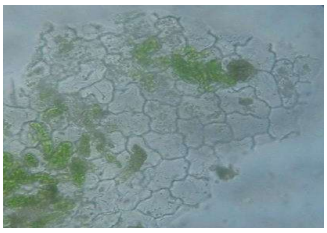

Figure. 20 Fragment of upper epidermal

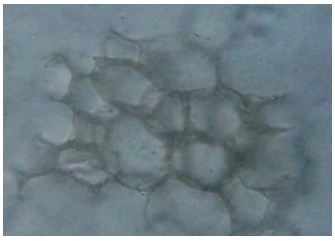

Figure.21 Fragment of parenchymatous cells

Figure.22 Spiral vessels (X 400)

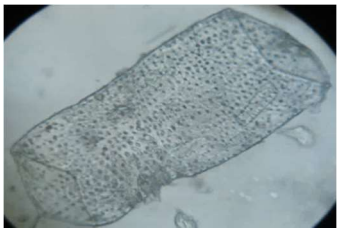

Figure.23 Pitted vessels (X 400)

Figure.24 Stomata (X 400)

Figure.25 Annular vessels (X 400)

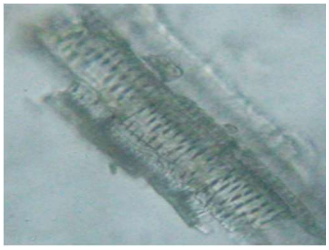

Figure.26 Scalariform vessels

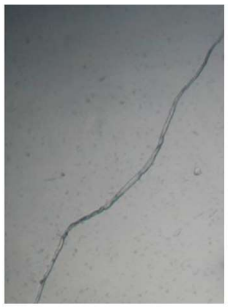

Figure.27 Fibres (X 400)

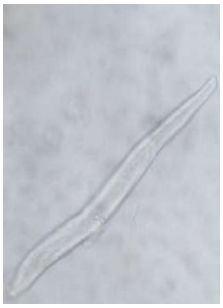

Figure.28 Tracheids (X 100)

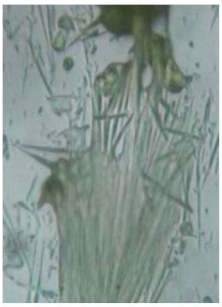

Figure.29 Raphides (X 400)

Discussion and Conclusion

In this research, the identification and microscopical characters of *Mirabilis jalapa* L. were carried out. The plant is perennial herbs. This characters are agreement in those of The wealth of India, (1962), Hutchinson, (1967), Kirtikar and Basu, (1975), Jain, (1991), Subrahmanyam, (1999) and Qi-ming, (2007). Stems is an erect, much branched, swollen at node. These characters are agreement with those described by The wealth of India, (1962), Jain, (1991), Dassanayake, (1999), Subrahmanyam, (1999), Rawat and Bhatt,

(2002) and Qi-ming, (2007). The leaves are opposite. This characters are agreed with those mentioned by Hutchinson, (1967), Subrahmanyam, (1999) and Qi-ming, (2007).

The inflorescences are terminal cymes and perianth various coloured such as white, yellow, red, purple and variegated colour. These characters are agreed with those mentioned by Backer *et al*., (1963), Jain, (1991) and Qi-ming, (2007). Flowers are bisexual. These characters are agreed with those mentioned by Saunders, (1939), Subrahmanyam, (1999) and Qi-ming, (2007). Stamen 5, shortly connate around the ovary. These characters are agreement with those mentioned by Hutchinson, (1967) Subrahmanyam, (1999) and Qi-ming, (2007).

Carpel one, monocarpellary, basal placentation and the ovary superior. These characters are agreed with those of Subrahmanyam, (1999). Fruit globose. This character is agreed with those of Hutchinson, (1967) Dassanayake, (1999), Subrahmanyam, (1999) and Qi-ming, (2007). Seed-testa adherent to the pericarp; embryo hooked. This character is agreed with those of Hutchinson, (1967).

In microscopical study, the leaves of this plant is dorsiventral. Anticlinal walls of lower surface of lamina are more waiver than upper. Anomocytic stomata are present only lower epidermis. Vascular bundle of laminas, midrib and petiole are collateral type and medullary bundles are also present in the middle of midrib and petiole. Raphides present in mesophyll layers of lamina and cortex layers of midrib and petiole.

In stem, cortx layers are found below the epidermal cells and composed of collenchymatous, chlorenchymatous and parenchymatous cells. Only one layer of endodermis is present. Vascular bundles are collateral types and medullary vascular bundles are scattered in the ground tissue. Raphide are present in cortical region.

In rhizome, periderm layers present. Vascular bundles are scattered throughout the cortical region. Bundles of raphide and starch grains are present. In root, cortex layer is below epiblema layer. Only one layer of endodermis and pericycle are present. Vascular bundle is diarch in young root and it is changed into anomalous structure in mature root. Raphides are present within the pericycle layer in a circular manner. These characters are agreed with those of Metcalfe and Chalk, (1950), Pandey, (1993) and Pandey and Chadha, (1996).

Multicellular trichome, fragment of upper surface, stomata, spiral vessel, pitted vessel, annular vessels, fibres, tracheids and raphides were found in the powdered sample.

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STUDY ON MORPHOLOGICAL AND HISTOLOGICAL CHARACTERS OF *FLEMINGIA STROBILIFERA* (L.) R. BR.

Swe Zin Soe[1], Sandar Myint[2]

Abstract

Flemingia strobilifera (L.) R. Br. known as Goung- on- sa in Myanmar was collected. The morphological and histological characters were studied in flowering and fruiting period during 2014-2015. Morphological characters of *Flemingia strobilifera* (L.) R. Br. are shrub, cylindrical stem, unifoliolate leaves, axillary paniculate cymes are enclosed by concave bracts, two-seeded fruits and globoid seeds. In histological study, the leaves possess paracytic stomata on lower surface of lamina, uniseriate and glandular trichomes are present on both surfaces of lamina, midrib, petiole and stem. Vascular bundles are collateral type. Fragments of epidermal cells and stomata, trichomes, fibers, tracheids, pitted vessels, scalariformed vessels and calcium oxalate substances are found in the powdered leaves, stems and roots. Starch grains are abundantly found in roots.

Keywords: *Flemingia strobilifera* (L.) R. Br., Morphological and Histological characters

Introduction

The plant *Flemingia strobilifera* (L.) R. Br. belongs to the family Fabaceae and subfamily Papilionaceae, which is known as Wild hops in English and Gaung-own-sar or Say-laik-pya or Pa-lan-phyu in Myanmar. This genus consists of 40 species in the World, 30 species in tropical Asia and Africa; 15 species in China and India and 14 species in Myanmar (Kress, 2003). *F. strobilifera* (L.) R. Br. is distributed from Bengal to south India, China, Indonesia, Loas, Malaysia, Myanmar, Philippines, Sri Lanka, Thailand, Ceylon and Vietnam (Chopra; 1956).

A decoction of the leaves is administered after childbirth and is used for bathing the body and rheumatism (Burkill, 1935). Leaves are used in the treatment of anthelmintic, tonic, rubefacient, tuberculosis and a postpartum medicine. Dried bracts are used for stuffing pillows and cushions. Roots are used in epilepsy, hysteria, to cure body pain, fever and indigestion.

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The Assamese take a small portion of the root in order to induce sleep (Chopra; 1956). In Nepal, root juice is used for diarrhoea and dysentery. In Trinidad and Tobago, it is used for kidney problem (Ghalot *et al*., 2013). It is also against human leukemia cell line (Yuri, 2005).

In Myanmar, the roots are used to treat in epilepsy and insomnia (Ashin-nargathein, 1972).

Materials and Methods

The specimens of *Flemingia strobilifera* (L.) R. Br. were collected from naturally growing plants in Bago Region during the flowering and fruiting period from July to March in 2014-2015. The collected fresh specimens of both vegetative and reproductive parts of the plant were identified by using Hooker (1879), Kirtikar and Basu (1975), Backer (1963), Key to the families of the flowering plants (1994) Dassanayake (1991) and Ren Sa and Michael (2010). Taxonomic descriptions were accompanied with the photograph of natural habitats, L.S of flower, T.S of ovary and parts of the plant with measurement. Herbarium specimens were also prepared and kept in the herbarium of the Department of Botany, University of Yangon.

Histological characters of leaves, stems and roots from ***Flemingia strobilifera*** (L.) R. Br. were examined by preparing free hand sections, the diagnostic characters of dried powdered of leaves, stems and roots have been examined and studied under microscope.

Results

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

Annual erect shrubs, about 3m high. Stems cylindrical. Leaves alternate, unifoliolate, stipulate, petiolate; stipules (0.4-0.9 x 0.1-0.2) cm; petiole cylindrical (0.9-1.3 x 0.1-0.4) cm; petiolule (0.2-0.3) cm long; the laminae ovate-lanceolate (4.5-8.9 x 1.9-3.2) cm, the upper surfaces rough, green, the lower surfaces smooth, pale-green, the base slightly cordate, the margins entire, the tips acute. Inflorescences axillary paniculate cymes, (6.8 x 10.5) cm. Flowers greenish yellow, papilionaceous, bracteates, bracteolate, pedicellate, complete, bisexual, irregular, zygomorphic, pentamerous, cyclic, hypogynous; bracts enveloped by large foliaceous, (2.3-4.2 x 0.5-2.3)cm, broadly rounded, shortly stalked, a short apical point, persistent: bracteole

minute, about 0.2 cm long, pedicel cylindrical, (0.1- 0.2) cm long, pale green, hairy; sepals (2+2+1), synsepalous, valvate, sepaloid, persistent, inferior; petals 1+2+(2), papilionaceous, the standard about (0.6x0.5) cm, the wing (0.3x0.4)cm, the keel (0.3x0.50) cm, imbricate; stamens 1+(9), diadelphous, stamina tube about (0.6x0.3) cm, pale green, the filaments equal, about (0.1) cm long, the anther dithecous, dorsifixed, longitudinal dehiscence; ovary superior, monocarpellary, unilocular, marginal placentation, two ovules in the locule, the styles terminal, about (0.5) cm long, pale green, the stigmas simple. Fruits two seeded capsule. Seeds globoid, dark-brown marble Fig (1-9).

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

Figure.1 Habit

Figure.2 Ventral view of leaf

Figure.3 Dorsal view of leaf

Figure.4 Inflorescence

Figure.5 Bract open out to show flower

Figure.6 L.S of flower

Figure.7 Fruit with 2-seeds

Figure.8 Fruits

Figure.9 Seeds

Histological Characters of Leaves, Stems and Roots of *Flemingia strobilifera* (L.) R. Br.

Leaves

In surface view, the epidermal cells of both surfaces were thin-walled parenchymatous. The upper epidermal cells were polygonal and more or less rectangular in shape. The both surfaces were wavy. Stomata were not observed on the upper surface and abundant on lower surface only. They were paracytic types, oval in shaped. The guard cells were reniform in shaped and contained many chloroplasts. Uniseriate trichomes with a short basal cell and glandular trichomes were present on both surfaces. The transverse section of the leaves, the cuticles were present on both surfaces. Single layer of epidermal cells were thin-walled, parenchymatous, rectangular to oval in shaped. Epidermal cells of both surfaces were wavy. Palisade cells 1-2 layers thick, elongated, chloroplast present and spongy cells with arm 4-5 layers, irregular, intercellular spaces present, thin-walled parenchymatous cells. Vascular bundles were collateral type Fig (10-12).

Midrib

The lamina has a straight, strongly developed midrib and was convex in both surfaces. In surface view, the epidermal cells of both surfaces were thin-walled parenchymatous and elongated along the length of the midrib. In transverse section, the midrib was covered by smooth cuticle. Both upper and lower epidermal cells were barrel-shaped. Uniseriate and glandular trichomes

were located on both surfaces of the midrib. The bundles were collateral, about 7 -12 in numbers.

The xylem were arranged in rows. Each row consists of 3- large metaxylem vessels elements. The xylem were exarch. There is a large pith at the center of the vascular bundles, composing of large, thin-walled, isodiametric parenchymatous cells without intercellular spaces. There are 3-7 layers of parenchyma cells, above and below the vascular bundles. Under this about 3-5 layers of rounded or oval collenchymatous cells are found Fig (13).

Petiole

In the surface view, the epidermal cells were thin-walled, rectangular to polygonal in shape and elongated along the axis. Stomata was absent. The trichomes present were similar to those found in the lamina and the midrib. They were unicellular trichomes with short basal cells and long tapering end.

In the transverse section of the petiole, 5-angle in outline. The thin cuticle, the epidermal cells one layer and barrel shaped. Collenchymatous cells 2-3 layered. Parenchymatous cells 3-5 layers. Sclerenchymatous bundle sheath 3-6 layered. Vascular bundles were 7-12 in number, phloem 4-7 layered, xylem arranged in 2-8 radial rows, each row had 2-5 cells and accessory bundles present in the ridges. The prismatic crystals were abundantly distributed throughout the cortex Fig (14-17).

Stem

In the surface view, the thin cuticle, the epidermal cells were parenchymatous thin-walled and rectangular to polygonal in shaped and elongated along the length of the stem. Uniseriate and glandular trichomes were present.

In transverse section of the stem, young stem were more or less triangular or irregular in shaped. The epidermal cells were one layer, barrel-shaped, thin-walled. Collenchyma cells were 2-4 layers irregular in shaped and thin-walled. Parenchymatous cells were 2-5 layers. Sclerenchymatous cells were present as a bundle cap. Vascular bundles were collateral type Fig (18-20).

Root

In the surface view of the epidermal cells were thin-walled and rectangular in shape. In transverse section of root, more or less circular in outline. Phellem 2-4 layers, thin-walled, tabular cells. Phellogen 8-12 layers and parenchymatous cells. Phelloderm 8-15 layers, thick-walled and lignified. Vascular bundles were radial type. Medullary rays were bi- multiseriate. Strach grains were present. Pith was absent. Prismatic calcium oxalate crystals were present Fig (21-24).

Histological Examination of Powdered Leaves, Stems and Roots

Powdered leaves, stems and roots include fragments of epidermal cells and stomata, trichomes, fibers, tracheids, pitted vessels, scalariformed vessels and calcium oxalate crystals and starch grains Fig (25-38).

Table (1) Sensory Characters of Powdered Leaves, Stems and Roots

Sample / Sensory characters	Leaves	Stems	**Roots**
Colour	Green	Light green	Light yellow
Odour	A little pungent	A little pungent	Pungent
Taste	Slightly bitter	Slightly bitter	Slightly bitter
Texture	Fibrous	Fibrous	Fibrous

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

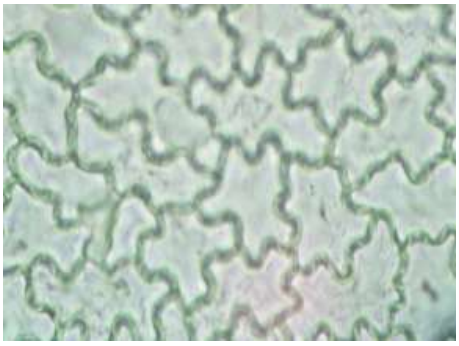

Figure.10 Surface view of upper epidermis (x400)

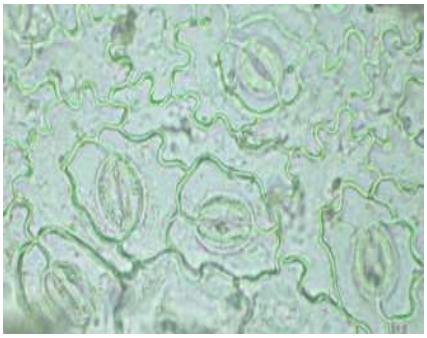

Figure. 11 Surface view of lower epidermis (x400)

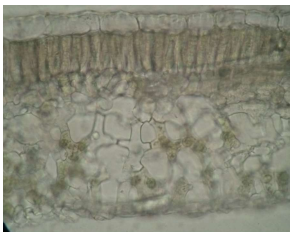

Figure.12 Lamina (x100)

Figure.13 T.S. of midrib (x100)

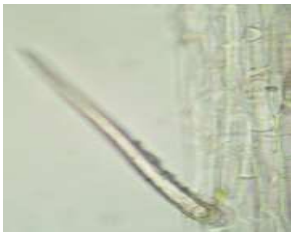

Figure.14 Unicellular trichome of the petiole(x100)

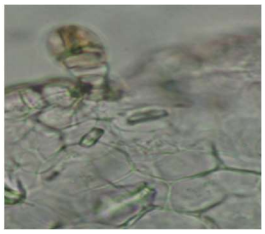

Figure.15 Petiole showing glandular trichome and prismatic crystals (x400)

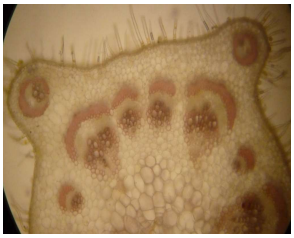

Figure.16 Close up view of Petiole, upper portion (x200)

Figure.17 Close up view of Petiole, lower portion (x200)

Figure.18 T.S of stem(x100)

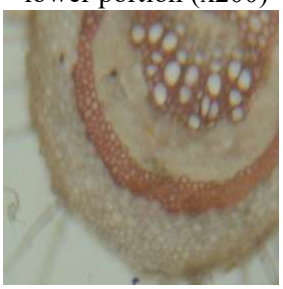

Figure.19 Close up view Vascular bundle (x100)

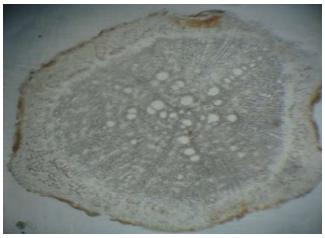

Figure.20 T.S. of root (x100)

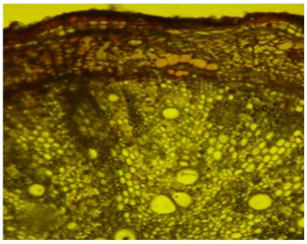

Figure.21 Close up view of root (x400)

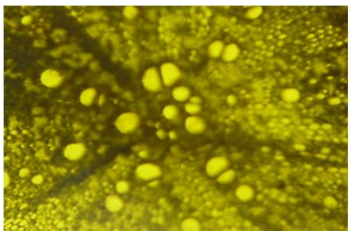

Figure.22 Close up view of Root (central portion) (x400)

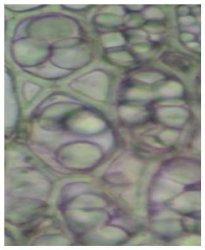

Figure.23 Parenchyma cell with starch grains (x400)

Diagnostic Characters of Powdered Leaves, Stems and Roots

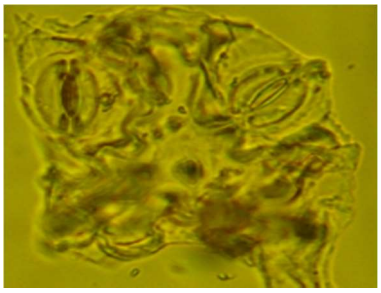

Figure.24 Spongy cells(x400)

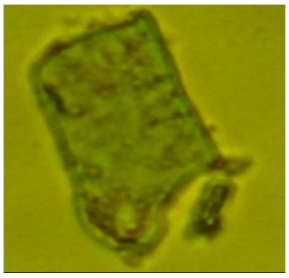

Figure.25 Pitted vessels (x400)

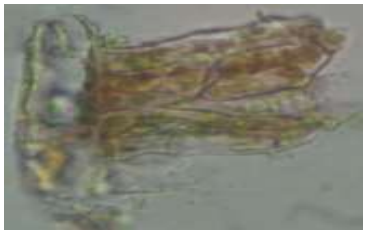

Figure.26 Fragment of upper epidermis and mesophyll (x400)

Figure.27 Spiral vessels(x400)

Figure.28 Parenchymatous cell (x400)

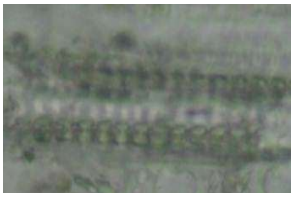

Figure.29 Scalariform vessel(x400)

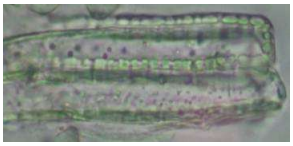

Figure.30 Phloem (x400)

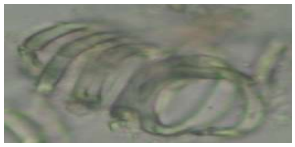

Figure.31 Spiral vessel(x400)

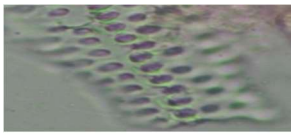

Figure.32 Pitted vessel(x400)

Figure.33 Pitted parenchyma cell (x400)

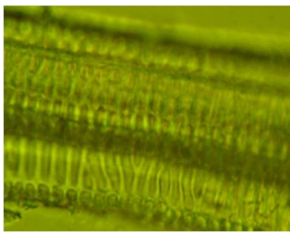

Figure.34 Scalariform vessel (x400)

Figure.35 Spiral vessel (x400)

Figure.36 Fibre (x400)

Figure.37 Unicellular trichome

Discussion and Conclusion

The medicinal plant *Flemingia strobilifera* (L.) R. Br. were collected from Bago region. In the present investigation, the morphological studied on both vegetative and reproductive parts of the plants were carried out. In the histological studied, the fresh and dried powdered of leaves, stems and roots had been undertaken.

In this research, the plant *Flemingia strobilifera* (L.) R. Br. is erect much branched shrub. The leaves are alternate and simple. This characters are agreed in those of Henderson (1949), Backer (1963), Kirtika and Basu (1975), Dassanayake (1991) and Ren Sa and Michael (2010). The inflorescences are 4-40 cymes (Backer, 1963) and branched racemes (Kirtika and Basu, 1975). However, the inflorescences are axillary and terminal, paniculate (Dassanayake, 1991). The bracts are broadly ovate, enclosing the inflorescence and persistent. This characters are agreed in those of Hooker (1879), Henderson (1949), Cooke (1958), Backer (1963) and Chopra (1975).

Pods are oblong, turgid with short beak. Seeds are two, dark-brown marble. This characters are agreed with those mentioned by Cooke (1958), Kirtika and Basu (1975) and Dassanayake (1991).

In histological study, the leaves of this plants are dorsiventral. In surface view, the cell walls of the epidermic are wavy. Paracytic type of stomata are present only on lower epidermis.

Uniseriate and glandular trichomes are found in petioles, lamina, midrib and stems. These characters are agreed with Metchalfe and Chalk, 1950. Prismatic calcium oxalate crystals are found in the surface view of midrib, petiole and stem. These characters are agreed with Betty (1974).

Starch grains are found abundantly in the roots of parenchymatous ray cells. These characters are agreed with Metchalfe and Chalk (1950), Pandey (1993) and Madan *et al*. (2013).

Fibers, pitted, spiral and scalariform vessels, fragments of cork, pieces of stomata and epidermal cells, trichomes, starch grains and calcium oxalate crystals are observed in powdered leaves, stems, roots of *Flemingia strobilifera* (L.) R. Br. (Betty and Snowdon, 1974).

In conclusion, morphological and histological characters of both fresh and dried powdered of leaves, stems and roots and their sensory characters and diagnostic characters would assist the identification and evaluation of powdered drugs of *Flemingia strobilifera* (L.) R. Br..

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MORPHOLOGICAL, HISTOLOGICAL STUDY OF LEAVES AND PHYTOCHEMICAL ANALYSIS OF LATEX OBTAINED FROM *CALOTROPIS GIGANTEA* (L.) R.BR AND *THEVETIA PERUVIANA* (PERS.) SCHUM.

Yin Yin Khaing[1], Myat Myat Moe[2]

Abstract

Two latex producing plants were used in present study. The first plant,*Calotropis gigantea* (L.) R. Br is collected from Dagon University Campus and its local name is 'Mayoe-gyi' (oleander in English) and belongs to the family Asclepidaceae. The second one *Thevetia peruviana* (Pers.) Schum.is taken from the area of North Dagon Township. Myanmar people call it 'Set-hnit-ya-thi' (Madar in English) and belongs to the family Apocynaceae. The collected plants were subjected in the plantidentification using the available literatures at the Botany Department, Dagon University.In morphological study the *Calotropis gigantea* (L.) R. Br. was perennial shrub. Leaves were usually simple and milky latex present. *Thevetia peruviana* (Pers.) Schum.leaves were simple, spirally arranged and also produce milky latex. The histological study, free hand sections of fresh specimen of leaves were studied under the microscope. The upper epidermal cells of *Calotropis gigantea* (L.) R. Br. were polygonal in shaped, the stomata were paracytic type and found on both surfaces. In *Thevetia peruviana* (Pers.) Schum., the upper surface view of cells were wavy in shaped, the stomata were anomocytic type and found on the lower epidermis of the leaf. In transverse section, vascular bundles of the two plants were bicollateral type and crescent shaped. The preliminary phytochemical properties were also examined from the latex of these two plants by using the methods of Central Council for Research in Unani Medicine.The presence of glycoside was mostly found in phytochemcial examination. Latex in nature is a milky fluid found in 10% of all flowering plants. The latex of many species can be processed to produce many materials.

Kew words : latex, paracytic, anamocytic, biocollateral, crescent

Introduction

Medicinal herbs are the local heritage with global importance. Medicinal plants as herbs are the great importance to the health of individual

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and communities. Among these, plant latex has much more attention in the research area because of its dazzling features in plant defense mechanism.

The medicinal properties of *Calotropis gigantea* (L.) R. Br. are known in this country from the earliest timealso known as Mayoe-gyi in Myanmar, belongs to the family Asclepiadaceae. *Calotropis gigantea* (L.)R. Br. is an erect perennial shrub, growing chiefly in waste lands. It ascends to an altitude of 3,000 ft. on the Himalayas, and extends from the Pumjab to south India, *Calotropis gigantea* (L.) R.Br. plant is a shrub or small tree 8-10 ft, milky latex which is present in all parts of the plant (Backer, 1965 and Huber, 1983). The milky juice is also used for tanning and dyeing. *Calotropis* juice is caustic when applied to unbroken skin or mucous membranes. All parts of the plant are considered to have valuable alterative properties when taken in small doses. Madar juice is also given internally or applied locally to procure abortion. In some parts of India, it is also used as a cattle poison (Chopra, 1982).This plant is popularbecause it produces a large quantity of latex. The plant has potential pharmacological properties. The abundance of latex in the green parts of the plantreinforces the idea that it produced and accumulated latex as a defense strategy against organisms such as bacteria, fungi and insects (Kumar *et.al.,* 2012).

Thevetia peruviana (Pers.) Schum. is an evergreen, tropical shrub or small tree in the family Apocynaceae. It is a close relative of *Nerium oleander* L. giving it one common name as yellow oleander and is also called lucky nut in the West Indies.This plant is cultivated as an ornamental plants. The leaves are willow- like, linear- lanceolate, and glossy green in color. They are covered in waxy coating to reduce water loss. The stem is green turning sliver gray as it ages. Flowers bloom summer to fall. The long funnel-shaped sometimes fragrant yellow flowers are in few flowered terminal clusters. *Thevetia peruviana* (Pers.) Schum. contains a milky sap containing acompound called thevetin that is used as a heart stimulant but in its natural form is extremely poisonous, as are all parts of plants, especially the seeds (Backer, 1965 andDutta, 1987).

Thus, the aim of this study is to examine medicinal plants scientific to know its medicinal values. The main objectives are to verify and confirm the morphology characteristics of vegetative and reproductive parts, to ascertain

thephytochemical test of latex from *Calotropis gigantea* R. Br and *Thevetia peruviana* (Pers.) Schum.

Materials and Methods

Botanical Studies

1. Collection and identification

The specimens of*Calotropis gigantea* (L.) R.Br., and *Thevetia peruviana*(Pers.) Schum.were collected from North DagonTownship, Yangon Region, especially during the flowering and fruiting periods from February, 2017 to June, 2017.After the collection, the specimens were identified with the help of available literatures Hooker,1885; Kirtikar and Basu,1935; Dutta,1979; Dassanayake,1983and Huber, 1983. Both the vegetative and reproductive parts of the specimens were used for the morphological studies.

2. Histological study

For histological studies, leaves and stem were examined by preparing free hand sections from the fresh specimens, according to the methods of Esau,1965; Trease and Evans, 1978; Pandey,1981 and Tandon,2011.

The samples were washed and dried at room temperature and then crushed into powder to study the powdered characteristic. The observation of the powdered drugs was made by using the powders of the vegetative plant parts. The powders were cleared in chloral hydrate solution on a glass slide and observed under the compound microscope.

Chloral hydrate solution was used as a clearing reagent. The presence of calcium oxalate crystals and prisms were tested by 80% sulphuric acid. Solution of phloroglucinol with concentrated hydrochloric acid was tested for lignin.

3. Chemical Studies

The preliminary phytochemical studies on the latex of *Calotropis gigantea* (L.) R.Br., and *Thevetia peruviana*(Pers.) Schum.had been undertaken. The experiment was carried out to determine the presence or absence of alkaloid, α -amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, starch, steroids, terpenoids and

tannin, according to the method of Central Council of Research in Unani Medicine,1987.

Preliminary phytochemical test (Extraction)

For preliminary phytochemical investigation, the collected plant parts were washed repeatedly with tap water. Then, latex were taken from the leaves and stored in air tight container for chemical analysis.2.5 mLof latex was extracted with 50 mL of two different solvents like ethanol and distilled water respectively.

Results

Morphological Characters of *Calotropis gigantea* (L.)R.Br., and *Thevetia peruviana* (Pers.) Schum.

Morphological characters of *Calotropis gigantea* (L.)R.Br.

Scientific name : *Calotropis gigantea* (L.)R.Br.
Myanmar name : Mayoe-gyi
English name : Crown flower
Family : Asclepiadaceae
Flowering and fruiting period : Throughout the year

Perennial shrubs, milky latex present (Fig. 1.1). Leaves simple, opposite and decussate, exstipulate, short, petiolate, lamina broadly ovate, the bases cordate, the margins entire, the tips acuminate, the upper and lower surface silvery hairs (Fig. 1.2-1.3). Inflorescence axillary, umbelloid cymes (Fig. 1.4). Flowerpurple in colour, ebracteate, ebracteolate, pedicellate, complete, bisexuals, actinomorphic, 5 merous, cyclic, hypogynous (Fig. 1.5). Sepals 5, synsepalous, imbricate, petaloid (lightgreen). Petals 5, apopetalous, connate at the base, valvate, petaloid (purple), inferior. Stamens 5, adnate or adherent to the gynoecium to produce a gynostegium, anther 2-celled, dithecous, adnate fixation, corona of 5 fleshy organs adnate to a coloum with an upcurved spur and auriculate at the base (Fig. 1.6). Ovary oblong, 2 carpelled, 2 loculed, style slender, stigma apex pentagonal, marginal placentation, superior(Fig. 1.7). Fruit ovoid, oblong follicles, seeds with a tufted micropylar coma of long silky hairs, the embryo large, the endosperm thin and small.

Morphological characters of *Thevetia peruviana* (Pers.) Schum.

Scientific name : *Thevetia peruviana* (Pers.) Schum.

Myanmar name : Set- hna- ya-thi

English name : Yellow oleander

Family : Apocynaceae

Flowering and fruiting period : throughout the year

Small tree, perennial, milky latex present (Fig. 2.1).Leaves simple, whorled, sub-sessile, exstipulate (Fig.2.2-2.3).Inflorescence axillary or terminal, dichasial cyme (Fig. 2.4).Flower bracteate, bracteolate, pedicellate, complete, bisexual, regular, actinomorphic, pentamerous, cyclic, hypogynous (Fig. 2.5). Sepal 5, aposepalous, quancuncial, sepaloid, persistent, inferior; Petal (5), synpetalous, fennel-shaped, twisted, petaloid (yellow),inferior; Anther (5), alternate, petalostomonous, filament very short, anther dithecous, introrse, basifixed, longitudinal dehiscence, inferior (Fig. 2.6). Carpel (2), bicarpelly, syncarpous, four-locular due to present of false septum, two ovules in each locule, axile placentation, style long, stigma bilobed, disc present, superior (Fig. 2.7). Fruit sub-globose.

Figure 1.1 Habit

Figure 1.2 Upper surface view of leaves

Figure 1.3 Lower surface view of leaves

Figure 1.4 Inflorescence

Figure 1.5 Flowers

Figure 1.6 L.S of flower

Figure 1.7 T.S of ovary

Figure 1. Morphological characters of *Calotropis gigantea* (L.) R.Br.

Figure 2.1 Habit

Figure 2.2 Upper surface view of leaves

Figure 2.3 Lower surface view of leaves

Figure 2.4 Inflorescences

Figure 2.5 Flower

Figure 2.6 L.S of flower

Figure 2.7 T.S of ovary

Figure 2. Morphological characters of *Thevetia peruviana* (Pers.) Schum.

Histological Characters of *Calotropis gigantea* (L.)R.Br., and *Thevetia peruviana* (Pers.) Schum.

Microscopical characters of leaves of *Calotropis gigantea* (L.)R.Br.

Lamina

In surface view, the upper and lower epidermal cells were parenchymatous, polygonal in shape, cell compact and anticlinal wall straight. Stomata were present on both surfaces and abundant in lower surfaces. The types of stomata were paracytic. Multicellular trichomes were present on both surfaces (Fig.3.1- 3.2).

In transverse section the lamina was dorsiventral and thick cuticle layer was present on both surfaces. The epidermal cells were one layer on both sides, cells were more or less rectangular in shaped. The mesophyll layer composed of palisade and spongy parenchyma. Palisade parenchyma cells are found on upper side and three-layered, the cells vertically erect, compact, spongy parenchyma cells lie internal the lower epidermis consisted of 5-7 layers of cells, irregular to isodiametric shaped and loosely arranged (Fig. 3.3).

Midrib

In surface view, the epidermal cells were parenchymatous and compactly arranged and irregular. Multicellular trichomes were present (Fig. 4.1).

In transverse section, convex at lower side and concave at upper sides covered with thin cuticle. Both epidermal cells were rounded or oval shaped. Below the epidermis, the cortex was differentiated into outer collenchyma and inner thin-walled parenchyma cells. The collenchymatous cells were 5-6 layers in thickness towards the upper surface and 4-5 layers in thickness towards the lower surface. They were polygonal to isodiametric in shaped. The parenchyma cells were 22-25 layers in thickness above the vascular bundle and 26-28 layers in thickness below the vascular bundle. They were thin-walled and rounded or oval in shaped. Intercellular spaces and crystals of calcium oxalate were present in parenchymatous cells. The vascular bundle was crescent-shaped in outline, bicollateral and closed type (Fig. 4.2- 4.6).

Petiole

In surface view, the epidermal cells were parenchymatous, thin-walled and mostly rectangular in shape and elongated along the length of the petiole(Fig. 5.1).

In transverse section, the petiole was semi circular shape in outline. The cuticle layer was thick. The epidermal cells were barrel-shaped. Muticellular trichomes (unicinate) were present. The cortex was made up of two different types of tissues, outer collenchymatous and inner parenchymatous tissues. The collenchymatous tissues below the epidermis 5-6 layers in thickness, oval shape, the parenchymatous tissues 11-14 layers in thickness, thin walled, rounded or oval in shape. Intercellular spaces and

calcium oxalate crystals (druses) were present. The vascular bundles were crescent shaped in outline and embedded in the parenchymatous tissues. Vascular bundles were bicollateral and closed type (Fig. 5.2- 5.4).

Microscopical characters of Leaves of*Thevetia peruviana* (Pers.) Schum.

Lamina

In surface view, the epidermal cells of both surfaces are parenchymatous cells. The upper epidermal cells are wavy in shaped and the lower epidermal cells are also slightly wavy walls. Stomata are numerous anomocytic types and present on lower surface (Fig. 6.1- 6.2).

In transverse section, the arrangement of the lamina tissue is dorsiventral. Both the upper and lower epidermis are covered with thin layer of cuticle. Both the upper epidermal cells are made up of parenchymatous and rectangular cells.The mesophyll layer consists of palisade and spongy parenchyma cells. The palisade mesophyll are found below the upper epidermis and make up of one layers and vertically elongated at right angle to the surface. They are tightly packed with one another and contained numerous chloroplasts. The spongy mesophyll consists of 10-11 layers of cells which are irregular to more or less rounded cells in shape and loosely arranged. The vascular bundles of lateral vein are embedded in mesophyll cells(Fig. 6.3).

Midrib

In surface view, the epidermal cells were parenchymatous and compactly arranged and polygonal in shaped (Fig. 7.1).

