FLORISTIC DIVERSITY, FOREST STRUCTURE AND ABOVEGROUND BIOMASS OF MANGROVE FOREST IN KANYIN CHAUNG COASTAL AREA, DAWEI DISTRICT, TANINTHARYI REGION, MYANMAR*

San Mon Thu[1] and Thi Thi Moe[2]

Abstract

This research conducted in the mangrove forests of Kanyin Chaung coastal area, Thayet Chaung Township, Dawei District, Tanintharyi region. To study the floristic diversity, forest structure and aboveground biomass of mangrove forest of Kanyin Chaung coastal area, twelve sample plots (20m x 20m) were established and observed during 2018. The diversity index of Kanyin Chaung coastal area was H=3.49, D= 0.90 and E= 0.92 (i.e Shannon-Wieners index (H), Simpsons index (D) and Shannon-Wieners index (E). Ecological successful species with the highest Importance Value Index were *Rhizophora mucronata* (44.02 %), *Xylocarpus moluccensis* (36.53 %) and *Avicennia marina* (31.45 %) in Kanyin Chaung coastal area. Forest density and basal area were 539 stem ha^{-1} and 14.11 m^2 ha^{-1} in the study area. The total mean aboveground biomass and carbon stocks of the study area were estimated 111.19 ton ha^{-1} and 53.37 C ton ha^{-1}. Aboveground biomass was significantly correlated with study stand ($p<0.01$) of the study area.

Keywords: Diversity, Forest Structure, Biomass, Mangrove forest

Introduction

In Myanmar, Latitude 20˚ N and 10˚ N Longitude 94˚ E and 98˚ E, from East to West 936 km and from North to South 2051 km, Coastal length 2300 km in Rakhine, Ayeyarwady delta and Tanintharyi with forest covering 52%. Tanintharyi Region lies at the southern end of Myanmar. The Region has common borders with Thailand on the east and south-east, Mon State on the north, and Andaman Sea on the west. The area of the Region is 16,735 square miles. Out of about 1,000 islands along Myanmar's coastline over 800 are in Tanintharyi coast. Myanmar hosts 32 species of mangrove trees of which *Rhizophora*, *Sonnertia*, *Aviccennia, Bruguiera* and *Xylocarpus* spp. are dominant (FAO, 2010).

Mangroves are salt-tolerant trees and shrubs that fringe intertidal areas of tropical and sub–tropical coastlines. They are keystone coastal ecosystems that are of economic, ecological and environmental importance to millions of people in the tropics. Mangroves provide important habitats and feeding grounds for a range of benthic and pelagic marine animals and bird species (Saenger, 2002; FAO, 2007a; FAO, 2007b), providing commercial fisheries resources and nursery grounds for coastal fisheries (Costanza *et al.*, 1997). As much as 75% of commercial fish species in the tropics spend part of their life cycle in mangroves environment (Mumby *et al.*, 2008). Mangroves are also important in climate regulation, nutrient cycling, habitat provisioning, shoreline protection and the provision of building materials and fuel wood. The value of mangrove goods and services worldwide has been estimated at US $ 1.6 billion each year (FAO, 2007b).

Forest structural characteristics such as canopy height, tree density, and biomass accumulation may be influenced primarily by climatic factors such as rainfall and by nutrient input (Golley *et al*., 1975; Smith 1992 and 2001). The architecture of a mangrove forest structure

[1] Dr, Assistant Lecturer, Department of Botany, University of Yangon
[2] Lecturer, Department of Botany, Dawei University
* Best Paper Award Winning Paper in Botany (2019)

is influenced by the magnitudes and periodicities of such forcing functions as tides, nutrients, hydroperiod, and stressors such as hurricanes, drought, salt accumulation, and frost. These in turn determine the basal area of the stem, the height, overall density and the species diversity of the forest stand (Lugo and Snedaker, 1974).

In the content of global warming, carbon absorption by mangrove forest ecosystem receives considerable attention now. Allomatery is a powerful tool for estimating tree weight from independent variable such as trunk diameter and height that are quantifiable in the field (Komiyama *et al.*, 2005). Mangrove forests are characterized by high productivity, high biomass and litter production (Alongi 2009; Boto & Bunt 1981; Mann 1982; Odum & Heald 1972).

Residents search for crab and prawns in the Kanyin Chaung mangrove forest. As long as they don't cause damage to the forest, they are allowed to extract its natural resources to generate income. The objective of the present study is to determine species diversity, forest structure and biomass accumulation of tree trunk weight in mangrove forest the study area in order to support sustainable mangrove forest management.

Methodology

Description of study area

This study area was carried out in the natural mangrove forest situated in the coast of Khanyin Chaung village, Thayet Chaung Township, Dawei District. It lies 98˚ 25' 45.87" E longitude and 13˚ 31' 35.92" N latitudes. Khanyin Chaung coastal mangrove area is 207.6 ha and protected since 1970. The Kanyin Chaung mangrove forest is bordered on one side by a 6.4 kilometer beach with beautiful *Casuarina* trees lining the shore. It was "unique" mangrove forest for establishing a community-based tourism project. The location map of the study area is as shown in figure 1.

The climate condition of the study area is warm and wet tropical climate for 2008-2018. The highest amount of rainfall is observed during August while April is the driest. The mean annual precipitation (MAP) is 5408 mm while the mean annual temperature (MAT) is 27°C.

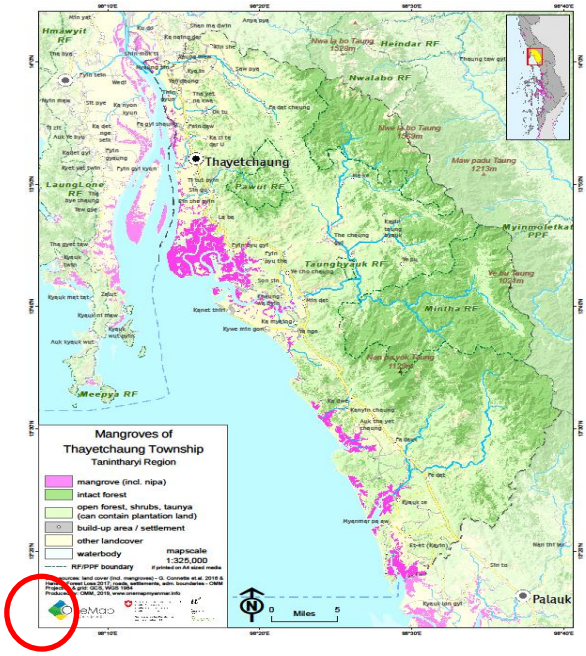


Figure 1 Location and land-cover map of Kanyin Chaung Coastal Area

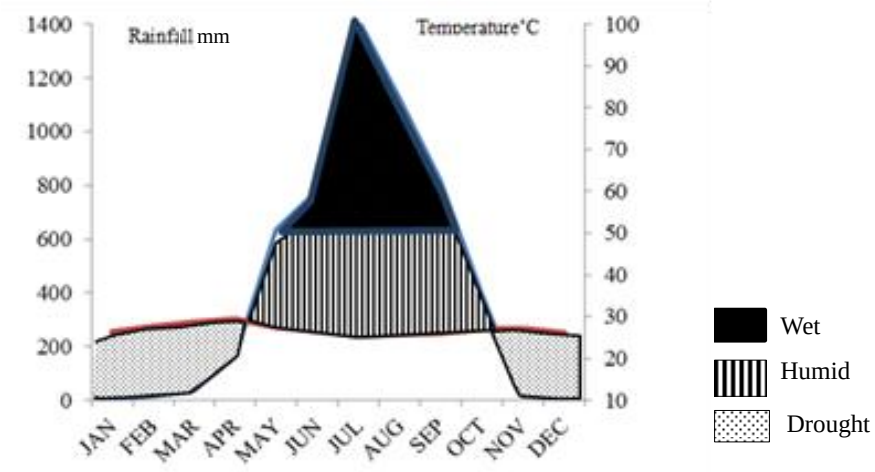


Figure 2 Climatic diagram of the study area (2008-2018)

Data collection

Each twelve plots of size 20 x 20 m were established through a nondestructive quadrat sampling technique to determine the species diversity, forest structure and aboveground biomass in the study area. The plots were laid depending on vegetation characteristic and landscape. Inside each plot, all trees with at least 5 cm girth breast height (GBH) were identified and measured the truck diameters (cm) and total height (m). We measured the truck diameter at 15 cm above the highest prop root for *Rhizophora* species, whereas the rest were measured at GBH (130 cm).

Data analysis

Forest inventory data were processed using standard analysis procedures as described by Cintron and Novelli (1984) to derive forest stand characteristics: stand frequency distribution, density (stems ha^{-1}), basal area (m^2 ha^{-1}), relative density, relative frequency, and relative dominance. Ecological importance values index (IVI) of each species was determined by summing the respective relative density, relative frequency and relative dominance. Importance value index measures relative dominance of species by criteria of how often it occurred, number of species, and area it occupies in a community. The species that attained the highest IVI was considered the principal species:

$$\text{Relative density} = \frac{100ni}{\sum_{i=1}^{m} ni}$$

$$\text{Relative frequency} = \frac{100Fi}{\sum_{i=1}^{m} Fi}$$

$$\text{Relative dominance} = \frac{100\ Bai}{Ba}$$

Species diversity is a measure of both species richness and evenness of a community. Species diversity varies greatly from one community to another. The diversity indices are better measures of the species diversity of a forest than the species density and mixture ratio and more

information than species counts alone (Weident, 2000). Species diversity is often expressed by the Shannon-Wiener index (H), Evenness (E) and Simpson's index (D) (Magurran, 1988).

Floristic diversity index, determined in this study using the Shannon-Wiener's Index (Shannon & Weaver, 1963), indicates a quantitative description of mangrove habitat in terms of species distribution and evenness. This species diversity index was used in several studies (Gevaña & Pampolina, 2009; Sharma *et al.*, 2010; Lumbres *et al.*, 2012) and was calculated using the following form:

$$H = -\sum P_i \ln P_i$$

Where, H is Shannon-Wiener diversity index, S is the number of species, and P_i is proportion of total sample belonging to the i^{th} species.

Shannon-Wiener diversity index places more weight on the rare species while Simpson's diversity index emphases on the common species (Weidelt, 2000).

Simpson's Index (D)

$$\mathbf{D} = 1 - \sum_{i=1}^{s} (\mathbf{P_i})^2$$

Where, D is Simpson's diversity index, S is the number of species, and P_i is proportion of species i^{th} in the community.

Evenness (*E*)

Species evenness is the relative abundance of individuals within a species in an area. Evenness is how evenly organisms are among species. Evenness gives an impression of the species distribution in a stand. The value E is regard as a suitable dimension for recording the second diversity component evenness. E is between 0 and 1. The value 1 represents all species as equally abundant. The value of E gradually goes down to 0 when the number of species decreases. Increasing evenness values mean a rise in diversity. Evenness was calculated by Shannon-Wiener function (1963), as follow:

$$E = \frac{H}{H_{max}} \qquad H_{max} = log_2 S$$

Where, E is the Shannon's evenness (evenness measure, range 0 - 1), H is the Shannon Wiener diversity index, H_{max} is the species diversity under conditions of maximal equitability, and S is the number of total species found in the sample plot.

Aboveground biomass and carbon stocks

Estimation of above-ground biomass (AGB) in live trees used commom allometric equations for trunk weight of mangroves was developed by (Komiyama *et al*. 2005):

$$W_s = a\ \rho\ (D^2\ H)^b$$

Where D is Diameter at breast height, H is Height, ρ is wood density of trunk and a and b are constant (a=0.0696, b=0.931)

Statistical Analysis

All statistical analysis for comparing the value of aboveground biomass and environmental factors were performed by SPSS version 16.0. Mean values was subjected to Pearson's correlations analysis at significant level of 0.01 and 0.05 to find the differences aboveground biomass, stem density, salinity, PH, soil fertility and soil texture between forest stands.

Results

Species Diversity

The diversity index of Kanyin Chaung coastal area was H=3.49, D= 0.90, E= 0.92 (i.e Shannon-Wieners index (H), Simpsons index (D) and Shannon-Wieners index (E) (Table 1). Species richness of the study area was 14.00 respectively. As a result of Shannon Wiener evenness (0.92) was evenly distributed among the species (Table 1).

Among the species recorded in the mangrove stand, *Rhizophora mucronata* was found dominating the mangrove forest with an IVI of 44.02 %; 11.01 % of relative density occurred in the study area (Table 2 and Figure 3). It was followed by *Xylocarpus moluccensis* (36.53 %) and *Avicennia marina* (31.45 %). All species with the highest importance values belonged to the family Rhizophoraceae.

Table 1 Species diversity in Kanyin Chaung coastal area

Description	**Kanyin Chaung coastal area**
Species richness	14.00
Shannon-Wiener index (H)	3.49
Simpson index (D)	0.90
Evenness (E)	0.92

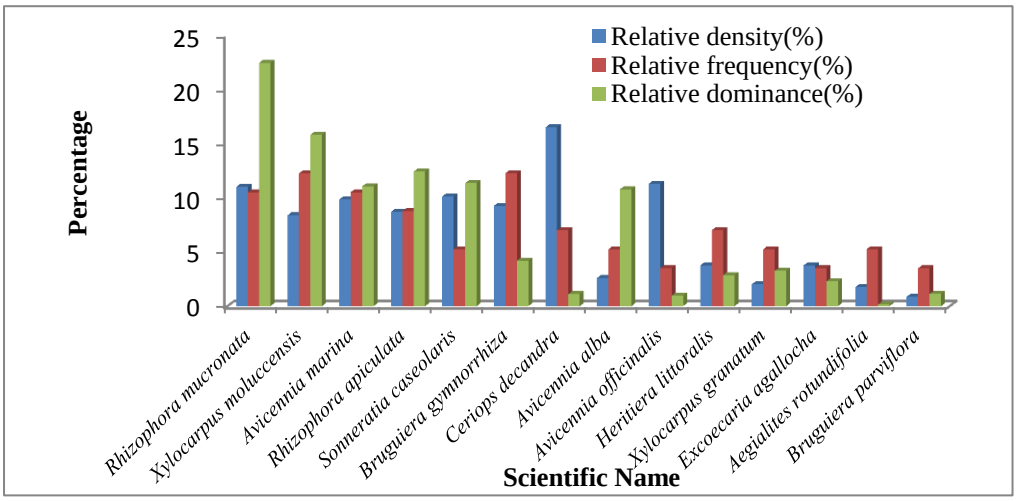


Figure 3 Relative density, frequency and dominance in Kanyin Chaung coastal area

Table 2 Ranking of importance value index in Kanyin Chaung coastal area

No.	Scientific name	RD(%)	RF(%)	RDm(%)	IVI(%)
1	*Rhizophora mucronata*	11.01	10.53	22.48	44.02
2	*Xylocarpus moluccensis*	8.41	12.28	15.84	36.53
3	*Avicennia marina*	9.86	10.53	11.07	31.45
4	*Rhizophora apiculata*	8.70	8.77	12.45	29.91
5	*Sonneratia caseolaris*	10.14	5.26	11.38	26.78
6	*Bruguiera gymnorrhiza*	9.28	12.28	4.21	25.77
7	*Ceriops decandra*	16.52	7.02	1.11	24.65
8	*Avicennia alba*	2.61	5.26	10.80	18.68
9	*Avicennia officinalis*	11.30	3.51	0.96	15.78
10	*Heritiera littoralis*	3.77	7.02	2.87	13.66
11	*Xylocarpus granatum*	2.03	5.26	3.26	10.55
12	*Excoecaria agallocha*	3.77	3.51	2.28	9.56
13	*Aegialites rotundifolia*	1.74	5.26	0.15	7.15
14	*Bruguiera parviflora*	0.87	3.51	1.13	5.51
	Total	**100**	**100**	**100**	**300**

Forest Structure

Horizontal and vertical structure

The GBH of mangrove species ranging from 5 cm to > 100 cm, total height vary from < 3 m to > 12 m. Tree density and basal area of the highest GBH classes of >100 cm in Kanyin Chaung area was 50 stem ha^{-1} and 7.42 m^2 ha^{-1} (Table 3 and Figure 4). The total basal area of mangrove species per hectare in Kanyin Chaung area was 14.11 m^2 ha^{-1} (Table 3). *Sonneratia caseolaris*, *Xylocarpus moluccensis* and *Rhizophora apiculata* registered the largest girth while *Avicennia alba*, *Rhizophora apiculata* and *Xylocarpus moluccensis* were the tallest. The average density of mangrove in the study area was 539 stem ha^{-1} (Table 3).