In transverse section, the midrib is convex in the upper surface andconcave in the lower surface in outline. Both surfaces are covered with thin cuticle. The epidermal cells are one layer, barrel shaped and compactly arranged. The lower epidermal cells are similar in shape and size to the upper epidermal cells. The cortex is made up of collenchymatous and thin wall parenchymatous cells. The collenchymas cells are 5-6 layers in thickness toward the upper surface and 6-7 layers in thickness toward the lower surface. The parenchyma cells are 18-19 layers in thickness above the vascular bundle and 20-21 layers in thickness below the vascular bundle. Every portion of the parenchymatous cells are thin walled, irregular rounded in shaped and inner cellular spaces are numerous among them. The vascular bundle is crescent in shaped and bicollateral types (Fig. 7.2- 7.3).

Petiole

In surface view, the epidermal cells were parenchymatous and compactly arranged and rectangular in shaped (Fig. 8.1).

In transverse section, the petiole is crescent shape in outline. The cuticle layer is thin. The epidermal cells are barrel shaped of parenchymatous cells and compactly arranged on both surfaces.The cortex is made up of two different types of tissues and below the epidermis. The collenchymatous tissues are towards the peripheral regions and thin- walled parenchymatous cells are towards the inner regions. The outer collenchymatous cells below the epidermis consists of 2-3 layers in thickness on the upper sides and 3-4 layers in thickness on the lower sides. The parenchymatous cells consists of 26-27 layers in thickness above the vascular bundle and 20-21 layers of parenchymatous layers in thickness below the vascular bundle.Vascular bundle is crescent shaped present in the central region and bicollateral types. Xylem present between the inner and outer phloem of vascular bundles (Fig. 8.2- 8.3).

Diagnostic Characters of powdered leaves of *Calotropis gigantea* (L.)R.Br, and *Thevetia peruviana* (Pers.) Schum.

Diagnostic characters of powdered leaves of*Calotropis gigantea* (L.) R.Br

The epidermal cells were parenchymatous, thin walled and wavy in surface view. Paracytic types of stomata were present. The lignified vessels were found in the form of pitted. Tracheids, fibers and fiber- tracheids were also found. Solitary and prismatic crystals of the calcium oxalates were present(Fig. 9).

Diagnostic characters of powdered leaves of*Thevetia peruviana* (Pers.) Schum.

The epidermal cells were parenchymatous, thin walled and wavy in surface view. The lignified vessels were found in the form of annular and pitted. Tracheids, fibers and fiber- tracheids were also found. Calcium oxalates were present (Fig. 10).

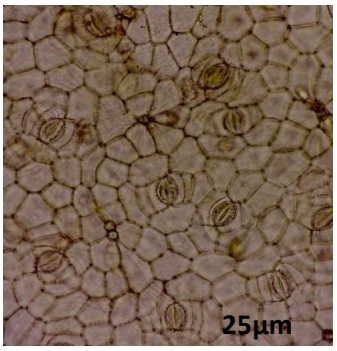

Figure 3.1 Surface view of upper epidermis showing epidermal cells and stomata

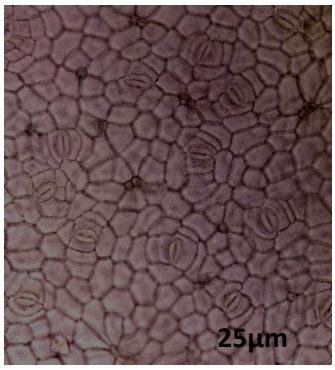

Figure 3.2 Surface view of lower epidermis showing epidermal cells and stomata

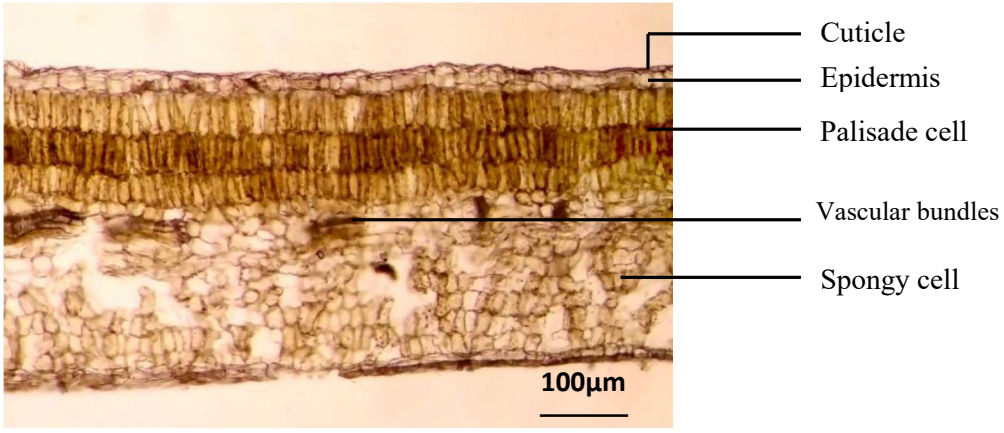

Figure 3.3 T.S of lamina

Figure 3. Microscopical characters of lamina of *Calotropis gigantea* (L.)R.Br.

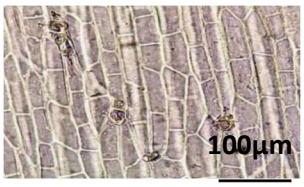

Figure 4.1 Surface view of midrib

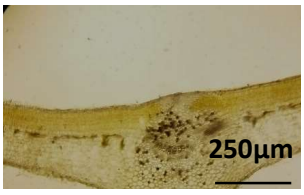

Figure 4.2 T.S of midrib (tip)

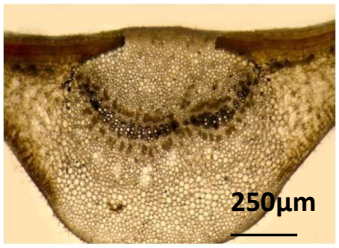

Figure 4.3 T.S of midrib (middle)

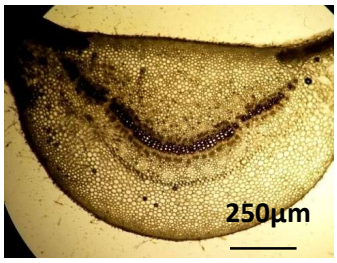

Figure 4.4 T.S of midrib (basal)

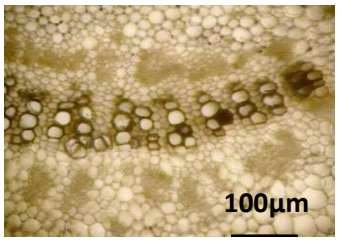

Figure 4.5 Closed up view of vascular bundles

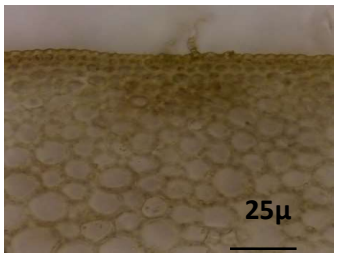

Figure 4.6 Epidermal cells with trichome and cortical region

Figure 4. Microscopical characters of midrib of *Calotropis gigantea* (L.)R.Br.

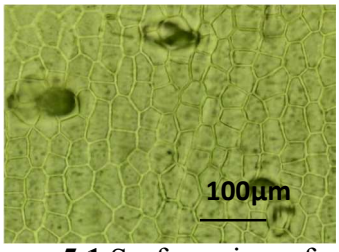

Figure 5.1 Surface view of petiole

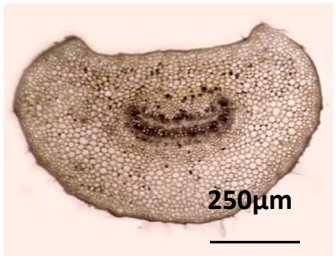

Figure 5.2 T.S of petiole

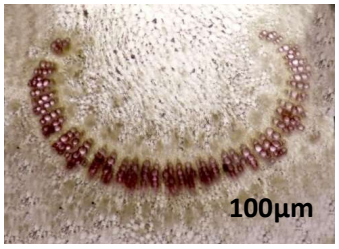

Figure 5.3 Closed up view of vascular bandles

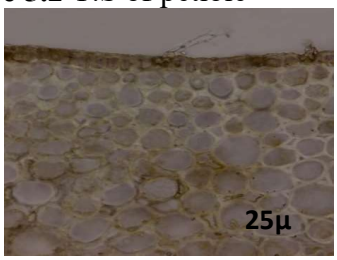

Figure 5.4 Epidermal cells with trichome and cortical region

Figure 5. Microscopical characters of petiole of *Calotropis gigantea* (L.)R.Br.

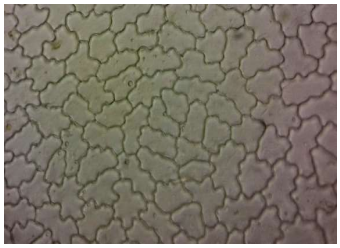

Figure 6.1 Surface view of upper epidermis showing epidermal cells

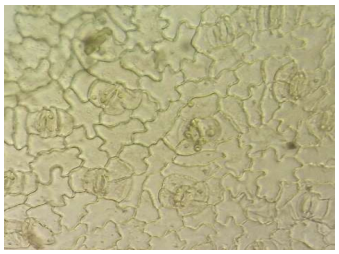

Figure6.2S Surface view of lower showing epidermal cells and stomata

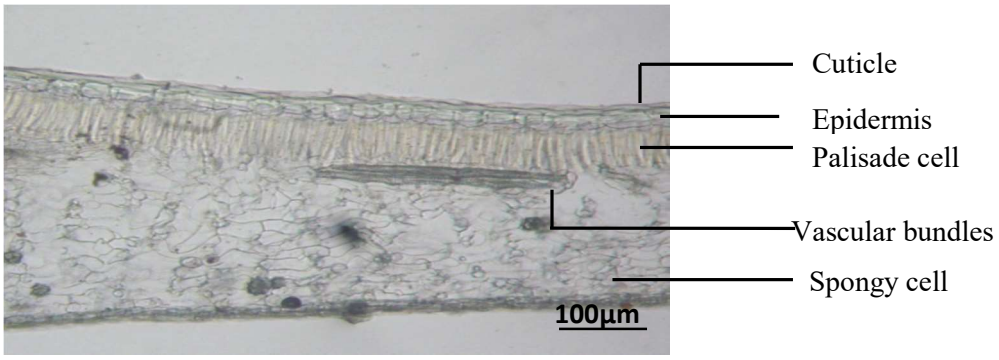

Figure 6.3 T.S of lamina

Figure 6. Microscopical characters of lamina of *Thevetia peruviana* (Pers.) Schum.

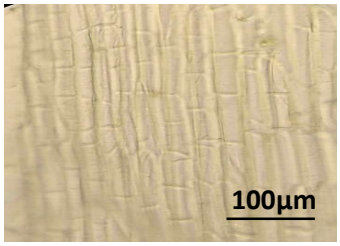

Figure 7.1 Surface view of midrib

Figure 7.2 T.S of midrib (tip)

100µm

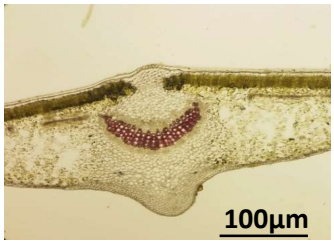

Figure 7.3 T.S of midrib (middle)

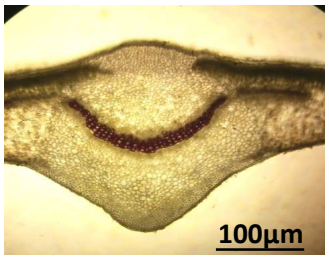

Figure 7.4 T.S of midrib (basal)

Figure 7. Microscopical characters of midrib of *Thevetia peruviana* (Pers.) Schum.

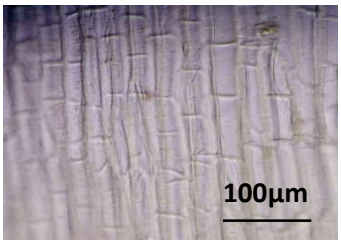

Figure 8.1 Surface view of petiole

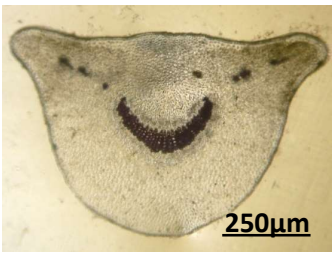

Figure 8.2 T.S of petiole

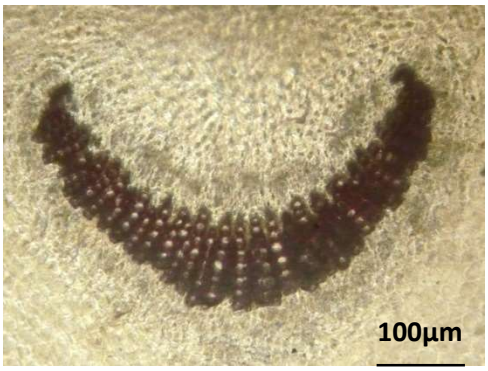

Figure 8.3 Closed up view of vascular bundles

Figure 8. Microscopicl characters of petiole of *Thevetia peruviana* (Pers.) Schum.

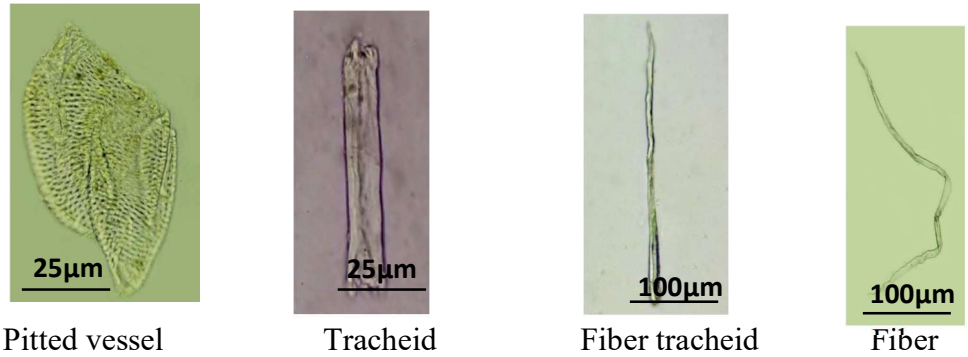

Figure 9. Diagnostic characters of powdered leaves of *Calotropis gigantean*(L.) R.Br.

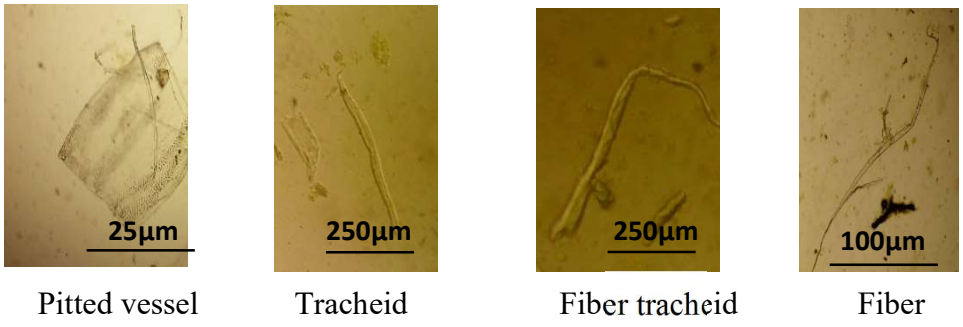

Figure 10. Diagnostic characters of powdered leaves of *Thevetia peruviana* (Pers.) Schum.

Chemical Studies

Preliminary phytochemical test of latex from *Calotropis gigantea* (L.)R.Brand *Thevetia peruviana* (Pers.) Schum.

The preliminary phytochemical test of latex indicated the presence of alkaloid, glycoside, reducing sugars and tannin.

Table (1) Preliminary phytochemical test of water extract of latex from *Calotropis gigantea* (L.) R.Br.and *Thevetia peruviana* (Pers.) Schum.

No.	Chemical constituents	Reagent	Observation	Results	
				Calotropis gigantea (L.) R.Br.	*Thevetia peruviana* (Pers.) Schum.
1.	Alkaloids	Mayer's reagent Dragendroff's reagent	White ppt Orange ppt	+ +	- +
2.	α-amino acid	Ninhydrin reagent	Pale orange ppt	-	-
3.	Carbohydrates	10 %, α-napthol +H_2SO_4(conc:)	Brown ppt	-	-
4.	Phenolic compounds	4 % $FeCl_3$, solution	Green ppt	-	-
5.	Reducing sugars	Benedict's solution	Blue green ppt	+	+
6.	Starch	I_2 solution	Bluish pp	+	-
7.	Steroids/ Terpenoids	H_2SO_4	White ppt	-	-
8.	Flavonoids	Mg/HCl(conc:)	Pale brown ppt	-	-
9.	Glycosides	10 % lead acetate solution	White ppt	+	+
10.	Saponins	Distilled water	Foaming	+	-
11.	Tannins	1% $FeCl_3$ solution	Brown yellow ppt	+	+

(+) present; (-) absent

Discussion and Conclusion

In the present research, taxonomical studies on both vegetative and reproductive parts and the histological studies of *Calotropis gigantea* (L.)R.Br., and*Thevetia peruviana* (Pers.) Schum. had been undertaken.

In the morphological study, the plant of *Calotropis gigantea* (L.) R.Br. was perennial shrubs, milky latex which was present in all parts of plant. The

leaves were simple, opposite and decussate, exstipulate. The inflorescences were cymes. The flowers were peduncled, often many- flowered cymes. The stamens 5, mostly inserted at the base of the corolla, corona-scales 5, inserted in the staminal tube, filament separate or connate in the tube. The ovary 2 carples, style slender. These characters were in agreement with those mentioned by Backer, 1965; Chropa, 1982; Huber, 1983;Kirtika & Basu,1935; Kumar *et al*, 2012. In the histological study, multicellular, uniseriate trichomes were present on both surfaces of the leaves. The stomata were distributed on both surfaces of the leaves and paracytic types. Vascular bundles of midrib and petiole were bicollateral and crescent in shaped. These characters were in agreement with those mentioned by Eusa, 1965 and Metcalfe and Chalk, 1950.

The plant of *Thevetia peruviana* (Pers.) Schum. was erect shrubs or small tree and milky latex present. The leaves are whorl, simple and exstipulate. The inflorescences are cymose and flowers are bisexual. These characters are agreements with those reported by Backer, 1965 and Datta, 1987.The epidermal cells of upper surface are wavy in shaped and lower surface are slightly wavy. The stomata are anomocytic types and occur in the part of lower epidermis. In transverse section, vascular bundles of midrib and petiole are crescent shaped. These characters were agreements with those mentioned by Metcalfe & chalk, 1950 and Eusa, 1965.

In preliminary phytochemical tests of distilled water extracts of the two latex plants indicated that the latex of *Calotropis gigantea* (L.) R. Br. and *Thevetia peruviana* (Pers.) Schum.contained alkaloids, glycosides, reducing sugars and tannins.These characters were agreed with those mentioned bySarkar*et al*., 2013.

In conclusion, the collected samples were verified by using available literature as family Asclepiadaceae and Apocynaceae respectively. The morphological, histological characters and phytochemical studies of latex can give valuable information of *Calotropis gigantea* (L.) R.Br., and of *Thevetia peruviana* (Pers.) Schum. Therefore, some activities of these two plants for physicochemical test and antimicrobial test should be needed to investigate for the future research work.

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MORPHOLOGICAL CHARACTERS, HISTOLOGICAL CHARACTERS AND NUTRITIONAL VALUES OF *POLYGONUM CHINENSE* L.

Aye Thida Aung[1], Tin Maung Ohn[2]

Abstract

Polygonum chinense L. is a medicinal plant which was collected from Yangon area (Botanical garden, Yangon University, Kamayut Township and Yankin Township).The plant verification was carried out. The morphological characteristics such as Inverted "v" shaped deep purple spot present on the surface of the leaf, ochreate stipule, inflorescence of terminal paniculate corymbose cymes, elliptic bract, apetalous flower, eight fertile stamens, tri-fid style, tri-gonous ovary were apparently observed in the species. Microscopic characteristics of the leaves and powdered drug were examined. Microscopic study showed that stomata were anomocytic type which was abundant in lower surface of the leaf, calcium oxalate crystals and tannin occurred here and there in the leaf. Nutritional values of the leaves showed that carbohydrate was the main component followed by vitamin C while fat and vitamin B_1, were also found. The leaf parts are used for boiling leaves to eat with Ngapi and put that leaves in fish curry. This plant can be used for the disease of rheumatism. It also be used for future research investigation of a such kind of disease.

Keywords: Morphological characters, Histological characters, nutritional values of *Polygonum chinense* L.

Introduction

Traditional medicine has been practiced in Myanmar from time immemorial. Native people have used herbal medicine for their care system. Medicinal plants are important for pharmacological research and drug development. *Polygonum chinense* L. belonging to family Polygonaceae. The plant is commonly called Ma-har-gar-kyan-sit, Wetkyein or Bokhtaung in Myanmar (Hundley and Chit Ko Ko, 1961; Nagathein; (1972) and Kress et al, 2003) and Chinese knotweed in English (website 1).

Herbal medicine is a major remedy in traditional medicine system, which is largely based on the use of plant parts, Medicinal plants are

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important for pharmacological research and drug development. Traditional medicine is defined as the therapeutic practices that have been in existence, of ten for hundred of year, before the development and spread of modern are still continued to be an important therapeutic aid for alleviating cilment of humans.

Leaves are used for curing skin diseases and can be eaten as vegetables (Wealth of India, 1959), *P.chinense* L. has tonic, vulnerary and antiscorbutic properties (Kirtikar and Basu, 1975). It is also used for dysentery, gastroenteritis, larynogopharyngitis. Poultice of dried material for furuncle and abscesses, decoction as external wash for dermatitis, eczema, pruritus, poisonous snake bites, chronic gastritis, duodenal ulcers, wound, pain, hemorrhage and irregular menstruation (website 2).

Theoretically all plants may be used in traditional medicine. In practice, however, only the relatively small number of plant heal been recorded in Myanmar. In Myanmar, *Polygonum chinense* L. is not only used in food as salad, but also applied in treatments of skin diseases and inflammatory in traditional medicine.

Aims and objectives are to verify the morphological characters, histological characters of leaves of *Polygonum chinense* L. and to study the nutritional values.

Materials and Methods

Polygonum chinense L. was chosen in the present work and collected from the Botany garden, University of Yangon, Kamayut Township, and also fromYankin Township.

The morphological characters of this plants were identified with the help of available literatures (Backer *et al.* (1963); Hooker (1885) and Kirtikar and Basu (1975). The histological characters of samples were examined by preparing free hand section of fresh leaf. To prepare leaf powder, the leaves were cleaned and dried in shade for 14 days. The dried leaves were grind into fine powder. The powder was observed for sensory characters and cleaned in chloralhydrate solution on glass slide to observe the microscopical characters. The Concentrated: H_2SO_4 was used for the examination of calcium oxalate crystals.

Starch was tested by iodine reagent, tannin by 5% $FeCl_3$ and lignin by Phluroglucinol B.P followed by conc: HCl.Chloralhydrate and Sodium hypochloride solution were used for clearing and bleaching.The nutritional

values of powdered leaves of *Polygonum chinense* L. were measured by Slurometer and Phytoflurometer. Determination of vitamin contents was carried out according to British pharmacopoeia (1965). The experimental work for the nutritional values was carried out at National Nutrition Center, Department of Health, Ministry of Health.

Results

Morphological characters

Habit-perennial herbs, stems herbaceous, cylindrical.Leaves-alternate, simple, unicostate, laminae ovate, purpled coloured patches present in the central portion of leaf blade, petioles glabrous, stipules ochreate, lanceolate towards at the upper portion and sheathing at the base. Inflorescences-terminal paniculate corymbose cymes, the peduncle cylindrical. Flowers-white, complete, actinomorphic,penta-merous, hypogynous; perianth 5-lobed, fused, ovoid, quincuncial calyx tubes glabrous, deciduous. Stamens 8, epiphyllous, filaments long, inserted, anthers dithecous, oblongoid, purple-coloured, dorsifixed, introrse, glabrous; Carpel (3), syncarpous, 1-loculed, one ovule in each locule, basal placentation, style tri-fid, stigma capitate. Fruits-nutlets, tri-lobed and conical shaped.Seeds-Black with thin testa (Fig. 1 to 15).

Figure.1 Habit of *Polygonum chinense* L.

Figure. 2 Leaves of *Polygonum chinense* L.

Figure. 3 Upper Leaf surface of *Polygonum chinense* L.

Figure. 4 Lower Leaf surface of *Polygonum chinense* L.

Figure. 5 Inflorescence

Figure.6 Close up view of Inflorescence

Figure. 7 Close up view of flower

Figure. 8 L.S of flower

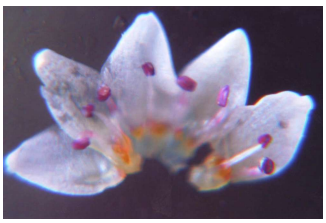

Figure. 9 Close up view of Androecium

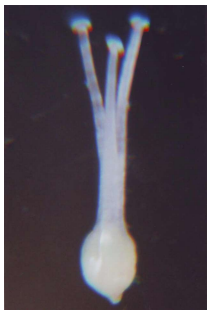

Figure.10 Close up view of Gynoecium

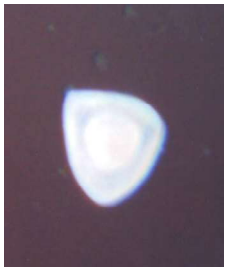

Figure. 11 T.S of ovary

Figure. 12 Close up view of a fruit

Figure. 13 Close up view of a seed

Figure. 14 Fruits

Figure. 15 Seeds

Microscopical characters of leaves of *Polygonum chinense* L.

Lamina

In surface view, cuticle present on upper epidermal cells was striated. Epidermal cells of both surfaces were parenchymatous, thin-walled. Upper epidermal cells were polygonal in shape and lower epidermal cells were irregular. In the upper epidermal cells, were straight and in the lower epidermal cells, they were wavy. Stomata of upper surface are anomocytic type and a few in number, those of lower surfaces are abundant, similar to upper stomata in shape and type (Fig. 16 and 17).

In Transverse section of upper portion of lamina, culticles are thick and slightly wavy on both surfaces. The epidermal cells of the upper surfaces are polygonal in shape and those of the lower surfaces are oval, upper epidermal cells are more larger than the lower ones, palisade cells below the upper epidermis is one layer thick, loosely arranged, intercellular spaces present, vertically erect, elongated in shape with numerous chloroplasts, spongy mesophyll cells are 5-6 layers, the cells are irregularly and loosely arranged (Fig. 18).

In Transverse section of middle portion of lamina, cuticles are slightly thick and slightly wavy on both surfaces. The epidermal cells of the upper surfaces are polygonal in shape and those of the lower surfaces are more or less rectangular in shape, upper epidermal cells are more larger than the lower ones, palisade cells are two layers; upper layers is more longer than the lower, spongy mesophyll cells are 5-6 layers; the cells are irregular (Fig. 19).

In Transverse section of basal portion of lamina, cuticles are slightly thick and slightly wavy on both surfaces. The epidermal cells of both surfaces are irregularly and loosely arranged, thin wall, parenchymatous cells and oval to rounded in shape, palisade cell short, more broad, 2-3 layers, irregular in shape; spongy mesophyll cells are 4-6 layers (Fig. 20).

In all portions of Transverse section of lamina, calcium oxalate crystals (druses) were abundant, vascular bundles are inconspicuous because lateral viens are narrow and few.

Microscopical characters of leaves of *Polygonum chinense* L.

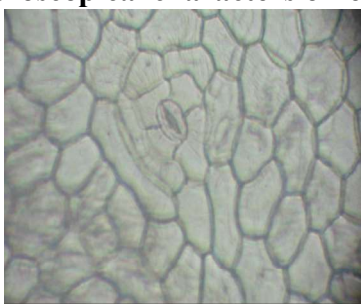

Figure.16 Surface view of upper epidermis (X 400)

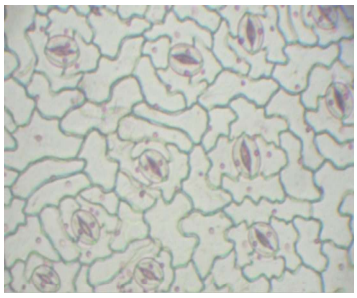

Figure.17 Surface view of lower epidermis (X 400)

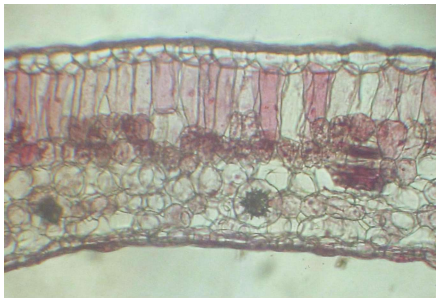

Figure.18 Tip portion of Lamina (X 400)

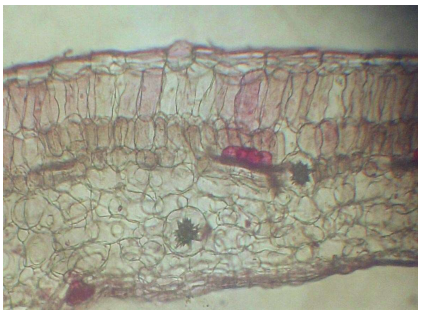

Figure. 19 Middle portion of Lamina (X 400)

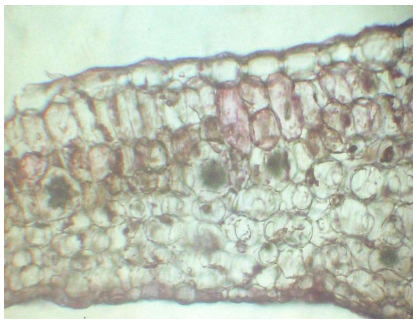

Figure. 20 Basal portion of Lamina (X 400)

Midrib

In surface view, both epidermal cells thin walled parenchymatous, rectangular to polygonal in shape. The cells are elongated along the length of the midrib. Lower epidermal cells are longer than upper ones (Fig. 21 and 22).

In Transverse section, upper and tip portion of midrib is and funnel shaped; middle portion is rectangular and basal portion is shield shape. The cuticle layers of the midrib are thin-walled (Fig. 23, 24 and 25).

Upper epidermal ells are oval to rounded; lower epidermal cell are round. Below the upper epidermis are 2-4 layers of collenchymatous cells., the cells are rounded in shape. Above the lower epidermis is 1-2 layers of thick collenchyma, the cells are also rounded. Vascular bundles are 8 in number at basal portion of midrib, one was larger than the other bundles. Collateral vascular bundles were observed in all portions of mindrib. Phloem lies towards the outer portions and xylem lies towards the inner portion. Phloem consists of sieve tube, companion cell and phloem parenchyma. Xylem consists of vessel, tracheicls and xylem parenchyma. At the upper and middle portion of the midrib, two vascular bundles were fround. At the tip of the midrib, only one vascular bundle is observed.

All portions of midrib, vascular bundles are sheathed by collenchymas instead of sclerenchyma. Calcium oxalate crystals were present in all portions of midrib (Fig.

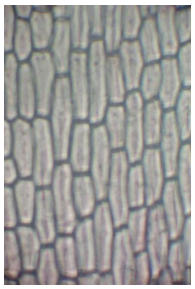

Figure.21 Upper surface view of midrib (X400)

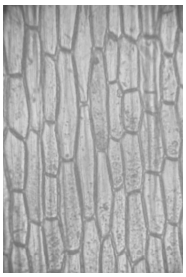

Figure.22 Lower surface view of midrib(X 400)

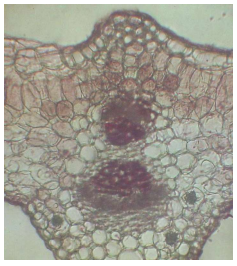

Figure. 23 T.S of midrib (apical) (X 400)

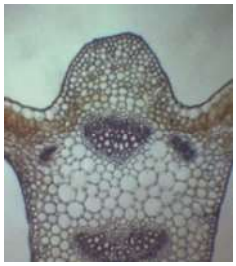

Figure. 24 T-S of midrib (middle) (X 400)

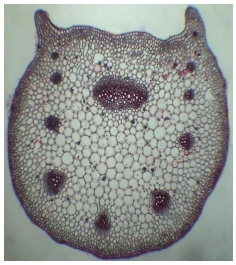

Figure. 25 T-S of midrib (basal) (X 400)

Petiole

In surface view, both epidermal cells are thin walled parenchymatous and rectangular to polygonal in shape (Fig. 26 and 27).

In Transverse section of petiole is semi-circular shape in outline. The epidermal cells of upper portion are thin wall parenchymatous, polygonal in shape. Epidermal cells of lower portion are rounded. Beneath the epidermis are collenchymatous and parenchymatous cells.

Collenchyma cells of upper portion are 3-5 layers; the cells are in shape and collenchyma cells of lower portion is 2-4 layers; the cells are also rounded. At the parenchyma cell beneath the collenchyma extend to the center, the cells are isodiametric in shape. Vascular bundles are about 10 in number, collateral type, are vascular bundle in the upper portion is largest. Phloem lies towards the outer portion and xylem towards the inner portion. Calcium oxalate crystals (druses) are abundant (Fig. 28 and 29).

Figure. 26 Upper surface view of petiole (X 400)

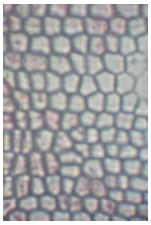

Figure. 27 Lower surface view of petiole (X 400)

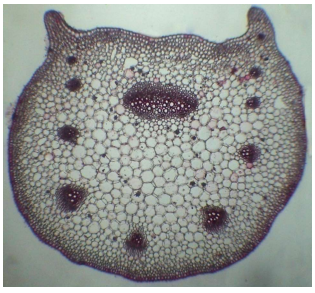

Figure.28 Transverse section of petiole (X 400)

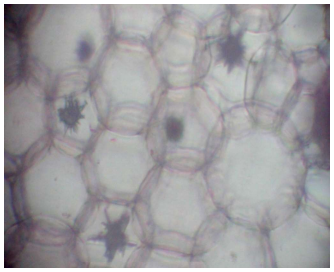

Figure.29 Transverse section of petiole (calciumoxalate crystals) (X 400)

Microscopical characters of powdered leaves of *Polygonum chinense* L.

Lower fragment of upper epidermal cells and lower epidermal cells with anomocytic stomata are found. Stomata are abundant in lower epidermal cells. Calcium oxalate crystals are abundant. Scalariform vessel, pitted vessel,

pitted tracheid, fiber tracheid, fiber, xylem parenchyma were also found (Fig. 30 to 37).

Table 1. Sensory characters of powdered leaves of *Polygonum chinense* L.

No	Characters	*Polygonum chinense* L.
1	Colour	Pale brown
2	Odour	Slightly pungent
3	Taste	Sour
4	Texture	Granular

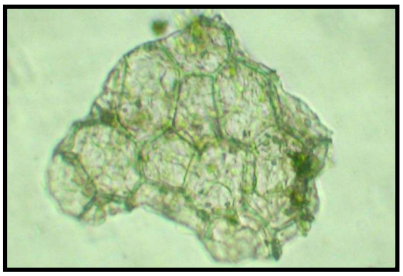

Figure.30 Surface view of upper epidermal cells (X 400)

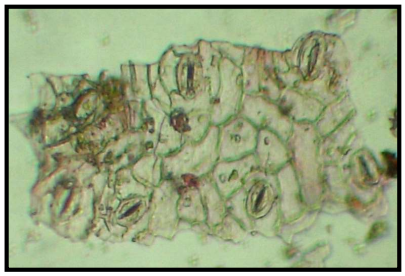

Figure.31 Surface view of lower epidermal cells (X 400)

Figure.32 Scalariform vessel (X 400)

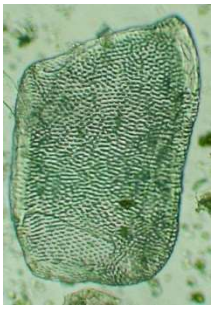

Figure.33 Pitted vessel (X 400)

Figure.34 Pitted tracheid (X 400)

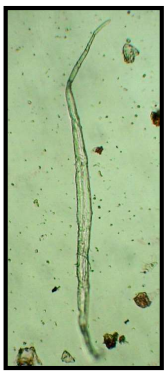

Figure.35 Fiber tracheid (X 400)

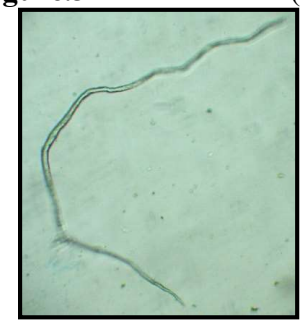

Figure.36 Fiber (X 400)

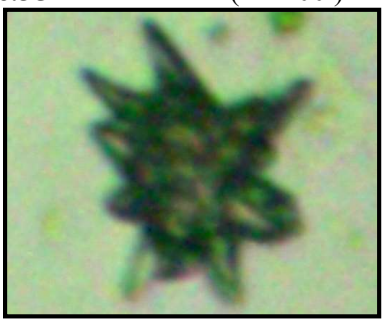

Figure.37 calciumoxalate crystal(X 400)

Nutritional values of leaves of *Polygonum chinense* L.

In the present work, In nutritional values of leaf powdered of *Polygonum chinense* L. carbohydrate, vitamin C, protein, fat, fibre and vitamin B_1 were present.The carbohydrate content is highest amount and vitamin B_1 is the lowest. The results are shown in (Table 2 and Fig 38).

Table 2. Constituents and nutritional values of the leaves of *P. chinense* L.

No.	Constituents	Nutritional values (mg/100g)
1.	Carbohydrates	63.36
2.	Protein	11.16
3.	Fat	1.76
4.	Fibre	1.12
5.	Vitamin B_1	0.46
6.	Vitamin C	28.78

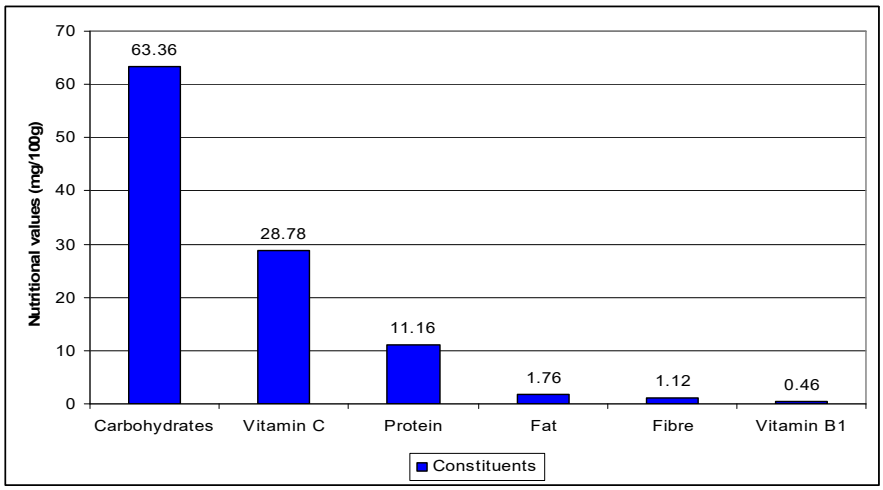

Figure. 38 Nutritional values of leaves of *P. chinense* L.

Discussion and Conclusion

Botanical study plays a vital role in identification of plants. There is a number of literature in which characters of family Polygonaceae, genus *Polygonum* and the species named *Polygonum chinense* L. were described. The morphological characters of *Polygonum chinense* L. in the present study revealed that its habit was perennial herbs, about 1.5 ft in hight. (Burkill, 1935), Wealth of India, (1959).Cylindrical glabrous stems were observed in the present work. Leaf shape observed in the present work was ovate. It is more or less similar to the finding of Bailey (1939) who stated that the leaves were ovate or even broader and (website 2) mentioned that the leaves are ovate to oblong. Dutta (1969) stated that the diagnostic character of family Polygonaceae is having ochreate stipules. A distinctive character of *Polygonum chinense* L. is that an inverted 'v' shaped spot occurs on the upper surface of the leaf (website 2). Purple coloured patches are found to be present at the central portion of leaf blade in the present study.

Inflorescence observed in *Polygonum chinense* L. was terminal paniculate corymbose cymes. This is agreed with Hooker (1885), who stated that inflorescence was head panicle or corymbose. White colour of flower observed in the present work was more or less similar to finding of previous workers; white or light reddish in colour. (Website 2) white or pink (Kirtikar and Basu, 1975). Polygonaceae is an apetalous family. Perianths are petaloid.

The segments or whole flora parts are called calyx in some literature while others mention as perianth. Perianth are fused and 5-lobed, perianth 5-cleft (Bailey, 1939); perianth lobes 5 (Cooke, 1958). In Dutta (1969) stamens are 5-8 and that was the same as Hooker (1885). This character of the species supported the finding of the present work in which 8 stamens are observed. Ovary trigonous, style tri-fid in the present study that agree with those of Kirtikar and Basu (1935), Ridely (1924) . Observation of nutlets as trigonous fruit, is in agreement with that of Hooker (1885); Cooke (1958); Kirtikar and Basu (1935).

In the histological characters, the microscopical characters was in agreement with work of previous authors especially Metcalfe and Chalk (1950). These are 2-4 layers of palisade tissue in leaf, vascular bundles are sheathed by collenchymas instead of sclerenchyma and collateral type of vascular bundles and abundant calcium oxalate crystals and anomocytic stomata.

In the powdered characters, relatively large and abundant calcium oxalate crystals, and wide distribution of tannin, scalariform vessels, pitted vessels, pitted tracheids, fiber tracheids and druses in powder of leaves. It can standardization of the drug that used of the leaf of *Polygonum chinense* L. in traditional medicine formulation.

According to the nutritional value the leaf may support human health care because of having minerals, protein, carbohydrates, fat and vitamins. In conclusion, plant parts and its products are important therapeutic agents.

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Website 1 http://www.ars-grin.gov/egi-bin/npgs/htm/tax search.pl?polygonum%20chinense

Website 2 http://plants.usda.gov.

MORPHOLOGICAL AND ANATOMICAL CHARACTERISTICS OF *VIGNA RADIATA* (L.) WILCZEK. IN MANDALAY REGION

Nilar Soe[1], Win Win Khaing[2] , Thein Kywe[3]

Abstract

Morphological and anatomical characters of leaves, stems and roots of *Vigna radiata* (L.) Wilczek. belonging to family Fabaceae (Subfamily-Papilionoideae) were studied. The specimens were collected from Myingyan Township, Mandalay Region. In morphological characters, the plants were observed annual erect herb with brown hirsute. Leaves were pinnately trifoliolate compound and sparsely pilose. The flowers were bisexual, zygomorphic, pentamerous, hypogynous and papilionaceous. Pods were dehiscent, sparely pale brown hirsute and seeds greenish or yellowish green, glabrous. In anatomical characters, paracyctic type of stomata was observed on both surfaces of laminae. The opposite system of two vascular bundles was observed in the midrib. The vascular bundles of stem were showed continuous circular ring. The vascular bundles of petioles, lamina, midribs and stems were collateral type. The vascular bundles of roots were found tetrarch to polyarch.

Key words: *Vigna radiata* (L.) Wilczek., Morphology, Anatomy

Introduction

The Leguminosae are one of the most economically important families. They provide food, fodder, dyes, gums, resins, oils and ornament (Lawrence 1964 and Zhengyi & Raven 2013).

Vigna radiata (L.) Wilczek. is belonging to Papilionoideae ranks high among the pulse crops in India. The seeds are highly nutritious and good source of protein. Sprouted seeds are eaten and sometimes seedlings are candied (Pandey 2000).

Vigna radiata (L.) Wilczek. (mung bean) is native to Bangladesh, India, and Pakistan. Mung beans are mainly cultivated in India, China,

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Thailand, Philippines, Vietnam, Indonesia, Myanmar, Australia, Iran, Eastern Africa and Bangladesh. Mung beans are commonly used in Chinese, as well as in Myanmar, Thailand, Japan, Korea, Philippines, Pakistan, India, and Southeast Asia. They are generally eaten either whole or as bean sprouts (Anonymous 2013).

In Myanmar, mung bean is grown in Kachin, Kayin, Kayar, Sagaing, Bago, Magwe, Mandalay, Mon, Shan (North), Yangon and Ayeyarwady. In Myanmar, about 90% of total production of mung bean is exported to India, China, Indonesia, Malaysia, UAE, etc. Export volumes are about 340000 tonnes in 2006-2007, over 340000 tonnes in 2007-2008, about 320000 tonnes in 2008-2009, over 320000 tonnes in 2009-2010 and about 170000 tonnes in 2010-2011 (Anonymous 2013).

Anatomical structure is most likely to provide evidence concerning the interrelationships of larger groups such as families, or in helping to establish the real affinities of genera of uncertain taxonomic status. Anatomy sometimes proves very helpful for individual identification. For example, microscopical methods are of great value in establishing the identity of herbarium specimens which are not accompanied by flower or fruits (Metcalfe & Chalk 1979).

Various medicinal uses and planting techniques of mung bean were studied by other researchers. However, the anatomical study of *Vigna radiata* (L.) Wilczek. is scanty. It is for this reasons, it is needed to study morphological and anatomical characteristics of *Vigna radiata* (L.) Wilczek.

The aims and objectives of this research are to study and describe the morphological and anatomical characters of leaves, stems and roots of *Vigna radiata* (L.) Wilczek. and to provide the specific information of morphological and anatomical characteristics for identification.

Materials and Methods

The specimens of *Vigna radiata* (L.) Wilczek. were collected from Myingyan Township, Mandalay Region from June to December 2016. The collected specimens were studied and identified in Department of Botany, University of Mandalay with the help of literatures Backer (1965), Hooker (1883), Dassanayake (1991), Qi-ming & Nian-he (2008) and Zhengyin & Raven (2013).

Results

Morphological Studies

Vigna radiata (L.) Wilczek., Fl. Congo Belge 6: 386. 1954.

Family	-	Fabaceae
Sub- family	-	Papilionoideae
Myanmar Name	-	Pe tesein
English Name	-	Mung bean; green gram
Flowering period	-	June and July

Annual erect herbs, 35.0 – 50.0 cm high; stems and branches brown hirsute. Leaves pinnately trifoliolate compound, alternate; stipules ovate, 0.2 – 1.5 cm by 0.2 – 0.9 cm, dorsifixed, pubescent; petioles 1.6 – 18.5 cm long, pubescent; stipels lanceolate, 0.2 – 0.7 cm long, pubescent; leaflets ovate, entire along the margin, acuminate at the apex, sparsely pilose on both surfaces, cuneate or rounded at the base; terminal leaflets 2.7 – 10.7 cm by 2.1 – 9.6 cm; lateral leaflets 2.0 – 10.5 cm by 1.8 – 9.5 cm. Inflorescenes axillary or terminal racemes; peduncles terete, 0.8 – 2.5 cm long, pubescent. Flowers bisexual, zygomorphic, cyclic, pentamerous, hypogynous, yellow; pedicel about 0.3 cm long, bracts ovate – lanceolate, 0.3 – 0.5 cm long, caducous; bracteoles linear, 0.3 – 0.6 cm long, persistent. Calyx campanulate, 5- lobed; tube 0.4 – 0.6 cm long, pubescent; lobes about 0.2 cm long, two lobes connate into a bifid one, pubescent. Corolla papilionaceous; standard orbicular, 1.0 – 1.3 cm by 1.4 – 1.7 cm, apex emarginated, without appendages, greenish yellow, glabrous; wings ovate, 1.5 – 1.8 cm by 0.4 – 0.7 cm, short clawed, yellow, glabrous; keels falcate, 1.3 – 2.0 cm by 0.4 – 0.9 cm, yellow, glabrous, beak incurved. Stamens 10 (9+1), diadelphous, free from the petals,

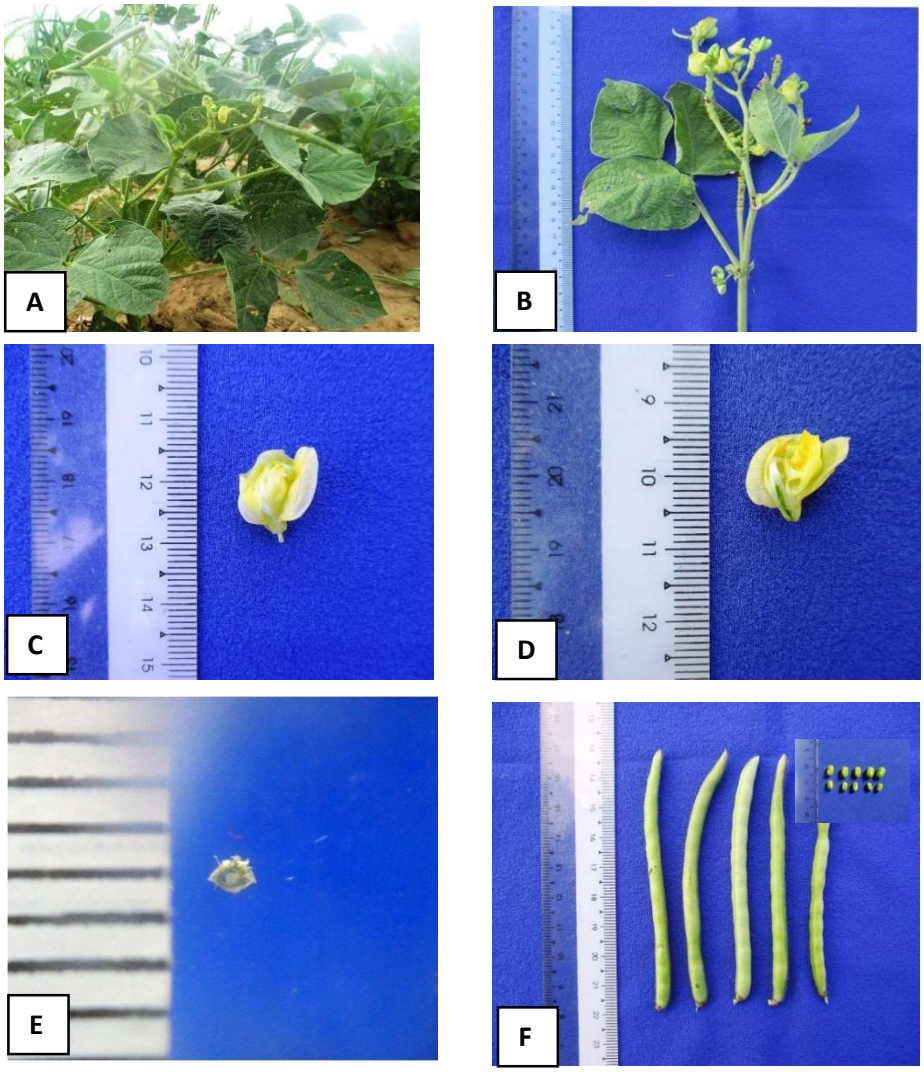

Figure 1 Morphological characters of *Vigna radiata* (L.) Wilczek.

A. Habit

B. Inflorescence

C. Flower

D. L.S of flower

E. T.S of ovary

F. Fruits and Seeds

inserted; free filament filiform, 1.0 – 1.5 cm long, yellow, glabrous; anthers dithecous, dorsifixed, yellow, dehiscing longitudinally. Carpel 1; ovary superior, linear, 0.8 – 1.5 cm long, unilocular, many ovules in the locule on the marginal placentae, pubescent; style flat, 1.0 – 1.5 cm long, twisted, with long hairs below the stigma; stigma globose. Pods straight, turgid, 6.0 – 9.4 cm long, 0.3 – 0.5 in width, 8- to 13- seeded, dehiscent, sparely pale brown hirsute. Seeds oblong – rounded, 0.3 – 1.0 cm by 0.2 – 0.6 cm, greenish or yellowish green, glabrous (Figure 1).

Anatomical Studies

Internal structure of petiole (Figure 2 A)

In transverse section, the petiole of *Vigna radiata* (L.) Wilczek. studied was oval shape in outline with prominent wing at the adaxial side, 1612.0 – 2062.5 µm in length, 1437.5 – 2000.0 µm in width. Distinguishable into dermal, ground and vascular tissue systems (Figure 2 A).

Dermal Tissue System: Composed of epidermal cells. In transverse section, epidermis 1 - layered on both surfaces, cell barrel in shape, compact, 11.25 – 25.00 µm in length 10.0 – 27.5 µm in width, outer and inner wall convex, anticlinal walls straight.

Ground Tissue System: Composed of collenchymatous and parenchymatous tissues. Collenchymatous cells 2 to 5 - layered, the layers 35.0 – 100.0 µm thick, cells polygonal in shape, 10.0 – 32.5 µm in length, 10.0 – 33.75 µm in width; parenchymatous cells below the collenchymatous cells, 3 to 6 - layered, the layers 56.25 – 112.5 µm thick, cells oval or rounded in shape, 11.25 – 23.75 µm in length, 15.0 – 26.25 µm in width, intercellular spaces present.

Vascular Tissue System: Vascular bundles embedded in the ground tissue, bundles arranged in a ring, consists of 5 large bundles alternate with 4 small bundles, collateral type, accompanied by 2 accessory bundles present in adaxial 2 lateral prominent wings, each bundle oval in shape, 125.0 – 312.5 µm in length, 125.0 – 687.5 µm in width; phloem lying outside and xylem lying inside; phloem composed of 4 to 8 -layered, the

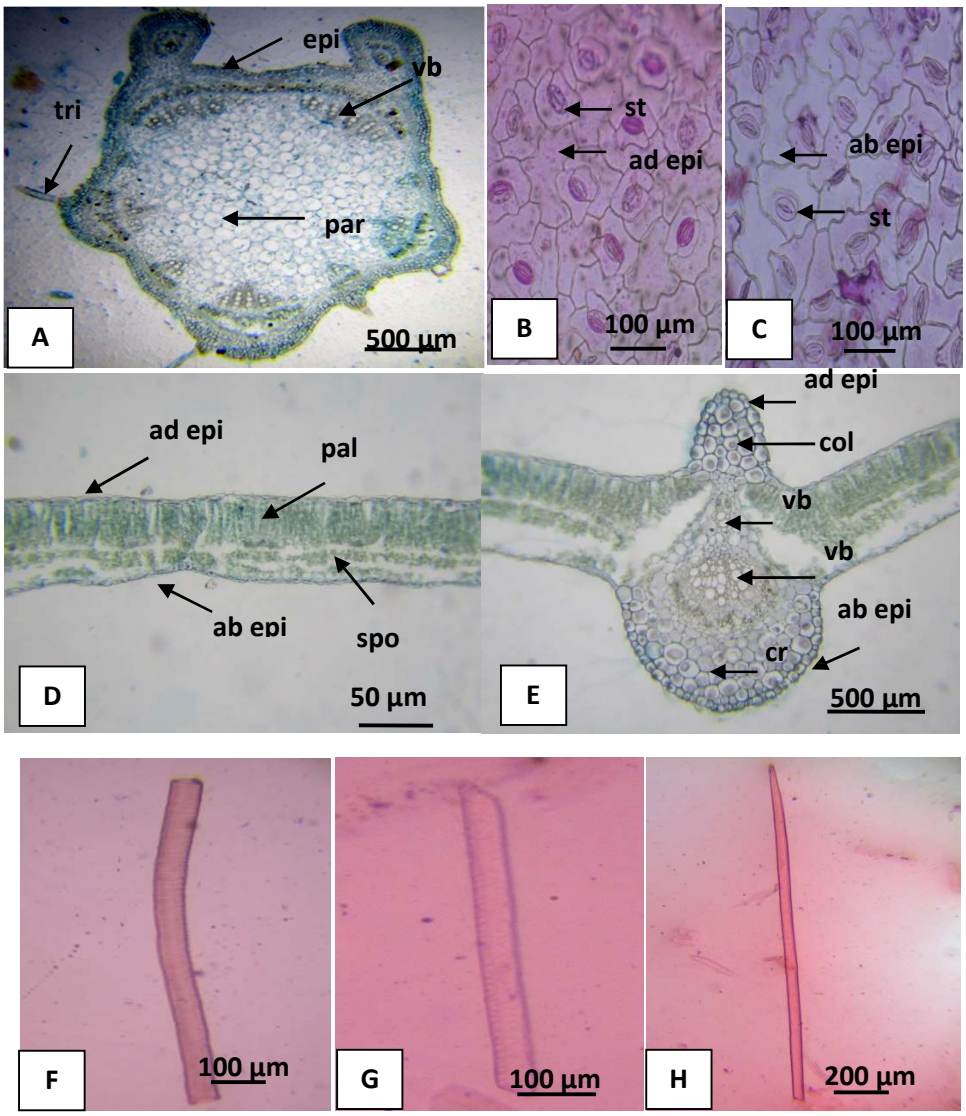

Figure 2 Internal structures of leaves of *Vigna radiata* (L.) Wilczek.

A. T.S of petiole
B. Adaxial surface view of lamina
B. Abaxial surface view of lamina
D. T. S of lamina
E. T.S of midrib
F. Vessel element
G. Tracheary element
H. Fibre

(ab epi = abaxial epidermal cell, ad epi= adxial epidermal cell, col = collenchyma cell, cr = cortex, epi= epidermal cell, par = parenchyma cell, pal = palisade parenchyma cell, ph = phloem, spo= spongy parenchyma cell, st = stoma, vb= vascular bundle, xy = xylem)

layers 31.25 – 68.75 µm thick, cells polygonal in shape, 6.25 – 11.25 µm in radial diameter, 3.75 – 11.25 µm in tangential diameter, phloem composed of sieve tubes, companion cells, phloem parenchyma cells and phloem fibres; xylem composed of 1 to 4 -layered, the layers 20.0 – 125.0 µm thick, cells polygonal in shape, 12.5 – 37.5 µm in radial diameter, 10.0 – 37.5 µm in tangential diameter, xylem composed of vessel elements, tracheids, xylem parenchyma and xylem fibres. Vessel elements thick walled, lateral walls straight, end walls oblique or transverse, thickening spiral or scalariform, perforation plates simple, 65.0 – 650.0 µm (mean 271.0 µm) in length, 10.0 – 75.0 µm (mean 30.3 µm) in width; tracheids elongate, lateral walls straight, end walls bluntly acute, thickenings spiral, 50.0 – 320.0 µm (mean 138.0 µm) in length, 10.0 – 50.0 µm (mean 18.6 µm) in width; fibres long, lateral walls straight, end walls acute, 250.0 – 3175.0 µm (mean 1190.0 µm) in length, 10.0 – 40.0 µm (mean 23.2 µm) in width, the pits slit-like; xylem parenchyma cells rectangular or irregular rectangular, pits simple.

Internal structure of lamina (Figure 2 B - D)

In transverse section, the lamina of *Vigna radiata* (L.) Wilczek. studied was dorsiventral with reticulate venation, 175.0 – 220.0 µm thick. Distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System: Composed of epidermal cells, guard cells of stomata, subsidiary cells and trichomes. In surface view, adaxial epidermal cells parenchymatous, polygonal in shape, 22.5 – 82.5 µm in length, 18.75 – 77.5 µm in width, cell walls wavy; abaxial epidermal cells parenchymatous, polygonal in shape, 18.75 – 100.0 µm in length, 13.75 – 81.25 µm in width, cell walls more wavier than adaxial cells; stomata paracyctic type; guard cells on adaxial surface 21.25 – 30.0 µm in length, 18.75 – 77.5 µm in width; guard cells on abaxial surface 18.75 – 31.25 µm in length, 5.0 – 8.75 µm in width; subsidiary cells on adaxial surface 27.5 – 70.0 µm in length, 6.25 – 22.5 µm in width; subsidiary cells on abaxial surface 25.0 – 77.5 µm in length, 2.5 – 21.25 µm in width. In transverse section, both upper and lower epidermis 1-layered; adaxial epidermal cells barrel shaped, 7.5 – 18.75 µm in length, 10.0 – 62.5 µm in width, anticlinal walls straight, outer and inner walls convex; abaxial epidermal cells barrel shaped, 8.75 – 21.25 µm in length,

18.75 – 31.25 µm in width, anticlinal walls straight, outer and inner walls convex; cuticle thin on both surfaces.

Ground Tissue System: Mesophyll differentiated into palisade and spongy parenchyma. Palisade parenchyma 2 or 3 - layered, the layers 62.5 – 87.5 µm thick, cells elongated, 22.5 – 40.0 µm in length, 6.25 – 12.5 µm in width, compact; spongy parenchyma 2 to 6 -layered, the layers 65.0 – 90.0 µm thick, cells oval or rounded in shape, 6.25 – 16.25 µm in length, 6.25 – 20.0 µm in width, chloroplast abundant, intercellular spaces present.

Vascular Tissue System: Vascular bundles of lateral veins were embedded in the mesophyll tissues. They were collateral 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, companion cells, phloem parenchyma and phloem fibres; xylem composed of vessel elements, tracheids, xylem parenchyma and xylem fibres. Vessel elements thick walled, lateral walls straight, end walls oblique or transverse, thickening spiral or scalariform, perforation plates simple, 70.0 – 260.0 µm (mean 157.0 µm) in length, 10.0 – 45.0 µm (mean 27.2 µm) in width; tracheids elongate, lateral walls straight, end walls bluntly acute, thickenings spiral, 10.0 – 200.0 µm (mean 78.0 µm) in length, 10.0 – 400.0 µm (mean 89.5 µm) in width; fibres long, lateral walls straight, end walls acute, 390.0 – 1425.0 µm (mean 690.0 µm) in length, 15.0 – 35.0 µm (mean 22.7 µm) in width, the pits slit-like; xylem parenchyma cells rectangular or irregular in shape, pits simple.

Internal structure of midrib (Figure 2 E)

In transverse section, the midrib of *Vigna radiata* (L.) Wilczek. studied was oval shaped in outline, with convex at the abaxial side and prominent protrude at the adaxial side, 937.5 – 1087.5 µm in radial diameter, 562.5 – 812.5 µm in tangential diameter. Distinguishable into dermal, ground and vascular tissue system.

Dermal Tissue System: Composed of epidermal cells and trichomes. In transverse section, both upper and lower epidermis 1- layered, cells barrel shaped, 15.0 – 36.25 µm in length, 6.25 – 43.75 µm in width, outer and inner walls convex, anticlinal walls straight.

Ground Tissue System: Composed of collenchymatous and parenchymatous tissues. Collenchymatous cells below the adaxial epidermis 3 to 6 - layered, the layers 115.0 – 250.0 µm thick, cells polygonal in shape, compact, 21.25 – 46.25 µm in length, 25.0 – 47.5 µm in width; collenchymatous cells above the abaxial epidermis 1 or 2 - layered, the layers 25.0 – 40.0 µm thick, cells polygonal in shape, 18.75 – 37.50 µm in length, 75.00 – 33.75 µm in width; parenchymatous cells above the vascular bundle, 4 to 6 - layered, the layers 50.0 – 75.0 µm thick, cells oval or rounded in shape, 10.0 – 35.0 µm in length, 8.75 – 31.25 µm in width; parenchymatous cells below the vascular bundle, 3 to 5 - layered, the layers 125.0 – 150.0 µm thick, cells oval or rounded in shape, 15.0 – 56.25 µm in length, 35.0 – 66.25 µm in width, intercellular spaces present.

Vascular Tissue System: Vascular bundles embedded in the ground tissue, composed of opposite systems of two bundles, with their xylem groups abutting on one another; phloem lying outside and xylem lying inside, collateral type. One large bundle situated above the abaxial side, oval in shape, 200.0 – 260.0 µm in radial diameter, 225.0 – 410 µm in tangential diameter; phloem 3 to 7 - layered, the layers 37.5 – 100.0 µm thick, cells compact, 3.75 – 8.75 µm in length, 3.75 – 8.75 µm in width; xylem 2 to 6 - layered, the layers 50.0 – 175.0 µm thick, cells polygonal in shape, 20.0 – 40.0 µm in length, 15.0 – 35.0 µm in width. One small bundle situated at the adaxial side, oval shaped, 100.0 – 150.0 µm in radial diameter, 60.0 – 150.0 µm in tangential diameter; phloem 3 to 6 - layered, the layers 31.25 – 75.0 µm thick, cells compact, 3.75 – 8.75 µm in length, 3.75 – 8.75 µm in width; xylem 2 to 4 - layered, the layers 65.0 – 150.0 µm thick, cells polygonal in shape, 15.0 – 30.0 µm in length, 10.0 – 20.0 µm in width. Phloem composed of sieve tubes, companion cells, phloem parenchyma cells and phloem fibres; xylem composed of vessel elements, tracheids, xylem parenchyma and xylem fibres. Vessel elements thick walled, lateral walls straight, end walls oblique or transverse, thickening spiral or scalariform, perforation plates simple, 55.0 – 275.0 µm (mean 147.5 µm) in length, 15.0 – 50.0 µm (mean 29.7 µm) in width; tracheids elongate, lateral walls straight, end walls bluntly acute, thickenings spiral, 45.0 – 250.0 µm (mean 128.0 µm) in length, 10.0 – 45.0 µm (mean 18.6 µm) in width; fibres long, lateral walls straight, end walls acute, 300.0 – 1525.0 µm (mean 690.0 µm) in length, 15.0 – 40.0 µm (mean

24.35 µm) in width; xylem parenchyma cells rectangular or irregular in shape, pits simple.

Internal Structure of Stem (Figure 3)

In transverse section, the stem of *Vigna radiata* (L.) Wilczek. studied was circular in outline, 2937.5 – 3625.0 µm in length, 3875.0 – 5062.5 µm in width. Distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System: In transverse section, epidermal cells 1- layered, cells oval or barrel in shape, 15.0 – 25.0 µm in length and 15.0 – 31.25 µm in width, outer wall convex, anticlinal walls straight; trichome uniseriate, 1 or 2-celled; cuticle thin.

Ground Tissue System: Composed of cortex, endodermis, pericycle and pith. The cortex differentiated into outer collenchymatous tissue and inner parenchymatous tissue. Collenchymatous cells forming a continuous sheath, 1 to 7 - layered, the layers 25.0 – 250.0 µm thick, the cells polygonal or oval in shape, 12.5 – 50.0 µm in length, 18.75 – 62.5 µm in width, thickening angular. Parenchymatous cells occur below the collenchymatous cells, 5 to 13 - layered, the layers 85.0 – 275.0 µm thick, the cells rounded or oval shaped, 10.0 – 50.0 µm in length, 17.5 – 50.0 µm in width, intercellular space present. Endodermis and pericyclic layer is inconspicuous. Pith cellular large, 2875.0 – 3750.0 µm in diameter, the cells parenchymatous, oval or rounded or polygonal in shape, 35.0 – 150.0 µm in length, 40.0 – 200.0 µm in width, thin-walled, intercellular spaces present.

Vascular Tissue System: Vascular bundles embedded in the ground tissue and arranged in a continuous circular ring, collateral type, the bundles 250.0 – 1125.0 µm thick; phloem lying outside and xylem lying inside; phloem 4 to 13 - layered, the layers 22.5 - 100 µm thick, the cells oval or irregular in shape, 5.0 – 12.5 µm in length, 8.75 – 10.0 µm in width, phloem composed of sieve-tube elements, companion cells, phloem parenchyma and phloem fibres; xylem arranged in radial rows, 1 to 6 - layered, the layers 30.0 – 200.0 µm thick, the cells rounded or polygonal in shape, 13.75 – 43.75 µm in length, 7.5 – 37.5 µm in width, xylem composed of vessel elements, tracheids, fibres and xylem parenchyma.

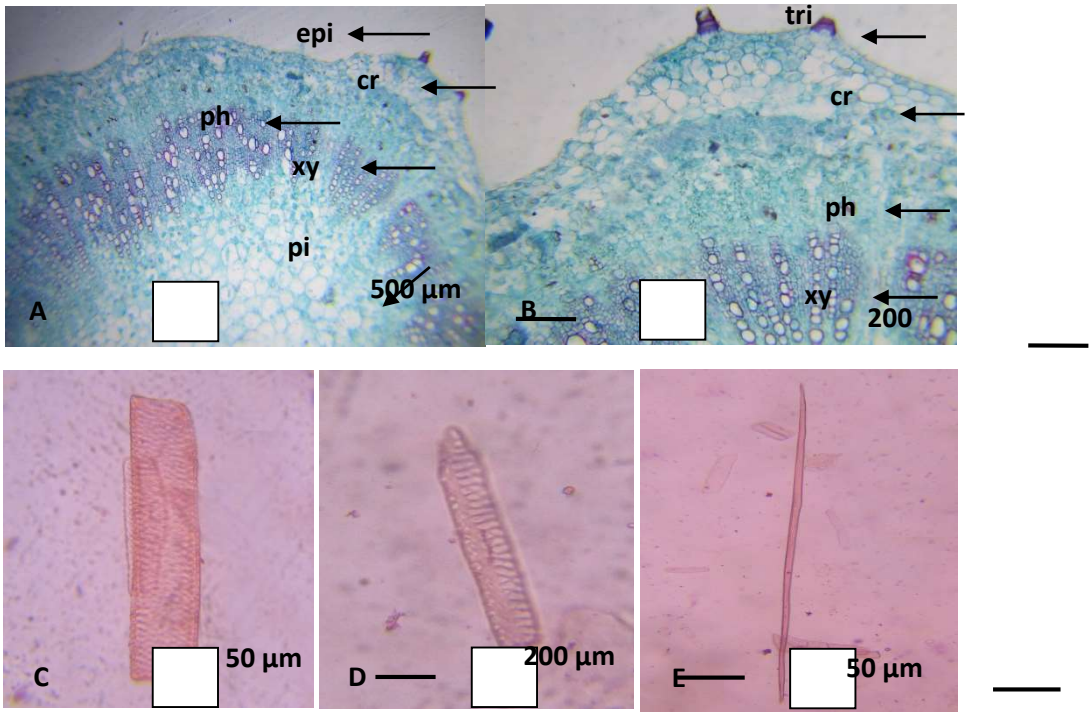

Figure 3 Internal structure of stem of *Vigna radiata* (L.) Wilczek.
A. Transverse section of stem
B. Close up view cortex layer and vascular bundle
C. Vessel element
D. Tracheary element
E. Fibre
(cr= cortex, epi= epidermis, pi = pith, ph= phloem, tri=trichome, xy = xylem)

Vessel elements thick walled, lateral walls straight, end walls oblique or transverse, thickening spiral or scalariform, perforation plates simple, 70.0 – 425.0 µm (mean 237.0 µm) in length, 20.0 – 75.0 µm (mean 42.0 µm) in width; tracheids elongate, lateral walls straight, end walls bluntly acute, thickenings spiral, 75.0 – 450.0 µm (mean 164.5 µm) in length, 10.0 – 30.0 µm (mean 18.2 µm) in width; fibres long, lateral walls straight, end walls acute, 350.0 – 2500.0 µm (mean785.0 µm) in length, 10.0 – 25.0 µm (mean 16.9 µm) in width, the pits slit-like; xylem parenchyma cells rectangular or irregular in shape, pits simple.

Internal structure of root (Figure 4)

In transverse section, the root of *Vigna radiata* (L.) Wilczek. studied was circular in outline, 1687.5 – 3625.0 µm in length, 1812.5 – 3437.0 µm in width. Distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System: The epiblema 3 to 4 - layered, the layers 15.0 – 56.25 µm thick, parenchymatous, the cells irregularly rectangular in shape, 8.75 – 33.75 µm in length, 20.0 – 81.25 µm in width.

Ground Tissue System: Composed of cortex, endodermis and pericycle. Cortex homogenous parenchymatous cells, 4 to 14 - layered, the layers 115.0 – 350.0 µm thick, parenchymatous, cells oval or barrel or irregular in shape, 7.5 – 27.5 µm in length, 10.0 – 50.0 µm in width. Endodermis and pericyclic layers are inconspicuous. In the central portion of vascular strand, hollow pith present, 375.0 – 1050 in diameter.

Vascular Tissue System: Vascular bundles occurs as radial type, vascular cylinder tetrarch to polyarch, the bundle 562.5 – 1662.5 µm thick; phloem distributed at the periphery of the xylem, 8 to 17 - layered, the layers 300.0 – 525.0 µm thick, the cells 15.0 – 35.0 µm in length, 10.0 – 15.0 µm in width; xylem strands, 400.0 – 800.0 thick, cells polygonal or rounded in shape, 15.0 – 95.0 µm in length, 15.0 – 80.0 µm in width; phloem composed of sieve-tube elements, companion cells, phloem parenchyma and phloem fibres; xylem composed of vessel elements, tracheids, fibres and xylem parenchyma. Vessel elements thick walled, lateral walls straight, end walls oblique or transverse, thickening spiral or scalariform, perforation plates simple, 75.0 – 245.0 µm (mean 156.5 µm) in length, 25.0 – 160.0 µm (mean 100.5 µm) in width; tracheids elongate, lateral walls straight, end walls bluntly acute, thickenings spiral, 75.0 – 340.0 µm (mean 153.5 µm) in length, 10.0 – 40.0 µm (mean 21.0 µm) in width; fibres long, lateral walls straight, end walls acute, 350.0 – 257.0 µm (mean 825.0 µm) in length, 10.0 – 25.0 µm (mean 14.7 µm) in width, the pits slit-like; xylem parenchyma cells rectangular or irregular in shape, pits simple.