Population density of total individual mangrove species by the height classes of the Kanyin Chaung area was 37.10% (Table 4). While the height classes of >12 m in Kanyin Chaung area was 11.01% (Table 4). Stratification or vertical structure of the community determines the different growth forms. This stratification is determined by the species diversity and age structure of a site, and affects the tree growth due to competition for light and other resources.

Table 3 Forest structure of the study area showing basal area and stem numbers per hectare in different girth classes

GBH classes	Density (stem ha^{-1})	BA/ha (m^2 ha^{-1})
5 - 19.9 cm	227	0.34
20 - 39.9 cm	125	0.91
40 - 59.9 cm	45	0.93
60 - 79.9 cm	55	2.17
80-99.9 cm	38	2.34
> 100 cm	50	7.42
Total	**539**	**14.11**

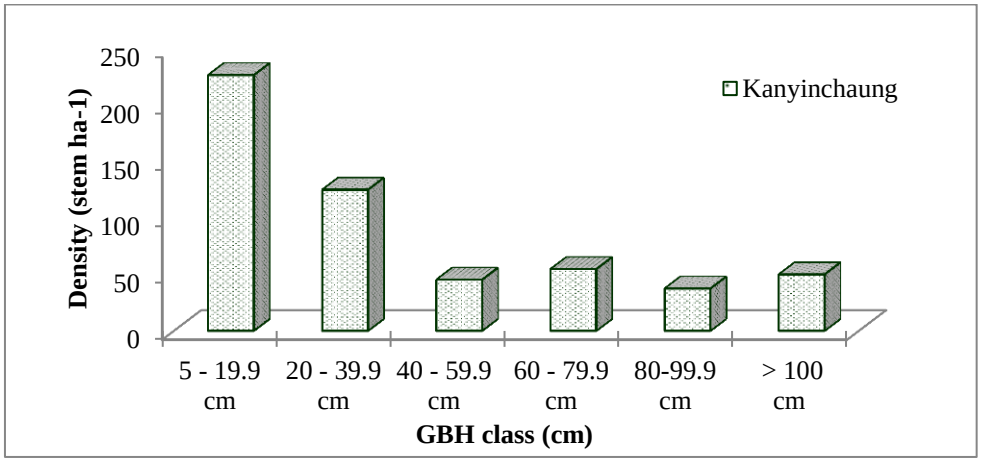


Figure 4 Stand structure of Kanyin Chaung coastal area

Table 4 Population density of mangrove species across height classes interval in Kanyin Chaung coastal area

Height Classes	No. of species	No. of Individual	% of total individual
< 3m	10	74	21.45
3 - 6m	13	128	37.10
6 - 9 m	11	66	19.13
9 - 12 m	10	39	11.30
>12 m	8	38	11.01
Total	52	345	100

Aboveground biomass and carbon stock of tree- trunk weight

On the study area**,** the Kanyin Chaung mangrove forest has a total mean biomass and carbon stock of 111.19 ton ha^{-1} and 53.37 C ton ha^{-1}. The total biomass C-stock 373.59 C ton ha^{-1} varied from 134.14 C ton ha^{-1} to 13.84 C ton ha^{-1} of each stand (Table 5 and Figure 5). Among the established sample plots, the highest huge quantities of biomass and stored carbon of

Rhizophora mucronata stand (279.45 ton ha^{-1}) and (134.14 C ton ha^{-1}) was estimated with large tree girth and high species wood density.

The results of Pearson's correlation between aboveground biomass and the environmental factors of the study area are shown in Table 6. According to the Pearson's correlation, significant negative correlations were found between study stand and total nitrogen ($p<0.05$), between study stand and soil texture (Clay) ($p<0.05$). The significant positive correlations were found between stem density and salinity ($p<0.05$), between stem density and available phosphorus ($p<0.01$). Aboveground biomass was significantly correlated with study stand ($p<0.01$) of the study area.

Table 5 Aboveground biomass and carbon stocks of each stand

Stand Name	Biomass (ton ha^{-1})	Carbon Stock (ton C ha^{-1})
Rhizophora mucronata	279.45	134.14
Xylocarpus moluccensis	129.46	62.14
Avicennia marina	127.29	61.10
Avicennia alba	98.73	47.39
Sonneratia caseolaris	83.36	40.01
Xylocarpus granatum	31.20	14.98
Bruguiera gymnorrhiza	28.83	13.84

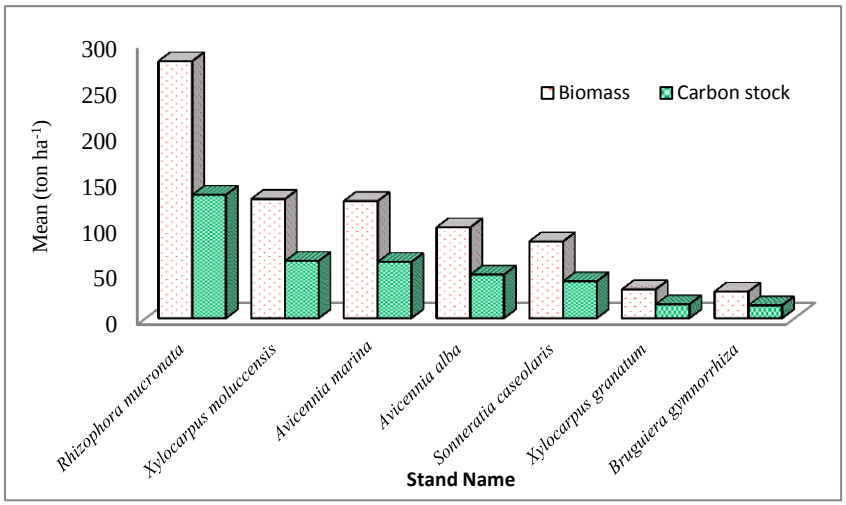


Figure 5 Aboveground biomass and carbon stocks of each stand

Table 6 Pearson's correlations between aboveground biomass and environmental factors of the study area

Variables	Study stand	Salinity	PH	N	P	K	Sand	Silt	Clay
Study stand	1	-0.621	-0.457	**-.811***	-0.541	**.830***	0.653	0.439	**-.823***
ABG Biomass (ton ha^{-1})	**.904****	0.596	0.333	0.646	0.63	-0.295	-0.547	-0.395	0.675
Stem density (n ha^{-1})	-0.408	**.794***	-0.431	0.023	**.958****	0.153	0.17	-0.362	0.071

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.
Note: pH = Soil pH, N = Total nitrogen, P = Available phosphorus, K = Potassium

Discussion and Conclusion

The species richness diversity index of Kanyin Chaung area was relatively higher than the Pantin-In area, Long-Lone Township (Thanda Soe, 2016) analysed by the method of Shannon-Wieners index (H), Simpsons index (D) and Shannon-Wieners index (E). Kirui *et al*., (2012) reported that changes in species richness in mangrove forest were likely to reduce resilience of mangrove ecosystem and make it vulnerable to natural and anthropogenic activities. Weidelt (2000) suggested that species diversity indices are better measure of the species diversity of a mangrove forest and more information than species counts alone.

Fourteen mangrove species were found in the study area with *Rhizophora mucronata*, *Xylocarpus moluccensis* and *Bruguiera gymnorrhiza* having high relative frequency compared to other species. Hamad *et al*., 2014 reported that high frequency of these species might be attributed to their high regeneration capacity despite their high use preference for building pole and fire wood. Mangrove species dominance value index indicated *Rhizophora mucronata* cover large area in the study site (Table 2). This might be attributed to the fact that most of *Rhizophora mucronata* species large in size, an indication that the species is less preferred for cutting as compared to the species of the family Rhizophoraceae and therefore has opportunities to grow into large tree.

The basal area of the highest GBH classes in Kanyin Chaung area (14.11 m^2 ha^{-1}) was higher than Pantin-In, Long-Lone area (Thanda Soe, 20016) (5.78 m^2 ha^{-1}). According to Bundotich *et al*., (2009), the observed basal area was standard of a healthy forest. The highest height classes of >12 m in Kanyin Chaung area (11.01%) was higher than the Long-Lone area (0.38 %) (Thanda Soe, 2016). Stratification or vertical structure of the community determines the different growth forms. This stratification is determined by the species diversity and age structure of a site, and affects the composition of the understory as well as tree growth due to competition for light, climatic factors and other resources.

The total mean aboveground biomass for the study area (111.19 ton ha^{-1}) within 50 years was compared to those reported the total aboveground biomass for Long-Lone Township (89.62 ton ha^{-1}) (Thanda Soe, 2016) and estuarine along the Bay of Bengal, India (60.0 ton ha^{-1}, Kathiresan *et al.*, 2013) which was lower than the study area. Moreover, the above-ground mean carbon stocks estimated in Southern China (50.0 ton C ha^{-1}, Chen *et al.*, 2012) was lower compared to that of the present study area due to Kanyin Chaung mangrove forest are easily accessible from forest age, stem density and trunk diameter with relatively less effort required to

harvest the products such as firewood and poles etc. These results agreed with the size range of trunk diameters and stem density in this present study was the sample diameter range of Komiyama *et al.*, (2005). According to Pearson's correlation, aboveground biomass was strongly significant between stands ($p < 0.01$). Stem density was significantly correlated with salinity ($p < 0.05$) as well as soil fertility ($P < 0.01$).

This study aimed at investigating the diversity, structure and aboveground biomass accumulation rates in Kanyin Chaung mangrove forest and the environmental factors. A high diversity index was observed in the study area attributed to the dominance of species, those belonging to the family Rhizophoraceae and Avicenniaceae. Nonetheless, because of the large tree girth and high density of species observed in this forest, it has the potential to sequester and store large amount of atmospheric carbon. Climatic factors, particularly rainfall, are important determinants of species richness, stand structure, and biomass of tree trunk weight in mangrove forests. It will be valuable for the restoration, conservation and management of natural mangrove forest resources. The restoration of natural mangrove forest is beneficial for balance of natural environment and local peoples' requirement. Mangroves ecosystem may be developed as sources of high value commercial products and fishery resources and as sites for an ecotourism industry.

Recommendation

This study presented findings that demonstrate the forest structure and biomass densities are key elements to view of carbon market and carbon trading as significant climate change mitigation opportunity, it is recommended that (a) assessment and monitoring should be done to assess mangrove cover changes overtime and predicts extents of human impacts on mangrove forest, (b) permanent plots should be established for MRV system of mangrove forest carbon stocks and (c) collaborative management should be done by harmonizing rules and regulations across stakeholders.

Acknowledgement

I would like to express our sincere thanks to Professor Dr Myint Aung, Department of Botany, University of Yangon for their invaluable advice and encouragement. I would like to mention to One Map Myanmar for helping land cover map.

References

Alongi, D. M. (2009). **The Energetics of Mangrove Forests.** Springer, Dordrecht.

Boto, K. G. & J. S. Bunt. (1981). **Tidal export of particulate organic matter from a northern Australian mangrove system**. *Estuarine Coastal and Shelf Science* **13:** 247-255.

Bundotich ,G., M. Karachi, E. Fondo and J.G. Kairo. (2009). **Structural inventory of mangrove forest in Ngomeni, in Advances in Coastal Ecology**: People Processes and Ecosystem in Kenya, J. Hoorweg and N. Muthiga, Eds., pp. 111-121, African studies centre, Leiden, Netherlands.

Chen, L., X. Zeng, N.F.Y. Tam, W. Lu, Z. Luo, X. Du and J. Wang. (2012). **Comparing carbon sequestration and stand structure of monoculture and mixed mangrove plantations of *Sonneratia caseolaris* and *S. apetala* in Southern China.** *For. Ecol. Manage.*, 284: 222-229.

Cintron, G and Y. S. Novelli. (1984). "**Methods for studying mangrove structure,"** in The Mangrove Ecosystem: Research Methods, S. C. Snedaker and J. G. Snedaker, Eds., pp. 91–113, UNESCO, Paris, France.

Costanza R., R. d'Arge, R. de Groot, S. Farber, M. Grasso, Hannon B., Limburg K., Naeem S., O'Neill R. V., Paruelo J., Raskin R. G., Sutton P., M. van den Belt. (1997). T**he value of the world's ecosystem services and natural capital.** *Nature* **387**:253-260.

FAO, (2007a). **State of the world's forests**. Food and Agriculture Organization of the United Nations, Rome. 237.

FAO, (2007b). **The World's Mangroves 1980-2005**. Forestry Paper 153, FAO, Rome. ix + 77 pp.

FAO. (2010). **Global Forest Resources Assessment 2010 Main Report** (FRA 2010); Food and Agriculture Organizations of the United Nations: Rome, Italy, 2010.

Gevaña, D.T. and N.M. Pampolina. (2009). **Plant diversity and carbon storage of a Rhizopora stand in Verde Passage, San Juan, Batangas, Philippines**. *J. Environ. Sci. Manage.*, 12: 1-10.

Golley, F. B., J. T. McGinnis, R. G. Clements, G. I. Child, and M. I. Duever. (1975). **Mineral cycling in a tropical moist forest ecosystem**. University of Georgia Press, Athens.

Hamad, H. M., I. S. Mchenga, and M. I. Hamisi. (2014). **"Status of exploitation and regeneration of mangrove forests in Pemba Island, Tanzania,"** *Global Journal of Bio-Science and Biotechnology*, vol. 3, no. 1, pp. 12–18.

Kathiresan, K., R. Anburaj, V. Gomathi and K. Saravanakumar. (2013). **Carbon sequestration potential of *Rhizophora mucronata* and *Avicennia marina* as influenced by age, season, growth and sediment characteristics in southeast coast of India.** *J. Coast Conserv.*, DOI 10.1007/s11852-013-0236-5.

Kirui, B. Y. K., J. G. Kairo, M. W. Skov, M. Mencuccini, and M. Huxham. (2012). **Effects of species richness, identity and environmental variables on growth in planted mangroves in Kenya.** *Marine Ecology Progress Series*, vol. 465, pp. 1–10.

Komiyama, A., S. Poungparn and S. Kato. (2005). **Common allometric equations for estimating the tree weight of mangroves**. Journal of Tropical Ecology 21: 471-477. 2005 Cambridge University Press.

Lumbres, R.I.C., J.A. Palaganas, S.C. Micosa, K.A. Laruan, E.D. Besic, C.W. Yun and Y.J. Lee. 2012. **Floral diversity assessment in Alno communal mixed forest in Benguet, Philippines**. *Landsc. Ecol. Eng.*, DOI 10.1007/s11355- 012-0204-5.

Lugo, A. E., S. C., Snedaker. (1974). **The ecology of mangroves.** *Annual Review of Ecology and Systematics* **5** 39-64.

Magurran, A. E. (1988). **Ecological diversity and its measurement**. Chapman and Hall, London. 179 pp.

Mann, K. H. (1982). **Ecology of Coastal Waters:** *A Systems Approach*. University of California Press, Berkley, USA.

Mumby P. J., Broad K., Brumbaugh D. R., Dahlgren C. P., Harborne A. R., Hastings A., Holmes K. E., Kappel C. V., Micheli F., Sanchirico J. N., (2008). **Coral reef habitats as surrogates of species, ecological functions, and ecosystem services**. *Conservation Biology* **22**:941-951.

Odum, W. E. & E. J. Heald. (1972). **Trophic analysis of an estuarine mangrove community.** *Bulletin of Marine Science* **22:** 671-738.

Saenger, P. (2002). **Mangrove Ecology, Silviculture and Conservation.** Kluwer Academic Publishers, Dordecht, The Netherlands.

Shannon, C. E, and Wiener, W. (1963). **The Mathematical Theory of Communication**, University of Illinois Press, Urbana, USA.

Sharma, C.M., N.P. Baduni, S. Gairola, S.K. Ghildiyal and S. Suyal. (2010). **Tree diversity and carbon stocks of some major forest types of Garhwal Himalaya, India.** *For. Ecol. Manage.*, 260: 2170-2179.

Smith, T. J. (1992). Forest structure. Pp. 101-136. In A. I. Robertson and D. M. Alongi (eds.), **Tropical Mangrove Ecosystems.** American Geophysical Union, Washington, D.C.

Smith, R.L and T.M Smith (2001). **Ecology and Field Biology**.6th edition. Addison Wesley Longman, San Francisco.

Thanda Soe. (2016). **Phytosociological Study of Mangrove Vegetation in Laung-Lone Township**, **Tanintharyi Region.** PhD Thesis, University of Yangon.

Weidelt, H.J. (2000). **Lectures Notes on Tropical Silviculture I and II.** Institude of Silviculture, Sec.II, Tropical Silvicultue, Gottingen University, Germany (Unplished).

STUDY ON THE FERMENTATION CONDITIONS OF SELECTED ENDOPHYTIC FUNGUS, SL-26 FROM *PRUNUS PERSICA* (L.) BATSCH

Su Su Latt[1], Zar Zar Yin[2] and Kay Thi Mya[3]

Abstract

The plant samples were collected from Mogok Township, Mandalay Region. These selected plants were identified as Rosaceae family - *Pyrus communis* L. (Thit-taw), *Eriolobus indica* Schnied. (Makhauk), *Prunus armeniaca* L. *(*Met-man), *Prunus persica* (L.) Batsch (Met-mon), and *Cydonia oblonga* Mill. (Chin-shaw-khar), during June to August, 2017. Isolation of endophytic fungi was done by surface sterilization procedure. The antimicrobial activity of these fungi was performed by agar well diffusion method with six kinds of test organisms. Ten fungal strains showed antimicrobial activity. Among them, three fungal strains exhibited the antimicrobial activity on four test organisms. Especially, SL-26 exhibited the moderate antibacterial activity (22.61 mm) against *Escherichia coli* at 5 days. Therefore, SL-26 was selected and the fermentation parameters of this fungus were optimized by the proper age and size of inoculum, effect of carbon and nitrogen sources on antibacterial metabolites production against *E coli*. According to the results, 120 hours age and 25% inoculum size were the most suitable condition for SL-26. In the effect of maximum antibacterial activity on various carbon and nitrogen sources, SL-26 showed the best activity on lactose and asparagine. the comparison of shaking culture and static culture, the antibacterial activity of static culture was more than that of shaking culture. And then the effect of temperature and pH were studied and the best activity was found at 30℃ and pH 6.5. Moreover, thirteen kinds of fermentation media (FM) and fermentation period were investigated. The highest antibacterial activity of SL-26 was obtained in FM-11 and the 5th days to be optimum fermentation incubation period. Endophytic fungi are the group of organism with very good potential for application in plant improvement and disease control.

Keywords: Endophytic fungi**,** antimicrobial activity, fermentation

Introduction

Endophytes are microorganisms in living plant tissues, apparently without inflicting negative effects (Carroll, 1989). Endophytes are presumably ubiquitous in the plant kingdom, some of which can improve the ecological adaptability of hosts (Miller, 2002).

Endophytic fungi are found in all kinds of plants, i.e. trees, grasses, algae and herbaceous plants. These microorganisms could improve the level of resistance to disease and abiotic stress, as well as favoring the growth of crop plants (Waller *et al*., 2005), producing bioactive substances (Lin *et al.,* 2010, Selim *et al*., 2011) and exhibiting antagonistic (Rocha *et al*., 2011) and antimicrobial activity (Kharwar *et al*., 2010).

Endophytic fungi form the promising source for the production of novel products with biological activity (Pimentel *et al.,* 2011). But the production of these metabolites is largely influenced by the nutritional as well as environmental factors associated with the fungi. The nutritional factors include carbon source, nitrogen source etc. and environmental factors include pH, temperature, incubation period (Thakur *et al.,* 2009). Optimization of such factors is necessary for obtaining highest yield of secondary metabolites. The aim and objectives of this research were to isolate the endophytic fungi from five selected plants of Rosaceae family, to evaluate the antimicrobial activity of isolated endophytic fungi, to investigate the optimum age and size of inoculum, to screen the effect of carbon and nitrogen sources, to compare the

[1] Assistant lecturer, Department of Botany, Pathein University
[2] Associate Professor, Department of Botany, Pathein University
[3] Professor and head, Department of Botany, Pathein University

antibacterial activity on shaking culture and static culture, to optimize the effect of pH and temperature on bioactive metabolites production and to observe the effect of sutible synthetic fermentation medium of selected endophytic fungus (SL-26).

Materials and Methods

Study Area and Collection of Plant Samples

These plant samples, *Pyrus communis* L. (Thit-taw), *Eriolobus indica* Schnied. (Makhauk), *Prunus armeniaca* L. *(*Met-man), *Prunus persica* (L.) Batsch (Met-mon), and *Cydonia oblonga* Mill. (Chin-shaw-khar) were collected from Mogok Township, Mandalay Region from June to August, 2017. The identification of these plants were referred by (Flora of Hong Kong, 2009 and Hundley and Chit Ko Ko, 1987) at Department of Botany, Pathein University.

Isolation of Endophytic Fungi (Espinosa *et al*., 1991)

In the isolation procedure of endophytic fungi, the leaves were washed in running tap water for 15 minutes and were cut into about 0.3 cm pieces. Then, these parts were sterilized by soaking in 95% ethanol for 15 minutes. And again, these parts were cut into smaller pieces and dried on sterilized tissue paper. After drying these pieces were placed on as Malt Extract Agar (BMEA) medium, Czapek-Dox Agar (CZA) medium, Malt Extract Agar (MEA) medium, Glucose Ammonium Nitrate Agar (GAN) medium, Dichloran-Rose Bengal-Chloramphenicol Agar (DRBC) medium and Potato Dextrose Agar (PDA) medium plate and incubated at room temperature. When hypal tips grow out, they were transferred into Malt Extract Agar (BMEA) medium.

Screening for Antimicrobial Activity (NITE 2005)

The isolated fungi were grown on BMEA medium at room temperature for 5 days. After incubation period, these fungi inoculated into the seed medium (glucose 0.5 g, peptone 0.3 g, yeast extract 0.3 g, $MgSO_47H_2O$ 0.01 g, K_2HPO_4 0.01 g, $CaCO_3$ 0.01 g, DW 100 mL at pH 6.5) for 3 days at room temperature. After three days, the seed medium (25%) was transferred into the fermentation medium (glucose 1.0 g, peptone 0.5 g, yeast extract 0.5 g, $MgSO_47H_2O$ 0.01 g, $K_2HPO_4$0.01 g, $CaCO_3$0.01 g, DW 100 mL at pH 6.5) and carried out for 3- 10days and evaluated the antimicrobial activity by agar well diffusion method.

Screening of Antimicrobial Activity by Agar Well Method (Collins, 1965)

1 day old culture test broth (0.2 mL) was added to 25 mL warm assay medium (glucose 1.0 g, peptone 0.3 g, KNO_3 0.1 g, DW 100 mL, agar 1.8 g) and thoroughly mixed and poured into plate. After solidification, the agar was left to set Cork borer was used to make the wells (8 mm in diameter). And then, the fermented broth (20 µL) was carefully added into the well and incubated at room temperature for 24-48 hours. The diameter of the zones of inhibition around each well measured and recorded after 24-48 hours incubation.

Test Organisms

Escherichia coli AHU 5436, *Bacillus subtilis* IFO 90571, *Bacillus pumilus*, *Candida albicans* NITE 09542, *Pseudomonas fluorescens*, IFO 94307 *Staphylococcus aureus* AHU 8465 were obtained from NITE (National Institute of Technology and Evaluation, Kisarazu, Japan).

Study on the Effects of Ages of Inoculum on Fermentation (Cruger, 1989)

The selected fungus SL-26 was grown on BMEA medium at room temperature for 5 days and then was transferred into seed medium. Incubation period 3 to 10 days were used for the production of antibacterial metabolite and the procedure of seed culture medium was also used as the previous method. And then, seed culture was transferred to 100 mL conical flask containing of fermentation medium and incubated at room temperature. The inoculum age of fermentation were studied by 48, 60, 72, 84, 96, 108, 120, and 132 hr.

Study on the Effects of Sizes of Inoculum on Fermentation (Cruger, 1989)

The selected fungus SL-26 was grown on BMEA medium for 5 days at room temperature. After 5 days incubation period, this fungus was inoculated into 100mL seed medium. For the size of inoculum, seed culture (5%, 10%, 15%, 20%, 25%, 30% and 35%) were transferred into the each flask of 100 mL fermentation medium. All fermentation media were carried out 5 days and antibacterial activity was investigated by agar well diffusion method.

Effect of Carbon Sources

Carbon sources (each 1.0 g or 1.0 mL) such as lactose, glycerol, dextrose, D-mannitol, arabinose, fructose, glucose, maltose, tapioca powder, sucrose, rice, soluble starch, molasses, oat, corn, carrot and potato were used. Fermentation were incubated at room temperature for 5 days.

Effect of Nitrogen Sources

Nitrogen sources (each 0.05 g or 0.05 mL) such as asparagine, malt extract, peptone, fishcake, milk, yeast extract, gelatin, soybean, casein, sodium nitrate, meat, urea, ammonium nitrate, potassium nitrate, rice bran, peanut, ammonium chloride and ammonium sulphate were also used. Fermentation medium were incubated at room temperature for 5 days.

Comparision of Static Culture and Shaking Culture (Hassan *et al*., 2017)

250 mL conical flask containing 100 mL of the fermentation medium was incubated on the shaker (100 rpm) for 5 days. At the same time, another those fermentation medium was incubated under static condition without shaking. These shaking culture and static culture were compared by using agar well diffusion method.

Effect of Incubation Temperature (Cazar *et al*., 2004)

The optimization temperature for antibacterial metabolite production was carried out at six different incubation temperatures viz. 20, 25, 30, 35, and 40 and 45°C. The fermentation medium was carried out 5 days and antifungal activity was studied by agar well diffusion method.

Effect of pH (Furtado *et al*., 2005)

The optimization of pH of the fermentation broth for antibacterial metabolite production was done by carrying out the fermentation at seven different pH values viz. 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0. For each pH value, desired pH by using either 0.1M NaOH or 0.1M HCl was adjusted into fermentation medium

Effect of Different Carbon and Nitrogen on Fermentation Medium

In this study five different of carbon such as lactose, glycerol, D-Mannitol, arabinose and fructose and five different of nitrogen were used such as asparagine, malt extract, peptone,

fishcake and milk. Fermentation medium FM1- (lactose and Asparagine), FM2- (lactose and malt extract), FM3- (lactose and peptone), FM4- (lactose and fishcake), FM5 (lactose and milk), were used as well as, FM6-(Asparagine and Glycerol) FM7-(asparagine and D-Mannitol), FM8-(asparagine and arabinose), FM9-(asparagine and fructose), FM10-(lactose, glycerol and asparagine), FM11-(lactose, glycerol, and malt extract), FM12- (lactose, asparagine and malt extract) and FM13-(glycerol, asparagine and malt extract) were applied.

Media Used in Fermentation (NITE, 2005)

Fermentation was undertaken by the suitable conditions of 25% sizes and 120 hrs ages of inoculum with thirteen different media. Fermentation was carried out for 5 days and antibacterial activity test was carried out 24 hrs.

Effect on Fermentation Period of SL-26

The optimal fermentation parameters period of isolated fungus SL-26 was studied such as 72 hour seed culture, 25% inoculum size, temperature 30˚C, pH-6.5 and static culture of fermentation, the antibacterial activity against *E. coli* was observed 3 days and 10days.

Results

Isolation of Endophytic Fungi

A total of thirty three fungi were isolated from five selected species of Rosaceae family. Isolated fungi were designated as SL. Six isolates (SL-1 to 6) were obtained from *Pyrus communis* L. and the other strains (SL-7 to 17) were isolated from *Eriolobus indica* Schnied. Another strains (SL-18 to 24) were obtained from *Prunus armeniaca* L. and SL-25 to 28 were isolated from *Pyunus persica* (L.) Batsch and (SL-29 to SL-33) were obtained from *Cydonia oblonga* Mill. These results were shown in Table 1 and Figure 1

Table 1 Isolated endophytic fungi

Scientific Name	Myanmar Name	English Name	Fungi
***Pyrus communis* L.**	Thit-Taw	Pear	SL-1 to SL-6
***Eriolobus indica* Schnied.**	Mat-Khauk	Crabapple	SL-7 to SL-17
***Prunus armeniaca* L.**	Met-man	Apricot	SL-18 to SL-24
***Pyunus persica* (L.) Batsch**	Met-mon	Peach	SL-25 to SL_28
***Cydonia oblonga* Mill.**	Chin-shaw-khar	Quince fruit	SL-29 to SL-33

Table 2 Morphological colour of isolatedc Endophytic fungi

No.	Isolated fungi	Surface colour	Reverse colour
1	SL-1	White	White
2	SL-2	Black	Yellow
3	SL-3	Pale brown	Yellow
4	SL-4	Black	White
5	SL-5	Brown	Pale brown
6	SL-6	Pale green	Yellow
7	SL-7	Brown in the center, White at the end	Yellow
8	SL-8	Black	Pale Yellow
9	SL-9	Black	Yellow
10	SL-10	Brown	Brown

No.	Isolated fungi	Surface colour	Reverse colour
11	SL-11	Pale orange	Red
12	SL-12	White	Pale Yellow
13	SL-13	Dark brown	Yellow
14	SL-14	White	White
15	SL-15	Pale brown	White
16	SL-16	Green	White
17	SL-17	Pale yellow	Yellow
18	SL-18	Green in the center, White at the end	Green
19	SL-19	Green	Yellow
20	SL-20	Cream	Yellow
21	SL-21	Brown	Pale Yellow
22	SL-22	Yellow	Cream
23	SL-23	Pale brown	Yellow
24	SL-24	Yellow	Pale Yellow
25	SL-25	Black	White
26	SL-26	Blue green	Cream
27	SL-27	Yellow	Yellow
28	SL-28	Pale green	Yellow
29	SL-29	Pale brown	Black
30	SL-30	Brown	Yellow
31	SL-31	Dark green Brown	Brown
32	SL-32	Cream	Pale brown
33	SL-33	White	White

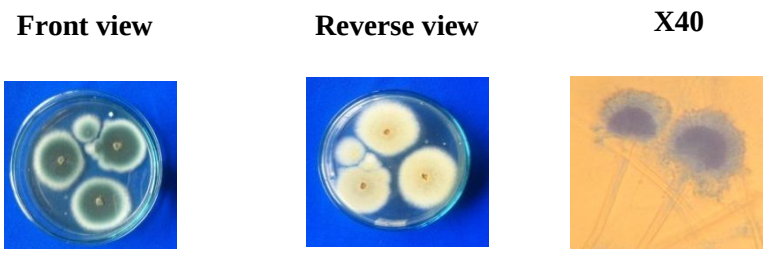


Figure 1 Morphology and microscopical character of selected fungus SL-26

Antimicrobial Activity of Endophytic Fungal Strains

Three endophytic fungi were tested for antimicrobial activity with four test organisms. Agar well diffusion method were employed for assay performance. Three isolated fungal strains exhibited the morderate antimicrobial activity. Among potent strains, SL-26 showed strong antibacterial activity against *Escherichia coli* (22.61mm) in 5 days fermentation period, than other fungal strains SL-6 and SL-19. Therefore, SL-26 was selected for further research.

Table 3 Antimicrobial Activity of Endophytic Fungus SL-26

Fermentation period (days)	**Test organisms and antimicrobial activity (mm)**			
	1	**2**	**3**	**4**
3	15.05	16.22	15.67	16.13
4	18.81	16.40	17.89	17.11
5	22.61	18.02	20.27	17.22
6	-	15.92	17.12	17.43

Antimicrobia; activity of isolated fungi against
(1) *Escherichia coli* (2) *Bacillus subtilis*
(3) *Bacillus pumilus* (4) *Candida albicans*

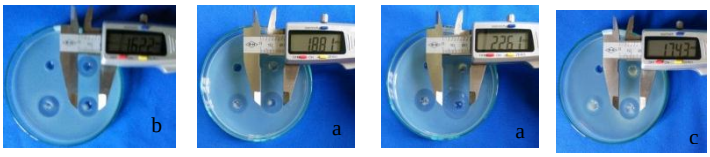


Figure 2 Antimicrobia; activity of isolated fungi against
(a) *Escherichia coli* (b) *Bacillus subtilis*
(c) *Bacillus pumilus* (d) *Candida albicans*

The Effect on Age of Inoculums of SL-26

In the effect of age of inoculum, the antibacterial activity of SL-26 was investigated by using 48, 60, 72, 84, 96, 108, 120, and 132 hrs old culture age of inoculums on *E. coli*. The results showed that 120 hrs age of inoculum gave the highest activities (20.51mm) followed by 20.11 mm at 108 hrs and 19.60 mm at 132 hrs age of inoculum.

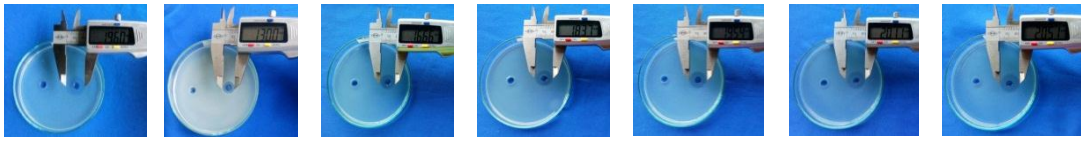


Figure 3 The effect on age of inoculum of SL-26 on *E. coli*

The Fffect on Sizes of Inoculum of SL-26 on *E. coli*

In this research work, the effect of size of inoculum was studied by using 5%, 10%, 15%, 20%, 25%,30% and 35 % inoculum. The highest antibacterial activity was obtained by using 25% inoculum (23.23mm), followed by 20% (23.10mm) and 15% (22.91 mm) respectively.