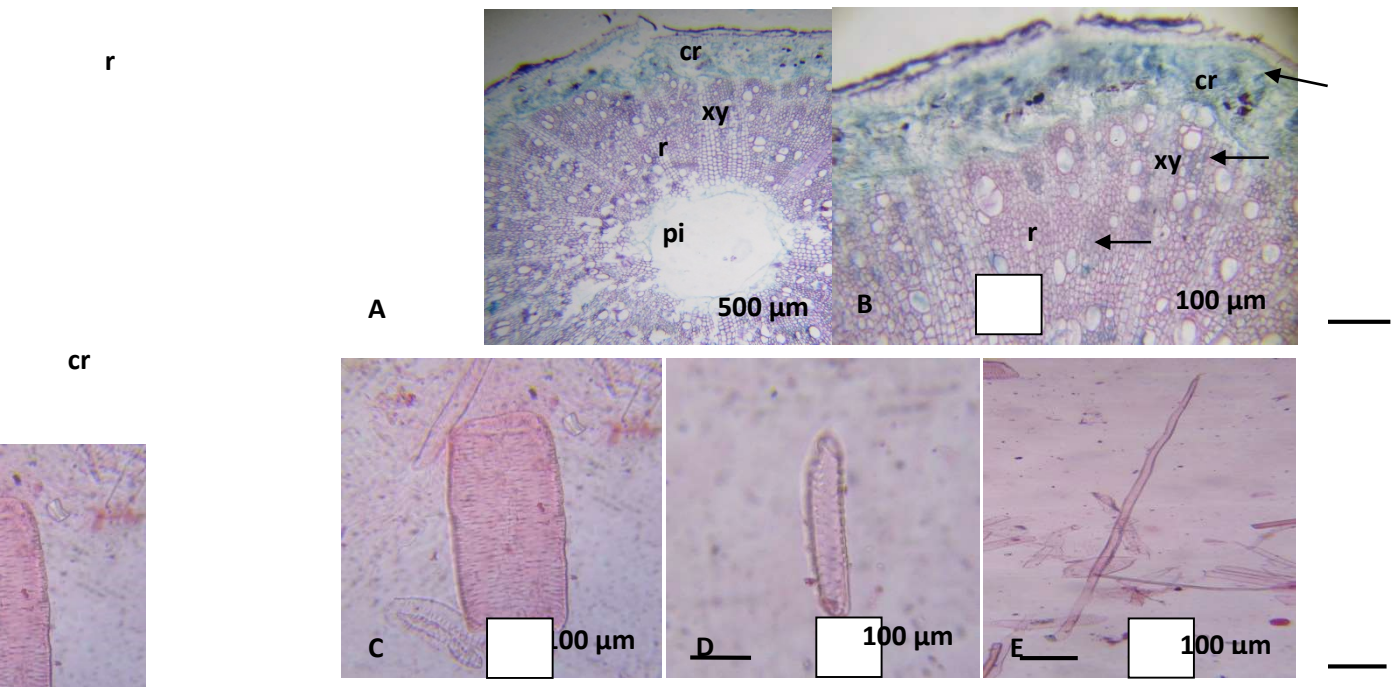

Figure 4 Internal structure of root of *Vigna radiata* (L.) Wilczek.

A. Transverse section of root
B. Close up view cortex and vascular bundle
C. Vessel element
D. Tracheary element
E. Fibre

(cr= cortex, pi = pith, ph= phloem, r = ray, xy = xylem)

Discussion and Conclusion

Vigna radiata (L.) Wilczek. (mung bean) has been consumed as common food in China for more than 2000 years. It is well known for its detoxification activities and is used to refresh mentality, alleviate heat stroke, and reduce swelling in the summer. Mung beans contain balanced nutrients, including protein and dietary fiber, and significant amounts of bioactive phytochemicals. Mung beans to be the main contributors to the antioxidant, antimicrobial, anti-inflammatory and antitumor activities (Tang *et al*. 2014).

Vigna radiata (L.) Wilczek. is belonging to family Fabaceae (Subfamily Papilionoideae) were studied. The plants were observed an annual erect herb with brown hirsute. The leaves were ovate, leave bases cuneate or

rounded and leaves tips were acuminate. The surfaces of leaves were sparsely pilose. These characters were in agreement with those mentioned by Hooker (1879), Dassanayake (1991) and Qi-ming & Nian-he (2008).

The petiole of *Vigna radiata* (L.) Wilczek. is irregular shape and the epidermal cells is uniseriate with rectangular shaped cells and covered with simple, unicellular and unbranched trichomes. One layer of circular collenchymas cells is located under the epidermis. The cortex consists of orbicular parenchymatous cells. The stele is clearly divided into large two adaxial bundles and smaller three abaxial bundles forming main trace and collateral type. The pith is composed of polygonal parenchymatous cells with intercellular space. In transverse section of leaves, the upper and lower leaf epidermis layers are composed of uniseriate with rectangular cells and buliform. The type of stomata observed is paracytic and they occur on the surface of both sides being more abundant on the lower surface. The midrib is well developed and vascular bundles are collateral type (Siapoosh *et al.* 2015).

The inflorescences were terminal and axillary racemes. The flowers were bisexual, zygomorphic, cyclic, hypogynous, pedicellate and yellow colour. These characters were agreed with Qi-ming & Nian-he (2008).

The calyx were campanulate, 5 – lobed; corolla papilionaceous. The stamens were diadelphous, free from the tepals and inserted, anthers dithecous, dorsifixed, dehiscing longitudinally. The ovaries were superior, linear, unilocular, marginal placentae. The fruits were dehiscent. These characters were agreed with those mentions by Hooker (1879), Dassanayake (1991) and Qi-ming & Nian-he (2008).

In anatomical characteristics of petioles, laminae, midribs, stems and roots were composed of dermal tissue system, ground tissue system and vascular tissue system.

In transverse section, petioles were oval shape in outline with prominent wing at the adaxial side. Vascular bundles arranged in a ring, collateral type, accompanied by 2 accessory bundles present in adaxial 2 lateral prominent wings. These characters were agreed with Metcalfe & Chalk (1950).

In surface view of laminae, stomata were paracyctic type, these characters were in agreement with Metcalfe & Chalk (1950) and Siapoosh *et al.* (2015).

In transverse section, midribs were oval shaped in outline with prominently protrude in ad-axial side. The two vascular bundles were observed, one large bundle and one small bundles lying opposite each other with their xylem groups abutting on one another. These characters were accordant with Metcalf and Chalk (1950) and Nassar (2013).

In transverse section, stems were circular in outline with wavy ridges. Pith cellular large and composed of thin walled parenchymatous cell. The vascular bundles were collateral type. These characters were in agreement with Metcalfe & Chalk (1950) and Siapoosh *et al.* (2015).

In the transverse section of root, epiblema was 3 or 4 - layered, parenchymatous; ground tissue system composed of cortex, endodermis and pericycle. The cortex composed of homogenous parenchymatous cells. The endodermis and pericycle was inconspicuous. The vascular tissue system was radial type, tetrarch to polyarch, phloem alternate with the xylem strands. Hollow pith was observed in the centre of root. These characters were agreed with Metcalf & Chalk (1950) and Nassar (2013).

In conclusion, the present research can provide the information of morphological and anatomical characters of *Vigna radiata* (L.) Wilczek. It is hoped that these finding were useful in species confirmation. The position of vascular bundles in petioles and midribs will provide the useful for the diagnostic characters of identification of this plant.

Acknowledgements

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INVESTIGATION OF PHYTOCHEMICAL CONSTITUENTS, PHYSICOCHEMICAL PROPERTIES AND ANTIMICROBIAL ACTIVITIES FROM THE LEAVES OF *SENNA AURICULATA* (L.) ROXB.

Ei Theingi Win[1], Aung Kyaw Min[2]

Abstract

The plant *Senna auriculata* (L.) Roxb., belong to the family Fabaceae (former Caesalpiniaceae). It is widely distributed in Myanmar. This plant is one of the famous medicinal plants that found in Mandalay, Magway and Sagaing Regions. In this paper, the preliminary phytochemical tests, physicochemical properties, nutritional value and antimicrobial activities of *Senna auriculata* (L.) Roxb. have been described. In Morphological study, this plant is a shrub which has cylindrical stem, brown in colour with several branches. The leaves are paripinnately compound with auriculate stipules. Inflorescences terminal or axillary, corymbose racemes, flowers are bright yellow. The fruits are oblongoid pods and flattened. The powdered leaves were tested for the phytochemical constituents and physicochemical properties. Alkaloid, glycoside, carbohydrate, α-amino acid, phenolic compound, flavonoid, terpenoid, steroid, tannin and reducing sugar were present but cyanogenic glycoside, starch and saponin were absent in samples. According to physicochemical examination, the powder of leaves were most soluble in water and moderately soluble in ethanol and methanol. The nutrient content of leaves were also studied. It revealed that the presence of protein, crude fiber and crude fat. Antimicrobial activities of *Senna auriculata* (L.) Roxb. were also tested by using agar-well diffusion method with six pathogenic microorganisms. In this experiment, acetone extract of leaves showed the most effective activities against *Pseudomonas aeruginosa* , *Bacillus pumalis* and *Escherichia coli* . The ethanol extract showed more antimicrobial activities against *Bacillus subtilis* and *Candida albicans. Senna auriculata* (L.) Roxb was observed to have antimicrobial activity and can be used for medicinal purposes.

Keywords: *Senna auriculata* (L.) Roxb., Phytochemical Test, Physicochemical properties, Nutritional values and Antimicrobial activities.

Introduction

Myanmar is well known for its wealth of natural plant resources for there are still many valuable plant materials to be explored. Among these, *Senna auriculata* (L.) Roxb., is also included. *Senna auriculata* (L.) Roxb.,

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which has enormous traditional uses against various diseases. The specimens were collected from Nyaung Oo Township, Mandalay Region. . This plant is located in North Latitude 21˚15'75" and East Longitude 94˚87'71". The medicinal plant, *Senna auriculata* (L.) Roxb. belong to the family Fabaceae and subfamily Caesalpinioideae. This subfamily has 27 genera and 124 species in Myanmar (John Kress, 2003) and 152 genera and nearly 2,800 species, mostly tropical and sub-tropical regions of the world (Purseglove, 1969).

In Myanmar, most of plants are growing wild and some species are cultivated as ornaments, but they are best known as medicinal plants. It has a long history of use as a folk medicine and its therapeutic efficacy is well recognized. In local use of *Senna auriculata* (L.) Roxb., some people cooked the fresh leaves of this plant as medicine. The dried leaves and flowers are boiled in water and then used to control the blood sugar level for diabetic patients.

Most of the Myanmar people familiar with the *Senna* as a popular genus that not only used as traditionally medicinal plant but also the leaves are used as vegetables since immemorial time (Kirtikar and Basu, 1933). This plant is native of tropical regions Southeast Asia and Africa. The leaves are used in the form of “Ceylon tea”, or “Matara tea”; the bark is “Avaram bark” important in tanning; the plants are used medicinally and as ornamentals (Dassanayake, 1991).

Plants could be considered as biosynthetic laboratory of chemical compounds like glycoside, alkaloids, terpenoids, phenols, resins and tannins etc. The plants have not only therapeutic potential but also provide carbohydrates, proteins and lipids as food for man. These are synthesize or deposited in specific parts of the plant body (Harbone, 1984).

Phytochemicals are nonnutritive plant chemicals that have protective or disease preventive properties. Plants produces these chemicals to protect itself, that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each works differently (Murugan and Wins, 2013). Many plant extracts have been shown to inhibit the growth of microorganisms. These extracts consist of chemicals and are usually considered to play a role in defence reactions of plants against infections by pathogenic microorganisms (Fawcett and Spencer,1976).

Senna auriculata (L.) Roxb. commonly known as Tanners sanna, is distributed throughout hot deciduous forests of India. The plant has been reported to possess antipyretic, hepatoprotective, antidiabetic, antiperoxidative and microbicidal activity (www. Pelagiaresearchlibiary.com & Journal's URL:www.bepls.com).

According to the Ashin Nargathein (1972), the root is used antidote, skin diseases, fever, asthma and diseases of urinary system. The leaves have laxative properties, which cures constipation. Leaves are anthelmintic and also used to treat ulcers, skin diseases, diabetes and leprosy. The dried flowers and flower buds are used as substitute for tea in case of diabetes patients and to improve the complexion in women. The bark and seed are to give relief in rheumatism, eye diseases, gonorrhea, diabetes and gout.

Senna auriculata (L.) Roxb. is used in the traditional system of medicine for urinary disorders, diabetes to control the blood sugar level, leprosy, worm infestation, diarrhoea, conjunctivitis; bark as astringent and used in skin conditions; leaves, flowers and fruits as anthelmintic; seeds for eye troubles, diabetes (www.ijpbs.net). For these valuable information, *Senna auriculata* (L.) Roxb., was selected, analyzed, tested and evaluated its activities.

The aim of the study is to know the valuable information of *Senna auriculata* (L.) Roxb. and medicinal uses. To achieve this aims, the objectives are to verify the morphological characters of *Senna auriculata* (L.) Roxb., to investigate the preliminary phytochemical and physicochemical properties, to determine the nutritional values and to examine the antimicrobial activities from leaves of *Senna auriculata* (L.) Roxb.

Materials and Methods

For morphological study, the plant materials were collected from Nyaung Oo Township, Mandalay Region, during the flowering period from September to February, 2016. The collected specimens were photographed to record the data and identified by using available literatures; (Kurz, 1877), (Hooker, 1879), (Kirtikar and Basu, 1933), (Burkill, 1935), (Backer, 1963), (Lawrance, 1964), (Purseglove, 1969), (Dassanayake, 1991), (Key to the Family of the Flowering Plants, 1994). The leaves of *Senna auriculata* (L.) Roxb. used in this research were collected from Nyaung Oo Township,

Mandalay Region from July to August, 2016. The leaves were washed thoroughly and air-dried in room temperature for two weeks. After that, the dried samples were pulverized by grinding to get powder and stored in air tight containers .

Preliminary Phytochemical investigation of *Senna auriculata* (L.) Roxb.

The preliminary phytochemical investigation has been undertaken on the leave of *Senna auriculata* (L.) Roxb**.,** to determine the presence or absence of organic constituents such as alkaloid, glycoside, reducing sugar, saponin, steroid, terpenoid, carbohydrate, tannin, flavonoid, cyanogenic glycoside, phenolic compound, starch and α-amino acid. The tests were carried out according to the standard method of (British Pharmacopoeia, 1968), (Marini Bettolo *et al*., 1981), (Harbone, 1984), (Central Council for Research in Unani Medicine, 1987) and (Trease and Evans, 2002). Preliminary phytochemical examination was carried out at the Pharmaceutical Research Department (PRD).

Physiocochemical properties of *Senna auriculata* (L.) Roxb.

Physicochemical investigation was conducted at the Department of Chemistry, West Yangon University. In this analysis, moisture content, total ash, acid insoluble ash, water soluble ash, water soluble matter and extractive values of leaves from *Senna auriculata* (L.) Roxb. were undertaken by using non-polar and polar solvents such as petroleum ether, chloroform, acetone, ethyl acetate, methanol, ethanol and distilled water. These values were determined according to the standard procedure given in (British Pharmacopoeia, 1968) and (Trease and Evans, 2002).

Nutrient Values of *Senna auriculata* (L.) Roxb.

Nutritional values of leaves were investigated according to the method for food analysis (AOAC, 2000). The analyses of nutritional values were performed at Food Industries Development Supporting Laboratory (FIDSL), Myanmar Food Processors and Exporters Association (MFPEA), Yangon.

Antimicrobial activities of *Senna auriculata* (L.) Roxb.

The powder was extracted by using pet-ether, chloroform, acetone, methanol, ethyl-acetate, ethanol and water. The various solvent extracts were

tested against six pathogenic microorganisms *(Bacillus subtilis,, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albican and Escherichia coli)* by using agar-well diffusion method (Cruickshank *et.al*., 1975). This test was conducted at the Pharmaceutical Research Department (PRD).

Table 1. Types of test organisms, their respective code numbers and effects.

No.	Type of test organisms	Code number	Effects
1.	*Bacillus subtilis*	N.C.T.C -8236	Pathogenic group, food poisonous, anthrax in man and animals.
2.	*Staphylococcus aureus*	N.C.P.C -6371	Boils, abscesses, wound, sepsis, burns, food poison, staphylococal pneumonia
3.	*Pseudomonas aeruginosa*	N.C.T.C -6749	Urinary tract infection, bone and joint infection, soft tissue infection, burns, ear infection, surgical wounds, ocular and gastro intestinal infection, respiratory system infections, chronic lung.
4.	*Bacillus pumalis*	N.C.I.B-8982	Eye infection, soft tissue infections
5.	*Candida albicans*	I.F.O-1060	Pneumonia like illness, meningitis, cardiac infection, sinus irritation, sores, ringworm
6.	*Escherichia - coli*	N.C.I.B-8134	Urinary - tract infections, dysentery, abscess, wounds and bed-sores

Results

Morphological characters of *Senna auriculata* (L.)Roxb.

Perennial shrubs up to 1.5-2.5 m high; younger stems cylindrical and pubescent (figure 1). Leaves are alternate, 5.0-12.0 cm long, 1.5-3.0 cm wide, unipinnate and paripinnately compound; petioles stout, cylindrical, 0.7-1.5 cm

long and 0.5-1.5 mm in diameter, slightly canaliculate above, tomentose; the racheae 5.5-9.0 cm long, 0.5-1.0mm in diameter, slightly canaliculated above, tomentose, glands present in between each pair of the leaflets, filiform, yellow or brown; leaflets 8-12 pairs, oblong to elliptic- oblong , 1.5-3.0 cm long, 0.5-1.4 cm wide, mucronate, the margins entire, the bases rounded or oblique, the upper surfaces rigidly subcoriaceous, both surfaces pubescent; petiolules about 1 mm long, dark brown, tomentose; stipules auriculate or lunate-reniform, 1.3-2.5 cm long and 1.0-1.8 cm wide, with pointed appendages curved towards the leaves, the appendages 0.1-0.4 cm long, green, veins distinct, reticulate, pubescent, persistent are shown in figures (2 and 3). Inflorescences terminal or axillary, corymbose racemes, 5 to 10 flowers, 4.5 - 7.0 cm long and 4.0-5.0 cm wide, the peduncles 5.0-7.0 cm long and 1.0-2.0 mm in diameter, pubescent, bracts ovate-acuminate, 5-7 mm long, 3-4 mm wide, green, pubescent, persistent, pedicels 2.0-3.5 cm long and 0.8-1.0 mm in diameter, pubescent; bracteoles linear, 3.0-3.5 mm long and about 2 mm wide, green, pubescent, caduceus are shown in figures (4 and 5). Flowers bright yellow, 2.5-3.0 cm long, 2.5-3.5 cm wide, complete, bisexual, irregular, zygomorphic, 5 merous, cyclic, hypogynous are shown in figures (6 ,7 and 8). Calyx; sepals-5, aposepalous, the two outer sepals obovate, cucullate, 7-10 mm long, 4-5 mm wide, the two inner ones ovate oblong, hooded, 1.2-1.4 cm long and 8-10 mm wide, the remaining one obovate, 1.0-1.2 cm long and 8.0-9.0mm wide, imbricate, brownish-yellow, coriaceous, glabrous, persistent, inferior (figure 9). Corolla; petals 5, apopetalous, rosaceous, the posterior petal broadly ovate or oblicular, the limbs 1.5-1.8 cm long, 1.0-1.4 cm wide, the claws 2.5- 3.0 mm long, the two lateral ones obovate oblong, the limbs 1.5- 1.8 cm long and 1.3-1.4 cm wide, the claws 4.0-5.0 mm long, the two anterior ones ovate- oblong, the limbs 1.8-2.0 cm long and1.2-1.5 cm wide, the claws 5.0- 6.0 mm long, valvate, bright yellow, membranous, veins reddish brown, reticulate, glabrous (figure 10). Stamens 10, apostamenous, 7 fertile and 3sterile, the fertile stamens 3 long and 4 short, the long filaments 1.2-1.5 cm long, the short ones 8.0-9.0 mm long, the larger anther lobes oblongoid, sickle shaped, 0.8-1.0 cm long, 1.2-2.0 mm in diameter, curved, the small ones rectangularly oblongoid, 5-6 mm long, 1.0-1.5 mm in diameter, straight or slightly curved, the anther tips truncate, brown or reddish-brown, dithecous, introse, basifixed, dehiscence by terminal pores, glabrous, the sterile filaments 2-2.5 mm long, the another lobes rounded and flattened,

2-3 mm long, about 2mm wide, light brown or yellowish-brown, glabrous, inferior are shown in figure (11). Pistil monocarpellary, unilocular (figure 12), the ovary oblongoid ,0.9-1.2 cm long, 0.8-1.0 mm in diameter, one ovule in each locule, marginal placentation , ovary superior, light brown, tomentose, hairs white; the styles 5-10 mm long, slender, curved, glabrous; the stigma filiform, 0.8-1.5 mm long, pubescent; the gynophores present are shown in figures (13 and 14). The pods dehiscent, oblongoid, flattened, 7-15 cm long, 1.2-1.8 cm in diameter, the tips mucronate, dark green, flexible, glabrous (figure 15). Seeds 10-20, ellipsoid, 8.0-12.0 cm long, 3.0-5.0 cm in diameter, brown to dark brown, hard and glabrous (figure 16).

Morphological characters of *Senna auriculata* (L.) Roxb.

Figure 1. Habit of flowering plant

Figure 2. Leaves

Figure 3. Leaves with stipules and glands

Figure 4. Inflorescence as seen

Figure 5. Inflorescence

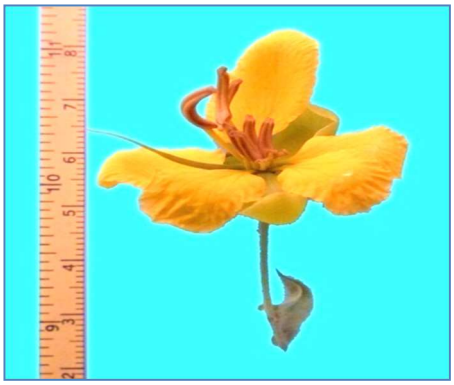


Figure 6. Flower with bract

Figure 7. Flower

Figure 8. L.S of Flower

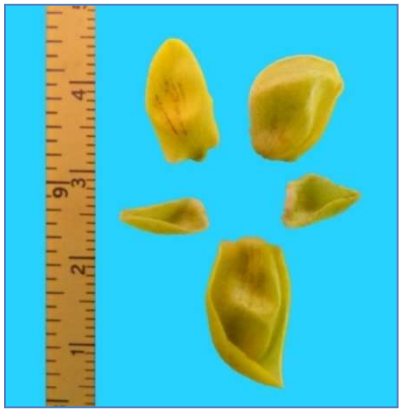


Figure 9. Sepals

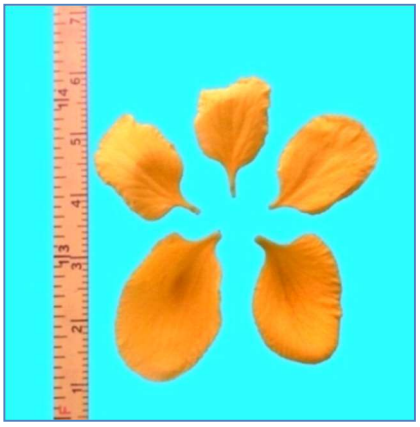


Figure 10. Petals

Figure 11. Stamens

Figure 12. Pistil

Figure 13. T.S of Ovary

Figure 14. L.S of Ovary

Figure 15. Fruits

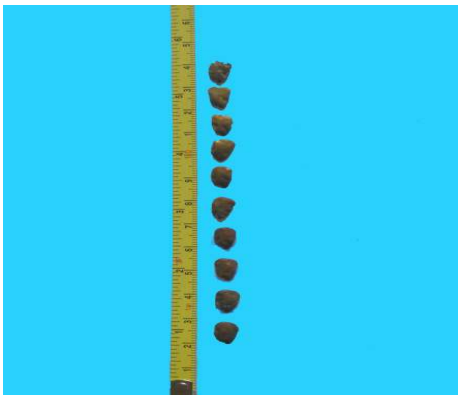


Figure 16. Seeds

Preliminary phytochemical investigation of *Senna auriculata* (L.) Roxb.

In preliminary phytochemical investigation, alkaloids, glycosides, reducing sugars, α-amino acids, carbohydrate, tannin, steroid, terpenoid, flavonoid, phenolic compound were observed in leaves. The tests have shown that cyanogenic glycoside, saponin and starch were absent. The results were shown in Table 2.

Table 2. Preliminary phytochemical investigation on leaves of *Senna auriculata* (L.) Roxb.

No.	Test	Extract	Test reagent	Observation	Results
1	Alkaloid	1 % HCl	1. Mayer's reagent 2. Dragendorff's reagent 3. Wagner's reagent 4. Hager's reagent	White ppt. Yellowish brown ppt. Deep blue ppt. Yellow ppt.	+ + + +
2	Glycoside	H_2O	10 % Lead acetate solution	White ppt.	+
3	Cyanogenic glycoside	H_2O	1. H_2O, Conc; H_2SO_4 2. Sodium picrate paper	No colour change	-
4	Saponin glycoside	H_2O	Distilled water	No persistent foam	-
5.	Starch	H_2O	Iodine solution	Brown ppt.	-
6	α-amino acid	H_2O	Ninhydrin reagent	Purple colour	+
7	Carbohydrate	H_2O	1. 10 % α naphthol , Conc: H_2SO_4	Red ring	+
8	Reducing sugar	H_2O	1. Fehling's solution	Brick red ppt.	+ +
9	Tannin	H_2O	1% gelatin & 10% NaCl solution	White ppt.	+

No.	Test	Extract	Test reagent	Observation	Results
10	Phenolic Compound	H_2O	5% $FeCl_3$	Brownish green colour	+
11	Flavonoid	70% EtOH	Conc: HCl/ Mg ribbon	Pink colour	+
12	Steroid	PE	Acetic anhydrite and conc: H_2SO_4	Bluish green colour	+
13	Terpenoid	PE	Acetic anhydrite and Conc: H_2SO_4	Pink colour	+

(+) = present, (-) = absent, ppt = precipitated

Physicochemical properties from *Senna auriculata* (L.) Roxb.

In physicochemical properties, moisture content, total ash, acid-insoluble ash, water-soluble ash content were determined and recorded. The extractive values of powdered leaves was investigated by using different solvents such as acetone, chloroform, ethyl acetate, petroleum ether, methanol, ethanol and distilled water. The leaves were most soluble in water and moderately soluble in ethanol and methanol as shown in Table 3.

Table 3. Physicochemical properties of *Senna auriculata* (L.) Roxb

No	Physicochemical characters	Contents (%)
		Leaves
1.	Moisture content	7.48
2.	Total ash	2.38
3.	Acid insoluble ash	6.43
4.	Water soluble ash	24.66
5.	Pet-ether soluble matter	2.77
6.	Chloroform soluble matter	0.74
7.	Ethyl acetate soluble matter	4.68

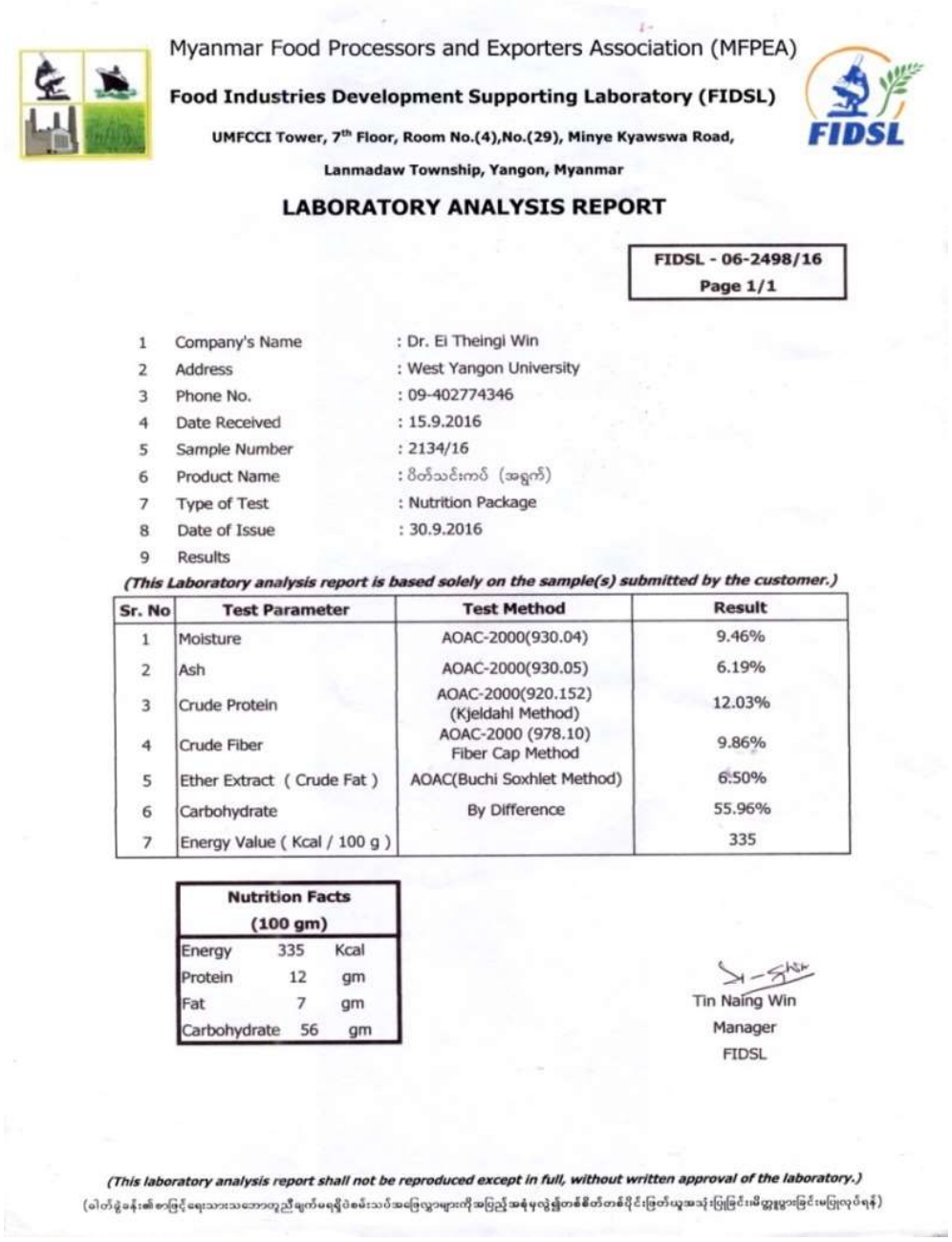
No	Physicochemical characters	Contents (%)
		Leaves
8.	Acetone soluble matter	4.11
9.	Ethanol soluble matter	10.14
10.	Methanol soluble matter	9.69
11.	Water soluble matter	15.35

Nutritional values on leaves of *Senna auriculata* (L.) Roxb.

The Nutritional value such as moisture, ash, crude protein, crude fiber, crude fat and carbohydrate values in the leaves of *Senna auriculata* (L.) Roxb. were found. It was also observed that energy value was 335 Kcal per 100g. The results were shown in Table 4.

Table 4. Nutritional values on leaves of *Senna auriculata* (L.) Roxb.

No	Test parameter	Amount of contents (%)
1.	Moisture	9.46
2.	Ash	6.19
3.	Crude protein	12.03
4.	Crude fiber	9.86
5.	Ether Extract (Crude Fat)	6.50
6.	Carbohydrate	55.96
7.	Energy value (Kcal / 100g)	335



Myanmar Food Processors and Exporters Association (MFPEA)

Food Industries Development Supporting Laboratory (FIDSL)

UMFCCI Tower, 7th Floor, Room No.(4),No.(29), Minye Kyawswa Road,

Lanmadaw Township, Yangon, Myanmar

LABORATORY ANALYSIS REPORT

FIDSL - 06-2498/16
Page 1/1

1	Company's Name	: Dr. Ei Theingi Win
2	Address	: West Yangon University
3	Phone No.	: 09-402774346
4	Date Received	: 15.9.2016
5	Sample Number	: 2134/16
6	Product Name	: ဗိတ်သင်းကပ် (အရွက်)
7	Type of Test	: Nutrition Package
8	Date of Issue	: 30.9.2016
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(930.04)	9.46%
2	Ash	AOAC-2000(930.05)	6.19%
3	Crude Protein	AOAC-2000(920.152) (Kjeldahl Method)	12.03%
4	Crude Fiber	AOAC-2000 (978.10) Fiber Cap Method	9.86%
5	Ether Extract (Crude Fat)	AOAC(Buchi Soxhlet Method)	6.50%
6	Carbohydrate	By Difference	55.96%
7	Energy Value (Kcal / 100 g)		335

Nutrition Facts (100 gm)		
Energy	335	Kcal
Protein	12	gm
Fat	7	gm
Carbohydrate	56	gm

Tin Naing Win
Manager
FIDSL

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(ဓါတ်ခွဲခန်းဆိုင်ရာဖြင့် ရေးသားသောအာတူညီချက်မရရှိပဲစမ်းသပ်အဖြေလွှာများကိုအပြည့်အစုံမဟုတ်ဘဲစီတိုင်းဖြတ်ယူအသုံးပြုဖြင့်းမြဖွယ်ရှားဖြင့်းမပြုလုပ်ရန်)

Figure 17. Nutritional value from Leaves of *Senna auriculata* (L.) Roxb

Antimicrobial activities of different solvent extracts from Leaves of *Senna auriculata* (L.) Roxb.

In this study, the different solvent extracts of leaves were tested with six pathogenic microorganism such as *Bacillus subtilis, Staphylococus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albicans and Escherichia coli* by using agar well diffusion method. In this experiment of leave extracts, chloroform, acetone, methanol, ethyl acetate and ethanol showed antimicrobial activities against all tested microorganisms but acetone extracts was most effective activities against *Pseudomonas aeruginosa, Bacillus pumalis and Escherichia coli* . The ethanol extract showed more antimicrobial activities against *Bacillus subtilis* and *Candida albicans.* Petroleum ether extract showed weak activity against *Escherichia coli* and did not show any against other tested microorganisms. The results were shown in Table (5) and Figures (18 and 19).

Table 5. Antimicrobial activities against different solvent extracts from leaves of *Senna auriculata* (L.) Roxb.

Solvents	Organisms					
	Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus pumalis*	*Candida albicans*	*Escherichia coli*
Pet-ether	-	-	-	-	-	11 mm
Chloroform	12 mm	13 mm	12 mm	11 mm	13 mm	13 mm
Acetone	15 mm	14 mm	17 mm	17 mm	14 mm	18 mm
Methanol	16 mm	17 mm	15 mm	14 mm	14 mm	15 mm
Ethyl acetate	13 mm	13 mm	13 mm	13 mm	13 mm	14 mm
Ethanol	17 mm	15 mm	15 mm	15 mm	16 mm	15 mm
Water	11 mm	-	-	-	-	11 mm

Agar well - 10 mm

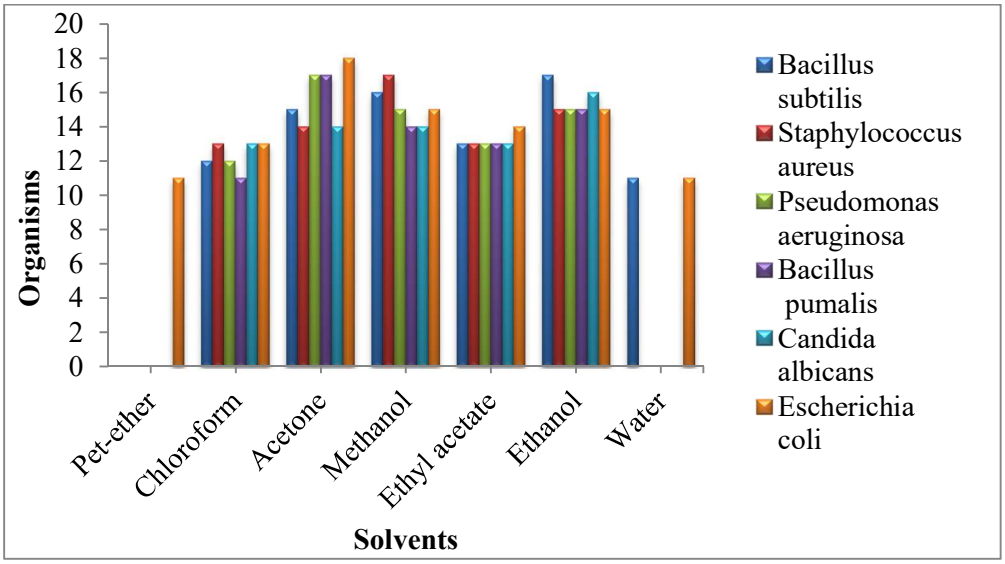
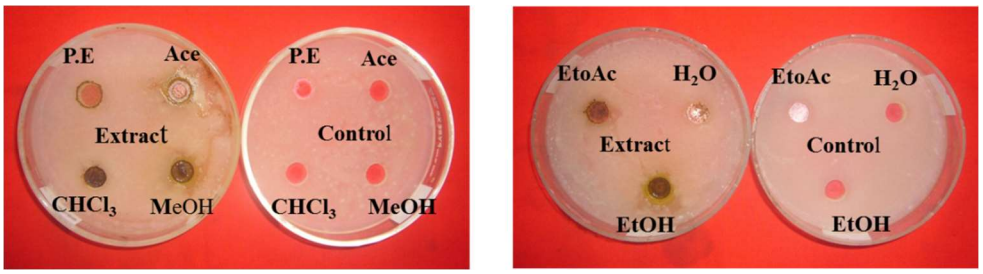
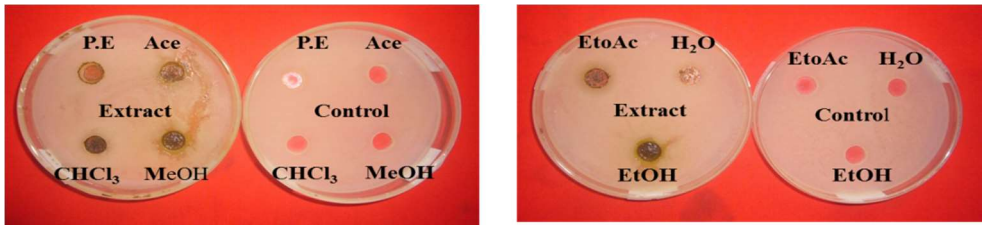


Figure 18. Antimicrobial activities against different solvent extracts from leaves of *Senna auriculata* (L.) Roxb.