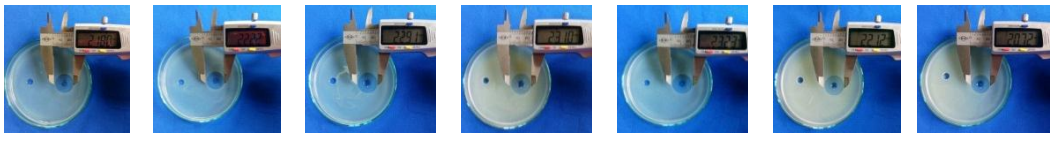


Figure 4 The effect on size of inoculum of SL-26 on *E. coli*

Effect of Carbon and Nitrogen Utilization on the Antibacterial Activity

The significant inhibition zone (22.14 mm and 21.53 mm) were obtained in glycerol and lactose as amended media. The moderate inhibition zone were found on D-mannitol (20.56 mm), arabinose (20.51 mm), fructose (20.39 mm), glucose (20.30 mm), and maltose (20.26 mm). Tapioca powder (19.69 mm), sucrose (19.55 mm), rice (19.23 mm), soluble starch (18.88 mm), molasses (18.56 mm), oat (17.78 mm), corn (17.41 mm), carrot (16.87 mm) and potato (15.58 mm) were regarded as poor inhibition zone. Similarly, the addition of asparagine displayed the greatest activity (23.65 mm), followed by malt extract (23.05 mm), peptone (22.46 mm), fish cake (21.61 mm), milk (21.31 mm), yeast extract (20.62 mm), gelatin (20.48 mm), soybean (20.33 mm), casein (19.94 mm), sodium nitrate (19.80 mm), meat extract (19.42 mm), urea (19.40 mm), ammonium nitrate (18.99 mm), potassium nitrate (18.28 mm), rice bran (17.67 mm) and peanut (17.62 mm). There were no activities when NH_4Cl and $(NH_4)_2SO_4$ were used as nitrogen source. These results were shown in Figure 5 and 6.

Table 4 Effect of carbon and nitrogen utilization on fermentation

Sr. No	Carbon sources	Antibacterial Activity(mm)
1	Lactose	22.14
2	Glycerol	21.53
3	D-Mannitol	20.56
4	Arabinose	20.51
5	Fructose	20.39
6	Glucose	20.30
7	Maltose	20.26
8	Tapioca powder	19.69
9	Sucrose	19.55
10	Rice	19.23
11	Soluble starch	18.88
12	Molasses	18.56
13	Oat	17.78
14	Corn	17.41
15	Carrot	16.87
16	Potato	15.58

Sr. No	Nitrogen sources	Antibacterial Activity (mm)
1	Asparagine	23.65
2	Malt extract	23.05
3	Peptone	22.46
4	Fishcake	21.61
5	Milk	21.31
6	Yeast extract	20.62
7	Gelatin	20.48
8	Soy- bean	20.33
9	Casein	19.94
10	Sodium nitrate	19.80
11	Meat extract	19.42
12	Urea	19.40
13	Ammonium nitrate	18.99
14	Potassium nitrate	18.28
15	Rice bran	17.67
16	Peanut	17.62
17	Ammonium chloride	No activity
18	Ammonium sulphate	No activity

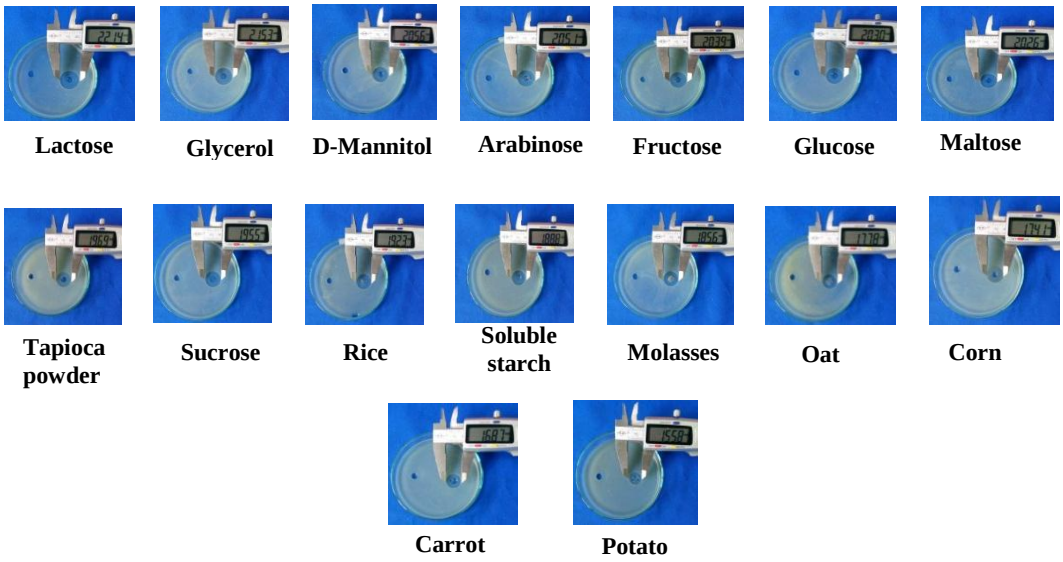


Figure 5 Effectof carbon utilization on the antibacterial activity of SL-26

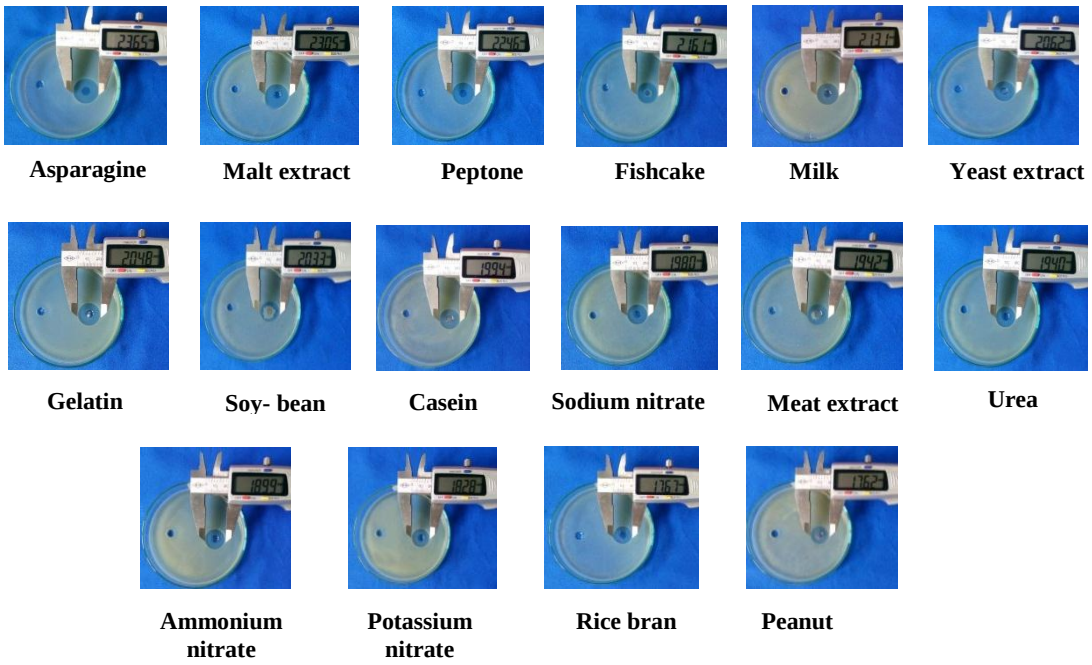


Figure 6 Effect of nitrogen utilization on the antibacterial activity of SL-26

Comparision of Static culture and Shaking Culture

When comparing the static culture and shaking culture of fermentation medium, antifungal activity from static culture is better than (20.52 mm) than that of shaking culture (18.30 mm).

Figure 7 Comparison on static culture and shaking culture of SL-26

Effect of Temperature and pH

Maximum antibacterial activity was recorded at 30˚C (20.59mm), followed by 35˚C (18.76 mm) and 25˚C (17.28 mm). There was a gradual decrease in antibacterial activity when the temperature was increased from 35˚C to 40˚C. Maximum inhibitory zone was occurred in pH 6.5 (20.76mm), followed by pH 7 (20.58 mm), pH 6 (19.89 mm) and pH 7.5 (19.87 mm). Under this conditions, minimum inhibitory zone was observed at pH-5 (17.15 mm), pH-4.5 (15.71mm) and pH-4 (14.98mm) respectively.

Table 5 Effects of different temperature and pH on the antibacterial against *E. coli*

Temperature range	Inhibition zone (mm)	pH range	Inhibition zone (mm)
20ºC	16.53	4	14.98
25ºC	17.28	4.5	15.71
30ºC	**20.59**	5	17.15
35ºC	18.76	5.5	18.53
40ºC	14.10	6.0	19.89
45ºC	13.57	6.5	20.76
		7	20.58
	Static culture	7.5	19.87
		8.0	18.35

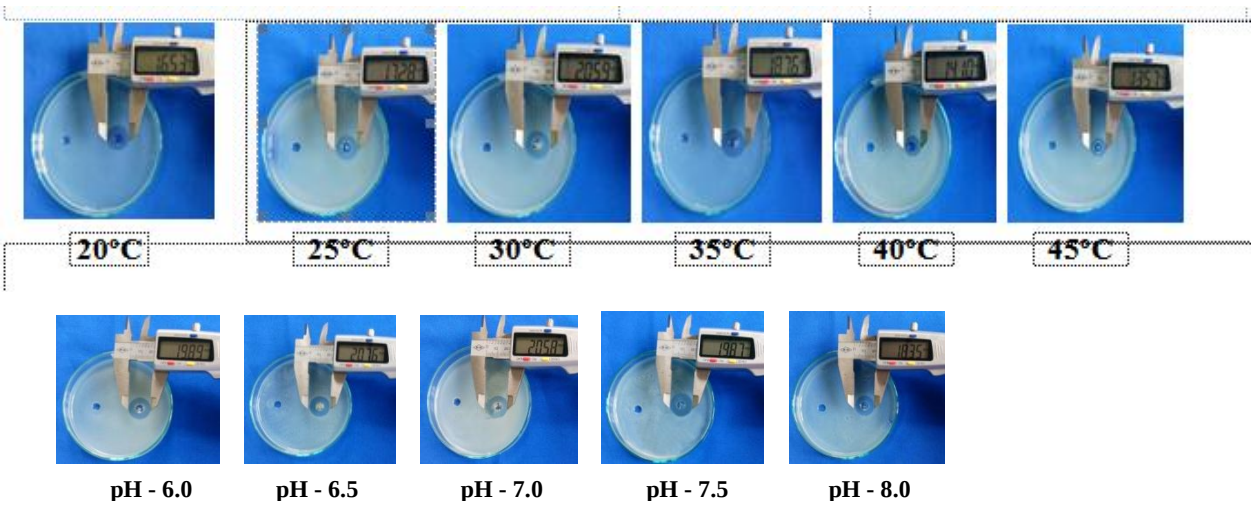


Figure 8 Effects of different temperature and pH on the antibacterial against *E. coli*

Antibacterial Activity of SL-26 on Thirteen Fermentation medium

In this study, FM-11 showed remarkable result (24.15mm) followed by FM-4 (23.76 mm) , FM-10 (23.25 mm), FM-8 (23.18mm) and FM-6 (23.16 mm) were also studied.

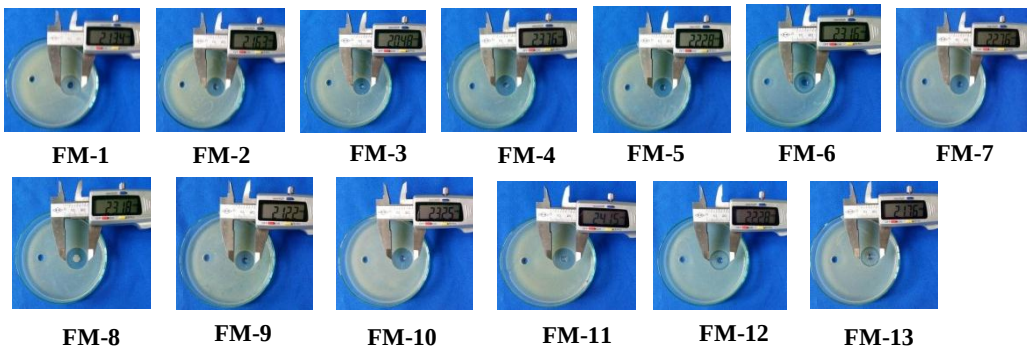


Figure 9 Antibacterial activity of SL-26 on thirteen fermentation medium

Effect of Fermentation Period of SL-26

The optimal fermentation parameters such as 72 hour seed culture, 25% inoculum size, temperature 30˚C, pH-6.5 and static culture of fermentation, the antibacterial activity against E. coli was observed 3 days and 10days. In 5 days fermentation period, the highest antibacterial metabolite of SL- 26 was obtained (26.12mm). And there was gradually decrease antibacterial activity in 6 days to 10 days fermentation period (24.77 mm, 22.76 mm, 20.49mm, 18.71mm and 18.68 mm) respectively.

Table 6 Antibacterial activity on suitable fermentation medium against *E.coli*

Fermentation period	Inhibitory zone
3	19.90
4	20.98
5	26.12
6	24.77
7	22.76
8	20.49
9	18.71
10	18.68

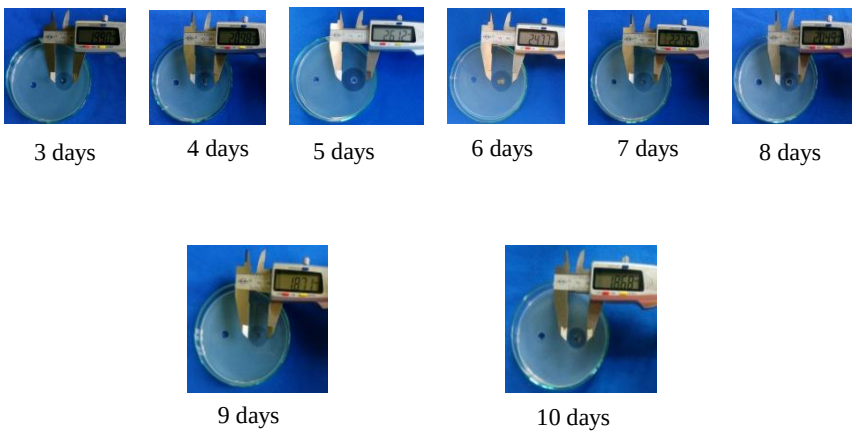


Figure 10 Antibacterial activity on suitable fermentation medium against E.coli

Discussion and Conclusion

The numerous species of fungal endophytes made an ecological niche in the inner space of plants. These ubiquitous fungi interact positively with their environment. Isolation of endophytic fungi from medicinal and other plant results to produce bioactive compound which has greater activity against various pathogenic microbes (Crueger, 1989).

The isolated fungi (SL-6, 19 and 26) could display the antimicrobial activity inhibiting the four test organisms. Among the potent strains, antibacterial activity of isolated fungus SL-26, isolated from *Pyunus persica* (L.) Batsch showed the maximum inhibitory zone of 22.61 mm against *E.coli* in 5 days fermentation period. Similarily, Catherin, 2007, demonstrated that highest zone of inhibition from endophytes of *Camellia sinensis* was observed on *E. coli* and *Staphylococcus aureus*.

Modifying fermentation parameters such as time, temperature, pH, and nutrients can help expanding the range of secondary metabolites (Pfefferle *et al*., 2000). In determining the most suitable size for production antibacterial compounds, 25% inoculum size reached the highest activity (23.23 mm) so that 25% size of inoculum regarded as the most suitable size. In investigation of the effect of carbon, maximum inhibition zone reached up (22.14 mm and 21.53 mm) in lactose and glycerol. Mao *et al.,* 2005 reported the effects of various carbon sources like lactose, sucrose, glucose, fructose, glactose, maltose and xylose. The maximum production of antibacterial metabolite in SL-26 was observed in the presence of asparagine and malt extract (23.65mm and 23.05mm) as nitrogen source. Pimentel *et al*., 2011, reported that the maximum antibacterial activity was obtained when media was supplement with asparagine.

Maximum antibacterial activity was found at pH 6.5 as the diameter of zone of inhibition was 20.76 mm. Maximum inhibitory activity was recorded at the incubation temperature of 30˚C (20.59mm). Compaore 2016, reported that the temperature 30ºC and pH-6.5 were observed to be optimum temperature and pH for antimicrobial activity. According to these results,30˚C is the most suitable temperature for the antimicrobial metabolite production. In this study, under static shaking culture, the diameter of inhibitory zone was more higher than under shaking culture. Fermentation medium (FM-11) was showed significant result by using lactose, glycerol and malt extract. Therefore FM-11 was chosen as a selected fermentation medium.

It was concluded that the present study was to observe the antibacterial activity of three isolated fungi and to investigate the optimum fermentation conditions of selected fungus SL-26 against *E. co*li.

Acknowledgements

Firstly, I wish to express our gratitude to Professor Dr Si Si Hla Bu, Rector, University of Pathein for providing me an opportunity to do this work. I also extended my thank to Professor Dr Than Tun and Dr Nilar Myint, Pro-Rectors, University of Pathein, for their valuable instruction and guidance. I would like to record my deep thank to Professor Dr Kay Thi Mya, Head of Botany Department, University of Pathein and Professor Dr Wah Wah Lwin, Department of Botany, University of Pathein for their suggestion and kind understanding during this study. Many thanks are due to my supervisor Dr Zar Zar Yin, Associate Professor, Department of Botany, University of Pathein, for her valuable instructions, encouragement and overall supervision for the successful completion of this research paper.

References

Alberts, J. F., Gelderblom, W. C. A., Thiel, P. G., Marasas, W. F. O., Vanschalkwyk, D. J. and Behrend, Y**.** (1990).**Effects of Temperature and Incubation Period on Production of Fumonisin B1 by *Fusarium moniliforme*. Applied and Environmental Microbiology**, 56 (6): 1729-1733.

Ando , K ., M. Suto and S. Inaba,(2004) . **Sampling and isolating methods of fungi**, Workshop at University of Pathein.

Catherine A.F. (2007). Tr**opical Biomedicine**, 22(2), 165-170.

Carroll G, (1988). **Fungal endophytes in stems and leaves: from latent pathogens to mutualistic symbiont.** Eco; 69: 2-9.

Cazar, M. E., Schmeda-Hirschmann, G. and Astrudillo, L., (2004). **Antimicrobial Butyrolactone I Derivatives from the Ecuadorian Soil Fungus *Aspergillus terreus* Thorn. Var *terreus*.** World J. Microbiol. Biotechnol. 21: 1067-1075.

Crueger, W., & Crueger, A. (1989): **Methods of fermentation**, **in Biotechnology**, **A Textbook of Industrial Microbiology, Internal Student Edition**.; 64-74

Espinosa –Garcia , F . J. and J.H. Langenhein .(1991). **The endophytic fungal community in leaves .** New Phyto. 116: 89 – 97.

Furtado, N. A. J. C, Fonseca, M. J. V. and Bastos, J., (2005). **The potential of an *Aspergillus fumigatus* Brazilian strain to produce antimicrobial secondary metabolites**. Braz. J. Microbiol. 36: 357-362.

Hassan, S. A. A., Bakhiet & S. E. A., (2017). **Optimization of antibacterial compounds production by A*spergillus fumigatus* isolated from sudanese indigenous soil**. Autumn, Vol 3, No 4.

Hundley , H.G. and Chit Ko Ko, (1987). **List of trees, Shrubs, Herbs and Principal Climbers,** etc, 4 ed., Swe Daw Oo Press, Yangon Myanmar.

Kress, J., *et al.,* (2003). **A checklist of the Tree, Shrubs, Herbs and Climbers of Myanmar**, Department of Systematic Biology Botany- National Museum of Natural History, Washington DC, USA.

Mao, X.B, Eksriwong, T., Chauvatcharin, S. and Zhong, j. j. (2005). **Optimization of carbon source and carbon and nitrogen ratio for cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*.** Process biochem 40:1667-1672.

Miller JD, Mackenzie S, Foto M, Adams GW, Findlay JA (2002). **Needles of white spruce inoculated with rugulosin-producing endophytes contain srugulosin reducing spruce budworm growth rate.** Mycol Res; 106: 471-479.

NITE (National Institute of Technology and Evaluation) (2004). **Media for fermentation to produce the metabolites**.

Pandy, B.P., (1994). **Angiosperms.** S. Chand & Company L.TD. Rm Nagar, New Delhi.Hu Qi- ming *et. Al*., 2008. **Flora of Hong Kong.** Vol II agriculture, Fisheries and Conservation Department Government of the Hong Kong Special Administrative Region.

Petrini, O. (1986). **Taxonomy of endophytic fungi of aerial plant tissue**. In: Fokkema NJ, van den Huevel J, eds. Microbiology of the phyllosphere. Cambridge, UK: Cambridge University Press, 175-187.

Peighamy-ashnaei S, Sharifi-Tehrani A, Ahmadzadeh N, Behboudi K, (2007). **Commin. Agric**. *Appl. Biol. Sci*., 72: 951-6.

Pfefferle ,C., Theobald, U. and Gurtler, H., (2000). **Improved secondary metabolite production in the genus *Streptosporangium* by optimization of the fermentation conditions**. J Biotechnol. 80:135-42.

Pimentel, M.R., Recco, M., Molina, G., Dion´ýsio, P., ostica Juniorm M.R. and Pastore, G. (2011). **The use of endophytes to obtain bioactive compounds and their application in biotransformation process** SAGE-Hindawi Access to Research. *Biotechnol. Res. Int.* Article ID 576286. 11

Rocha ACS, Garcia D, Ueanabaro APT, Carneiro RTO, Araujo IS, Mattos GRR, Goes- Neto A. (2011).- **Foliar endophytic fungi from *Hevea brasiliensis* and their antagonism on *Microcyclus ulei*.** Fungal Diversity 47, 75-84.

Sarika R. Deshmukh, Yogita K. Dhas and B.A.Patil, (2014). **Comparative account on medicinal importance of *Momordica charantia* and its endophytes**.Volume3, Issue 9, 632-640.

Selim KA, El-Bein AA, Abd El-Rahman TM, EL-Diwany AI. (2011). **Biodiversity and antimicrobial activity of endophytes associated with Egyptian medicinal plants.** Mycosphere 2, 669-678.

Thakur, DB, Bora, TC, Bordoloi, GN and Mazumdar, S. (2009). **Influence of nutrition and culturing conditions for optimum growth and antimicrobial metabolite production by *Streptomyces*** sp. 201. J Med Mycol, 19:161-167.

ISOLATION AND OPTIMIZATION OF ANTIBACTERIAL METABOLITE PRODUCTION OF ENDOPHYTIC FUNGI FROM THE LEAF OF *ANNONA SQUAMOSA* L.

Nwe Nwe Aye[1] and Phyu Phyu Win[2]

Abstract

In the course of the investigation of endophytic fungi were isolated from the leaf of *Annona squamosa* L. used PGA (Potato Glucose Agar) and LCA (Low Carbon Agar) media. After isolation, morphology of 7 days old culture were studied. According to this study, 3 different fungi (NA-01, NA-02 and NA-03) were isolated. In the study of antibacterial activities, endophytic fungus (NA-01) showed the highest antibacterial activities against *Micrococcus luteus* than other fungi. In the present investigation, endophytic fungi was isolated for antibacterial metabolite production, and effect of fermentation medium, pH and temperature variation were optimized for maximum antibacterial metabolite against *Micrococcus luteus.* The maximum production of antibacterial metabolite was observed in FM-1 medium (glucose, glycerol, yeast extract, peptone, K_2HPO_4, $CaCO_3$) at pH 5.0 and incubation temperature of 25ºC with shaking condition.

Keywords: *Micrococcus luteus*, *Annona squamosa* L., endophytic fungi

Introduction

Endophytic fungi, at the beginning were applied for any organism found within plant (Petrini, 1986).

Plants may serve as a reservoir of large number of microorganisms known as endophytes (Bacon and White, 2000). Endophytes are microorganisms (mostly fungi and bacteria) that inhabit plant hosts for all or part of their life cycle. They colonize the internal plant tissue beneath the epidermal cell layers without causing any apparent harm or symptomatic infection to their host, living within the intercellular spaces of the tissue and its seems that they may penetrate the living cells (Strobel and Daisy, 2003).

Microorganisms are capable of amazing array of different types of fermentation. Various media types were tested to provide growth of the particular microorganisms. These media also could be modified in order toestablish the optimal conditions for production of the active secondary metabolite (Casida, 1968).

Medium optimization is still one of the most critically investigated phenomenon that is carried out before any large scale metabolite production, and possess many challenges too. Before 1970s, media optimization was carried out by using classical methods, which were expensive, time consuming, involving plenty of experiments with compromised accuracy. Nevertheless, with the advent of modern mathematical/statistical techniques, media optimization has become more vibrant, effective, efficient, economical and robust in giving the result for designing a production medium, the most suitable fermentation conditions (eg., pH, temperature, agitation speed, etc.) and the appropriate medium components (eg., carbon, nitrogen etc.) must be identified and optimized accordingly. Further, by optimizing the above said parameters, maximum product concentration could be achieved (Gupte and Kulkarni, 2003; Franco-Lara *et al.*, 2006; Wang *et al.*, 2011).

[1] Assistant Lecturer, Department of Botany, Pathein University
[2] Lecturer, Department of Botany, Pathein University

In the present paper, the aim and objectives are (i) to isolate endophytic fungi from the leaf of *Annona squamosa* L., (ii) to study antibacterial activity of endophytic fungi (NA-01, NA-02 and NA-03) and (iii) to optimize the fermentation media, pH and temperature for the maximum production of antibacterial metabolite of endophytic fungus NA-01.

Materials and Methods

Medium used for isolation of fungi

LCA medium
(Low Carbon Agar Medium)
(Ando and Inaba, 2004)

Glucose	0.2 g
Sucrose	0.2 g
K_2HPO_4	0.1 g
KNO_3	0.1 g
KCl	0.05 g
Agar	1.8 g
DW	100 mL
pH	6.5

PGA medium
(Potato Glucose Agar Medium)

Potato	20 g
Peptone	0.3 g
Glucose	2.0 g
Agar	1.8 g
DW	100 mL
pH	6.5

Isolation Procedure of Endophytes (Ando and Inaba, 2004)

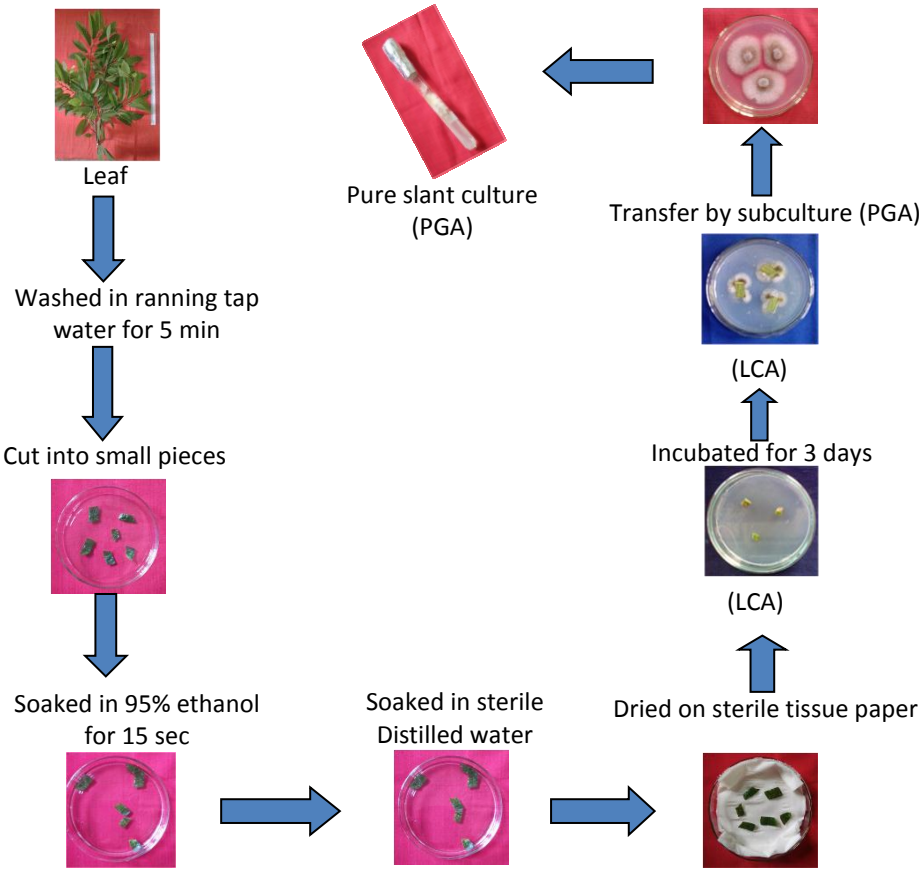


Figure 1 Isolation Procedure of Endophytes from Plant Leaves

Medium used antibacterial test (Ando and Inaba 2004)

Seed medium		**Fermentation medium**		**Assay medium used for test organism**	
Glucose	2.0 g	Glucose	2.0 g	Glucose	1.0 g
Peptone	0.3 g	Peptone	0.3 g	Peptone	0.3 g
KNO_3	0.1 g	K_2HPO_4	0.01 g	KNO_3	0.01 g
K_2HPO_4	0.01 g	$MgSO_4$	0.01 g	Agar	1.8 g
DW	100 mL	$CaCO_3$	0.1 g	DW	100 mL
pH	6.5	DW	100 mL	pH	6.5
		pH	6.5		

Screening for antibacterial activities by agar well diffusion assay (Mohanta, *et al.*, 2008)

1. The isolated fungi were grown at room temperature for 7 days on PGA medium for sporulation
2. The isolated fungi were inoculated on seed medium and incubated at room temperature for 3 days
3. Five mL of seed culture was transferred into the fermented medium and incubated at room temperature for 7 days
4. 0.2 mL of fermented broth was filled into the holes on assay plate containing test organisms incubated for 24-36 hours.

7 days old culture

100 mL conical flask containing 50 mL seed medium

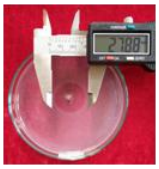


Figure 2 Procedure of antimicrobial activity test

Medium optimization for fermentation and production of antibacterial metabolite

FM-1		**FM-2**	
Glucose	2.0 g	Glucose	1.5 g
Glycerol	0.2 g	Yeast Extract	0.3 g
Yeast extract	0.3 g	Polypeptone	0.3 g
Peptone	0.3 g	K_2HPO_4	0.01 g
K_2HPO_4	0.01 g	$CaCO_3$	0.1 g
$CaCO_3$	0.1 g	DW	100 mL
DW	100 mL	pH	6.5
pH	6.5		

FM-3		**FM-4**	
Glycerol	1.8 g	Glycerol	0.2 g
Yeast extract	0.8 g	Glucose	1.0 g
Polypeptone	0.3 g	Yeast extract	0.3 g
K_2HPO_4	0.01 g	Peptone	0.3 g
$CaCO_3$	0.1 g	K_2HPO_4	0.01 g
pH	6.5	$CaCO_3$	0.1 g
		pH	6.5

Study on effect of pH

The optimization of pH of the fermentation broth for antibacterial metabolite production was done by carrying out the fermentation at six different pH values viz 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5. The medium was adjusted to the desired pH by adding 0.1 N NaOH or 0.1 N HCl (Naik *et al.*, 1988).

Figure 3 Effect of pH on the fermentation

Study on effect of incubation temperature on fermentation

The optimization temperature for antibacterial metabolite production on fermentation of fungus NA-01 was carried out at five different incubation temperatures viz. 20ºC, 25ºC, 30ºC, 35ºC, and 40ºC. The fermentation was carried out 7 days and antibacterial activity was studied by agar well diffusion assay method.

Figure 4 Different incubation temperature of (NA-01) for the fermentation

Comparison of activity of static and shaking culture

Two different flask containing 100 mL of (FM-1) fermentation medium with pH 5.0 at 25ºC were prepared. One fermented flask was incubated on the shaken (100 rpm) and another fermented flask was incubated under static condition (without shaking). These two culture were compared the antibacterial activity by using agar well diffusion method.

Figure 5 Flask with shaking and static culture medium on the fermentation

Results

Botanical name *Annona squamosa* L.

Myanmar name Awzar

Family Annonaceae

Outstanding characters

The plant is small tree. The leaves are simple, alternate, exstipulate, the margin entire, the tip acute. Inflorescence is solitary cymes. The flowers are greenish yellow colour, ebracteolate, pedicellate, bisexual. Fruit an aggregate of barriers, globose, fleshy. The seeds are large, brownish black.

Figure 6 *Annona squamosa* L.