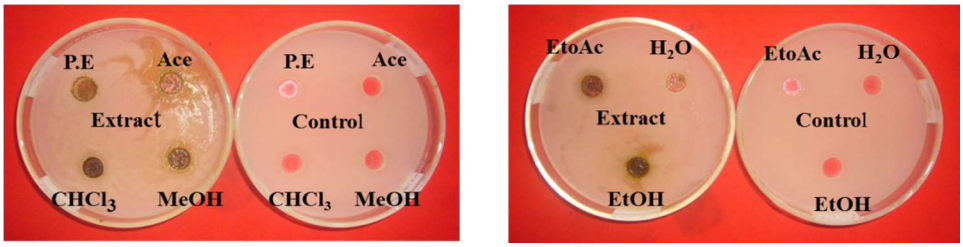
Antimicrobial activities of Leaves extracts from *Senna auriculata* (L.) Roxb.



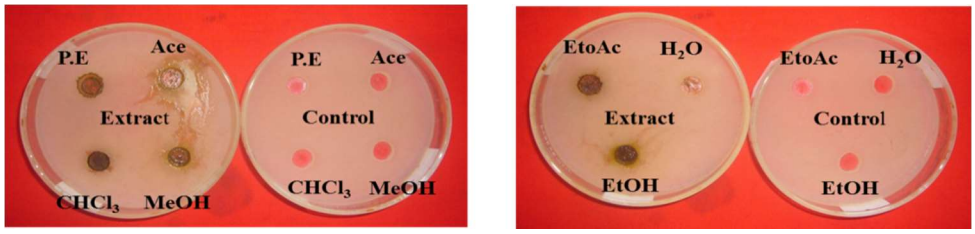
Bacillus subtilis



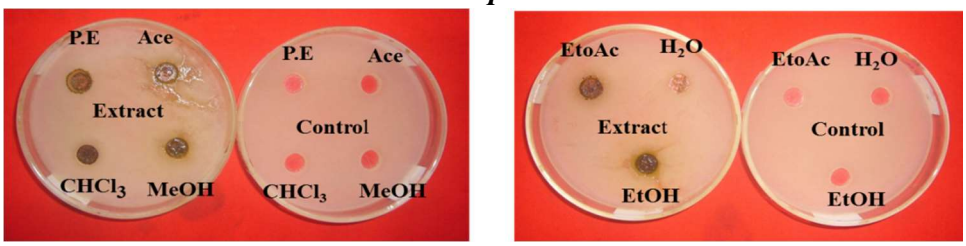
Staphylococcus aureus



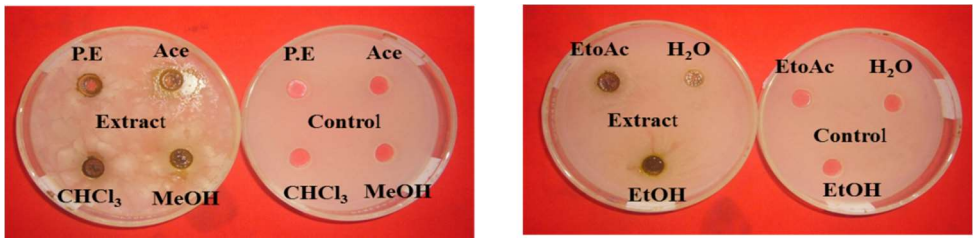
Pseudomonas aeruginosa



Bacillus pumalis



Candida albicans



Escherichia coli

Figure 19. Antimicrobial Activities from Leaves of *Senna auriculata* (L.) Roxb.

Discussion and Conclusion

The present study deals with the collection, identification and macroscopical studies of *Senna auriculata* (L.) Roxb. This plant belong to the family Fabaceae and commonly known as Mataran tea, Tanner's tea and locally known as Peik-thin-gat.

The plant are perennial shrubs, younger stems cylindrical and pubescent. The flowering time is September to February. The leaves are paripinnately compound, filiform glands present in between the leaflets and stipules auriculate or lunate-reniform. Inflorescences are terminal or axillary corymbose raceme, the flowers are bright yellow, bisexual and hypogynous. Stamens 10, 7 fertile and 3 sterile, dithecous, basifixed. The pistil monocarpellary, unilocular and gynophore present. The fruits are dehiscent pod, dark green and flattened; seeds ellipsoid (Figures 1 to 16). These outstanding characters are in agreement with those mentioned by (Hooker, 1879), (Kirtikar and Basu, 1933), (Burkill, 1935), (Cooke, 1958), (Backer, 1963), (Lawrence, 1964), (Purseglove, 1969), (Kurz, 1877) and (Dassanayake, 1991).

According to phytochemical tests (Table 2) , the results revealed that alkaloids, glycosides, phenolic compounds, reducing sugar, carbohydrates, tannins, flavonoids, terpenoids, steroids and α- amino acids were distinctly found and starch, cyanogenic glycosides and saponins were absent. The results showed that it consists of secondary metabolites have shown to be responsible for the therapeutic activity of plants (Trease and Evans, 2002).

The solubility tests were carried out to find the amount of total solid soluble in solvents. The powder of leaves were most soluble in water and moderately soluble in ethanol and methanol (Table 3). This results justifies it use in Myanmar Folkloric that the decoction of sample is remedy for the treatment of diabetes and skin diseases (Ashin Nargathein, 1972).

In the nutritional value evaluation, protein, crude fiber and crude fat were present in leaves of *Senna auriculata* (L.) Roxb. It was observed that energy value was 335 Kcal per100g (Table 4).

The antimicrobial activity was investigated by agar well diffusion method with six pathogenic microorganisms. Antimicrobial activities of extracts were evaluated by measuring the zone of inhibition. The acetone

extract and ethanol extract of leaves exhibit strong antimicrobial activity against all the tested microorganisms. The methanol extract showed good activity against all the tested microorganisms. The chloroform and ethyl acetate extracts showed moderate activity against on six pathogenic microorganisms. The aqueous extract of leaves showed weak activity against *Bacillus subtilis* and *Escherichia coli* and also the petroleum ether extract showed weak activity against *Escherichia coli* and did not show any against other tested microorganisms (Table 5 and Figures 18 and 19). The antimicrobial activity may be due to the presence of phytochemical constituents like terpenoid, flavonoid and phenolic compound present in the leaves of *Senna auriculata* (L.) Roxb. as secondary metabolites (Murugan and Wins, 2013).

In conclusion, The plant products plays an important role in the treatment of diseases without any side effects, there is a need to search new drugs from natural sources. Phytochemical screening revealed that the terpenoid and phenolic compounds might be responsible for the better inhibitory activity from the leaves extracts of *Senna auriculata* (L.) Roxb. The antimicrobical activity of *Senna auriculata* (L.) Roxb. leaves suggests that the extract contains the effective active phytochemicals responsible for the elimination of microorganisms. The inhibitory effect of the extract justified the medicinal use of *Senna auriculata* (L.) Roxb. and further study is required to find out the active component of medicinal value.

Acknowledgements

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PHYTOCHEMICAL AND PHYSICO-CHEMICAL STUDIES OF RIPE FRUIT PULP OF *BORASSUS FLABELLIFER* LINN. AND IT'S NATURAL COLOURANTS

Khin Win Kyi

Abstract

To day, the demand for natural colorants is increasing worldwide due to the increased awareness on therapeutic and medicinal properties and their benefits and also because of profound effects of synthetic dyes. This study was conducted to evaluate the food colourants from ripe fruit pulp of *Borassus flabellifer* L. (Htan), family Arecaceae. These plants are abundantly found in Myanmar and have many medicinal and economic values. The plant specimens used in this research were collected from Dalla Township, Yangon Region. Botanical identification of the plant was recorded with photo images. In this study, the colorants produced from fresh fruit pulp and powdered sample of *Borassus flabellifer* L. were used with powdered rice and agar to get rice cakes and agar desserts. The present study deals with the phytochemical and physicochemical screenings and nutritional values of ripe fruit pulp of *Borassus flabellifer* L. The phytochemical screenings revealed the presence of several phytochemicals. The phytochemical tests show the presence of alkaloids, glycosides, flavonoids, carbohydrates, reducing sugar and absence of cyanogenic glycosides. The physico-chemical properties of powdered ripe fruit pulp were investigated by using different solvents according to WHO guidelines. Nutritional value of ripe fruit pulp was investigated and shown that the presence of carbohydrate, sugar, protein, fats and vitamin C.

Key words: *Borassus flabellifer,* phytochemicals, natural colorants, nutritional values

Introduction

In recent years there has been an increasing demand for materials of natural origin, particularly regarding natural colourants (Trease and Evans, 2009). The present research work includes the extraction of natural colourants from a resource plant which is used in making of rice desserts in Myanmar.

Natural colourants are derived from naturally occurring sources such as plants, insects, animals and minerals. This study was carried out on

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carotenoids from fruit pulp in ripe fruits of *Borassus flabellifer*L. (Htan),used as food colourants in Myanmar. The sample used in this research were collected from Dalla Township,Yangon Region.

Borassus flabellifer L. is known as 'Htan' in Myanmar and belongs to the family Arecaceae. *Borassus* is one of the most widespread palm genera. Most species grow in low sandy Costal plains exposed to sun and wind. However some species such as *Borassus flabellifer* L. can occur in mountain districts of India and on river banks. In Ceylon, the soft yellow, pulpy tissue, under the outer skin of ripe fruit, is squeezed out and the juice is dried in thick layers into an edible preparation called punatoo (Dassanayake, 2000). The yellow coloured fruit pulp of *Borassus flabellifer* L. ripe fruit is applied externally in skin diseases (Kirtikar, 1975).

Natural dyes not only release medicinal properties but also improve the aesthetic value of the product and they are unique and ecofriendly (Grover and Panti, 2011). Carotenoids are natural pigments, comprising a class of hydrocarbons (carotenes) and their oxygenated derivatives (xanthophyll). Carotenoids are excellent singlet oxygen scavenger and are used as food colourants, food additives, cosmetics and nutraceuticals.

Carotenoids are a family of compounds of over 600 fat soluble plant pigments that provide much of the colour in nature. They are important nutritions for the human body owing to their provitamin A and antioxidant activity (Krinsky & Johson, 2005).

Dietary carotenoids are thought to provide health benefits in decreasing the risk of disease, particularly certain cancer and eye disease. In part, the beneficial effects of carotenoids may be due to their role as antioxidants.

The purpose of this research is to produce natural colourants from plant for use in pharmaceutical and food preparations. These colourants produced from natural plant sources, have less toxicity and more stability for human. Therefore the natural colourants of *Borassus flabellifer* L. were investigated in this research.

Aim and objectives of present research are (i) to verify the resource plant, *Borassus flabellifer* L. and investigation of its natural colourants, (ii) to examine the qualitative and quantitative analysis of ripe fruit pulp of *Borassus*

flabellifer L. and (iii) to explore the preparation of rice and agar dessert by using natural colourants from ripe fruit pulp of *Borassus flabellifer* L.

Material and Methods

Botanical Aspect

The specimens used in this research were collected from Dalla Township, Yangon Region. The ripe fruits of *Borassus flabellifer* L. were collected from June to September in 2013 and flowers were collected from December, 2013 to January, 2014. Fresh specimens were used for taxonomic identification with the help of available literatures such as Backer (1968), Dassanayake (2000), Hooker (1894).

The ripe fruits of *Borassus flabellifer* L. were washed, peeled and pulped. The pulp was extracted manually with a sieve. At the same day, fruit pulp (180 g) was extracted with various solvents and their solubility were observed. Some fruit pulp was dried in incubator at 30°C for one week. The dried samples were pulverized and stored in air-tight containers.

Chemical Aspect

Preparation of dessert by using natural colourants from ripe fruit pulp of *Borassus flabellifer* L.

Fresh fruit pulp (5 g) was mixed with rice powder (10 g) and 200 ml of water. The mixture was heated on stove. When the mixture become thick, it was poured into another container and heated with steam. Fresh fruit pulp (5 g) was mixed with agar powder (10 g) and added 200 ml of water. The mixture was heated on stove. When the mixture was become thick, it was poured into another container and let it cool. Powdered fruit pulp (1 g) was mixed with (2 g) rice powder and 40 ml of water. The mixture was heated on stove. When the mixture was become thick, it was poured into another container and heated with steam. Powdered fruit pulp (1 g) was mixed with (2 g) agar powder and 40 ml of water. The mixture was heated on stove. When the mixture was thick, it was poured into another container and let it cool.

Preliminary Phytochemical investigation of powdered samples of *Borassus flabellifer* L.

Preliminary phytochemical tests were made according to the method of Central Council for Research in Unani Medicine (1987) and Trease and Evans (2009).

Physico-chemical examination of ripe fruit pulp of *Borassus flabellifer* L.

Physico-chemical properties which includes moisture content, total ash, acid insoluble ash, water soluble ash and solubility in non polar and polar solvents such as pet-ether, ethyl acetate, acetone, ethanol, methanol and water soluble matter contents of powdered samples were carried out by the methods of British Pharmacopoeia (1968) and World Health Organization (1998).

Determination of some elements (EDXRF)

The elements were analyzed by using Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometer at Department of physics, University of Mandalay.

Quantitative determination of some elements by AAS

The total ash samples were used to study the constituents of elements by using Atomic Absorption Spectrophotometer (AAS) at Universities' Research Centre (URC).

Nutritional values of ripe fruit pulp

Protein, fibre, fat, carbohydrate and sugar contents have been determined at Food Industries Development Supporting Laboratory (FIDSL) by the method of A.O.A.C (Horwitz,1980).Vitamin C contents have been determined at Myanmar Scientific and Technological Research Department, according to the procedures given in the method of A.O.A.C (Horwitz, 1980).

Results

Botanical Aspect

Morphological characters of *Borassus flabellifer* L.

Figure 1. Habit

Figure 2. Fruits

Figure 3. Male flower

Figure 4. Pistilate flower

Habit of *Borassus flabellifer* L. are very tall, dioecious palms, trunk stout, unarmed. Leaves are terminal, fan-shaped, multifid; petiole deeply chanelled, armed with coarse irregular teeth. Spadices are very larges interfoliar, peduncle sheathed with open spathes; male spadix branched, rachillae large, cackin-like, closely imbricated bracts enclosed spikelets of flowers, sunken in cavity. Staminate flowers are minute. Sepals 3, connate; petals 3, connate; stamen 6, filament short, anther bilobed. Female spadix sparingly branched, bearing few scattered solitary flowers. Pistillate flowers are large, sepals 3,reniform, imbricate; petals 3; staminode 6, gynoecium rounded, tricarpellate, central basal placentation; stigma low and knob-like. Fruits are large, bearing 1 to 3 seeds, perianths persistent, epicarp smooth, mesocarp fibrous, endocarp composed of 3 pyrenes, seeds bilobed (Fig.1 to 4).

Chemical Aspect

Preparation of desserts by using natural colourants from ripe fruit pulp of *Borassus flabellifer* L.

The yellow coloured rice cakes and agar dessert was obtained by applying fresh ripe fruit pulp and pale yellow coloured desserts by applying powdered ripe fruit pulp (Fig.5-13).

Preparation of desserts from ripe fruit pulp of *Borassus flabellifer* L.

Figure 5. Ripe fruit

Figure 6. Ripe fruit pulp

Figure 7. Samples in incubator (air dry condition)

Figure 8. Semidried fruit pulp

Figure9. Powdered fruit pulp

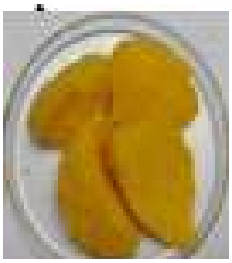

Figure 10. Fresh fruit Pulp rice cake

Figure 11. Fresh fruit pulp agar dessert

Figure 12. Powdered fruit pulp rice cake

Figure13. Powdered Fruit pulp agar dessert

Preliminary phytochemical investigation

In preliminary phytochemical investigation, the presence of alkaloid, glycoside, reducing sugar, saponin, phenolic glycoside, carbohydrate, flavonoid, terpenoid were observed but α-amino acid, tannin and cyanogenic glycoside were absent in ripe fruit pulp of *Borassus flabellifer* L. The results were shown in Table (1).

Table 1. Preliminary phytochemical tests on ripe fruit pulp of *Borassus flabellifer* L

No	Constituents	Extracted solvent	Test reagents	Observation	Results
1.	Alkaloid	3%HCL	1. Mayer's reagent	Whiteppt	+
			2. Dragendroff's reagent	Orangeppt	+
			3. Wagner's reagent	Reddish brownppt	+
2.	Glycoside	DW	10% lead acetate solution	White ppt	+
3.	Reducing sugar	DW	Fehling's solution	Brick red ppt	+
4.	Saponin	DW		Marked frosting	+
5.	Cyanogenic glycoside	DW	Sodium picrate, Conc. H_2SO_4	No colouration	-
6.	Phenolic compounds	DW	1% $FeCl_3$ solution	Deep blue colour	+
7.	α-amino acid	DW	Ninhydrin reagent	No colourchange	-
8.	Carbohydrate	DW	10% α-naphthol and conc. H_2SO_4	Pink ring	+
9.	Flavonoid	EtOH	Mg and conc. HCl	Pinkcolour	+
10.	Steroid	Benzene	Acetic anhydrate and conc. H_2SO_4	Pink colour	+
11.	Tannin	DW	0.1% $FeCl_3$ test solution	Green colour	-
12.	Terpenoid	Pet-ether	Acetic anhydrate and conc. H_2SO_4	Yellowish green	+

(+) = present (-) = absent ppt = precipitate

Physico-chemical investigation

Table 2. Physico-chemical properties of powdered ripe fruit pulp of *Borassus flabellifer* L.

No.	**Physico-chemical characters**	**Content (%)**
1.	Moisture content	15.74
2.	Total ash	5.63
3.	Acid insoluble ash	23.20
4.	Water soluble ash	5.67
5.	Water soluble matters	34.19
6.	EtOH soluble matters	37.10
7.	MeOH soluble matters	40.16
8	Acetone soluble matters	1.95
9.	EtOAc soluble matters	0.38
10.	Pet-ether soluble matters	0.11
11.	N-hexame soluble matters	0.62

Determination of some elements (EDXRF)

Table 3. Elemental analysis of ripe fruit pulp of *Borassus flabellifer* L. by using Energy Dispersive X-Ray Fluorescence spectrometer

No.	Elements	Concentration value (%)
1.	Cl (chlorine)	1.114
2.	K (Potassium)	0.917
3.	P (Phosphorus)	0.046
4.	Ca (calcium)	0.029
5.	S (Sulphur)	0.014
6.	Fe (Iron)	0.006
7.	Cu (Copper)	0.001
8.	Zn (Zinc)	0.001
9.	Rb (Rubidium)	0.001

Quantitative determination of some elements (AAS)

Table 4. Relative concentration of elements in ripe fruit pulp of *Borassus flabellifer* L. by using AAS

No.	**Elements**	**Concentration value (mg/L)**
1.	Cd (Cadmium)	0.089
2.	Cr (Chromium)	0.062
3.	Pb (Lead)	Not detected

Nutritional value of resource plant part

Table 5. Nutritional values of the ripe fruit pulp of *Borassus flabellifer* L.

No.	**Types of nutrients**	**Content (%)**
1.	Carbohydrate	**14.01**
2.	Sugar	**8.60**
3.	Protein	**0.73**
4.	Fat	**0.11**
5.	Fibre	**0.60**
6.	Vitamin C	**0.027**

Discussion

In this research, Morphological characters of *Borassus flabellifer* L. were in agreement with the character mentioned by Dassanayake (2000), Hooker (1894), Kirtikar and Basu (1957).The rice and agar dessert was prepared by using natural colourants of *Borassus flabellifer* L. The fresh natural colourants produced yellow coloured dessert and the powdered fruit pulp produced pale yellow coloured dessert. It was suggested that more interesting colour were obtained by using fresh fruit pulp.

In phytochemical test, the ripe fruit pulp of *Borassus flabellifer* L. indicated the presence of carbohydrate, reducing sugar, glycoside, alkaloids,

flavonoids, saponin, steroid and terpenoid except α amino acid, tannin and cyanogenic glycoside.

According to physico-chemical properties, the solubility of the powdered ripe fruit pulp of *Borassus flabellifer* L. was most soluble in acetone and moderately soluble in methanol and ethanol.These were in agreement with the findings of Vengalah *et.al*. (2015).

In ripe fruit pulp of *Borassus flabellifer* L., chlorine, potassium and phosphorus were found to be principal elements and the other elements such as calcium, sulphur, iron, chromium, copper, zinc and rubidium were trace elements.Minerals are important for the body to stay healthy. Minerals keep bones, muscles, heart and brain to work properly. Minerals which need for human body are calcium, phosphorus, magnesium, sodium, potassium, chloride and sulphur. Ripe fruit pulp of *Borassus flabellifer* L. contain chlorine 1.11 % and potassium 0.91%.

Chlorine is one of the most important electrolytes in the blood, along with sodium, potassium and calcium. Chloride is a highly important, vital mineral required for both human and animal life. Chloride is related to chlorine compounds in common salt NaCl. Chloride is a by-product of the reaction between chlorine and an electrolyte such as potassium, magnesium, or sodium, which are essential for human metabolism (Website 1).

Epidermiological and clinical studies show that a high potassium intake reduces cardiovascular disease mortality. This is mainly attributable to the blood pressure-lowering effects of potassium on the cardiovascular system. A high potassium diet may also prevent or atleast slow the progression of renal disease. The best way to increase potassium intake is to increase the consumption of fruits and vegetables (He, 2008).

The constituents of cadmium and chromium were determined as 0.089 % and 0.062% in ripe fruit pulp of *Borassus flabellifer* L. and lead is not detected.The poisonous level of cadmium and chromium were 0.3mg/ kg. Thus the constituents of Cadmium and chromium in ripe fruit pulp were not harmful for human health. The nutritional values of fresh ripe fruit pulp of *Borassus flabellifer* L. were determined as 14.01% carbohydrate, 8.6% sugar,0.73% protein, 0.11% fat and 0.60% fibre. The contents of carbohydrate were greater than others.

Carbohydrate is the most important food energy provider among the macronutrients, accounting for between 40 and 80 percent of total energy intake. The energy balance be maintained by consuming a diet containing at least 55 percent total energy from various sources of carbohydrates. It is engaging in regular physical activity. Although high- carbohydrate foods provide the full range of vitamin and mineral nutrients, some are also particularly rich in phytochemicals, many of which are antioxidants (FAO/WHO, 1998).

Conclusion

According to the present work is to distribute the apply knowledge of selection of natural colourants and preparation of desserts by using natural colourants with different vehicles (rice and agar) to local people in very simple methods. It is expected that the results of present research will contribute to useful information of natural colourants for applying in pharmaceuticals and food preparations as less toxic and more stable colourants for human.

This study is preliminary investigation of food colourants from plant sources. Further study for isolation and identification of compounds and shelf life of colourants will be needed to produce food colourants for health benefit.

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FIFTEEN SPECIES OF MEDICINAL PLANTS FROM KAWLIYA RESERVED FOREST IN DAIK-U TOWNSHIP, BAGO REGION

Naw Tharaphi Aung[1], Swe Swe Aye[2]

Abstract

The present study was carried out, to assess record and report the taxonomical and medicinal properties of wild plants from Kawliya Reserved Forest in Daik-U Township, Bago Region. Plants were collected from the research area during the flowering and fruiting period. All the specimens were classified and identified according to the standard literatures. Moreover, medicinal uses and other related information was confirmed by standard literatures. The present study comprises 15 species belonging to 12 families used by the local people. Among them 3 species are monocotyledons and 12 species are dicotyledons. The most common wild medicinal plants in Kawliya Reserved Forest are *Leea macrophylla* Roxb., *Desmodium triquetrum* (L.) DC., *Passiflora foetida* L., *Clerodendrum indicum* L. and *Thunbergia laurifolia* Lindl.. Most important families having medicinal importance are *Rauwolfia serpentina* (L.) Benth., *Gloriosa superba* L., *Senna alata* (L.) Roxb., *Zingiber zerumbet* (L.) Rosc. ex. J.E.Sm., *Justicia adhatoda* L., *Melastoma malabathricum* L. and *Costus speciosus* Sm. In this study area, *Rauwolfia serpentina* (L.) Benth. and *Gloriosa superba* L. are depleted due to over-exploitation of the local people.

Keywords: Kawliya Reserved Forest, Taxonomic and Medicine.

Introduction

Daik–U Township is located on the Yangon-Mandalay railroad and highway which lies to the West of Sittaung River in Southeasten part of Bago Region. It lies between North latitudes 17˚ 34′ and 17˚ 58′ and between East longitudes 96˚ 19′ and 96˚ 52′ (Mon Mon Htay, 2012). Forest coverage is 31% and it have Four Reserved Forest. These are Kawliya, Bainder, Auk-kanyin myaung and Salu Reserved Forest (Kyaw Zay Moe, 2008). Among them Kawliya Reserved Forest is the selected area for this research. The range of study area is 73898 acres. People in this study area rely on Reserved Forest

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in many ways such as timbers, fuel, food, fodder, medicinal plants, etc. The people in Daik-U Township are still forced to practice traditional medicine for their health care treatment. The knowledge of uses of plants are transmitted from one generation to the next. Over-exploitation and extension of new agriculture lands are a great impact of local human life as well as local human right. As a result a lot of medicinal plant habitats are degraded and valuable medicinal plant are becoming rarer.

This paper represented the taxonomic study and medicinal uses on 15 species from Kawliya Reserved Forest in Daik-U Township. The specimens were collected for the present study from July 2015-January 2016. In taxonomic study, the plants collected were classified and identified by using standard literatures. Such as (Lawrence, 1969), (Hooker, 1875), (Dassanayake, 1996) and (Hu Qi-ming, 2008 & 2009). Medicinal uses and other related informations was confirmed by standard literatures (Kyaw Soe & Tin Myo Ngwe, 2004 and Kirtikar & Basu, 1975). Elder people were real users and had a lot of information about the plants and their traditional uses.

The objectives of this work are to classify and identify some wild plants by using their morphological characters and to provide knowledge of medicinal plant uses of wild plants from Kawliya Reserved Forest.

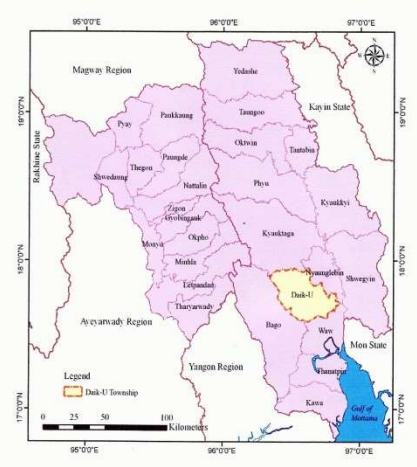

Figure 1. Location of Daik-U Township in Bago Region

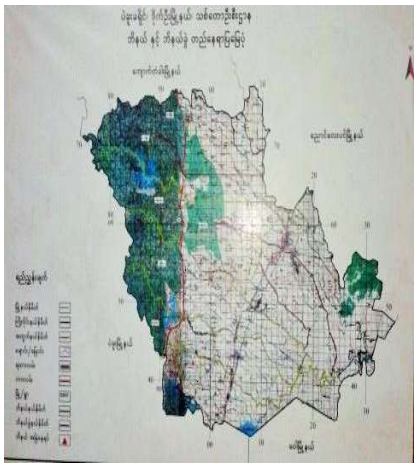

Figure 2. Location of Reserved Forest in Daik-U Township

Materials and methods

The present study was conducted during July 2015-January 2016. Work plan was prepared and general information about the area was gathered before the start of field work. Plants were collected from the research area during the flowering and fruiting period. Map were obtained from Forest Department, Daik-U Township, Bago Region, as shown in Figure 1 and 2.

Collection of medicinal Data

Frequent trips were arranged during raining and winter season during 2015-2016. Medicinal uses and other related information was confirmed by standard literatures (Kyaw Soe & Tin Myo Ngwe, 2004).

Morphological study of collected specimens

Plant were collected from various sites during the period of research work and documented according to (Lawrence, 1964).

The taxonomic descriptions are accompanied by the photographs of habits, a single branch with inflorescence, parts of the flower and fruit. Plants were classified and identified with the help of (Hooker, 1875), (Dassanayake, 1996) and (Hu Qi-ming, 2009). Classification system of selected species were arranged according to Angiosperm Phylogeny Group (APG-III).

Results

The collected species are arranged according to APG-III system as shown in Table 1.

Table 1. List of the 15 collected species of medicinal plants according to APG III.

Super order	Order	Family	Scientific Name
Lilianae	Liliales	Colchicaceae	(1) *Gloriosa superba* L.
	Zingiberales	Costaceae	(2) *Costus speciosus* Sm.
		Zingiberaceae	(3) *Zingiber zerumbet* (L.) Rosc. ex. J.E.Sm.
Rosanae	Vitales	Vitaceae	(4) *Leea macrophylla* Roxb.
	Fabales	Fabaceae	(5) *Senna alata* (L). Roxb.
			(6) *Desmodium gyrans* DC.
			(7) *Desmodium triquetrum* (L.) DC.
	Malpighiales	Passifloraceae	(8) *Passiflora foetida* L.
	Myrtales	Melastomataceae	(9) *Melastoma malabathricum* L.

Super order	Order	Family	Scientific Name
Caryophyllanae	Caryophyllales	Polygonaceae	(10) *Polygonum chinense* L.
Asteranae	Gentianales	Apocynaceae	(11) *Rauwolfia serpentina* (L.) Benth.
	Lamiales	Lamiaceae	(12) *Clerodendrum indicum* (L.) Kuntze.
		Acanthaceae	(13) *Thunbergia laurifolia* Lindl.
			(14) *Justicia adhatoda* L.
		Bignoniaceae	(15) *Markhamia stipulata* (Wall.) Seem. ex. K. Schum.

1. Scientific Name - ***Gloriosa superba*** L. (Semidauk)

Family - Colchicaceae

Morphological Character

Annual herbaceous climber. Stems cylindrical. Leaves alternate, sessile, laminae ovate-lanceolate, base cuneate, margin entire, tip modified into tendril. Inflorescence terminal and axillary, solitary to subcorymbose. Flower large and showy. Perianth segments 6, greenish yellow at the base with red tinge at the tips when young, becoming red after anthesis, margin crispy waved. Stamen 6, filament filiform, recurved. Carpel (3), trilocular, two ovule in each locule, axile plaecntation, style deflexed, stigma trifid. Fruits loculicidal capsules, distinctly 3-lobed. (Figure-3)

Flowering and Fruiting period : June to September

Parts used - Roots

Medicinal Uses - Toothace, stomach problem, scabies, ulcer, diarrhea, dysentery, leukorrhea, silkworm, piles, jaundice, ear disease, oxystocic, pustules, boils.

Location : N - 17° 53′ 27.7′, E - 096 ° 29′ 11.6′


Figure 3. Habit and Inflorescence of *Gloriosa superba* L.

2. Scientific Name - ***Costus speciosus*** Sm. (Hpalan-taung-hmwe)
Family - Costaceae

Morphological Character

Perennial herbs with underground rhizomes. Leaves simple, spirally arranged with the coriaceous sheath closed to the apex, laminae elliptic oblong, upper surface glabrous and lower one with silky hairs, base ovate, margin entire, tip acute. Inflorescence terminal cone like spikes with bright red bracts. Flower showy. Sepal (3), tubular. Petal (3), companulate. Stamen 1 fertile, epipetalous, lateral staminodes absent, other staminodes fused to form a labellum, infundibuliform, cream white with yellow center, filament of fertile stamen flattened, white with yellow apex. Carpel (3), 3-locule, many ovule in each locule, axile placentation, stigma protruding between the anther lobes. Fruits loculicidal capsule, ovoid, crowned by persistent calyx. (Figure-4)

Flowering and Fruiting period : June to October

Parts used - Stems, roots.

Medicinal Uses - Carminative, fever, cough, arthritis, bronchitis, inflammation, dyspepsia, rheumatism, earache.

Location : N - 17° 52′ 34.2′, E - 096 ° 29′ 16.3′

Figure 4. Habit and Inflorescence of *Costus speciosus* Sm.

3. Scientific Name - ***Zingiber zerumbet*** (L.) Rosc. ex. J.E. Sm. (Gan-eik)

Family - Zingiberaceae

Morphological Characters

Perennial rhizomatous herbs, leafy stem, pubescent. Leaves alternate and distinchous, simple, petiole long with sheathing bases, ligulate, laminae oblong-oblanceolate, base obtuse, margin enire, tip acuminate, both surface pubescent. Inflorescent borne separately from the leaves, peduncle up to 40 cm long, rounded at the apex, spikes. Flowers pale yellow, bracteate. Calyx 3-tooth, white tubular. Corolla 3-lobed. Stamen one fertile, other staminodes to form labellum, coloured as petal. Carpel (3), trilocular, 2-4 ovules in each locule, axile placentation, style filiform, stigma oblongoid, protruding between the anther lobes. Fruits not seen. (Figure-5)

Flowering and Fruiting period : June to September

Parts used - Rhizome

Medicinal Uses - Cough, asthma, worms, skin disease, laxative, stomachic, aphrodisiac, carminative, dyspepsia, inflammation, bronchitis, diarrhea.

Location : N - 17° 52′ 34.2′, E - 096 ° 29′ 16.3′


Figure 5. Habit and Inflorescence of *Zingiber zerumbet* (L.) Resc ex. J.E.Sm.

4. Scientific Name - ***Leea macrophylla*** Roxb. (Kya-hpetgyi)

Family - Vitaceae

Morphological Characters

Erect shrubs. Stems flexuose. Leaves alternate, simple, very large, laminae broadly ovate, base subcordate, margin coarsely serrate or sublobed, tip acute or acuminate, upper surface dark green and lower white, pubescent. Inflorescence terminal, much branched puberulous corymbose cymes. Flower small white, bracteates. Calyx (5), campanulate. Corolla (5), connate at the base and adhering to the steminal tube. Stamen (5), filament inserted between the lobes of the tube, anther connected laterally. Carpel (6), one ovule in each locule, style short, stigma thickened. Fruits berry, usually 3-6 lobes. (Figure-6)

Flowering and Fruiting period : July to November

Parts used	-	Leaves, roots
Medicinal Uses	-	Lymphadenitis, dry cough, tingling and numbness, septic wound, abortion, guinea-worm.
Location	:	N - 17° 53′ 25.0′, E - 096 ° 29′ 10.9′


Figure 6. Habit and Inflorescence of *Leea macrophylla* Roxb.

5. Scientific Name - *Senna alata* (L). Roxb. (Pwesay-mezali)

Family - Fabaceae (Caesalpinioideae)

Morphological Character

Perennial shrubs, horizontally branches. Stem furrowed. Leaves unipinately paripinnate compound, leaflets 10-15 pairs, bilateral symmetrical opposed and fold together at night, pulvinate, laminae oblong obtuse, base rounded oblique, margin entire, tip mucronulate. Inflorescence terminal and axillary racemose. Flower yellow cluster, bract large caducous. Sepals 5. Petals 5. Stamen 10, unequal, 7 fertile and 3 staminodes. Carpel 1, many ovule in the locule, style curved, stigma simple. Fruit black pod, thick flattened winged. (Figure-7)

Flowering and Fruiting period : November to February

Parts used - Leaves, pods.

Medicinal Uses - Constipation, dermatomytosis, scabies, tinea imbricate, arthritic.

Location : N - 17° 56′ 26.9′ , E - 096 ° 27′ 48.9′

Figure 7. Habit and Inflorescence of *Senna alata* (L). Roxb

6. Scientific name - ***Desmodium gyrans*** DC. (Say-kamyin)

Family - Fabaceae (Papilionoideae)

Morphological Characters

Shrubs. Stems cylindrical. Leaves alternate, trifoliolate compound, leaflets 1-2, unequal, laminae oblong-lanceolate, base rounded or obtuse, margin entire, tip obtuse, stipulate deltoid. Inflorescences terminal and axillary, racemes. Flower bluewish yellow, bracteates . Calyx campanulate, teeth deltoid, shorter than the tube. Standard orbicular, wings oblong-obovate, keels slightly beaked. Stamen 1+5, diadelphous. Carpel 1, monocarpellary, 4-7 ovule in the locule, style slender, stigma simple, gynophores present. Pods lomentum. Seeds 4-7, globose. (Figure-8)

Flowering and Fruiting period : December to February

Parts used - Leaves

Medicinal Uses - Antidote, cardiac-tonic, wound healing, rheumatism, pyrexia, dysentery, malaria, hepatitis, hemoptysis.

Location : N - 17° 52′ 45.6′, E - 096 ° 29′ 09.4′

Figure 8. Habit and Inflorescence of *Desmodium gyrans* DC.

7. Scientific Name - ***Desmodium triquetrum*** (L.) DC. (Lauk-thae)

Family - Fabaceae (Papilionoideae)

Morphological Characters

Erect shrubs. Stems triangular. Leaves alternate, unifoliolate compound, laminae lanceolae, base subcordate, margin ciliated, tip acute, both surface pubescent, petiole 1-1.5 cm long with broadly winged. Inflorescence axillary and terminal racemes. Flower violet, bracteates. Sepals (5), campanulate. Standard orbicular, wings oblong, keels slightly beaked. Stamen 1+(9), diadelphous. Carpel 1, monocarpellary, many ovule in the locule, ovary densely pubescent, stigma capitates. Fruits lomentum with calyx, flattened, 6-8 jointed. (Figure-9)

Flowering and Fruiting period : November to December

Parts used - Leaves

Medicinal Uses - Urinary infection, earache, helminthic, tuberculosis, lung disease, heatache, scabies.

Location : N - 17° 52′ 46.5′, E - 096 ° 29′ 09.4′

Figure 9. Habit and Inflorescence of *Desmodium triquetrum* (L.) DC.

8. Scientific Name - ***Passiflora foetida*** L. (Taw-suka)

Family - Passifloraceae

Morphological Characters

Tendrillar climbers. Stems herbaceous, cylindrical, pubescent. Leaves alternate, simple, stipules modified into tendrils, petioles with tomentose, laminae ovate with 3-lobed, bases cordate, margin ciliate, the tip acminate, both surface pilose. Inflorescence axillary and solitary cymes. Flower white, bracteoles 3, fimbriate, persistent. Sepals 5. Petals 5. Corona 2 rims, corona filament purple with white-tinged. Stamen 5, free, androgynophore present, the filament dilated, anther dithecous, dorsifixed. Carpel (3), trilocular, 2 ovule in each locule, parietal placentation, style 3, stigma capitates. Fruits berries. Seed numerous, flattened, arillate. (Figure-10)

Flowering and Fruiting period : March to November

Parts used - Leaves, fruits.

Medicinal Uses - Asthma, nervous anxiety, hysteria, giddness, skin disease with inflammation.

Location : N - 17° 53′ 24.8′, E - 096 ° 29′ 02.4′

Figure 10. Habit and Inflorescence of *Passiflora foetida* L.

9. Scientific Name - ***Melastoma malabathricum*** L. (Oboke-gyi)
Family - Melastomaceae

Morphological Characters

Strigose or villous shrubs. Stems cylindrical, short dense hair. Leaves opposite and decussate, laminae elliptic lanceolate, both surface pubescent, base ovate, margin entire, tip acute. Inflorescence terminal 3-7 flowered cymes. Flower mauve- purple, bract large elliptic narrowed into a stalk generally enclosing the buds. Sepals (5), deciduous. Petals 5. Stamem 5+5, outer whorl is sickle shaped and long, filament yellow. Carpel (5), many ovule in each locule, style simple, curved. Fruits loculicidal capsule with persistent calyx, oviod. Seeds numerous, oblongoid. (Figure-11)

Flowering and Fruiting period : Throughout the year

Parts used - Leaves, roots.

Medicinal Uses - Toothache, wound, stomach problems, diarrhea, dysentery, wash for ulcers to prevent scaring from smallpox, piles, leukorrhea, scabies, ulcers.

Location : N - 17° 56′ 33.9′, E - 096 ° 28′ 05.0′

Figure 11. Habit and Inflorescence of *Melastoma malabathricum* L.

10. Scientific Name - ***Polygonum chinense*** L. (Mahaga-kyan-sit)

Family - Polygonaceae

Morphological characters

Perennial herbs. Stems cylindrical and grooved at the bases. Leaves simple, alternate, stipules ochreate, usually sheathing the stem, laminae ovate oblong, base truncate or rounded, margin entire, tip acute. Inflorescence terminal, paniculate corymbose cymes. Flower white and small, bracteates. Perianth 5-lobed, connate up to half the length. Stamen (4+4), connate at the base, biseriate, the filament unequal, the anther ovoid, dithecous, dorsifixed. Carpels (3), unilocular, one ovule in each locule, basal placentation, style 2-3 cleft, stigma capitate. Fruits ovoid, nutlet with persistent style. (Figure-12)

Flowering and Fruiting period : November to May

Parts used - Whole plants

Medicinal Uses - Dysentery, gastroenteritis, hepatitis, wash for dermatitis, worm, scorpion bite, arthrtis, oedema.

Location : N - 17˚ 53′ 26.2 ″,E - 096˚ 29′ 11.4 ″

Figure 12. Habit and Inflorescence of *Polygonum chinense* L.

11. Scientific Name - ***Rauvolfia serpentine*** (L.) Benth (Bonma-yaza)

Family - Apocynaceae

Morphological Characters

Perennial undershrubs. Stems cylindrical. Leaves whorls, simple, green in upper surface, pale green in lower surface, the laminae ovate, the bases attenuate, the margins entire, the tips rounded or acute. Inflorescence terminal corymbose cymes. Flower pink, bracteae. Sepals (5), campanulate, lobe unequal. Petals (5), corolla tube long, white hair present in the tube. Stamens 5, epipetalous, filament very short. Carpels (2), bilocular, 2 ovule in each locule, style long and filiform, stigma bi-apiculate. Fruit a drupe, deeply 2-lobed. Seeds ovoid. (Figure-13)

Flowering and Fruiting period : October to March

Parts used - Roots

Medicinal Uses - Hypertension, helminthic infection,

Location : N - 17° 53′ 27.7′, E - 096 ° 29′ 11.6′

Figure 13. Habit and Inflorescence of *Rauwolfia serpentina* (L.) Benth.

12. Scientific Name - ***Clerodendrum indicum*** (L.) O. Ktze. (Nga-yant-padu)

Family - Lamiaceae

Morphological Characters

Shrubs. Stem quandrangular. Leaves 3-5 whorled, laminae oblong-lanceolate, base acute, margin entire, tip acuminate. Inflorescence terminal and axillary dichasial cymes. Flower white, bracteate. Sepals 5, connate at the base, cup-shaped, persistent. Petals 5-lobed, salverform. Stamens 4, epipetalous, didynamous,filament white with red top. Carpels 2, bicarpellary, 4 locules due to false septum, axile placentation, one ovule in each locule, style filiform, stigma bifid. Fruit a drupe, globose, persistent calyx. (Figure-14)

Flowering and Fruiting period- November to March

Parts used - Leaves, roots.

Medicinal Uses - Indigestion, piles, amenorrhoea, giddness, vomitng, improvement of memory due to neural deficit, white-patches.

Location : N - 17° 52′ 33.8′, E - 096 ° 29′ 16.2′

Figure 14. *Clerodendrum indicum* (L.) Kuntze.

13. Scientific Name - ***Thumbergia laurifolia*** Lindl. (Panye-sut)

Family - Acanthaceae

Morphological Characters

Woody vines. Stem subquandragular, node slightly tumid. Leaves opposite and decussate, laminae elliptic or oblong-lanceolate, base rounded to caudate, margin entire or slightly undulate, tip acuminate. Inflorescence terminal and axillary racemose. Flower purple, bracteoles large, more or less obliquely oblong, cream colour with reddish brown veins. Calyx annular ring unlobed. Corolla purple, lobes 5, subequal, tubular below, campanulate above. Stamens 4, didynamous, filament flattened, anther with hairy, epipetalous. Carpels (2), bilocular, two ovule in each locule, style slender, stigma bifid, funnel shaped. Fruits capsule. (Figure-15)

Flowering and Fruiting period : December to March

Parts used - Leaves, roots.

Medicinal Uses - Menorrhagia, ear ailments, deafness, alcoholic liver toxicity, scorpion bite, diabetic, anti-inflammatory, diarrhea.

Location : N - 17° 52′ 46.6′, E - 096 ° 29′ 09.4′

Figure 15. Habit and Inflorescence of *Thunbergia laurifolia* Lindl.

14. Scientific Name - ***Justicia adhatoda*** L. (Maya-gyi)

Family - Acanthaceae

Morphological Characters

Large shrubs. Stems cylindrical, swollen above the nodes. Leaves opposite and decussate, laminae elliptic, base obtuse, margin entire, tip

acuminate. Inflorescence terminal and axillary spikes. Flowers bilabiate, white with spot, bracteate, bracteolate, sessile. Sepals 5-tooth, campanulate. Petals bilabiate, limb 2-lipped, upper lip erect, shallowly 2-lobed, lower lip 3-lobes. Stamens 2, petalostemonous, the filament long. Carpels (2), bilocular, one ovule in each locule, the style long, stigma simple, disc present. Fruit not seen. (Figure-16)

Flowering and Fruiting period : January to May

Parts used - Leaves, roots.

Medicinal Uses - Haematemesis, melena, pulmonary disease, bleeding piles, dry cough, bronchitis, asthma, diabetes, tuberculosis, heavy menstrual bleeding.

Location : N - 17° 53′ 25.0′, E - 096 ° 29′ 10.9′

Figure 16. Habit and Inflorescence of *Justicia adhatoda* L.

15. Scientific Name - ***Markhamia stipulata*** (Wall.) Seem. ex K.Schum. (Ma-hlwa)

Family - Bignoniaceae

Morphological Characters

Perennial trees. Leaves unipinnately imparipinnate compound, leaflets 3-9 pairs, laminae ovate oblong, base slightly cordate, margin entire, tip acute. Inflorescence terminal racemose, rustly yellow pubescent, 4-10 flowered. Flower brownish yellow, bracteate, bracteolate. Sepals aestivaation closed, at flower time clef to the base on one side, spathaceous, brown hairy. Petals (5),

campanulate, lobe crisped crenate. Stamens 4, epipetalous, didynamous. Carpel 1, many ovule in the locule, style terminal, filiform, stigma bifid. Fruits capsule, linear-oblong, epicarp brown hairy. Seed including wing. (Figure-17)

Flowering and Fruiting period : December to April

Parts used - Roots, flowers.

Medicinal Uses - Skin disease, analgesic

Location : N - 17° 56′ 26.9′, E - 096 ° 27′ 48.9′

Figure 17. Habit and Inflorescence of *Markhamia stipulata* (Wall.) Seem. ex. K. Schum.

Discussion and conclusion

This paper provide comprehensive information on the native and the uses of medicinal plants in Kawliya Reserved Forest in Daik- U Township, Bago Region. The present investigation comprises the indigenous uses of 15 species belonging to 12 families from the study area. Among them 3 species are monocotyledons and 12 species are dicotyledons. Fabaceae has the highest number of 3 species followed closely by Acanthaceae with 2 species. The remaining families were represented with 1 species each. The most common wild medicinal plants in Kawliya Reserved Forest are *Leea macrophylla* Roxb., *Desmodium triquetrum* (L.) DC., *Passiflora foetida* L., *Clerodendrum indicum* L. *Thunbergia laurifolia* Lindl. and *Markhamia stipulata* (Wall.) Seem. The most important species having medicinal importance are *Rauwolfia serpentina* (L.) Benth. which use traditionally to reduce hypertension (Nargathein, 1972 and Bhattacharjee, 1998). The roots of *Costus speciosus*

Sm. is useful for dyspepsia, inflammation and rheumatism (Kirtikar & Basu, 1958). The treatment of caught, asthma and worm is used to *Zingiber zerumbet* (L.) Rosc ex. Sm. and diarrhea and dysentery is used to *Melastoma malabathricum* L. (Kirtikar & Basu, 1958). *Gloriosa superba* L. is useful for the commercial colchicine compound production (Ponglux et. al., 1987) and *Justicia adhatoda* L. have the bioactive compounds , amrinone and phytol (Jayapriya & Shoba, 2015). *Senna alata* (L). Roxb. is the most potent species for having significant antimicrobial activity (Nayak et. al., 2015). *Desmodium gyrans* DC. have anticoagulant activity (Vipin et. al, 2015) and the ethanol extract of *Polygonum chinense* L. against on *Escherichia coli* and *Pseudomonas aeruginosa* (Thomas et. al., 2012). Among them, *Rauwolfia serpentina*. (L.) Benth. and *Gloriosa superba* L. are depleting in study area due to over-exploitation by the local people. In summary, the conservation of the wealth of medicinal plant resources and transfer of plants knowledge should be established with the cooperation of local people otherwise the potential medicinal plants in Kawliya Reserved Forest may be lost forever and become extinct.

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TAXONOMIC STUDY ON EIGHT SELECTED SPECIES OF FAMILY ORCHIDACEAE IN SOUTHERN PART OF KALAMA TAUNG RESERVED FOREST, PAUNG TOWNSHIP, MON STATE

Thant Zaw Win[1], Moe Sandar Shein[2]

Abstract

The present research deals with a taxonomic study on family Orchidaceae in Southern part of Kalama Taung Reserved Forest, Paung Township, Mon State. This study feature was represented by eight species belong to eight genera of family Orchidaceae. There were two terrestrials and six epiphytic species. ***Eulophia zollingeri*** (Rchb. f.) J.J. Sm. and ***Malaxis versicolor*** Abeyw. are terrestrial and ***Coelogyne schilleriana*** Rchb. f., ***Bulbophyllum crassipes*** Hook. f., ***Dendrobium fimbriatum*** Hook., ***Liparis viridiflora*** (Blume) Lindl., ***Renanthera coccinea*** Lour. and ***Rhynchostylis retusa*** Blume are epiphytes. The collected eight species were identified and classified. Morphological characters, common names, flowering periods and Global Positioning System (GPS) were also presented.

Key words: Orchidaceae, Southern part of Kalama Taung Reserved Forest

Introduction

Myanmar occupies an area of 678,033 sq km in Southeast Asia. Mon State extends 12,155 sq km at the southeast of Myanmar. Southern part of Kalama Taung Reserved Forest covers 101.79 sq km in Paung Township, Mon State. It is situated between North-latitude 16° 36′ and 16° 50′ and East-longitude 97° 25′ and 97° 33′ (Fig. 2).

Up to 1000 genera and 15-20,000 species; some estimates run as high as 30,000 species (Cronquist, 1981). According to Kress *et al.* (2003), 131 genera and 738 species were recorded in checklist of Myanmar.

The family has a cosmopolitan distribution, and orchids may be found under nearly all conditions-as understory plants in dark, tropical lowland forests; at the top of all trees in the rain forest, where they are intermittently baked by the sun and then showered by torrential rain; in grassy and marshy

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areas; along the sides of the street; and as pioneering plants on landslides. They are only absent from extreme environments, such as the sea, the driest deserts, and the tops of the coldest mountains. The terrestrial habit prevails in temperate regions, but in the tropics most species are epiphytes (Heywood *et al*., 2007).

Some of the characters which support this family are: (1) Zygomorphy of flowers accompanied with different type of labellum formation and spur, (2) epigynous flowers, epigyny provides greater protection to the ovary, (3) majority of the plants are herbs, which may be epiphytes or saprophytes, (4) Constant reduction and suppression of the members of androecium and formation of pollinium, (5) Reduction in the stigmatic lobes which often develop into rostellum. Formation of gynostemium facilitates insect pollination, (6) Pollen is often sticky where there is no need of a thick sculptured exine and (7) Diversity in shape and size of flowers which may be of various types (Verma, 2011).

Orchids are never wind-pollinated. External agents such as ants, bees, wasps, flies and butterflies are responsible for the transfer of pollinia on to the stigmas in their search for nectar. Seeds of orchids are duct-like, exalbuminous and they are dispersed by the wind. The family is characterized by the absence of a root in the embryo. Hence, it is dependent on a symbiotic relationship with certain mycorrhizal fungi for its early supply of food during germination. In nature the environment plays an important part in the struggle for existence of this family (Jayaweera, 1981).

Orchid is suffering from an uncertain future through over exploitation, habitat loss due to human activities and impact of climate change. The future of orchid population is disturbing and the world will face the extinction of many species. In Asia, climate change occurs rapidly due to compound pressure on natural resources and the environment associated with rapid urbanization, industrialization and economic development (Barman and Devadas, 2013).

The aim of this research is to identify the morphological characters of collected species and to describe the outstanding characters of orchid species.

Materials and Methods

The specimens were collected from Southern part of Kalama Taung, Paung Township, Mon State during 2013-2016. The morphological characters of vegetative and reproductive parts and Global Positioning System (GPS) were recorded. The specimens were prepared and preserved based on the herbarium techniques of Lawrence (1964). Then, the herbarium specimens were deposited in Herbarium of Yangon University for references and other researchers. The collected plant specimens were identified based on Bartle (1966), Chen *et al*. (2009), Jayaweera (1981) and Seidenfaden (1992).

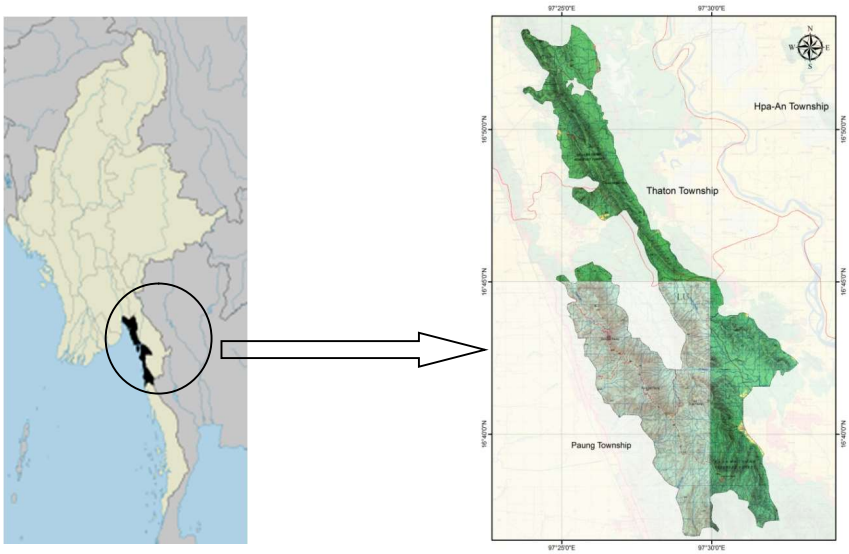
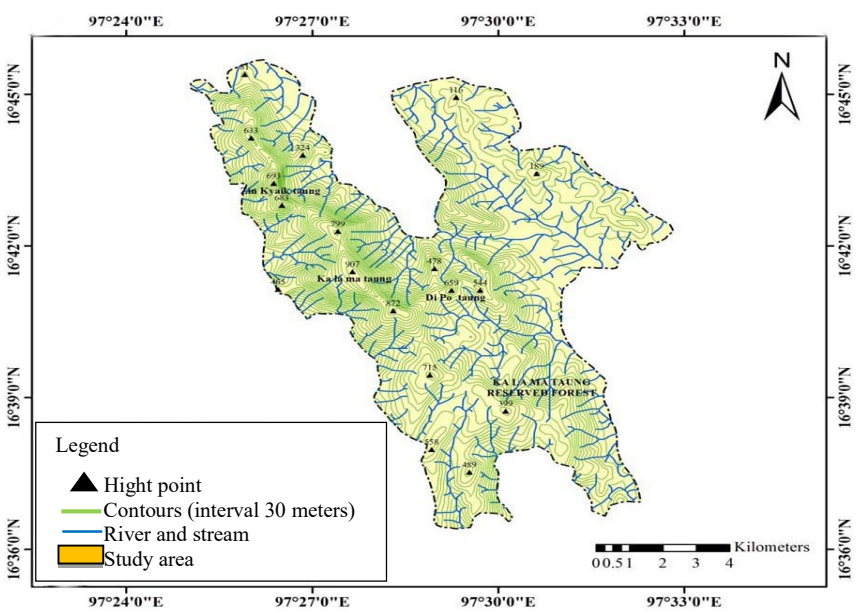


Figure 1. Map of Kalama Taung Reserved Forest in Thaton and Paung Township, Mon State in Myanmar



Source : UTM

Figure 2. Location Map of Southern part of Kalama Taung Reserved Forest

Results

The collected eight species belong to eight genera, five tribes, two subfamilies of family Orchidaceae were classified according to Dressler (1981).

Family	Subfamily	Tribe	Scientific Name
Orchidaceae	Epidendroideae	Coelogyneae	(1) *Coelogyne schilleriana* Rchb. f.
		Epidendreae	(2) *Bulbophyllum crassipes* Hook. f.
			(3) *Dendrobium fimbriatum* Hook.
		Malaxideae	(4) *Liparis viridiflora* (Blume) Lindl.
			(5) *Malaxis versicolor* Abeyw.
	Vandoideae	Cymbidieae	(6) *Eulophia zollingeri* (Rchb. f.) J.J. Sm.
		Vandeae	(7) *Renanthera coccinea* Lour.
			(8) *Rhynchostylis retusa* Blume

Key to the studied genera:

1. Plant terrestrial ----------2
1. Plant epiphyte or lithophyte ----------4
   2. Pseudobulb present; inflorescence with lax and medium flower --- 4. ***Eulophia***
   2. Pseudobulb absent; inflorescence with crowded and small flower ----- 3
3. Column short; lip non-resupinate ---------- 6. ***Malaxis***
3. Column long; lip resupinate ---------- 5. ***Liparis***
   4. Pseudobulb absent ---------- 5
   4. Pseudobulb present ---------- 6
5. Inflorescence erect, spreading; flowers lax ----------7. ***Renanthera***
5. Inflorescence pendent; flowers densely crowded ----------8.***Rhynchostylis***
   6. Leaves solitary; inflorescence appear from the base of the pseudobulb ---------- ----------1. ***Bulbophyllum***
   6. Leaves two or more; inflorescence axillary or terminal ---------- 7
7. Pseudobulb various shape; inflorescence pendent; flowers large and small-- 3.***Dendrobium***
7. Pseudobulb ovoid; inflorescence erect; flowers medium ------ 2.***Coelogyne***

1. ***Coelogyne schilleriana*** Rchb. f., Allg. Gartenz. 26: 189. 1858.

Epiphytic and lithophytic herb; pseudobulbs globosely ovoid, 1-2 cm, covered with leathery sheath. Leaves 2, at the end of undeveloped pseudobulb; leaf blade elliptic-lanceolate, 4.0-7.0 x 0.6-1.5 cm, margin entire, apex acuminate, coriaceous. Flowers solitary from between the leaves, 3-4 cm in diameter, tawny yellow; bracts sheathing imbricate, 1.0-1.5 x 0.4-0.7 cm, deciduous; peduncle 3-4 cm long. Dorsal sepal oblong-elliptic, 3.0-3.5 x 1.0-1.3 cm, apex acute; lateral sepals lanceolate, 2.8-3.3 x 0.6-0.8 cm, acuminate, deflexed. Petals linear, 2.8-3.3 x 2.0-4.0 mm, 1-veined. Lip erect, embracing the column, 3-lobed, 2.3-3.0 x 3.0-4.0 cm; lateral lobes oblong, apex rounded, parallel with the column; middle lobe very broad, wavy, crisped, notched at the tip, pale yellow with dark reddish blotches. Column concave, 1.5-1.8 cm long, winged on both sides, tip rounded. Anthers 2-celled; pollinia 4, in 2 pairs, waxy. Capsule obovate, 2.0-3.0 x 1.0-1.5 cm, pendulous, with narrowly winged.

Common name: Not known (Fig. 3)

Ecology : N Lat.- 16° 44′ 10.8″, E Long.- 097° 25′ 56.6″, Altitude- 608 m; Flowering and Fruiting period: February to May; epiphytic on *Artocarpus heterophyllus* Lam. and rocks.

Specimen examined: Min-taya-tapar Taung, Paung Township; 22 April, 2015; Thant Zaw Win, Coll. no. 004 (RGN-20037).

Habit Inflorescence Flower

Figure 3. *Coelogyne schilleriana* Rchb. f.

2. *Bulbophyllum crassipes* Hook. f., Fl. Brit. Ind. 5 (16): 760.1890.

Epiphytes with a creeping rootstock; pseudobulbs ovate, 2.0-4.0 x 1.5-2.5 cm. Leaf one, appear from each pseudobulb; petioles 1-2 cm long; leaf blade oblong, 10-19 x 2-3 cm, strap-shaped, apex obtuse and emarginate, thickly leathery. Scape arching, from the base of pseudobulb; raceme 2-7 cm long, densely many-flowered; floral bracts longer than pedicel with ovary, ovate-lanceolate, 5-8 x 2-4 mm, apex acute, pale brown. Flowers yellow, dense cylindrical spike; bracts 4-7 x 2-3 mm. Dorsal sepal oblong, concave, 4-6 x 2-3 mm, apex obtuse; lateral sepals ovate-lanceolate, 6.0-8.0 x 2.0-3.5 mm, much longer than dorsal sepal, apex acute, lower edges connate. Petals triangular, 2.0-3.0 x 0.5-1.0 mm, base oblique and decurrent to column foot, apex caudate. Lip ligulate, 2.5-3.5 x 1.5-2.0 mm, longer than petals, fleshy, margin finely papillate, grooved at adaxial base, with 2 auricles on both basal sides; auricles subsquare, about 1 mm long, apex truncate and slightly retuse. Column with deltoid teeth, 2.5-3.0 x 1.3-1.7 mm; foot about 2 mm long; anther cap glabrous; pollinia 4, cohering in pairs, oblong, the two inner pollinia smaller.

Common name: Not known (Fig. 4)

Ecology: N Lat.- 16° 35′ 51.0″, E Long.- 097° 29′ 09.0″, Altitude- 25 m; Flowering and Fruiting period: January to April.

Specimen examined: Mu-kyi Taung, Paung Township; 24 January, 2015; Thant Zaw Win, Coll. no. 002 (RGN-20036).

Habit Inflorescence Flower

Figure 4. *Bulbophyllum crassipes* Hook. f.

3. *Dendrobium fimbriatum* Hooker, Exot. Fl. 1: ad t. 71. 1823.

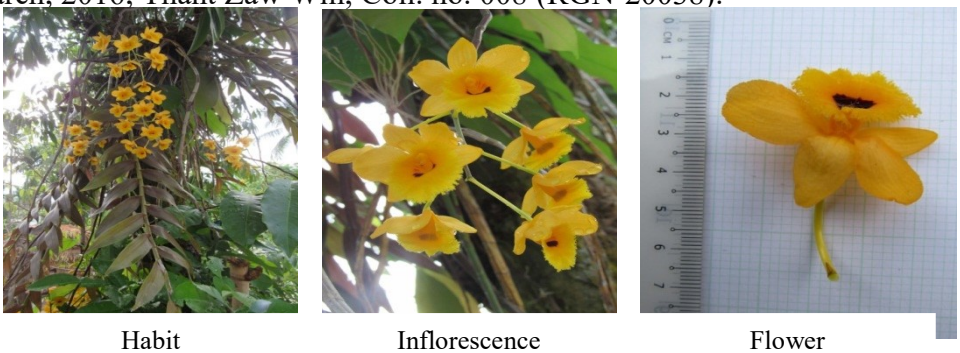
(Syn.: *Callista fimbriata* (Hook.) Kuntze, *C. oculata* (Hook.) Kuntze, *Dendrobium fimbriatum* var. *occulatum* Hook. f., *D. fimbriatum* var. *oculatum* Hook., *D. paxtoni* Paxton)

Epiphytic herb, 40-90 cm high, pendulous; internodes 1.5-4.0 cm long, longitudinally grooved. Leaves distichous along the stem; leaf blades oblong-lanceolate, 7-15 x 1-3 cm, base tightly clasping sheaths, apex acute, slightly bilobed, leathery. Inflorescences racemes, 5-15 cm long, laxly 6-15 flowered, pendulous; peduncle 2-4 cm long; rachis thin, curved; basal sheaths tubular, 3-10 mm, overlapping, membranous. Flowers golden yellow, fragrant, 3.5-4.5 cm wide, spreading, thinly textured; bracts ovate-triangular, 3-5 mm long, apex acute, membranous; pedicels and ovary 2.5-3.0 cm long. Dorsal sepal oblong, 1.3-2.5 x 0.6-1.1 cm, 5-veined, apex obtuse; lateral sepals ovate-lanceolate, as long as dorsal, 5-veined, base oblique, apex obtuse; mentum rotund, 2-5 mm long. Petals oblong-elliptic, 1.2-2.5 x 0.7-1.5 cm, margin erose, apex obtuse, 5-veined. Lip suborbicular, 1.5-2.5 x 2.0-3.0 cm, base narrowed into a claw about 3 mm long, margin compound fimbriate, adaxially densely pubescent, transversely lunate deep purple spot, with purplish red stripes on either side at base. Column about 2 mm; foot 4-6 mm long; anther cap conic, glabrous, front margin denticulate.

Common name: Ar-me-let-tan-shae (Fig. 5)

Ecology : N Lat.- 16° 44′ 10.0″, E Long.- 097° 24′ 59.4″, Altitude-74 m; Flowering and Fruiting period: February to May.

Specimen examined: Min-taya-tapar Taung, Paung Township; 14 March, 2016; Thant Zaw Win, Coll. no. 008 (RGN-20038).



Habit Inflorescence Flower

Figure 5. *Dendrobium fimbriatum* Dalzell

4. *Liparis viridiflora* (Blume) Lindl., Gen. Sp. Orch. Pl. 31. 1830.

(Syn.: *Cestichis longipes* (Lindl.) Ames, *Leptorkis longipes* (Lindl.) Kuntze, *L. viridiflora* (Blume) Kuntze, *Liparis longipes* Lindl., *L. pendula* Lindl., *L. pleistantha* Schltr., *L. simondii* Gagnep., *L. spathulata* Lindl., *Malaxis viridiflora* Blume, *Sturmia longipes* (Lindl.) Rchb. f.).

Tufted epiphyte; pseudobulbs densely arranged, ovoid, 1.0-6.0 x 0.8-2.0 cm, attenuate to apex, clothed in papery sheaths. Leaves 2, sessile, jointed continuing with pseudobulb; leaf blade oblanceolate, 4-24 x 1-3 cm, apex acuminate, drooping, subcoriaceous, 5-7 veined. Inflorescence terminal, dense-flowered raceme, longer than leaves, 14-18 cm long, pendulous; rachis terete, 5-130 cm long. Flowers greenish white, 2.0-3.5 mm in diameter; floral bracts narrowly lanceolate, 2.0-6.0 x 0.5-1.5 mm, apex acuminate, 1-veined, membranous; pedicels and ovary 4-7 mm long. Dorsal sepal oblong, 2-5 x 1-2 mm, margin revolute, apex obtuse; lateral sepals ovate-elliptic, recurved and reflexed, 1-veined, slightly wider than dorsal. Petals narrowly linear, 2-6 x 0.4-1.0 mm, margin revolute, apex rounded, 1-veined. Lip inferior, ovate-oblong, 2.0-3.5 x 1.5-2.0 mm, margin undulate, apex subacute, recurved about the middle, thickened, adnate to the base of the column. Column slender, incurved, 1.5-2.5 x 0.5-0.7 mm, base slightly enlarged. Anther terminal, 2-loculed; pollinia 4, waxy in two pairs, spindle-shaped. Capsule obovoid-ellipsoid, 4-6 x 3-4 mm, fruiting pedicel 3-6 mm long.

Common name: Tha-zin-bo (Fig. 6)

Ecology : N Lat.- 16° 36′ 20″, E Long.- 097° 28′ 30.″, Altitude- 300 m; Flowering and Fruiting period: December to March; epiphytes on *Dipterocarpus costatus* Gaertn.

Specimen examined: near Ah-me Stream, Mu-Kyi Taung, Paung Township; 3 January, 2016; Thant Zaw Win, Coll. no. 007 (RGN-20040).

Habit Inflorescence Flower

Figure 6. *Liparis viridiflora* (Blume) Lindl.

5. *Malaxis versicolor* Abeyw., Ceylon J. Sci., Biol. Sci. 2: 147. 1959.

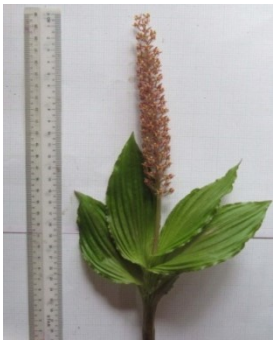
Terrestrial herb, 20-40 cm high; pseudobulbous annulate. Leaves sessile; leaf blade ovate, 10-16 x 5-8 cm, base continuous with the sheath, margin wavy, apex acuminate, plicate, membranous. Inflorescence terminal, cylindrical racemose, flowering bearing portion 10-17 cm long; peduncle 5-10 cm long. Flowers small, pink; floral bracts ovate, 3-6 x 1-3 mm, 1-veined; pedicels 0.7-1.0 cm long. Dorsal sepal ovate-subulate, 4-7 x 2-4 mm, apex acute, 3-veined; lateral sepals obliquely oblong, apex obtuse, deflexed, 3-veined. Petals lanceolate, 4.0-6.0 x 0.5-1.0 mm, apex truncate, 1-veined. Lip superior, subquadrate, 1.6-2.0 x 2-3 mm, base hollowed and adnate to the base of the column, with two large auricles close to the sides of the column; apex pectinate, 9-13 toothed, mid-tooth short, blunt and trifid at the apex. Column 2 suberect horns at the apex, 1.0-1.3 x 0.5-0.8 mm, narrowing towards the middle and again broadening towards the base. Anther terminal, 2-loculed; pollinia 4, cohering in two pairs, obovoid.

Common name: Not known (Fig. 7)

Ecology : N Lat.- 16° 36′ 33.7″, E Long.- 097° 29′ 02.0″, Altitude- 28 m; Flowering and Fruiting period: April to July; terrestrial at shady and humid places.

Specimen examined: Oak-ta-tar Taung, Paung Township; 28 May, 2015; Thant Zaw Win, Coll. no. 005 (RGN-20041).

Habit



Inflorescence

Flower

Figure 7. *Malaxis versicolor* Abeyw.

6. *Eulophia zollingeri* (Rchb. f.) J.J. Sm., Fl. Buitenz. 6: 228. 1905.

(Syn.: *Cyrtopera formosana* Rolfe, *C. papuana* Ridl., *C. rufa* Thw., *C. sanguine* Lindl., *C. zollingeri* Rchb. f., *C. rufum* (Thw.) Trimen, *C. sanguineum* (Lindl.) N.E. Br., *Eulophia carrii* C.T. White, *E. formosana* (Rolfe) Rolfe, *E. macrorhiza* Blume, *E. ochobiensis* Hayata, *E. papuana* (Ridl.) J.J. Sm., *E. sanguine* (Lindl.) Hook. f., *E. yushuiana* S.Y. Hu, *Graphorkis macrorhiza* (Blume) Kuntze, *G. papuana* (Ridl.) Kuntze, *G. rufa* (Thw.) Kuntze, *G. sanguine* (Lindl.) Kuntze)

Plants terrestrial herb with horizontal tuberous rootstock, 3-12 x 2-4 cm, with vermiform roots. Leaves produced after flowering. Inflorescence racemose, 40-80 cm long; peduncles 30-50 cm long, with several scattered and clasping sheaths. Flowers spreading, dull purple-red, 2.0-3.7 cm in diameter; bracts lanceolate, 1-2 cm long, persistent; pedicel and ovary 1.0-2.5 cm long. Dorsal sepals elliptic-oblong, 1.5-2.5 x 0.7-1.3 cm, apex acuminate; lateral sepals oblong, base oblique, acuminate, longer than the dorsal, inserted on the foot of the column, spreading; mentum short, about 5 mm long, conical and incurved. Petals oblanceolate, 1.5-1.8 x 0.7-1.0 cm, spreading, apex

mucronate. Lip inferior, 3-lobed, middle lobe broadly ovate, apex apiculate and recurved; lateral lobes obtuse, embracing to the column. Column 1.0-1.4 cm long, base produced into a foot. Anther terminal, umbonate, 2-loculed, pollinia 2, ovoid on a short strap.