Morphology of isolated endophytic fungi

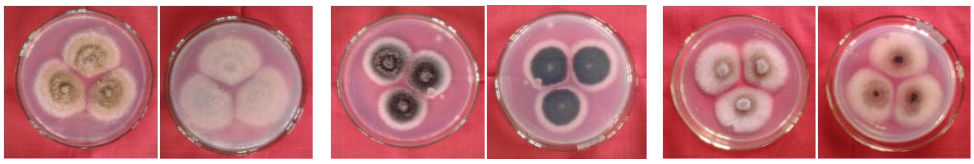
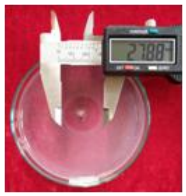


Figure 7 Morphology of isolated endophytic fungi isolated from *Annona squamosa* L.

Table 1 Antibacterial activities of isolate fungi from the leaves of *Annona squamosa* L.

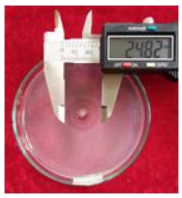
	Micrococcus luteus	*Agrobacterium tumefaciens*	*Staphylococcus aureus*	*Salmonella typhi*	*Escherichia coli*
NA-01	**27.88**	17.10	25.44	19.94	17.02
NA-02	16.70	-	**24.82**	-	-
NA-03	**20.28**	-	-	-	16.53

(5 days fermentation)

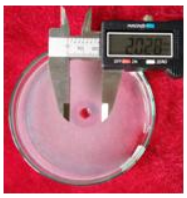


NA-01

Figure 8 Antibacterial activity of isolated fungus (NA-01) against *Micrococcus luteus*



NA-02



NA-03

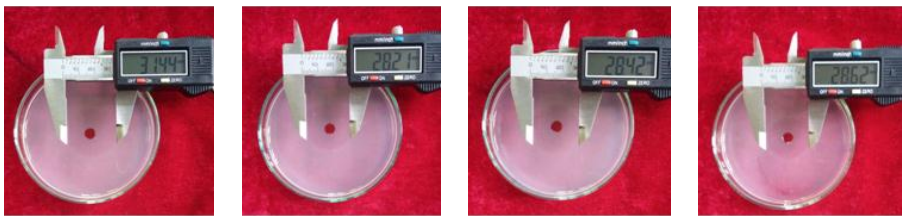
Figure 9 Antibacterial activity of isolated fungus (NA-02) against *Staphylococcus aureus* and (NA-03) against *Micrococcus luteus* (5 days fermentation)

The effect on media on the fermentation (size of agar well 8 mm)

Table 2 The effect of different media on the antibacterial activity of isolated endophyte NA-01

Medium	Activity (Clear zone, mm)
FM-1	**31.44**
FM-2	28.21
FM-3	28.42
FM-4	28.62

Test organism was *Micrococcu luteus*



FM-1 FM-2 FM-3 FM-4

Figure 10 The effects of fermentation medium for the production of antibacterial metabolite of (NA-01) against *Micrococcus luteus*

Effect of pH on fermentation

Table 3 The effect on pH on the fermentation (size of agar well 8 mm)

pH	Activity (Clear zone, mm)
4.0	26.28
4.5	27.28
5.0	**33.09**
5.5	25.59
6.0	23.68
6.5	22.83

Test organism was *Micrococcu luteus*

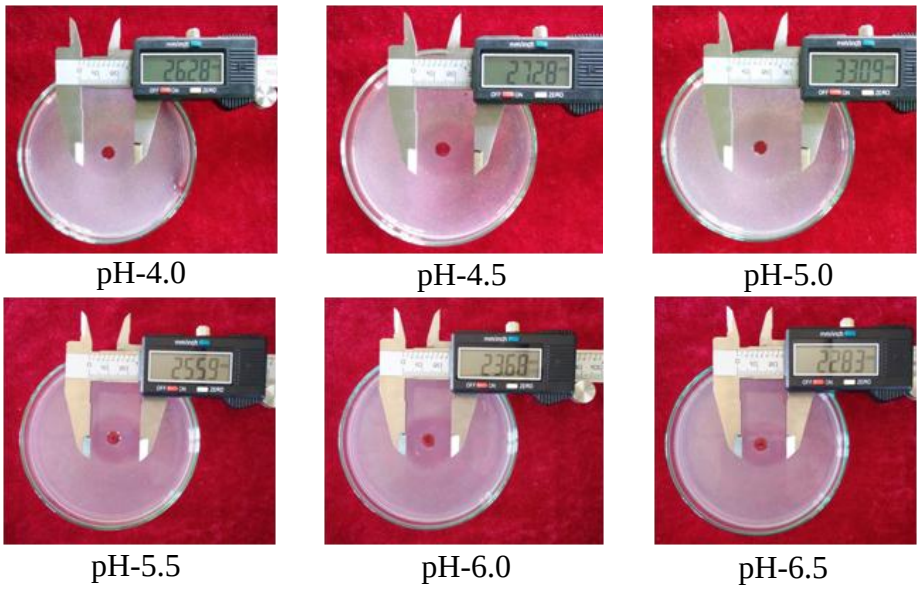


Figure 11 Effect of pH on the fermentation for the antibacterial metabolite of (NA-01) against *Micrococcus luteus*

Effect of temperature

Table 4 Effect of incubation temperatures on the fermentation for (NAO-01) against *Micrococcus luteus*

Temperature (°C)	Inhibitory zone (mm)
20°C	-
25°C	**31.24**
30°C	22.35
35°C	18.02
40°C	15.46

agar well size = 8 mm

Figure 12 The effects of incubation temperatures (25 ºC, 30 ºC, 35ºC and 40ºC) of NAO-01 against *Micrococcus luteus*

Comparison of activity of static culture and shaking culture

Figure 13 The effect of shaking and static culture of (NAO-01) against *Micrococcus luteus*

Maximum antibacterial activity of endophytic fungus NA-01

Maximum antibacterial activity was observed in fermentation medium-1 (FM-1) (glucose, glycerol, yeast extract, peptone, K_2HPO_4, $CaCO_3$), pH-5.0 at 25ºC with shaking condition. The maximum activity reached at 5 days fermentation as 33.86 mm clear zone on *Micrococcus luteus.*

Discussion and Conclusion

Endophytes are microorganisms that includes bacteria and fungi within plant tissue without causing and immediately negative effects, and has been found in every plant species examined to date and recognized as the potential source of novel natural products for exploitation in medicine, agriculture and industry with more bioactive natural products isolated from the microorganisms (Strobel and Daisy, 2003).

In the present study, isolation of endophytic fungi from the leaf of *Annona squamosa* L. According to result, 3 different endophytic fungi (NA-01, NA-02 and NA-03) were isolated. In the present, antibacterial activities of isolated endophytic fungi NA-01, NA-02 and NA-03 were studied. It was observed that endophytic fungus NA-01 showed the antibacterial activity on *Micrococcus luteus* (27.88 mm), *Agrobacterium tumefaciens* (17.10 mm), *Staphylococcus aureus* (25.44 mm), *Salmonella typhi* (19.94 mm) and *Escherichia coli* (17.02 mm). Endophytic fungus NA-02 showed the activity on *Micrococcus luteus* (16.70 mm) and *Staphylococcus aureus* (24. 82 mm) and endophytic fungus NA-03 showed the activity on *Micrococcus luteus* (20.28 mm) and *Escherichia coli* (16.53 mm) clear zone respectively.

According to this result, endophytic fungus (NA-01) showed the highest antibacterial activities against on *Micrococcus luteus* (27.8 mm) than other fungi. Therefore, NA-01 was selected for further investigation.

The constituents of a medium must satisfy the elemental requirements for cell biomass and metabolite production (Stanbury *et al.,* 1997). The yield of bioactive compound can sometimes be substantially increased by the optimization of physical (temperature, salinity, pH and light) and chemical factors (media components, precursors, and inhibitors) for the growth of microbes (Thakur *et al.,* 2009).

In the present study, medium optimization for fermentation and production of antibacterial metabolite by using four different fermentation media (FM-1, FM-2, FM-3 and FM-4). It was observed that fermentation medium (FM-1) is the best medium for the production of antibacterial metabolite.

The pH level of the growth medium has a marked effect on secondary metabolite production (Rizk *et al.,* 2007).

In the present study, pH 5.0 were best for the production of antibacterial metabolite.

Physical factors such as incubation temperature, can exert different on the growth and production phase of secondary metabolite (Rizk *et al.,* 2007).

In the present study the effect of incubation temperature values, it was observed that optimum temperature 25ºC (31.24 mm clear zone) was suitable for the production of antibacterial metabolite.

Aeration is critical factor for cell growth and metabolite production by aerobic microbial culture. The previous investigation showed that oxygen supply plays an important role in the cell

growth and production of bio-metabolite by the fungus (Shih, *et al.*, 2007; Fang and Zhong, 2002).

In the present study effect of different fermentation condition such as shake culture and stationary culture were studied. The results revealed that metabolite production was higher in shaking culture (31.43 mm clear zone) than static culture (27.10 mm clear zone).

The maximum production of antibacterial metabolite of endophytic fungus NA-01 could be achieved in fermentation medium (FM-1) (glucose, glycerol, yeast extract, peptone, K_2HPO_4, $CaCO_3$). Further process parameters like incubation temperature at 25ºC, pH 5.0 are found to be optimum for the maximum production of antibacterial metabolite. According to above conditions maximum activity was found to be (33.86 mm) clear zone on *Micrococcus luteus.*

The present study concluded that the optimum conditions require for the production of antibacterial metabolite by endophytic fungus NA-01 were determined and metabolites production showed the highest antibacterial activity against *Micrococcus luteus.* Hence further studies will be carried out on extraction, purification and identification of antibacterial metabolites of endophytic fungus NA-01.

Acknowledgements

Firstly, I wish to express our gratitude to Professor Dr Si Si Hla Bu, Rector, Pathein University for providing me an opportunity to do this work. I also extended my thank to Professor Dr Nilar Myint and Dr Than Htoon, Pro-Rectors, Pathein University, for their valuable instruction and guidance. I would like to record my deep thank to Professor Dr Kay Thi Mya, Head of Botany Department, Pathein University and Professor Dr Wah Wah Lwin, Department of Botany, Pathein University for their suggestion and kind understanding during this study. Many thanks are due to my supervisor Dr Phyu Phyu Win, Lecturer, Department of Botany, Pathein University, for her valuable instructions, encouragement and overall supervision for the successful completion of this research paper.

References

Ando, K. and S. Inaba. (2004). **Workshop on sampling and isolation method of fungi, Pathein University Biotechnology Development Centre.**

Backer, C.A and R.CB. Van Den Brink, (1965). **Flora of Java** (Vol-I & II). The Netherlands: N.V.P Noordhoff Groningen.

Bacon, C.W. and J.F.White. (2000). **Microbial Endophytes.** marcel Deker, New York, USA.

Casida, I.E. (1968). **Industrial microbiology.** John Wiley and Sons, New York

Fang Q.H. and J.J. Zhong (2002). **Two-stage culture process for improved production of ganoderic acid by liquid fermentation of higher fungus *Ganoderma lucidum*.** Biotechnol Prog 18:51-54.

Franco-Lara E., H. Link and D. Weuster-Botz (2006). **Evaluation of artificial neural networks for modelling and optimization of medium composition with a genetic algorithm.** Process Biochem. 41, 2200-2206. 10.1016/j.procbio.2006. 06.024

Gupte M., and P. Kulkarni. (2003). **A study of antifungal antibiotic production by *Thermomonospora* sp MTCC 3340 using full factorial design.** J. chem.. Technol. Biotechnol. 78, 605-610. 10.1002/jctb.818

Hundley, H.G., and Chit Ko Ko, (1987). **List of Trees, Shrubs, Herbs and Principal Climer etc.** Govt Printing and stationary, Burma.

Kress, J., *et. al., (*2003). **A Checklist of the Trees, Herbs and climbers of Myanmar.** Department of Systematic Biology. Botany National Museum of Natural History, Washington DC, USA.

Mohanta, J.K. Yayung and U.B. Mohanpatra, (2008). **Antimicrobial potentials of endophytic fungi inhabiting three Ethno-medicinal plant of Similipal Biosphere Reverve, India.** The Internal Journal Microbiology. Volume 5 Number 2.

Naik, M.K., P.C. Hiremath and R.K. Hegde. (1988). Mysore J. Agric. Sci., 22: 471-474

Petrini, O. (1986). **Taxonomy of endophytic fungi of aerial plant tissues.** In: Microbiology of the phylosphere. Fokkenna N.J.Van Den Heuvel J. (eds) Combridge University Press, Cambridge. pp. 175-187.

Rizk, M., T. Abdel-Rahman and H. Metwall. (2007). **Factor affecting growth and antifungal activity of some *Streptomyces* species against *Candida albicans.*** J. Food Agric. Environ. 5:446-449.

Shih, I.L., K.L. Tsai and C. Hsieh (2007). **Effects of culture conditions on the mycelial growth and bioactive metabolite production in submerged culture of *Cordyceps militaris.*** Biochem Eng J 33(3): 193-201.

Stanbury, P.F., A. Whitaker and S.J. Hail. (1997). **Principles of fermentation Technology.** Elsevier. London.

Strobel, G and B. Daisy (2003). **Bioprospecting for microbial endophytes and their natural products.** *Micro and Mol Bio Rev* 67: 491-502

Thakur, D.; T.C. Bora; G.N. Bordoloi and S. Mazumadar (2009). ***J. Med. Mycol.*** 19;161-167.

Wang Y., X. Fang, F. An, G. Wang and X. Zhang (2011). **Improvement of antibiotic activity of *Xenorhabdus bovienii* by medium optimization using response surface methodology.** Microb. Cell Fact, 10, 1-15. 10.1186/1457-2859-10-98

TAXONOMIC STUDY ON FIFTEEN SPECIES OF ORCHIDACEAE FOUND IN PINLAUNG TOWNSHIP, SOUTHERN SHAN STATE

Pa Pa Aung[1] and Nwè Nwè Yi[2]

Abstract

The present paper deals with taxonomic study on fifteen species of Orchidaceae found in Pinlaung Township, Southern Shan State of Myanmar. This area lies between 19° 40'-20° 30' N latitude and 96° 22'-96° 55' E longitude. The elevation of Pinlaung Township is 1465 m above sea level. The orchid species of this study area were investigated during 2017-2018. All the specimens were collected, and identified by referring to Hooker, Backer & Brink, Pedersen *et al*., Xinqi *et al*.. In this paper, 15 species belonging to 11 genera were presented. Among them, 11 species were epiphyte and 4 species were terrestrial. Two pollinia were found in 6 species and four pollinia in 9 species. The morphological characters of the individual species were presented with relevant photographs. An artificial key to the species was constructed. The valuable information of orchid species found in Pinlaung Township of Southern Shan State will provide to further researchers.

Keywords: Taxonomy, Orchidaceae, Pollinia, Pinlaung Township

Introduction

Taxonomy is one of the branch of botany which is an advanced subject that deals not merely with the identification and naming of plant but also with their classification and evolution. Plant taxonomic study has among its objective the learning of the kinds of plants on the earth and their names, of their distinctions and their affinities, their distribution and habitat characteristics, and the correlation of these facts of knowledge with pertinent scientific data contributed by research activities of related fields of botanical endeavor (Lawrence 1964).

Orchidaceae is the largest family of flowering plants and a cosmopolitan in distribution and consists of about 800 genera and 18,000 to 20,000 species (Heywood *et al.* 2007). Members of the Orchidaceae family are distributed in worldwide and consists of 700 to 800 genera and about 20,000 species (Simpson 2006). Xinqi *et al.* (2009) reported that Orchidaceae consists of about 800 genera and 25,000 species and worldwide in distribution. Kress *et al.* (2003) mentioned that there are 128 genera and 738 species in Myanmar.

The Shan State is the largest one among the seven States of Myanmar. Pinlaung Township is located in Southern Shan State of Myanmar. It lies between 19° 40'-20° 30' N latitude and 96° 22'-96° 55' E longitude. The elevation of Pinlaung Township is 1465 m above sea level. The total area is 3349.98 square kilometer. It is bounded by Naungshwe Township in the east, Pyinmana Township in the west, Pekhone Township in the south and Kalaw Township in the north.

The aim and objectives of this research are to identify and classify the natural orchid species of Pinlaung Township, to record the taxonomical characters of Orchidaceae, and to contribute the information of orchid species in the study area for further researchers.

[1] Assistant Lecturer, Department of Botany, Meiktila University
[2] Professor & Head, Department of Biology, Sagaing University of Education

Materials and Methods

The species of Orchidaceae were collected from Pinlaung Township of Southern Shan State during 2017-2018. All the collected specimens were recorded individually by photographs while flowering period. Then, the specimens were kept immediately into the plastic bags to identify and classify systematically.