Common name: Myae-thit-kwa (Fig. 8)

Ecology : N Lat.- 16° 41′ 39.4″, E Long.- 097° 27′ 37.4″, Altitude- 862 m; Flowering and Fruiting period: April to July; terrestrial at humid places.

Specimen examined: Kalama Taung, Paung Township; 30 May, 2015; Thant Zaw Win, Coll. no. 006 (RGN-20039).

Habit Inflorescence Flower

Figure. 8. ***Eulophia zollingeri*** (Rchb. f.) J.J. Sm.

7. ***Renanthera coccinea*** Lour., Fl. Cochinch. 2. 521. 1790.

Epiphytic herbs; stem elongate, 30-90 cm high, sending out long wiry roots. Leaves 2-rank; leaf blade oblong, 7-20 x 2-3 cm, margin entire, apex 2 lobed, rigid, thick and fleshy, veins parallel. Inflorescence borne opposite leaves, spreading panicles, lax-flowered; peduncle erect, 50-90 cm long. Flower medium-sized, scarlet color. Dorsal sepal erect, narrowly spatulate, 3-4 x 0.3-0.5 cm, margin conspicuous undulate, apex obtuse; lateral sepals 3.5-4.5 x 0.7-1.3 cm, margin conspicuous undulate, apex obtuse, deflexed, much longer than dorsal, widely spreading. Petals narrowly linear, 2.5-3.0 x 0.2-0.4 cm. Lip jointed on the base of the column, 3-lobed; lateral lobes of lip

subquadrate to orbicular. Column truncate, about 8 x 4 cm. Pollinia 4, in 2 pairs, reniform, slightly unequal, 2-grooved.

Common name: Pinle-thit-kwa-ni (Fig. 9)

Ecology : N Lat.- 16° 39′ 29.0″, E Long.- 097° 27′ 15.0″, Altitude- 30 m; Flowering and Fruiting period: April to July; epiphytic on *Mangifera longipes* Griff.

Specimen examined: Oak-ta-tar Taung, Paung Township; 20-April-2015; Thant Zaw Win, Coll. no. 003 (RGN-20042).

Habit Inflorescence Flower

Figure 9. *Renanthera coccinea* Lour.

8. *Rhynchostylis retusa* Blume, Bijdr. 286, pl. 49. 1825.

(Syn.: *Aerides retusa* (L.) Sw., *Epidendrum retusum* L.; *Gastrochilus retusus* (L.) Kuntze, *Limodorum retusum* (L.) Sw., *Saccolabium retusum* (L.) Voigt.)

Epiphytic herbs, about 20 cm high; stems stout and thick, non-pseudobulbs, roots aerial. Leaves distichous, sessile; leaf blade strap-shaped, 15.0-40.0 x 1.5-2.5 cm, base sheathing grooved, margin entire, apex unequally lobed, veins parallel, coriaceous. Inflorescence axillary, racemes, 15-30 cm long, drooping; peduncle 9-15 cm long, drooping. Flowers densely crowded into long cylindric racemes, tinged violet; bracts acute, 3-5 mm long, around the pedicel. Dorsal sepal ovate, about 11 x 6 mm; lateral sepals broadly ovate, about 12 x 8 mm. Petals oblong-ovate, about 11.5 x 5 mm. Lip conduplicate, 3-lobed, apex slightly retuse, clawed, with a saccate spur. Column prolonged into distinct foot, about 5 x 2.5 mm; rostellum shortly beaked. Anthers

terminal, 2-locule; pollinia 2, globose, waxy; caudicle slender, apex slightly dilated.

Common name: Kyauk-mi-tu (Fig. 10)

Ecology : N Lat.- 16° 35′ 50.1″, E Long.- 097° 29′ 05.6″; Altitude- 26 m; Flowering and Fruiting period: June to September; epiphytic on *Bouea burmanica* Griff. species.

Specimen examined: Mu-kyi Taung, Paung Township; 7 July, 2014; Thant Zaw Win, Coll. no. 001 (RGN-20043).

Habit Inflorescence Flower

Figure 10. *Rhynchostylis retusa* Blume

Discussion and Conclusion

According to Arthur Cronquist (1981), Orchidaceae family included up to 1000 genera and 15-20,000 species, some estimates run as high as 30,000 species. In China, about 800 genera and ca. 25,000 species (some estimates as high as 30,000 species): worldwide, except for Antarctica, most numerous in the humid tropics and subtropics; 194 genera and 1,388 species in five subfamilies (Chen *et al*. 2009). According to Kress *et al*. (2003), 131 genera and 738 species were recorded in checklist of Myanmar. In this study area, eight species that is possessed of one species in each genus were recorded.

According to Seidenfaden (1992), there are six subfamilies under family Orchidaceae, namely Apostasioideae, Cypripedioideae, Neottioideae, Orchidoideae, Epidendoideae and Vandoideae. Eight species, eight genera, five tribes under two subfamilies were collected. These collected species are

two terrestrial and six epiphytic orchid species (*Coelogyne schillerina* is both epiphytic and lithophytes).

The subfamily Epidendroideae includes three tribes (Coelogyneae, Epidendreae and Malaxideae) and five genera (*Coelogyne*, *Bulbophyllum*, *Dendrobium*, *Liparis* and *Malaxis*). The outstanding characters of ***Coelogyne schillerina*** are leaves 2, at the end of the undeveloped pseudobulb; flower solitary, with imbricated bracts at the base. This results were agreed with Bartle (1966). The outstanding characters of ***Bulbophyllum crassipes*** are densely-flowered racemes; basal auricles of lip subsquare on both sides, apex slightly retuse; floral bracts longer than pedicel with ovary; column with deltoid teeth. Those findings were agreed with Chen *et al.* (2009) and Seidenfaden (1992). The outstanding characters of ***Dendrobium fimbriatum*** are lip rounded, margin compound fimbriate, with 1 transversely lunate deep purple spot. These characters were agreed with Chen *et al*. (2009), Seidenfaden (1992) and Bartle (1996). The outstanding characters of ***Liparis viridiflora*** are epiphyte; pseudobulbs ovoid; leaves jointed on the sheath; racemes longer than leaves; flowers greenish white. These characters were agreed with Jayaweera (1981) and Seidenfaden (1992). The outstanding characters of ***Malaxis versicolor*** are leaves ovate; petals lanceolate, 1-veined; lip pectinate, subquadrate. This results were agreed with Jayaweera (1981).

The subfamily Vandoideae includes two tribes (Cymbidieae and Vandeae) and three genera (*Eulophia*, *Renanthera* and *Rhynchostylis*). The outstanding characters of ***Eulophia zollingeri*** are leafless at the time of flowering period; flowers are dull purplish-red; column produced into a foot. These findings were agreed with Jayaweera (1981). The outstanding characters of ***Renanthera coccinea*** are inflorescences borne opposite leaves; flowers scarlet color; lateral lobes of lip subquadrate to orbicular. This results were in agreement with Chen *et al.* (2009) and Seidenfaden (1992). ***Rhynchostylis retusa*** possess dense drooping racemes of pinkish-white flowers; apex of lip retuse with a saccate spur; column foot distinct. Those results were agreed with Chen *et al.* (2009), Jayaweera (1981) and Seidenfaden (1992).

This research paper is dedicated to help partially for publish of the Orchids of Myanmar.

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STUDY ON PALYNOLOGICAL, NUTRITIONAL VALUES AND ANTIMICROBIAL ACTIVITY OF BEE POLLEN FROM DIVISION OF APICULTURE, NAYPYITAW

Win Theingi Aung[1], Nay Zin Myint[2], Myat Myat Moe[3]

Abstract

Bee pollens of kapok and sunflower field sites were collected from Division of Apiculture, Naypyitaw during June-February, 2017. Bee pollens come from the pollen baskets of returning foragers of bee were gathered by bee house. Bee pollen grains were examined by light microscope and photomicrographs. The characters of each pollen grain were identified and classified by using literatures. Pollen grains of 12 species belonging to 10 genera of 8 families were found in the kapok and sunflower field sites. They are *Albizia*, *Mimosa*, *Ceiba*,*Celosia*, *Physalis* and *Vernonia*. And then, the pollen species of sunflower field sites were obtained *Albizia*, *Brassica*, *Richardsonia*, *Ocimum*, *Helianthus* and *Vernonia*. Among them, *Vernonia* pollen grains are found in both two sites. The pollen grains are monads, tetrads and polyads. Tetrad grain is observed in only one species *Mimosa*; polyads as in 2 species of *Albizia* and the remaining of 9 species are monads. The aperture type, number, position, size, sculptures of each grain were presented and varied. There are colpate, colporate and porate. The aperture types are 3 species of colpate (*Brassica*, *Richardsonia* and *Ocimum*), 3 species of colporate (*Ceiba*, *Physalis* and *Helianthus*) and 3 species of porate (*Mimosa*, *Celosia, Vernonia* and 2 species of *Albizia*). The smallest pollen (15.5 – 17.5 × 16.5 – 18.0 μm) is *Mimosa pudica* L. and the largest pollen (43.8 – 102.5 × 75.5 – 120.0 μm) is *Albizia saman* (Jacq.) Merr. The nutritional values were carried out at Department of Research and Innovation Analysis Department. According to the analysis of D.R.I, nutritional values of bee pollens were found carbohydrate (sugar) as major constituents and followed by protein. And then, antimicrobial activity was performed in the microbiological laboratory of Dagon University. The ethanolic and methanolic extracts of bee pollen had provided the best clear zone against *Escherichia coli* and *Pseudomonas aeruginosa* in microbial activity.

Key words: Pollen morphology, Bee plants, SuperFood and Medicine

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[2.] Assistant Lecturer, Department of Botany, Dagon University
[3.] Dr, Professor and Head, Department of Botany, Dagon University

Introduction

The relationship between bee, flowers and man is one of the wonders of the universe, being a living proof that the flora, fauna and man to live in harmony. Bees need flowers to feed themselves, plants need bees to be pollinated and to produce seed to ensure the perpetuation of plant species.

Palynology is the science of studying pollen collected from air, water, sediment deposits and not least the study of bee pollen. Palynology is an interdisciplinary science, biological sciences and particularly botany (Bhojwanii & Bhatnagar, 2005).

Pollen is a fine powder-like material of male reproductive cells. Pollen grains can be distinguished by different shape and color, by different content in nutrients, vitamins and biologically active substances of each plant (Bogdanov, 2012).

Bee pollen is a collection of pollen grains from various botanical sources, bees collected on the tibia of their hind legs by mix with nectar and secretion from the hypopharyngeal glands such as β-glycosidase enzymes (Carpes *et al*., 2009).

Bee pollen are natural raw materials include pollen and nectar. Pollen is the bee's major source of proteins. Nectar is a bee's source of carbohydrates. Nectar provides carbohydrates and pollen supplies the remaining dietary requirements such as protein, lipids, vitamins and minerals (Campos *et al*., 2008).

Bee pollen has also been used for many centuries in traditional medicine and supplementary nutrients, primarily because bee pollen has complete food and health benefits (Cheng *et al*., 2013).

Antimicrobial properties can protect the human body against both cellular oxidation reactions and pathogens (Senguel *et al*., 2009). In addition, bee pollen has antimicrobial effects (Haas, 1992).

The aims and objectives of this research work are to study pollen morphology for identifying the various pollen types of bee pollen present in field sites, to provide information that natural energizer can be obtained by taking bee pollen and to investigate antimicrobial activity in order to know what types of bacteria can be killed by taking bee pollen.

Materials and Methods

The research was conducted on two samples of bee pollen (Figure 1) harvested from different sites in Division of Apiculture, Pyinmana Township, Naypyitaw. The bee pollen specimens were kapok and sunflower field sites. These samples were obtained directly beekeepers in June-February, 2017 and were collected by using pollen collectors.

Pollen collection by bees

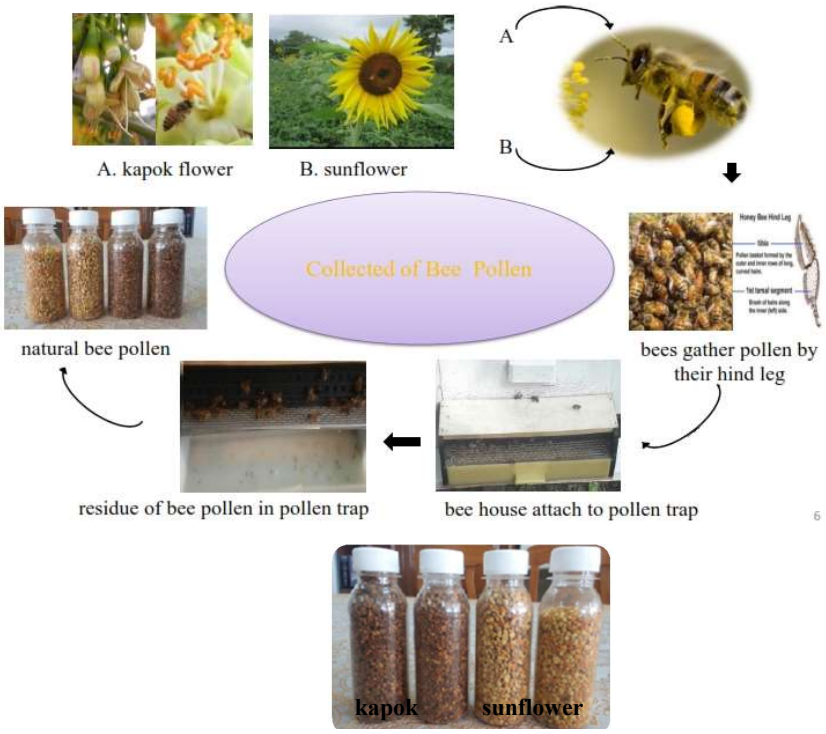
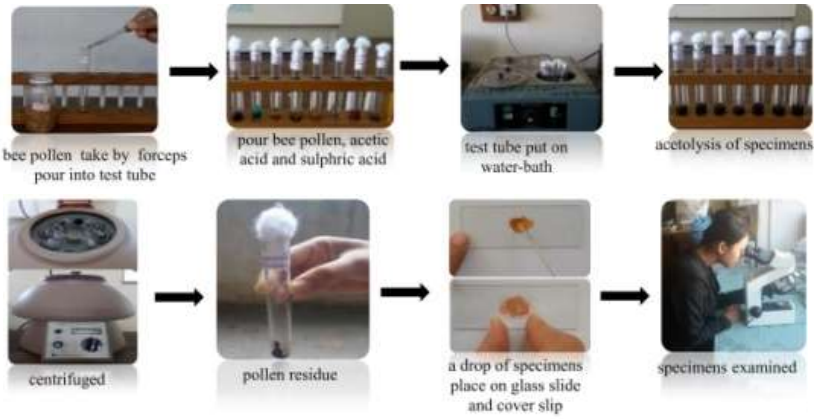


Figure 1. kapok and sunflower bee pollen

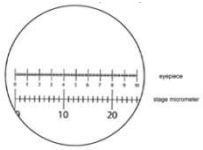
For studies of bee pollen morphology, acetolysis of pollen grains (Erdtman, 1952) and glycerine jelly of acetolysis pollen grains by Kisser's formula (Erdtman, 1952) were prepared. Pollens counting method were used by using ocular microscope grains (Brookes and Thomas, 1967).

Acetolysis of pollen grains (Erdtman, 1952) for studies of pollen morphology



Glycerine jelly of acetolysis pollen grains by Kisser's formula (Erdtman, 1952)

Pollen counting method by using ocular the microscope



Ocular

$$\text{Percentage of particular pollen grains} = \frac{\text{No. of particular pollen type}}{\text{Total No. of trapped pollen grains}} \times 100$$

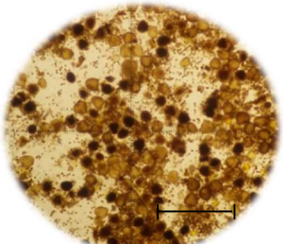
In nurtitional values, the analysis of bee pollens (kapok and sunflower) were determined by using A.O.A.C (Horwitz, 1997) method carried out at Department of Research and Innovation Analysis Department (D.R.I).

As antimicrobial activities, six solvent extracts of kapok and sunflower bee pollens were tested on seven pathogenic microorganisms by using paper disc diffusion method described by (Cruickshank, 1968).

Results

Pollen morphology of two samples of bee pollen (kapok and sunflower) has been studied from Division of Apiculture at Pyinmana Township, Naypyitaw. In pollen morphology, 12 species that belong to 10 genera of 8 families have been observed. Distribution of bee pollen sample in kapok field site is presented in Figure 2; Table 1 and Figure 3; Table 3 is sunflower field site. And then, the families of pollen morphology in the samples are listed according to APG III (2009) system and the genus according to alphabetical order as follow in Tables 1 and 3. Finally, the nutritional values of two bee pollens were included (Table 5 and Figure 7) and antimicrobial activity of diameter of each solvent extracts of two bee pollens were tested in Table 6 and Figure 9.

Microscopical images of kapok bee pollen field sites



Outline of pollen morphology (X10, Scale bar = 10 μ)

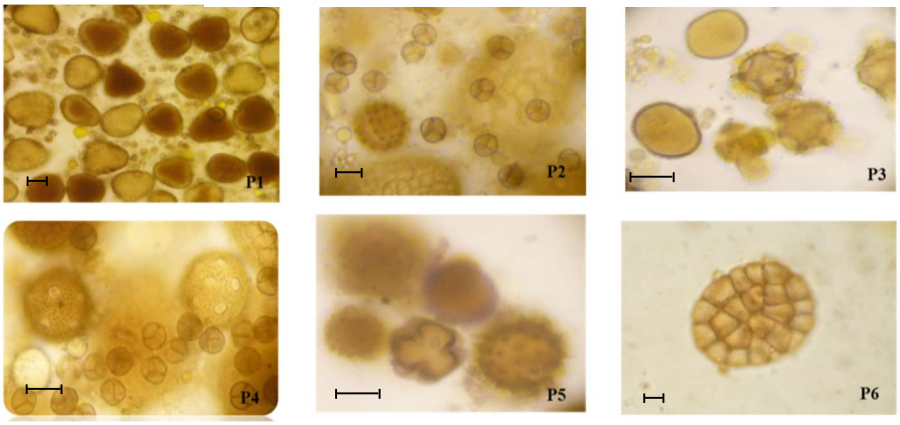
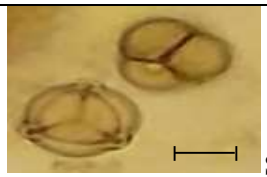
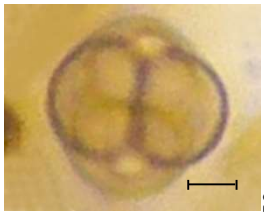


Figure 2. Pollen morphology of bee pollen P1-P6 in kapok bee pollen field sites

Table 1. Pollen type and its morphology of bee pollen in kapok field sites

Scale bar = 10 µ

No.	Pollen type	Plant name; Family; Myanmar name; English name and Pollen Morphology
P6.	 Surface view	-*Albizia saman* (Jacq.)Merr. -Fabaceae (Mimosoideae) -Myanmarkokko -Rain tree -Polyad, more than 16-celled, very large, 43.8 – 102.5 x 75.5 – 120.0 µm in length and breadth; amb subglobose; each grain triporate, very small, 2.2 x 1.5 – 2.5 µm in length and breadth; exine 1.5 – 1.8 µm thick, sexine as thicker as nexine; sculpturing faintly reticulate.
P2.	 Surface view  Surface view	-*Mimosa pudica* L. -Fabaceae (Mimosoideae) -Htikayon -Senistive plant -Tetrad, tetragonal, very small, 15.5 – 17.5 x 16.5 – 18.0 µm in length and breadth; amb rounded; each grain triporate, very small, 10.0 – 12.5 x 11.5 – 14.0 µm in length and breadth; exine 1.0 – 1.5 µm thick, sexine thicker than nexine; sculpturing psilate.

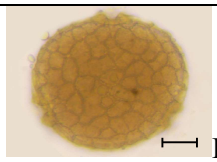
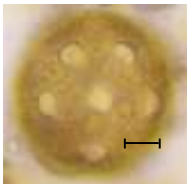
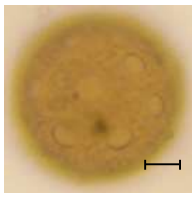
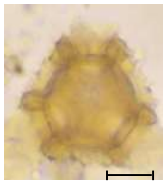
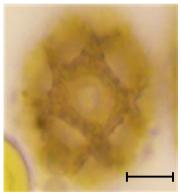
No.	Pollen type	Plant name; Family; Myanmar name; English name and Pollen Morphology
P1.	 Polar view  Equatorial view	-*Ceiba pentandra* (L.) Gaertn. -Malvaceae (Bombacoideae) -Thinbawletpan -Kapok -Tricolporate, oblate spheroidal, large, 39.5 – 41.5 x 52.5 – 55.0 µm in length and breadth; amb rounded; colpi longicolpate; pori lalongate; exine 1.5 – 2.0 µm thick, sexine thicker than nexine; sculpturing retipilate.
P4.	  Surface view	-*Celosia argentea*L. -Amaranthaceae -Tawkyetmauk -Wildcock's comb -Polyporate (about 10), spheroidal, medium,26.5–30.5µm in diameter; amb circular; pori circular; exine 1.5 – 2.0 µm thick, sexine thicker than nexine; sulpturing reticulate.
P5.	 Polar view  Equatorial view	-*Physalis minima*L. -Solanaceae -Baungpan -Wild gooseberry -Tricolporate, subprolate, medium, 23.5 – 26.2 x 22.5 – 25.0 µm in length and breadth; amb rounded triangular; colpi longicolpate; pori lalongate; exine 1.3 – 1.8 µm thick, sexine thicker than nexine; sculpturing reticulate.
P3.	  Surface view	-*Vernonia cinerea* (L.) Lees. -Asteraceae -Kadupyan -Purple ironweed -Triporate, spheroidal, medium, 21.5 – 29.5 µm in diameter; amb rounded; pori circular; exine 2.5 – 5.5 µm thick, sexine thicker than nexine; sculpturing echinolophate (lophoreticulate).

Table 2. Distribution of pollen morphology in Kapok bee pollen field sites

Code No.	Kapok field sites	Total No. of grains counted	Percentage
P1.	*Ceiba pentandra* (L.) Gaertn.	495	55.5%
P2.	*Mimosa pudica* L.	251	28.1%
P3.	*Vernonia cinerea* (L.) Lees.	80	9.0%
P4.	*Celosia argentea* L.	32	3.6%
P5.	*Physalis minima* L.	26	2.9%
P6.	*Albizia saman* (Jacq.) Merr.	8	0.9%

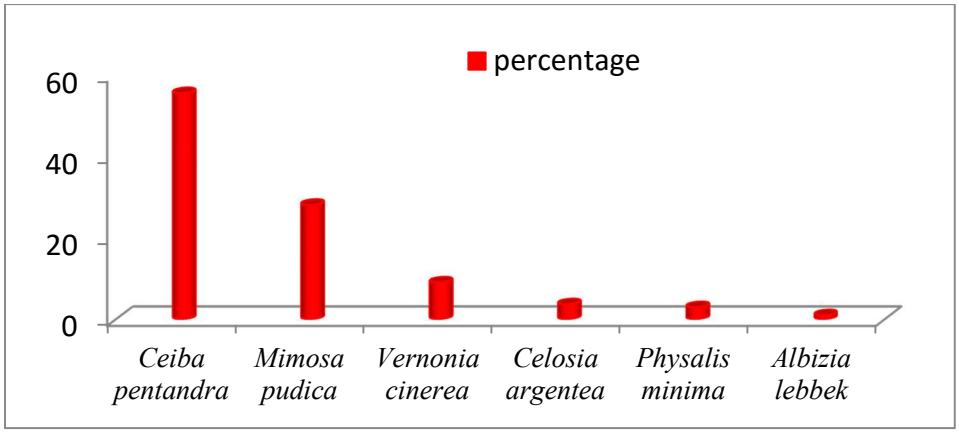
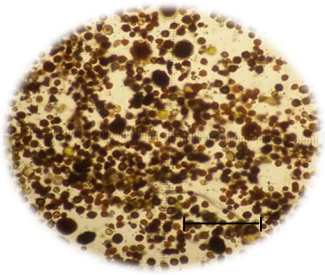


Figure 3. Distribution of pollen morphology in Kapok bee pollen field sites

Microscopical images of sunflower bee pollen field sites



Outline of pollen morphology (X10, Scale bar = 10 μ)

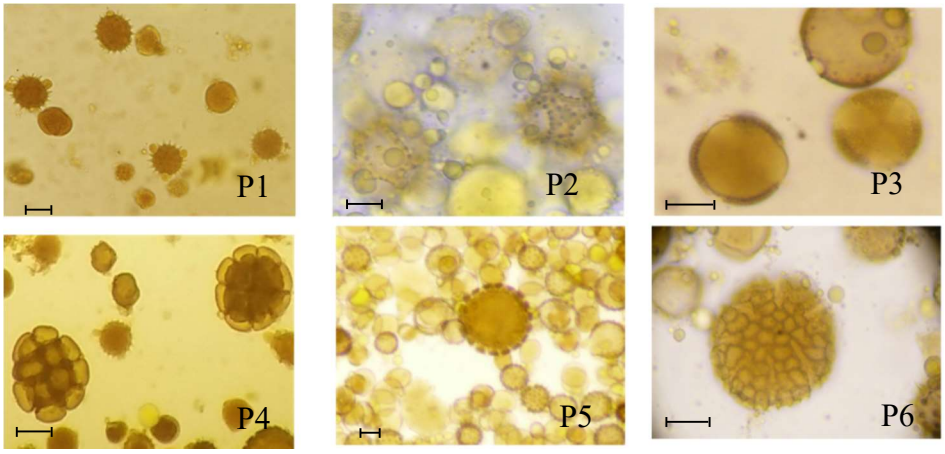
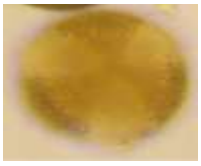
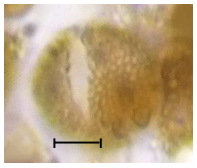
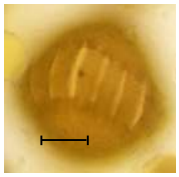
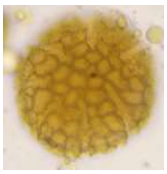


Figure 4. Pollen morphology of bee pollen P1-P6 in sunflower bee pollen field sites

Table 3. Pollen type and its morphology in bee pollen in sunflower field sites

Scale bar = 10 µ

No.	**Pollen type**	**Plant name; Family; Myanmar name;English name and Pollen Morphology**
P4.	 Polar view	-*Albizia lebbek* Benth. -Fabaceae (Mimosoideae) -Anyakokko -Sristree -Polyad, 16-celled, medium, 28.5 – 32.5 x 30.0 – 35.0 µm in length and breadth; amb subglobose; each grain triporate, small, 10.0 – 12.5 x 11.0 –13.0 µm in length and breadth; exine 1.5 – 1.8 µm thick, sexine thicker than nexine; sculpturing reticulate.

No.	Pollen type	Plant name; Family; Myanmar name;English name and Pollen Morphology
P3.	 Polar view  Equatorial view	-*Brassica campestris* L. -Brassicaceae -Monnyin -Mustard -Tricolpate, oblate spheroidal, small, 21.5 – 22.5 x 23.0 – 25.0 µm in length and breadth; amb rounded; colpi longicolpate; exine 1.3 – 2.0 µm thick, sexine thicker than nexine; sculpturing reticulate.
P5.	 Polar view  Equatorial view	-*Richardsonia brasiliensis* Gomes. -Rubiaceae -Notknown -Mexican clover -Polycolpate (about 18), oblate, large, 55.0 – 60.0 x 70.0 – 85.0 µm in length and breadth; amb circular; colpi brevicolpate; exine 2.3 – 3.0 µm thick, sexine thicker than nexine; sculpturing reticulate.
P6.	 Polar view  Equatorial view	-*Ocimum americanum* L. -Lamiaceae -Pinseinyaing -Wild ocimum -Hexacolpate, prolate spheroidal, medium, 32.5 – 42.5 x 30.0 – 37.5 µm in length and breadth; amb rounded; colpi longicolpate; exine 1.7 – 2.3 µm thick, sexine thicker than nexine; sculpturing reticulate.

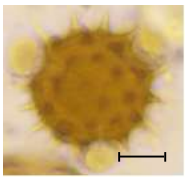
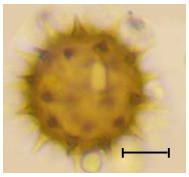
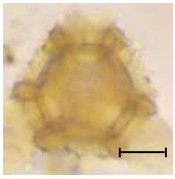
No.	Pollen type	Plant name; Family; Myanmar name;English name and Pollen Morphology
P1.	 Polar view  Equatorial view	-*Helianthus annuus* L. -Asteraceae -Neykyapan -Sunflower -Tricolporate, oblate spheroidal, small, 17.5 – 22.5 x 18.0– 23.0 µm in length and breadth; amb rounded; colpi brevicolpate; pori lalongate; exine 2.0–2.5 µm thick, sexine thicker than nexine; sculpturing echinate.
P2.	  Surface view	-*Vernonia cinerea*(L.) Lees. -Asteraceae -Kadupyan -Purple ironweed -Triporate, spheroidal, medium, 22.5 – 30.0 µm in diameter; amb rounded; pori circular; exine 2.5 – 5.5 µm thick, sexine thicker than nexine; sculpturing echinolophate (lophoreticulate).

Table 4. Distribution of pollen morphology in sunflower bee pollen field sites

Code No.	Sunflower field sites	Total No. of grains counted	Percentage
P1.	*Helianthus annuus* L.	630	49.9%
P2.	*Vernonia cinerea* (L.) Lees.	414	32.8%
P3.	*Brassica campestris* L.	96	7.6%
P4.	*Albizia lebbek* Benth.	63	5.0%
P5.	*Richardsonia brasiliensis* Gomes.	53	4.2%
P6.	*Ocimum americanum* L.	6	0.5%

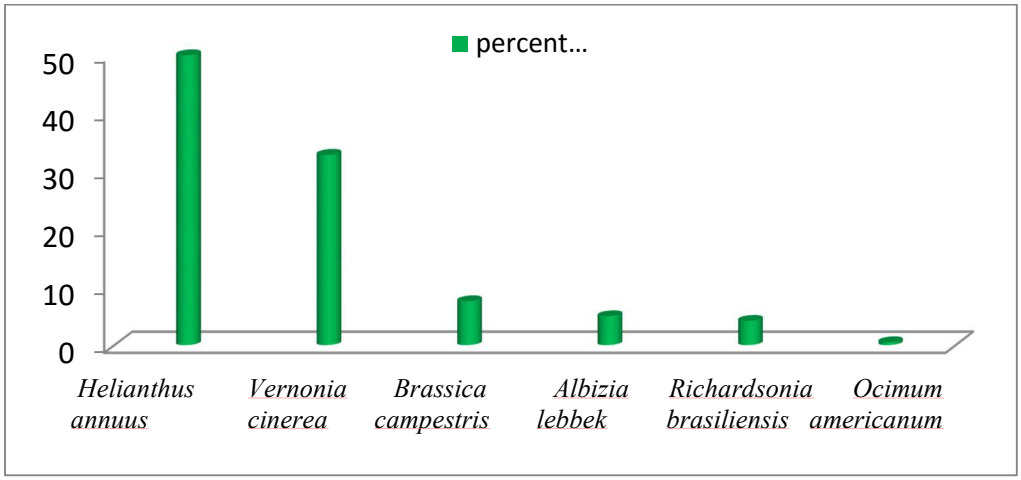


Figure 5. Distribution of pollen morphology in sunflower bee pollen field sites

The estimation of nutritional values of two bee pollen samples (kapok; sunflower)

The estimation of data was shown in Table 5 and Figure 7. The nutritional values of A.O.A.C methods contained carbohydrate, sugar, protein and fat for food and medicine.

Table 5. Nutritional values of two bee pollen samples

No.	Types of elements	Concentration (%)	
		Sunflower field site	Kapok field site
5.	Carbohydrate	41.71	45.95
6.	Sugar	37.70	28.04
1.	Protein	16.40	23.29
4.	Fat	2.55	4.02

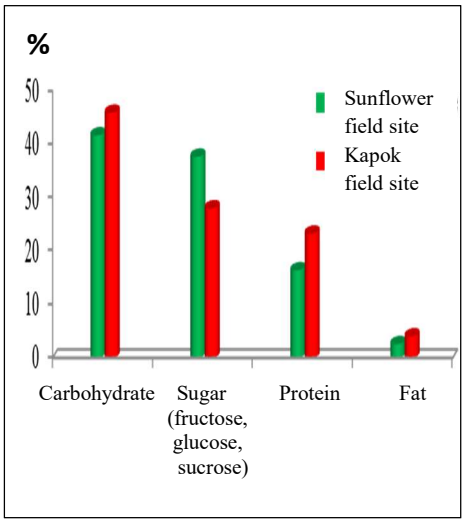
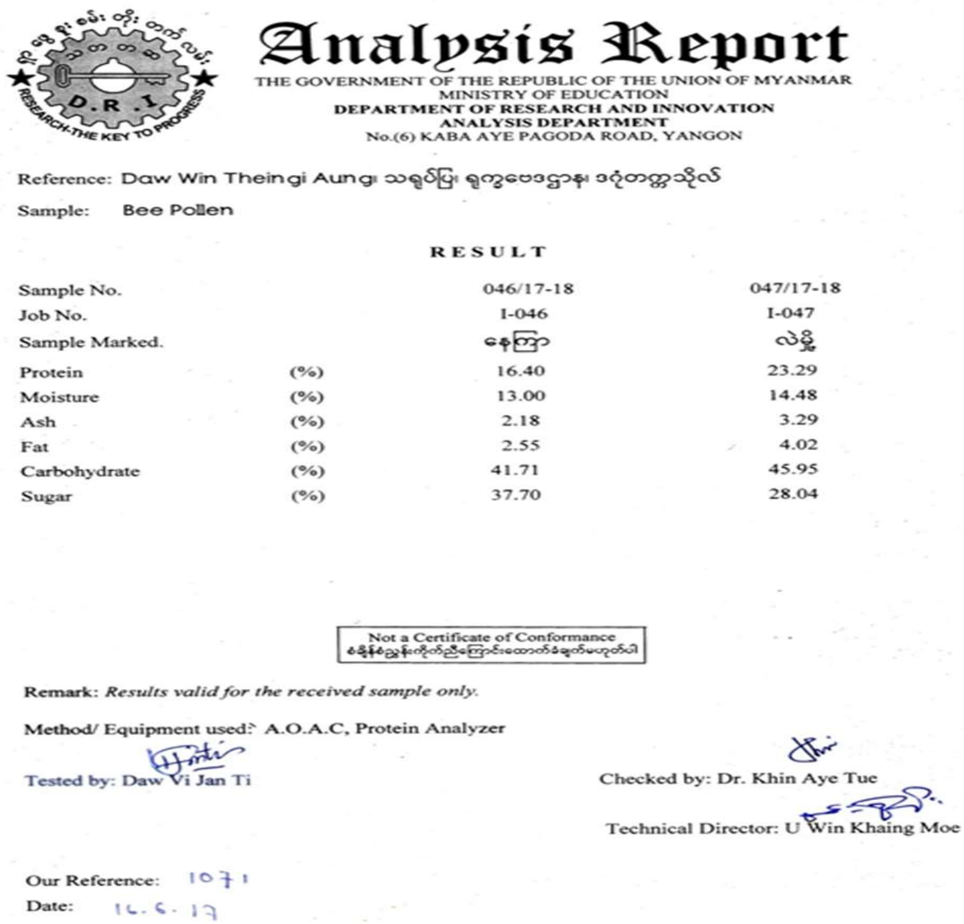


Figure 6. Nutritional values of two bee pollen samples



Analysis Report

THE GOVERNMENT OF THE REPUBLIC OF THE UNION OF MYANMAR
MINISTRY OF EDUCATION
DEPARTMENT OF RESEARCH AND INNOVATION
ANALYSIS DEPARTMENT
No.(6) KABA AYE PAGODA ROAD, YANGON

Reference: Daw Win Theingi Aung၊ သရုပ်ပြ၊ ရုက္ခဗေဒဌာန၊ ဒဂုံတက္ကသိုလ်

Sample: Bee Pollen

RESULT

Sample No.		046/17-18	047/17-18
Job No.		I-046	I-047
Sample Marked.		နေကြာ	လဲမှို
Protein	(%)	16.40	23.29
Moisture	(%)	13.00	14.48
Ash	(%)	2.18	3.29
Fat	(%)	2.55	4.02
Carbohydrate	(%)	41.71	45.95
Sugar	(%)	37.70	28.04

Not a Certificate of Conformance
စံချိန်စံညွှန်းကိုက်ညီကြောင်းထောက်ခံချက်မဟုတ်ပါ

Remark: *Results valid for the received sample only.*

Method/ Equipment used: A.O.A.C, Protein Analyzer

Tested by: Daw Yi Jan Ti

Checked by: Dr. Khin Aye Tue

Technical Director: U Win Khaing Moe

Our Reference: 1071

Date: 16.6.17

Figure 7. The nutritional values of two bee pollen samples (kapok;sunflower) in D.R.I

The determination of antimicrobial activity of bee pollen (kapok; sunflower)

Table 6. Antimicrobial activity of different solvent extracts of bee pollen samples

Paper disc=10 mm; -= negative

Bee pollen samples	Test organisms	Diameter of zone inhibition					
		Acetone extract	Ethanol extract	Ethyl-acetate extract	Methanol extract	Pet-ether extract	Water extract
kapok	*Bacillus subtilis*	23mm	25mm	12mm	33mm	-	-
	Candida albican	12mm	25mm	12mm	20mm	-	-
	Escherichia coli	13mm	**32mm**	12mm	**35mm**	-	-
	Pseudomonas aeruginosa	13mm	**33mm**	12mm	**36mm**	-	-
	Saccharomyces cerevisiae	12mm	26mm	-	20mm	-	-
	Staphylococcus aureus	25mm	30mm	12mm	27mm	-	-
	Micrococcus luteus	21mm	27mm	12mm	20mm	-	-
sunflower	*Bacillus subtilis*	21mm	27mm	12mm	37mm	-	-
	Candida albican	12mm	13mm	12mm	22mm	-	-
	Escherichia coli	13mm	**34mm**	12mm	**40mm**	-	-
	Pseudomonas aeruginosa	12mm	**35mm**	12mm	**40mm**	-	-
	Saccharomyces cerevisiae	12mm	26mm	16mm	23mm	-	-
	Staphylococcus aureus	16mm	27mm	12mm	30mm	-	-
	Micrococcus luteus	-	20mm	12mm	24mm	-	-

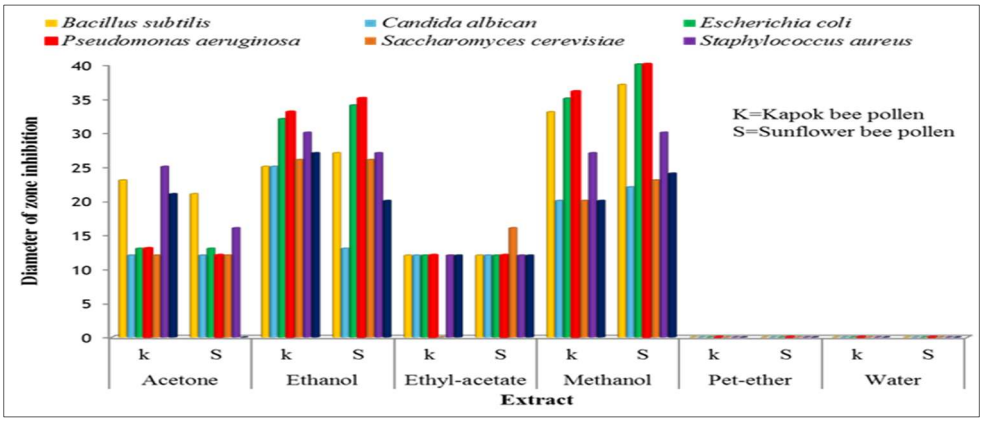
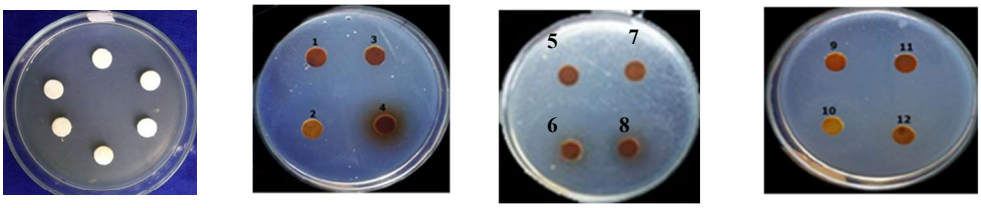
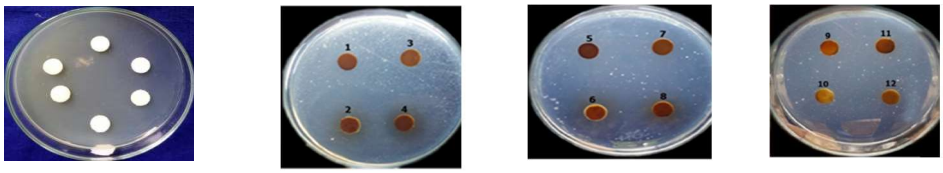


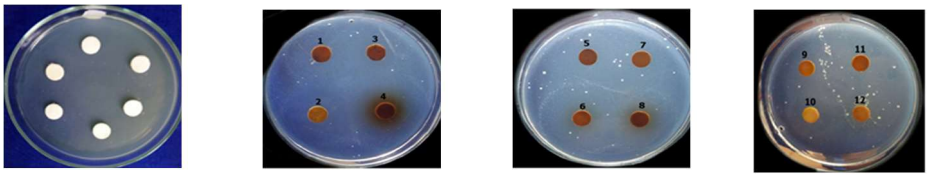
Figure 8. Antimicrobial activity of different solvent extracts of bee pollen samples



Control ***Bacillus subtilis***



Control ***Candida albican***



Control ***Escherichia coil***

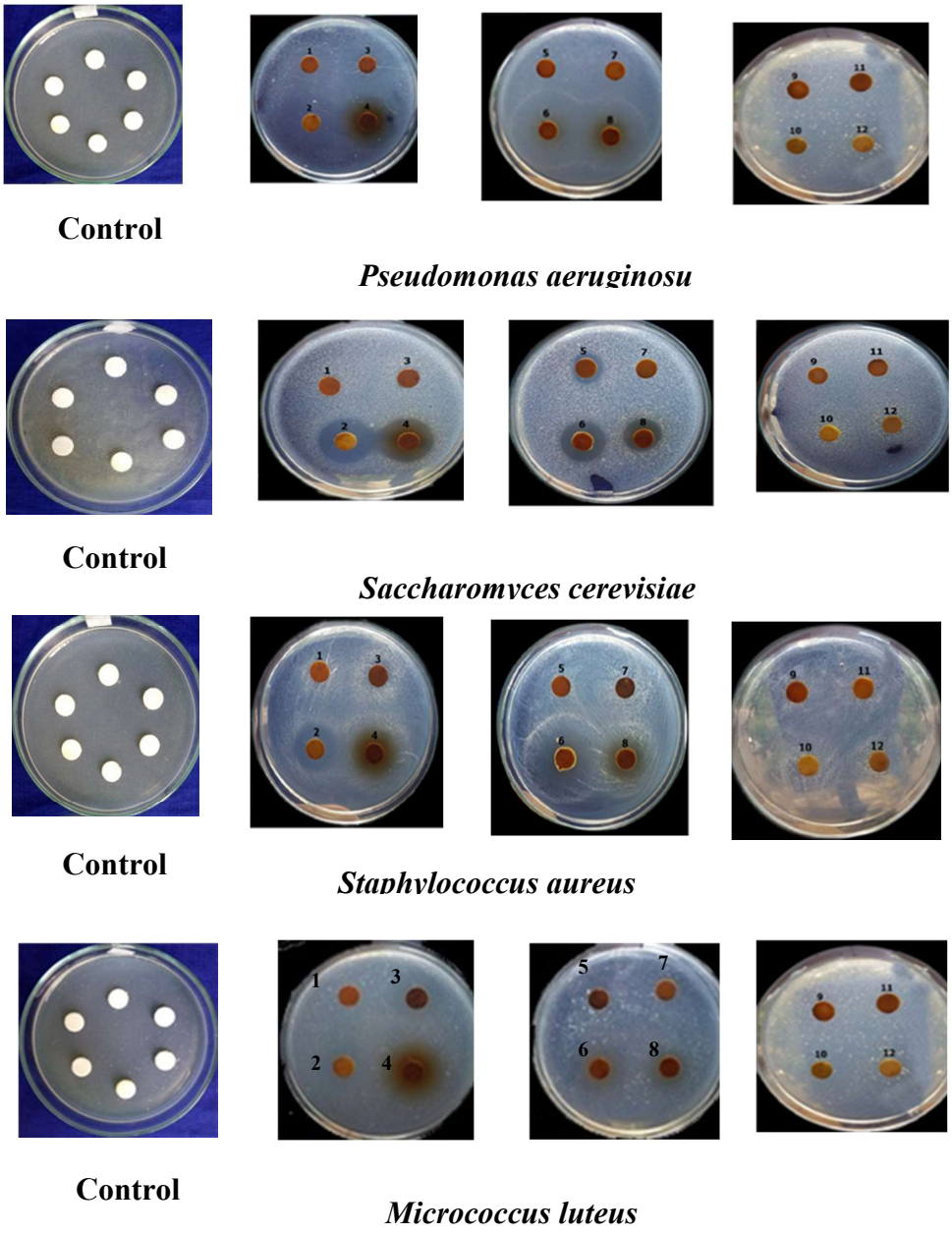


Figure 9. Diameter of zone inhibition of antimicrobial activity of two bee pollens

1, 2, 5, 6, 9, 10= sunflower bee pollen sample
3, 4, 7, 8, 11, 12= kapok bee pollen sample
1, 3 = acetone
2, 4= ethanol
5, 7= ethyl-acetate
6, 8= methanol
9, 11= pet-ether
10, 12= water

Discussion and Conclusion

This research, two samples of kapok and sunflower bee pollen collected from Division of Apiculture, Naypyitaw. According to palynological point of view, 12 species that belong to 10 genera of 8 families have been obtained in this study area. The types of pollen grains are monad, tetrad and polyads. The pollen type of *Mimosa* is tetrad and *Albizia* is polyads. The rest grains are monad.

Erdtman (1952) reported that, tetrad in *Mimosa* and polyads in *Albizia,* porate aperture in Mimosoideae. In this study, the pollen type of *Mimosa* and *Albizia* are agreed with Erdtman, 1952.

According to Sharma (1986), *Ceiba* pollen grain of Bombacoideae is tricolporate, retipilate and nexine thinner than sexine. In the study, pollen aperture of *Ceiba* species is in agreement with those given by Sharma, 1986.

Sharma *et al*. (1973) described that tricolpate and reticulate grain of *Brassica* species. In this research, *Brassica* pollen grain is agreed with Sharma *et al*., 1973.

On the basic of Chaturvedi *et al.* (1990), the pollen grains of *Celosia* are pantoporate; exine 1.5 - 5.0 μm thick. In this study, pollen character of *Celosia*is in agreement with those described by Chaturvedi *et al*., 1990.

Erdtman (1971) stated that Rubiaceae is eurpalynous family; *Richardsonia* pollen grain is 3 - polycolpate (about 12 - 18). In the study, pollen morphology of *Richardsonia*is agreed with Erdtman, 1971.

According to Ramakrishna *et al*. (2014), *Physalis* pollen grain is tricolporate and reticulates. In present work, pollen character of *Physalis* is in agreement with those given by Ramakrishna *et al*., 2014.

Solomon (1989) described that pollen grains of *Ocimum* are 3-6 colpate and reticulate. In this studied, pollen character of *Ocimum* is agreed with Solomon, 1989.

According to Paldat (2016), *Helianthus* grain is tricolporate and echinate. *Vernonia* grain is triporate and lophae by Stix, 1960. In this study, pollen morphology of *Helianthus* and *Vernonia* are agreed with Paldat, 2016 and Stix, 1960.

Hence, the studied of the pollen grain including bee pollen of two field sites are identical with these pollen characters of literatures as above.

As present in Figure 2, Kapok bee pollen field sites have 6 species. They are *Ceiba*, *Mimosa*, *Vernonia*, *Celosia*, *Physalis* and *Albizia*. In this site, *Ceiba* pollen grain is highest and *Albizia* is lowest. And then, *Vernonia*, *Celosia*, *Physalis* and *Albizia* are followed by moderate. So, *Ceiba* pollen grain was found to be the main sources in the kapok bee pollen field sites. Similarly, sunflower bee pollen field sites were found 6 species in Figure 3. They are *Helianthus*, *Vernonia*, *Brassica*, *Albizia*, *Richardsonia* and *Ocimum*. In these sites, highest pollen grain is *Helianthus* and *Ocimum* is lowest. *Vernonia*, *Brassica*, *Albizia* and *Richardsonia* are included by moderate. So, *Helianthus* pollen grain was found to be the main sources in the kapok bee pollen field sites. It can be inferred that asteraceae families with (*Vernonia*) were presented in two bee pollen of kapok and sunflower.

In this study, the nutritional values of bee pollen (sunflower; kapok) contains 41.71 and 45.95 percent of digestible carbohydrate, 37.70 and 28.04 percent of sugar (mainly fructose, glucose and sucrose), 16.40 and 23.29 percent of protein (including essential amino acids) and 2.55 and 4.02 percent of fat in bee pollen. It could be concluded that the percentage of rest constituents except sugar in kapok is more than in sunflower. But, the percentage of sugar in sunflower of bee pollen is distinctly more than in kapok. So, bee pollen of sunflower can be used as natural energizer for the body because of its sugar percentage (Table 5 and Figure 7).

As antimicrobial activities, the microorganism of *Bacillus subtilis*, *Candida albican*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Staphylococcus aureus* and *Micrococcus luteus* were studied with acetone, ethanol, ethyl-acetate, methanol, petroleum-ether and aqueous extracts by agar disc diffusion method. Ethanol and methanol extracts were more effective than acetone and ethyl-acetate. Among them, petroleum ether and aqueous extract provided no antimicrobial activity in present results.

In this experiment, the best antimicrobial effect of bee pollen (kapok; sunflower) extracts were found at ethanolic and methanolic extracts against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The highest antimicrobial activity was shown on *Escherichia coli* and *Pseudomonas aeruginosa* with ethanolic and methanolic extracts and displayed in Table 6 and Figure 9. It was concluded in this study, the ethanolic and methanolic extracts of sunflower bee pollen were more than that of kapok in the diameter of zone inhibition of microbial activity.

According to the reports of Carpes *et al*. (2007) also reported that ethanolic and methanolic extracts of bee pollen had provided the higher antimicrobial properties against *Bacillus subtilis*, *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The antimicrobial activities of different solvent showed such as acetone, ethanol, ethyl-acetate, methanol, pet-ether and water. Ethanol and methanol extracts were more effective than acetone and ethyl-acetate. Among them, petroleum ether and aqueous extract provided no antimicrobial activity. These results are also the same as recorded in present research work.

Haggstrom (2014) suggested that disease can be caused by *Escherichia coli* such as urinary bladder, intestines, kidneys and food poisoning activity and *Pseudomonas aeruginosa* such as mucous membrane, skin and urinary tract infection. Bee pollen can be effective in treatment of various allergic diseases, immune system for various body, asthma attack and urinary infection by Talbott, 2015.

As the conclusion, this research work was carried out to prove the origin of bee pollen (kapok; sunflower) by studying pollen morphology and to suggest that bee pollen should be taken as a superfood because it has nutritious foods such as carbohydrate, sugar, protein and fat. Bee pollen is an effective medicinal treatment product according to antimicrobial activity such as *Escherichia coli* of urinary bladder etc. *and Pseudomonas aeruginosa* of mucous membrane etc. In addition, it is necessary to research for the further study what benefits it will be effective for health by how much dose used daily and what cause will happen if people take bee pollen as overdose daily diet.

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IRRIGATION SCHEDULE OF ACTUAL VERSUS ESTIMATED EVAPOTRANSPIRATION ON VEGETABLE SOYBEAN (*GLYCINE MAX* L.) AGS - 292

Zin Moe Moe

Abstract

Scheduling of irrigation to crops is essential for efficient utilization of available water, saving of input and enhancing yield. The study was aimed to investigate the efficient water use based on soil moisture measuring methods and to evaluate the interactions between actual and estimated water requirement on vegetable soybean field of VFRDC. According to soil analysis, the soil type is sandy loam soil. Soil sample was collected daily for measuring actual water requirement of vegetable soybean by Gravimetric method. The estimated evapotranspiration (ET_0) of Blaney & Criddle was compared to CROPWAT method. The climate data had also recorded such as temperature, humidity, wind and sunshine hours. Crop water requirement was measured by the method of Blaney & Criddle (1950) which is suitable method for the most of crops in Myanmar. A comparison was made between estimated crop water consumption versus actual water consumption. The result of this study showed that in young stage of crop, actual crop water consumption and estimated crop water consumption were almost equal level, but when plants became mature, the actual crop water consumption was higher than the estimated one. Based on this investigation, it must be concluded that the temperature based method for estimated crop water requirement, Blaney and Criddle method, is the most suitable in Myanmar for irrigation schedule.

Introduction

Vegetable soybean belongs to the division Magnoliophyta, Class – magnoliopsida, Order – Rosales and Family Fabaceae, it involved has about 400-500 genera and 10,000 species. Vegetable soybean is botanically called *Glycine max* (L.). Soybean originated from China, and it had been one of the most economically important crops in the world. It was cultivated since 5000 years ago. Recently its cultivation extends from temperate to tropical countries (Xo, 1999). Soybean can be classified into two groups. The first group is grain type which is employed mainly in the bran and oil production, with medium grain size [one hundred seed weight (HSW) varying among 10 to 19 g], however, have undesirable flavor. The second group is denominated

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food type, with flavor taste, constituted by two subgroups, the first with HSW smaller than 10 g, consumed in the sprouts form and natto (fermented) and the second with HSW presenting 20 g or more, being consumed directly by human principally in the immature pod form (Fehr and Caviness, 1977), snack, being denominated vegetable soybean, green soybean or edamame; presenting also the subgroups denominated sweet soybean (Kuromame) and salad soybean. Vegetable soybean requires large amount of water for growth especially during flowering to pod filling stages. Maintaining proper soil moisture throughout the growing season is important for good quality pods. Insufficient water during this period will cause flowers and pods drop developed pods (Nguyen, 1997). However, excessive soil moisture will inhibit root activity resulting of slow growth and delay flower period, so flooding the plants should be avoided. Usually, first irrigation is needed within a week and seeds need moist soil but not wet for their germination, therefore, furrow irrigation up to half or three-quarters of the height is common practice. Depending upon weather and soil moisture conditions, the irrigation is continued at 7-10 day intervals until the pods are well developed. Irrigating the crop is essential at critical periods such as flowering and pod filling stages (Javier, 1991). Maintaining proper soil moisture throughout the growing season is important for good quality pods. The soil must be moist, but not wet, until the pods have matured. Water should be irrigated into 1–2 times/week. Most roots are in the upper 1 ft (30 cm) of soil but root penetration and water extraction take place to 1 to 3 inches (2.54-7.62 cm) depth (Srinives, 1989). Irrigation is one of the most important factors in world agricultural development. Irrigation water requirement using the Cropwat model is a FAO model for irrigation management designed by Smith which integrates data on climate, crop and soil to assess reference evapotranspiration (ETo), crop evapotranspiration (ETc) and irrigation water requirements. Water requirement depend mainly on the nature and stages of growth of the crop (Initial stage, Crop development stage, Mid-season stage and Late-season stage approach) and environmental conditions (Allen *et al.,* 1998). Crop Water Requirement (CWR) is defined as the depth of water needed to meet the water loss through evapotranspiration of a crop (FAO, 1984). Application depth means the amount of water used when irrigating. It is often expressed in millimeters. The control of infiltration and runoff, which is a common problem for farmers, is essential to effectively control the depths of the water

to be applied (Pereira, 1996). Irrigation scheduling techniques can be based on soil water measurement, meteorological data or monitoring plant stress. Conventional scheduling methods are to measure soil water content or to calculate or measure evapotranspiration rates. The moisture content of the soil is the most important factor to consider in irrigation scheduling. Soil moisture affects not only plant growth but also the success of seedling, cultivation and harvesting operations (Brady, 1974). There are many methods for measuring soil moisture, including gravimetric, tensiometric, electrical and soil feel methods. A standard method for measuring water content is the gravimetric method. Crop water use or consumptive use, also known as evapotranspiration (ET_c), is the water used by a crop for growth. The method for estimated calculation of crop water requirement has many methods. The most widely and suitable use of method is Blaney and Criddle method (1950).

The aims and objectives of this research paper are to investigate the efficient water use based on soil moisture measuring methods and to evaluate the interactions between actual and estimated water requirement on vegetable soybean under field cultivation.

Materials and Methods

Experimental Site

The experiments were conducted at the farm of Vegetables and Fruits Research and Development Centre (VFRDC), Hlegu Township, Yangon Region. It is located at 17° 5' North Latitude and 96° 15' East Longitude and its annual average rainfall is 8.95 inches.

Experimental Lay out

There are 20 plots in this experiment. One plot containing 4 rows and each row had 10 plants. The plot size was 360 cm x 180 cm. The spacing between plants and between rows were 30 cm each. Total experimental area was 1772633 cm^2 (Fig. 1).

Cultivation Practices

For vegetable soybean plantation, plough was done one month before planting. Furrow irrigation was done before sowing the seeds at three quarter of the height of experimental plots. Inorganic fertilizer (0:50:0) were applied as

basal during land preparation. Side dressing with NPK 50:0:50 were applied at 30 and 40 days after sowing.

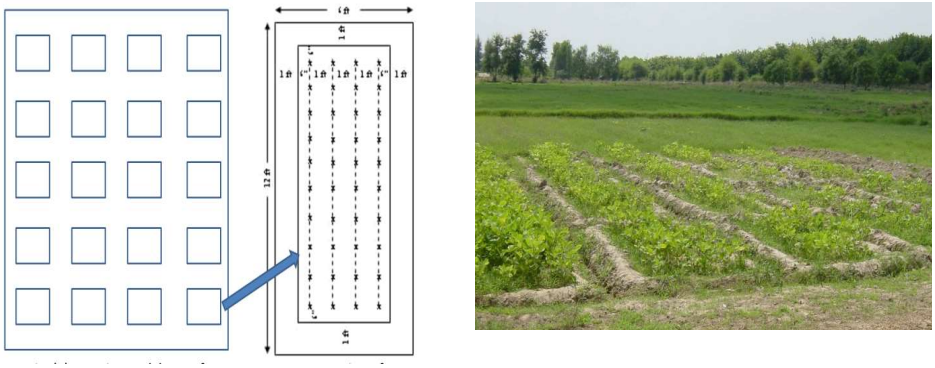

Fig 1. Experimental layout for crop water consumption of vegetable soybean

Culture Condition

The vegetable soybean was first germinated within the(90cm x 120cm) size polyethylene bag in the nursery. After one week, germinated seedlings were transferred to the experimental plot. Before transplanting the seedlings, the soil was irrigated.

Actual Crop Water Requirement

The soil samples were taken in every two days after watering by Gravimetric Sampling Method (Dastane, 1972). For determining bulk density, the soil samples were taken from every corner of each plot. These samples were weighed and placed in the soil sample container, dried in hot air oven at 104°C until the soil samples get constant dried weight and then reweighed.

The bulk density is computed using the following equation.

$$\text{Bulk density, g/cc} = \frac{\text{Dry weight}}{V}$$

where V = soil sample container volume.

For measurement of soil moisture content percent by weight, soil moisture content percent by volume and water depth, the soil samples were taken diagonally three times from each plot with boring stick at a depth of 1 ft (30 cm).These samples were weighed and then dried in hot air oven at 104°C

until the soil samples get standard dried weight and then reweighed (Dastane, 1972).

$$\text{Soil moisture content percent by weight} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Dry weight}} \times 100$$

Estimated Crop Water Requirement

Blaney and Criddle method is the widely and suitable method for crop water requirement in Myanmar. The following equations are used for estimation.

$$\mathbf{U = K f}$$

where, U = monthly evapotranspiration or consumptive use
K = crop coefficient
f = climatic coefficient

$$\mathbf{f = t p / 100}$$

where, t = temperature, °F
p = day time hour

$$\mathbf{K = K_t K_c}$$

where, K_t = temperature coefficient

$$\mathbf{K_t = 0.0173\ t - 0.314}$$

K_c = standard crop coefficient

CROPWAT method (Version 8.0)

Crop water requirement is also estimated by using CROPWAT computer program for irrigation planning and management. CROPWAT 8.0 for Windows is a decision support tool developed by the Land and Water Development Division of FAO in 2006. It is used for the calculation of crop water requirement and based on soil, climate and crop data.

Data Collection

Monthly data on temperature, humidity and average rainfall were collected. Soil sample was also collected 2 days after irrigation until the next irrigation (wilting point). Dry and wet weight of soil sample was measured and the moisture content and water depth was calculated in every sample collection time.

Results

The growing period can be divided into four distinct growth stages

(Fig. 2): initial, crop development, mid-season and late season.

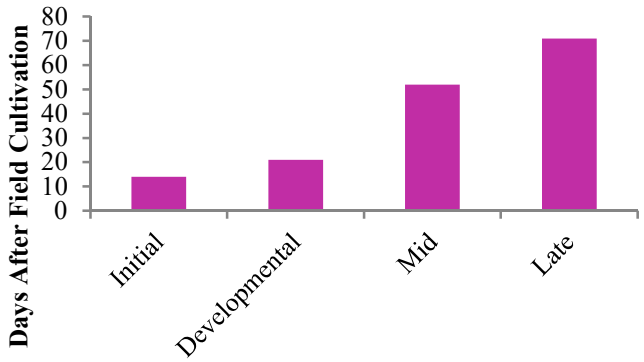

Fig 2. Growth stages of vegetable soybean

Crop water requirement at growth stages of vegetable soybean

Two days after irrigation, wet weight, dry weight, moisture content and water depth in the soil sample of the five plots were recorded until next irrigation time. The required data and the result of ET_c (actual crop water consumption) at initial, developmental, mid and late stages were shown in Tables (1, 2, 3 and 4).

Table 1. Soil sample collection for actual crop water consumption at initial stage (14-21 DAFC (Days after field cultivation)

Plot	Initial Stage (Mean)			
	Wet	Dry	MO	WD
1	48.25	44.45	12.24	1.47
2	50.03	46.10	12.21	1.42
3	49.00	45.05	12.56	1.51
4	49.58	46.08	10.88	1.31
5	50.03	46.28	11.60	1.39
Total	246.89	227.96	59.49	7.10
Mean	49.38	45.59	11.90	1.42

Wet = Wet Weight in Soil Sample MO = Soil Moisture Percent by VolumeDry = Dry Weight in Soil Sample WD = Water Depth, (inch)

Table 2. Soil sample collection for actual crop water consumption at developmental stage (21-50 DAFC)

Plot	Developmental Stage (Mean)			
	Wet	Dry	MO	WD
1	45.10	42.48	8.83	1.06
2	50.75	47.75	9.00	1.08
3	49.55	39.53	36.30	4.36
4	47.35	38.08	34.86	4.18
5	49.45	40.70	30.79	3.69
Total	242.20	208.54	119.78	14.37
Mean	48.44	41.71	23.96	2.87

Table 3. Soil sample collection for actual crop water consumption at mid stage (51-70 DAFC)

Plot	Mid Stage (Mean)			
	Wet	Dry	MO	WD
1	53.73	48.60	15.12	1.81
2	48.43	43.98	14.49	1.74
3	54.83	49.28	16.13	1.94
4	46.38	42.25	14.00	1.68
5	45.70	38.38	27.31	3.28
Total	249.07	222.49	87.05	10.45
Mean	49.81	44.50	17.41	2.09

Table 4. Soil sample collection for actual crop water consumption at late stage (71-90 DAFC)

Plot	Late Stage (Mean)			
	Wet	Dry	MO	WD
1	48.98	36.23	50.39	6.05
2	48.48	45.73	8.61	1.03
3	45.85	36.15	38.42	4.61
4	42.03	35.38	26.92	3.23
5	46.10	43.48	8.63	1.04
Total	231.44	196.97	132.97	15.96
Mean	46.29	39.39	26.59	3.19

Comparison of Actual water use and Estimated water use

Based upon the daily temperature, the estimated daily crop water consumption was calculated by Blaney and Criddle method. Actual crop water consumption or ET_c and estimated crop water requirement were also calculated by gravimetric method and Blandey and Criddle method (Table 5, Fig. 3 and 4).

Table 5. Comparison of Actual water use and Estimated water use of Gravimetric Method and Blaney & Criddle method

Growth Stage	Actual (mm)	Estimated (mm) (Blaney & Criddle)
Initial Stage	36.04	36.58
Developmental Stage	26.50	26.42
Mid Stage	43.65	36.58
Late Stage	28.83	20.24

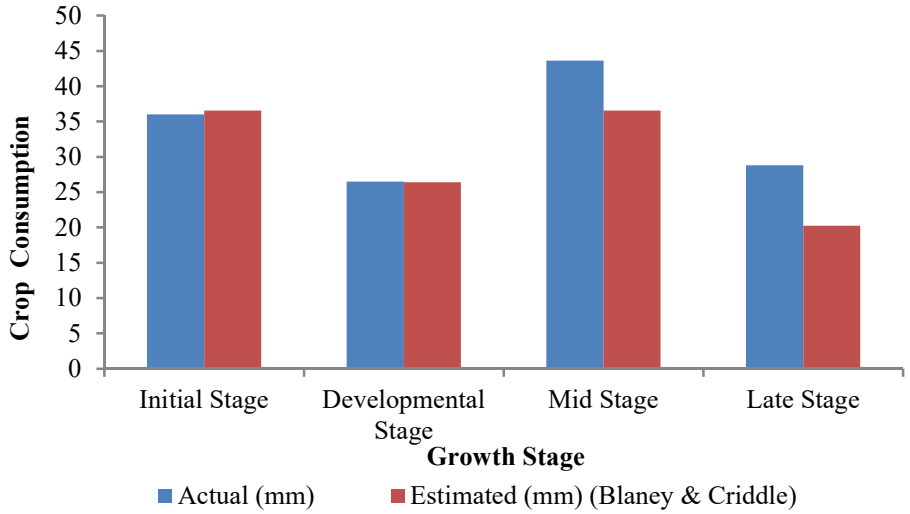

Fig 3. Comparison of crop water consumption of vegetable soybean at different growth stages by actual and Blandy and Criddle methods

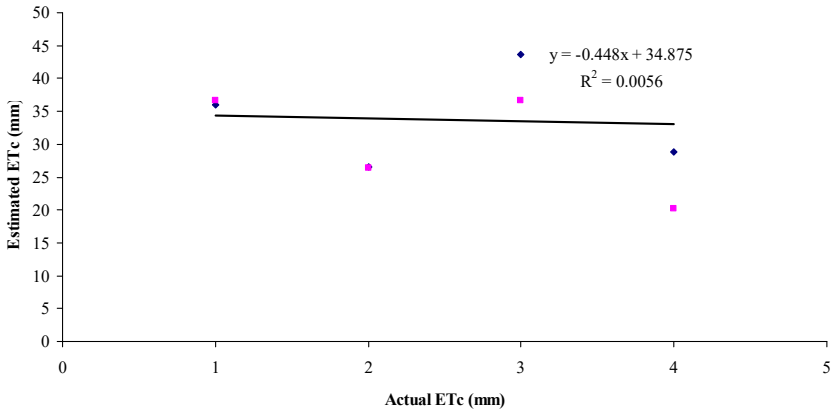

Fig 4. Linear regression graph showing the comparison of actual and estimated (Blaney & Criddle) ET_c (mm)

Comparison of CROPWAT and Blaney and Criddle for ET_c

Another methods used in this experiment are CROPWAT and Blaney and Criddle. These methods are used to measure for evapotranspiration rate of crop. When measure at the initial growth stage of crop, the Blaney and Criddle had crop water consumption of 36.58 mm and the CROPWAT had 30.25 mm. In developmental stage, Blaney and Criddle had 26.42 mm while in CROPWAT had 57.33 mm. Blaney and Criddle and CROPWAT had 36.58 mm and 50.50 mm at mid stage. At the late stage, Blaney and Criddle had higher ET_c (20.24 mm) than CROPWAT (17.60 mm). The difference was also presented in (Table 6 and Fig. 5).

Table 6. Comparison of Actual and Estimated (CROPWAT and Blaney and Criddle) Methods for water consumptive use (mm)

Growth Stage	Actual	CROPWAT	Blaney and Criddle
Initial stage	36.04	30.25	36.58
Developmental stage	26.50	57.33	26.42
Mid stage	43.65	50.50	36.58
Late stage	28.83	17.60	20.24

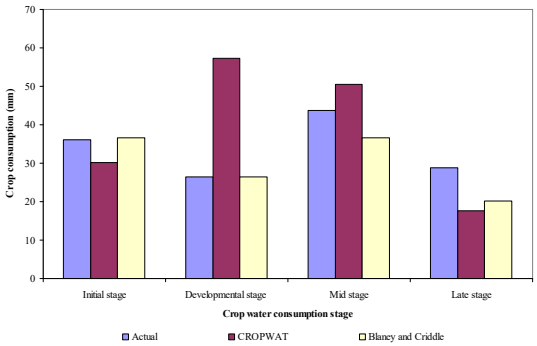

Fig 5. Comparison of crop water consumption of vegetable soybean at different growth stages by actual, CROPWAT and Blandy and Criddle methods

Discussion and Conclusion

The results showed that between two methods, the estimated water consumption of Blaney and Criddle method was more closed to the actual water consumption of vegetable soybean at any growth stage. These were 36.04 and 36.58 at initial stage; 26.50 and 26.42 at developmental stage; 43.65 and 36.58 at mid stage and 28.83 and 20.24 at the late stage while the value of CROPWAT was not associated with the actual water consumption of crop (30.25 at initial stage, 57.33 at developmental stage, 50.50 at mid stage and 17.60 at late stage). According to results of these measurements, CROPWAT had higher evapotranspiration rate than Blaney and Criddle method. The correlation of actual water consumption and estimated consumption of Blaney and Criddle was 0.005. Linear regression is correlated between -1 and 1 (http//brohrer.github.io). Therefore actual water consumption and estimated consumption of Blaney and Criddle was correlated. It is therefore suggested that the Blaney and Criddle method could substitute for actual water consumption of crop. The calculation of actual water consumption requires daily soil sample collection, drying of collected soil in an oven. If the consideration intended to the cultivators or to the arid areas which had scarcity of water, or to the required materials and so on, the Blaney and Criddle method was the most suitable and reliable method. Blaney and Criddle method is simple, using measured data on temperature only (www.fao.org). In CROPWAT, input data used are climatic data (temperature, humidity, sunshine duration, wind speed and rainfall, crop data and soil categories (www.thematrixit irrigationit lessons). The CROPWAT had higher

ET_c values when compared to Blaney and Criddle, there might be due to the climatic factor requirements. CROPWAT had higher climatic factor requirements but modified Blaney and Criddle is only T°-based method. Therefore, there has a suggested that if all the climatic data is available, CROPWAT formula can be used to measure ET_c. If temperature (T°) data is only available, the Blaney and Criddle formula is suitable to use to estimate ET_c. Crops are different in their response to water stress at a given growth stage. Crops summarized according to their sensitivity to water stress at various growth stages reveal the importance of these stages in making the irrigation decision (Karam *et al*., 2004). It can also express that if the mean temperature know, percent of annual day time hours can be calculated based on latitude data that is easily available for users. Mean temperature can be obtained easily at anywhere, therefore, temperature based modified Blaney and Criddle method can be used throughout the country. The user should be checked up with actual and estimates according to their location and eliminate condition. According to the observation, Blaney and Criddle method is well suited for vegetable soybean. It can be concluded that the irrigation schedule uses daily meteorological data as inputs to calculate evapotranspiration. It also contains a water balance ledger to keep track of water removed from the soil and water added to the soil. Irrigation is predicted according to how long it would take, to deplete the moisture remaining in the soil. The model will either save the farmer on water resources, energy and labour, or will increase his yield and income on less irrigated land. Vegetable soybean production in Myanmar is still low compare to other countries due to the lack of the applied methods. If the estimated methods can be used properly, the yield will be increased. Among methods, the Blaney and Criddle method is suitable for Myanmar because it is temperature based method and low equipment of other factors. It is therefore hoping to help the yield and income of cultivators which will also provide the national economy.

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(2) www.thematrixit irrigationit lessons)

(3) http//brohrer.github.io