Identification of genera and species were carried out by referring to Hooker (1894), Backer & Brink (1968), Pedersen *et al*. (2011), Xinqi *et al*. (2009). All of the nomenclatural studies were finalized by referring to the web sites of International Plant Name Index (IPNI) and online Botanical Database of Tropical Plant (TROPICOS). Myanmar names were referred to Hundley & Chit Ko Ko (1987) and Kress *et al*. (2003). The genera and species arrangements under the families were placed alphabetically. An artificial key to the species was constructed.

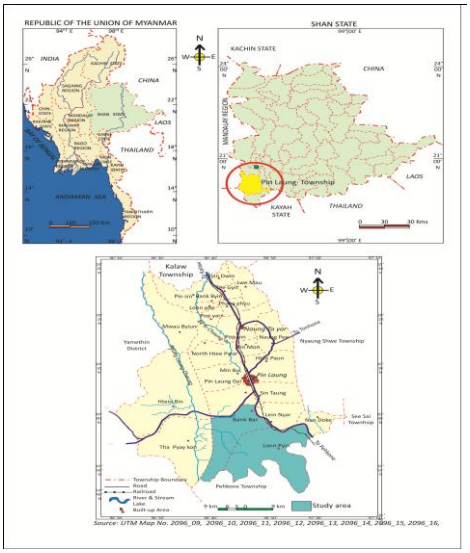


Figure 1 Location Map of Study Area in Pinlaung Township

Results

In this paper, the collected 15 species belonging to 11 genera of Orchidaceae were found in the study area. Then the genera and species were also arranged alphabetically as shown in Table 1.

Table 1 List of Collected Species from Pinlaung Township in Southern Shan State

Family	No.	Scientific Name	Myanmar Name
Orchidaceae	1	*Bulbophyllum odoratissimum* (Sm.) Lindl.	Unknown
	2	*Cleisostoma crochetii* (Guill.) Garay	Unknown
	3	*Dendrobium cariniferum* Rchb. f.	Mahar dewi; Payaung setku
	4	*Dendrobium crystallinum* Rchb. f.	Pan setku thitkwa
	5	*Habenaria chlorina* Parish & Rchb. f.	Unknown
	6	*Habenaria commelinifolia* (Roxb.) Wall. ex Lindl.	Unknown

Family	No.	Scientific Name	Myanmar Name
Orchidaceae	7	*Hemipilia cordifolia* Lindl.	Unknown
	8	*Holcoglossum kimballianum* (Rchb.f.) Garay	Yosetgale
	9	*Otochilus albus* Lindl.	Unknown
	10	*Peristylus prainii* (Hook. f.) Krzl.	Mya thein dan; Tamasok
	11	*Pholidota articulata* Lindl.	Unknown
	12	*Pholidota convallaria*e (E.C. Parish & Rchb. f.) Hook. f.	Unknown
	13	*Pholidota imbricata* Lindl.	Padi sint; Sin mi thitkwa
	14	*Sunipia scariosa* Lindl.	Unknown
	15	*Vanda coerulea* Griff. ex Lindl.	Moe lon hmaing

Outstanding Characters

1. *Bulbophyllum odoratissimum* (Sm.) Lindl., Gen. Sp. Orchid. Pl. 55: 1830. (Figure 2. A)

Stelis odoratissima Sm., Cycl. 34: Stelis n. 12. 1814.
Myanmar name : Unknown
Flowering period : April to June

Sympodial epiphytes; pseudobulbs subcylindric, one-jointed. Leaves simple, 1 leaf per pseudobulb; blades oblong. Inflorescences subumbellate racemes, erect, many-flowered. Flowers white, about 1.0 cm in diameter, fragrant. Dorsal sepals ovate-lanceolate; lateral sepals lanceolate. Lateral petals ovate; labellum ligulate. Pollinia 4.

2. *Cleisostoma crochetii* (Guill.) Garay, Bot. Mus. Leafl. 23: 170. 1972.

(Figure 2. B)

Sarcanthus crochetii Guill., Bull. Mus. Natl. Hist. Nat., ser. 2, 28: 238. 1956.

Myanmar Name : Unknown

Flowering period : July to August

Monopodial epiphytes. Leaves simple, alternate; blades oblong. Inflorescences racemes, many-flowered. Flowers whitish purple, about 1.2 cm in diameter. Dorsal sepals oblong; lateral sepals falcately-ovate. Lateral petals elliptic to ligulate; labellum 3-lobed. Pollinia 4.

3. *Dendrobium cariniferum* Rchb. f., Gard. Chron. 1869: 611. 1869. (Figure 2. C)

Myanmar name : Mahar dewi; Payaung setku

Flowering period : February to April

Sympodial epiphytes; pseudobulbs cylindrical, many-jointed. Leaves simple, alternate; blades oblong. Inflorescences racemes, 1- to 2-flowered. Flowers white, about 3.5 cm in diameter, fragrant. Dorsal sepals ovate-lanceolate; lateral sepals obliquely ovate-triangular. Lateral petals oblong-elliptic; labellum 3-lobed. Pollinia 4.

4. ***Dendrobium crystallinum*** Rchb. f., Gard. Chron. 572. 1868.
(Figure 2. D)

Myanmar Name : Pan setku thitkwa

Flowering Period : February to May

Sympodial epiphytes; pseudobulbs cylindrical, many-jointed. Leaves simple, alternate; blades oblong-lanceolate. Inflorescences racemes, 1- to 2-flowered. Flowers white, about 5.0 cm in diameter. Dorsal and lateral sepals oblong-lanceolate. Lateral petals oblong; labellum suborbicular. Pollinia 4.

5. ***Habenaria chlorina*** Parish & Rchb. f., Trans. Linn. Soc. London 30: 140. 1874. (Figure 2. E)

Myanmar name : Unknown

Flowering period : July to September

Sympodial terrestrials. Leaves simple, alternate; blades oblong-lanceolate. Inflorescences terminal spike, many-flowered. Flowers yellow, about 0.9 cm in diameter. Dorsal sepals ovate; lateral sepals ovate-lanceolate. Lateral petals falcately linear-lanceolate; labellum linear-lanceolate, distinctly 3 partites. Pollinia 2.

6. ***Habenaria commelinifolia*** (Roxb.) Wall. ex Lindl., Gen. Sp. Orchid. Pl. 325. 1835. (Figure 2. F)

Orchis commelinifolia Roxb., Hort. Bengal. 63. 1832.

Myanmar name : Unknown
Flowering period : September to December

Sympodial terrestrials. Leaves simple, alternate; blades oblong-lanceolate. Inflorescences terminal spike, 4- to 8-flowered. Flowers white, about 2.5 cm in diameter. Dorsal sepals broadly obovate; lateral sepals ovate. Lateral petals obliquely oblong; labellum suborbicular, distinctly 3-lobed. Pollinia 2.

7. ***Hemipilia cordifolia*** Lindl., Gen. Sp. Orchid. Pl. 296. 1835.
(Figure 3. A)

Myanmar name : Unknown
Flowering period : June to July

Sympodial terrestrial. Leaves simple, solitary; blades cordate. Inflorescences terminal racemes, many-flowered. Flowers pinkish purple, about 1.0 cm in diameter. Dorsal sepals ovate-lanceolate; lateral sepals falcately oblong-ovate. Lateral petals ovate; labellum obovate-oblong, obscurely 3-lobed. Pollinia 2.

8. ***Holcoglossum kimballianum*** (Rchb. f.) Garay, Bot. Mus. Leafl. 23(4): 182. 1972. (Figure 3. B)

Vanda kimballiana Rchb. f., Gard. Chron, ser. 3 5: 232. 1889.

Myanmar name : Yosetgale
Flowering period : October to December

Monopodial epiphyte. Leaves simple, alternate; blades terete. Inflorescences axillary racemes, many-flowered. Flowers pinkish white, about 4.5 cm in diameter. Dorsal sepals elliptic; lateral sepals obliquely ovate-falcate. Lateral petals elliptic; labellum 3-lobed. Pollinia 2.

9. *Otochilus albus* Lindl., Gen. Sp. Orchid. Pl. 35. 1830. (Figure 3. C)

Myanmar name : Unknown

Flowering period : May to July

Sympodial epiphytes; pseudobulbs tetragonal, many-jointed. Leaves simple, 2 leaves per pseudobulb; blades oblong. Inflorescences terminal racemes, pendulous, many-flowered. Flowers white, about 1.2 cm in diameter. Dorsal sepals oblong; lateral sepals oblong-lanceolate. Lateral petals narrowly oblong-lanceolate; labellum 3-lobed. Pollinia 4.

10. *Peristylus prainii* (Hook. f.) Kraenzl., Orchid. Gen. Sp. 1: 514. 1898.

(Figure 3. D)

Habenaria prainii Hook. f., Fl. Brit. India 6: 159. 1890.

Myanmar name : Mya thein dan; Tamasok

Flowering period : May to July

Sympodial terrestrial. Leaves simple, alternate; blades ovate-oblong. Inflorescences terminal spike, many-flowered. Flowers creamy white, about 0.3 cm in diameter. Dorsal sepals obovate; lateral sepals linear-oblong. Lateral petals broadly ovate; labellum narrowly obovate, slightly 3-lobed. Pollinia 2.

11. *Pholidota articulata* Lindl., Gen. Sp. Orchid. Pl. 38. 1830.

(Figure 3. E)

Myanmar name : Unknown

Flowering period : March to May

Sympodial epiphyte; pseudobulbs oblong, many-jointed. Leaves simple, mostly 2 leaves per pseudobulb; blades elliptic-lanceolate. Inflorescences terminal racemes, many-flowered. Flowers yellowish white, about 1.2 cm in diameter. Dorsal sepals oblong; lateral sepals ovate, oblique. Lateral petals oblong-lanceolate; labellum cymbiform. Pollinia 4.

12. *Pholidota convallariae* (E.C. Parish & Rchb. f.) Hook. f., Hooker's Icon. Pl. 19: ad pl. 1880. 1889. (Figure 3. F)

Coelogyne convallariae E.C. Parish & Rchb. f., Flora 55: 277. 1872.

Myanmar name : Unknown

Flowering period : April to May

Sympodial epiphyte; pseudobulbs narrowly ovoid, one-jointed. Leaves simple, mostly 2 leaves per pseudobulb; blades narrowly elliptic. Inflorescences basal racemes, many-flowered. Flowers creamy white; about 0.6 cm in diameter. Dorsal sepals ovate; lateral sepals obliquely ovate. Lateral petals ovate-elliptic; labellum shallowly saccate. Pollinia 4.

13. *Pholidota imbricata* Hook., Exot. Fl. 2:, ad pl. 138. 1825.

(Figure 4. A)

Myanmar name : Padi sint; Sin mi thitkwa

Flowering period : June to August

Sympodial epiphyte; pseudobulbs suboblong, one-jointed. Leaves simple, 1 leaf per pseudobulb; blades oblanceolate. Inflorescences basal racemes, pendulous, many-flowered. Flowers creamy white, about 0.4 cm in diameter. Dorsal sepals broadly ovate; lateral sepals ovate to cymbiform. Lateral petals linear; labellum ovate to panduriform. Pollinia 4.

14. *Sunipia scariosa* Lindl., Gen. Sp. Orchid. Pl. 179. 1833. (Figure 4. B)

Myanmar name : Unknown

Flowering period : December to May

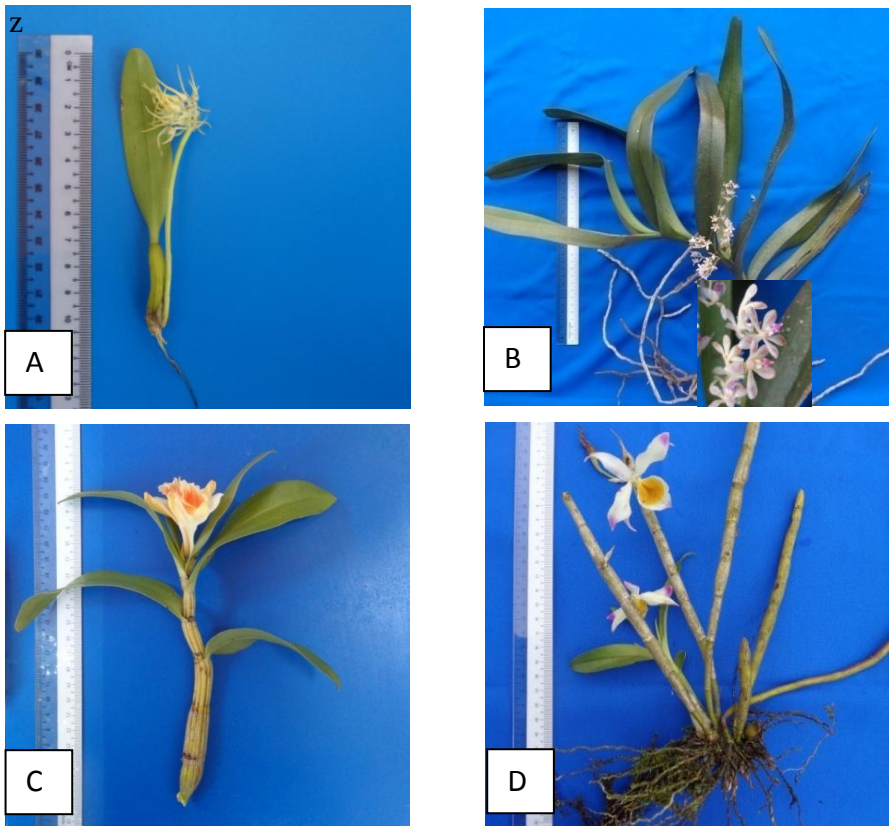
Sympodial epiphyte; pseudobulbs ovoid, one-jointed. Leaves simple, 1 leaf per pseudobulb; blades oblong. Inflorescences basal spike, pendulous, many-flowered. Flowers yellowish-green, about 0.8 cm in diameter. Dorsal sepals ovate; lateral sepals falcately lanceolate. Lateral petals suborbicular; labellum linguiform. Pollinia 4.

15. *Vanda coerulea* Griff. ex Lindl., Edwards's Bot. Reg. 33:, sub pl. 30. 1847. (Figure 4. C)

Myanmar name : Moe lon hmaing

Flowering period : July to December

Monopodial epiphyte. Leaves simple, alternate; blades oblong. Inflorescences axillary racemes, many-flowered. Flowers bluish purple, about 8.0 cm in diameter. Dorsal sepals suborbicular; lateral sepals obovate. Lateral petals broadly obovate; labellum linear-oblong, distinctly 3-lobed. Pollinia 2.



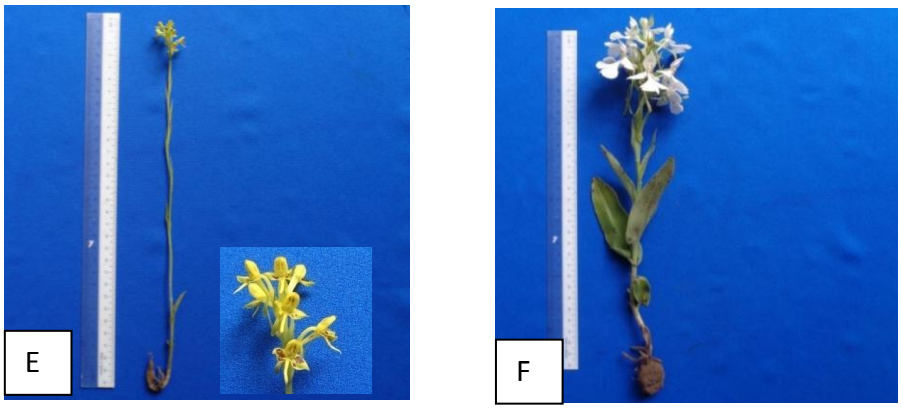


Figure 2 A. *Bulbophyllum odoratissimum* (Sm.) Lindl. B. *Cleisostoma crochetii* (Guill.)Garay
C. *Dendrobium cariniferum* Rchb. f. D. *Dendrobium crystallinum* Rchb. f.
E. *Habenaria chlorina* Parish & Rchb. f. F. *Habenaria commelinifolia* (Roxb.) Wall.

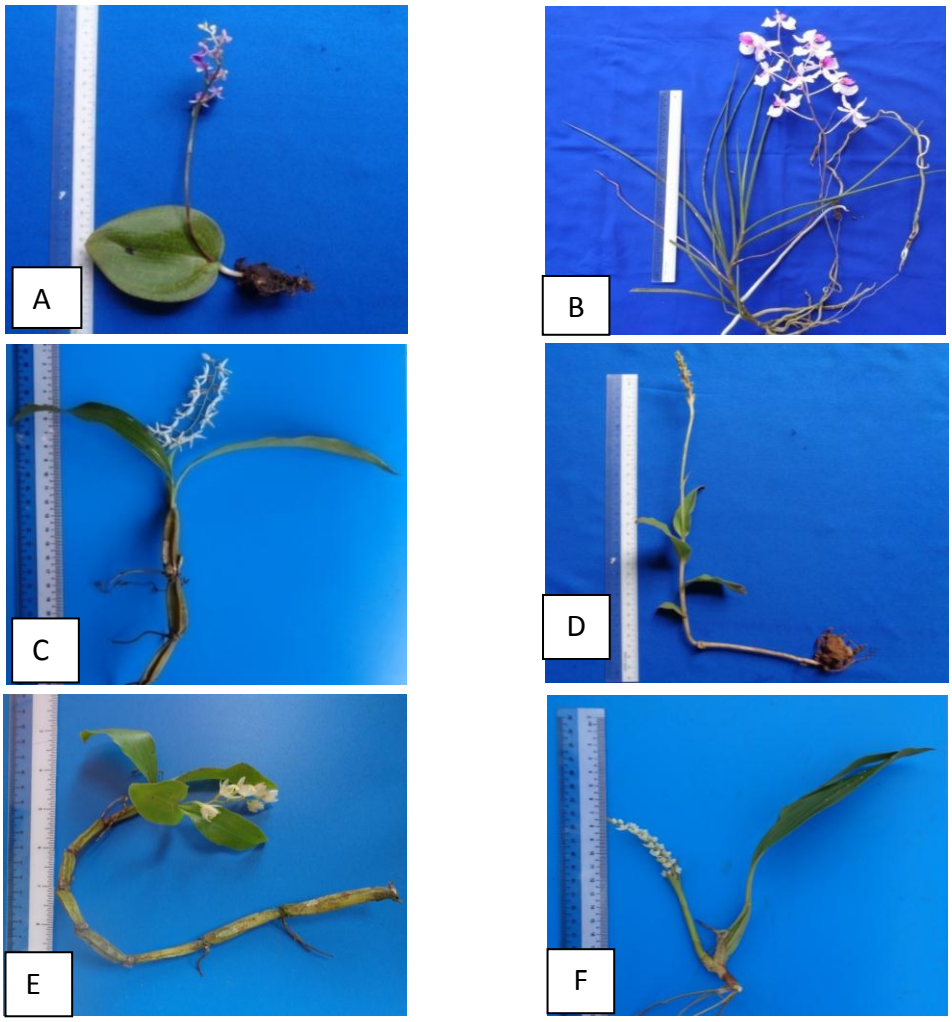


Figure 3 A. *Hemipilia cordifolia* Lindl. B. *Holcoglossum kimballianum* (Rchb.f.) Garay
C. *Otochilus albus* Lindl. D. *Peristylus prainii* (Hook. f.) Krzl.
E. *Pholidota articulata* Lindl. F. *Pholidota convallaria*e (E.C. Parish & Rchb. f.) Hook. f.

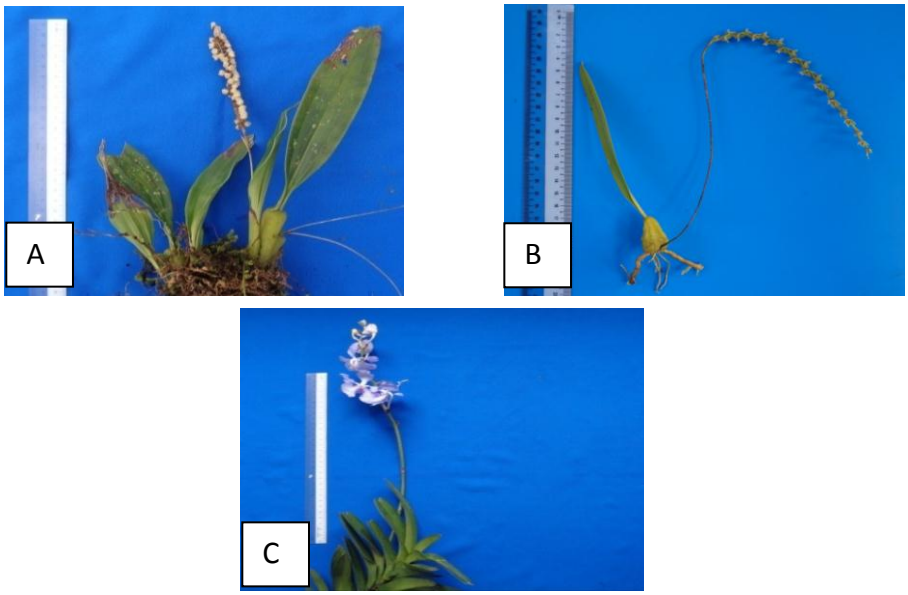


Figure 4 A. *Pholidota imbricata* Lindl. B. *Sunipia scariosa* Lindl.
C. *Vanda coerulea* Griff. ex Lindl.

An Artificial Key to the Studied Species

1. Terrestrials---------- ---------- 2
1. Epiphytes---------- 5
   2. Flowers white or creamy white---------- 3
   2. Flowers yellow or pinkish purple ---------- 4
3. Flowers about 2.5 cm in diameter; labellum suborbicular ----------
   ----------6. *Habenaria commelinifolia*
3. Flowers about 0.3 cm in diameter; labellum narrowly obovate ----------
   ----------10. *Peristylus prainii*
   4. Leafblades oblong-lanceolate; inflorescence spike ----------
      ----------5. *Habenaria chlorina*
   4. Leafblades cordate; inflorescence racemes----------
      ---------- 7. *Hemipilia cordifolia*
5. Monopodial epiphytes ---------- 6
5. Sympodial epiphytes ---------- 8
   6. Flowers less than 3.0 cm in diameter; pollinia 4 ----------
      ----------2. *Cleisostoma crochetii*
   6. Flowers more than 4.0 cm in diameter; pollinia 2 ---------- 7
7. Leafblade terete; lateral petals elliptic ----------
   ----------8. *Holcoglossum kimballianum*
7. Leafblade oblong; lateral petals broadly obovate ----------
   ----------15. *Vanda coerulea*
   8. Pseudobulbs one-jointed ---------- 9
   8. Pseudobulbs many-jointed ----------12
9. Leaves mostly 2 leaves per pseudobulb----------
   ----------12. *Pholidota convallariae*
9. Leaves one leaf per pseudobulb ---------- 10

10. Inflorescences erect; flowers fragrant---------- 1. *Bulbophyllum odoratissimum*
10. Inflorescences pendulous; flowers not fragrant---------- 11
11. Flowers creamy white; lateral petals linear ---------- 13. *Pholidota imbricata*
11. Flowers yellowish-green; lateral petals suborbicular ---------- 14. *Sunipia scariosa*
12. Inflorescences with many-flowered ---------- 13
12. Inflorescences with 1- to 2-flowered----------14
13. Pseudobulbs tetragonal; lateral sepals oblong-lanceolate---------- 9. *Otochilus albus*
13. Pseudobulbs oblong; lateral sepals ovate---------- 11. *Pholidota articulata*
14. Flowers fragrant; lateral sepals obliquely ovate-triangular ---------- 3. *Dendrobium cariniferum*
14. Flowers not fragrant; lateral sepals oblong-lanceolate----------4. *Dendrobium crystallinum*

Discussion and Conclusion

The present paper deals with taxonomic study on fifteen species of Orchidaceae found in Pinlaung Township, Southern Shan State of Myanmar. Altogether 15 species belonging to 11 genera of Orchidaceae were presented. Among them, the number of pollinia 2 and 4 were found in 6 species and 9 species respectively.

Xinqi *et al*. (2009) had been classified the Orchidaceae into 5 subfamilies. In this paper, 2 subfamilies were found such as Orchidoideae and Epidendroideae.

The Orchidoideae is a very large subfamily of highly successful terrestrial orchids which included *Habenaria chlorina* Parish & Rchb. f., *H. commelinifolia* (Roxb.) Wall. ex Lindl., *Hemipilia cordifolia* Lindl., and *Peristylus prainii* (Hook. f.) Krzl..

The subfamily Epidendroideae are the major orchid group, with more than half of all orchid species which included *Bulbophyllum odoratissimum* (Sm.) Lindl., *Cleisostoma crochetii* (Guill.) Garay, *Dendrobium cariniferum* Rchb. f., *D. crystallinum* Rchb. f., *Holcoglossum kimballianum* (Rchb.f.) Garay, *Otochilus albus* Lindl., *Pholidota articulata* Lindl., *P. convallaria*e (E.C. Parish & Rchb. f.) Hook. f., *P. imbricata* Lindl., *Sunipia scariosa* Lindl., and *Vanda coerulea* Griff. ex Lindl..

Among the 15 studied species, *Habenaria commelinifolia* (Roxb.) Wall. ex Lindl., *Peristylus prainii* (Hook. f.) Krzl., *Cleisostoma crochetii* (Guill.) Garay, *Dendrobium cariniferum* Rchb. f., and *Otochilus albus* Lindl. were abundantly found in the study area.

Orchids are one of the largest and most diverse groups of angiosperms. They can be easily distinguished from other flowering plants. The distinctive characters of this family are terrestrial or epiphytic herbs having trimerous, often resupinate flowers with labellum and the presence of pollinia.

Orchid species are famous for their beauty. There are natural orchid species and hybrid species in Myanmar. Some species of orchids have both economic value and medicinal value.

One third of a total area of Pinlaung Township is covered by forest vegetation. Therefore, the species of orchid growing naturally are found in this area. It is necessary in Myanmar to keep the valuable orchid species.

It is hoped that this paper will contribute valuable information about some orchid species found in Pinlaung Township.

Acknowledgements

I would like to express my gratitude to Dr Thandar, Professor and Head, Department of Botany, Meiktila University for her permission given to me to contribute this paper. I would like to offer my thanks to Dr Thidar Myint, Professor, Department of Botany Meiktila University for her advice.

References

Backer, C.A. & R.C.B.V.D. Brink. (1968). Flora of Java Vol. III. Rijksherbarium, Leyden, N.V.P. Noordhoof.

Heywood, V.H., R.K. Brummit, A. Culham & O. Seberg. (2007). Flowering plant of the families of the World. Firefly Books; Ontario, Canada.

Hooker, J.D. (1894). The flora of British India, Vol. V & VI, L. Reeve & Co, London.

Hundley, H.G. & Chit Ko Ko. (1987). List of trees, shurbs, herbs and principal climbers, etc. Fourth Revised edition Swe Daw Oo Press, Mayangon, Yangon, Myanmar.

Kress, J.W., A.D. Robert, F. Ellen & Yin Yin Kyi. (2003). A checklist of the trees, shrubs, herbs and climbers of Myanmar. Department of Systematic Biology-Botany. National Museum of Natural History, Washington DC. USA.

Lawrence, G.H.M. (1964). Taxonomy of vascular plants. The Macmillan Company. New York.

Pedersen, H.E., H. Kurzweil, S. Suddee & P. J. Gribb. (2011). Flora of Thailand. Vol 12 (1). The Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand.

Xinqi, C., L. Zhongjian, Z. Guanghua, L. Kaiyong, J. Zhanhe, L. Yibo, J. Xiaohua, P.J. Cribb, J.J. Wood, S.W. Gale, P. Ormerod, J.J. Vermeulen, H.P. Wood, D. Clayton & A. Bell. 2009. Flora of China (Orchidaceae). FOC Vol. 25.

ISOLATION OF SOIL FUNGI AND FERMENTATION CONDITIONS OF *FUSARIUM* SP. AGAINST *STAPHYLOCOCCUS AUREUS*

Thet Thet Wai[1], Yu Yu Tin[2] and Swe Swe Myat[3]

Abstract

The present study, soil samples were collected from six different places at Wundwin Township in Mandalay Region. The isolation of fungi were isolated by using physical treatment dilution method (Hayakawa and Kobayashi, 2005) and direct method (Ando,2004).Then, antimicrobial activities of soil fungi were tested by paper disc diffusion assay method. The fungal extract were tested for antimicrobial activity against test organisms *Staphylococcus aureus.* In the fifteen fungi screening, the isolated fungi TW-03 (22.57 mm) and TW-14(34.81mm)exhibited the activity against *Staphylococcus aureus*. In this result, TW-14 showed highest antibacterial activity. *Fusarium* sp. was identified from the selected fungus TW-14. Therefore, this fungus *Fusarium* sp. was selected for further investigation such as age, size, carbon sources and nitrogen sources for suitable fermentation condition. In the study of ages and sizes of inoculum, it was observed that 60hr and 10% seed cultured was best for the fermentation. Then, in this study of carbon and nitrogen sources utilization it was found that soluble starch and soybean gave the best activity on *Staphylococcus aureus*.

Introduction

All micro-organisms require water, sources of energy, carbon, nitrogen, mineral element and vitamin plus oxygen in their growth medium. Specific nutritional requirements of microorganisms used in industrial fermentation processes are as complex and varied as the microorganisms in question. Not only are the types of microorganisms diverse (bacteria, molds and yeast, normally), but the species and strains become very specific as to their respectively. (**Gutcho, Sydney, 1973)**

In the present study, it is an effort to understand the soil fungal diversity in Wundwin Township, Mandalay Region. In many cases the complex or natural media have to be supplemented with mainly inorganic nutrients to satisfy the requirements of the fermenting organism. **(Zabriski, *et al*., 1980)**

Fermentation nutrients are generally classified as: sources of carbon, nitrogen and sulfur, minerals and vitamins. **(Vogel, Henry 1983)**

Many microorganisms can use a single organic compound to supply both carbon and energy needs. Following the carbon source, the nitrogen source is generally the next most plentiful substance in the fermentation media. A few organisms can also use the nitrogen source as the energy source.**(Rhodes and Fletcher, 1966)** Nitrogen can be inorganic such as ammonium salts, or organic such as amino acids, proteins or urea. The carbon substrate has a dual role in biosynthesis and energy generation, with carbohydrates being the usual carbon source for microbial fermentation processes (**Stanbury *et al*. 1995**).

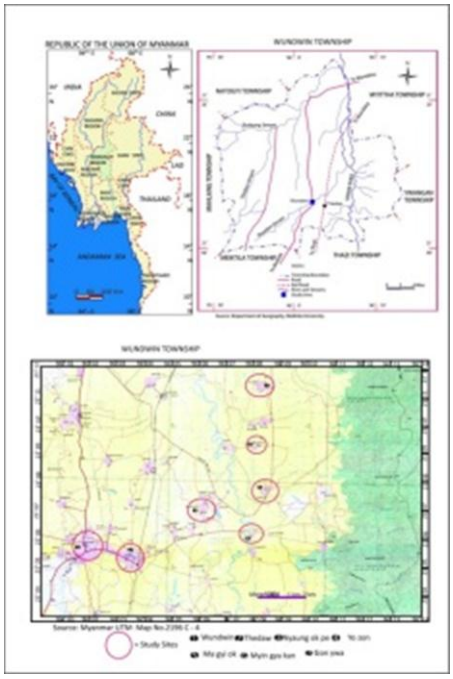
Therefore, the main object of present study is to investigate to find out ages and sizes of inoculum, effects of carbon and nitrogen sources for the production of antibacterial metabolite.

[1] Assistant Lecturer, Department of Botany, Meiktila University
[2] Dr, Associate Professor, Department of Botany, Meiktila University
[3] Dr, Lecturer, Department of Botany, Pathein University

Materials and Methods

Procedure for the effect of ages of inoculum

Isolation and screening soil fungi from different soil samples. Six different soil samples was collected from Wundwin Township, Mandalay Region (Figure 1 and Table.1). The isolation of soil microorganisms were referenced by the following methods. The isolation of fungi were carried out by the physical treatment dilution method (Hayakawa and Kobayashi, 2005) and direct method (Ando, 2005) as shown in Figure 2 and 3.



Source: Department of Geography, Meiktila University

Figure 1 Study Area of Wundwin Township

Table 1 Six different soil samples collected at six different places

Soil Samples No.	Collected Area	Texture	pH	Moisture	Location
1	Hitaw Mu Pagoda	Sandy Loam	5.4	9	N 21°04’55.361” E 096°08’48.250”
2	Ma Gyi Oak village	Loamy Sand	4.5	6	N 21°07’22.330” E 096°08’45.861”
3	Myin Kya Kan village	Sandy Loam	6.3	11	N 21°09’21.882” E 096°08’45.641”
4	Gyone Yar village	Sandy Loam	6.8	14	N 21°11’15.357” E 096°08’43.883”
5	Nyaung Oak Phee village	Loamy Sand	6.4	12	N 21°06’58.476” E 096°05’47.607”
6	Quarter(3), Wundwin	Sandy Loam	7.3	12	N 21°05’03.456” E 096°00’38.507”

Isolation By Physical Treatment dilution method (Hayakawa and Kobayashi, 2005)

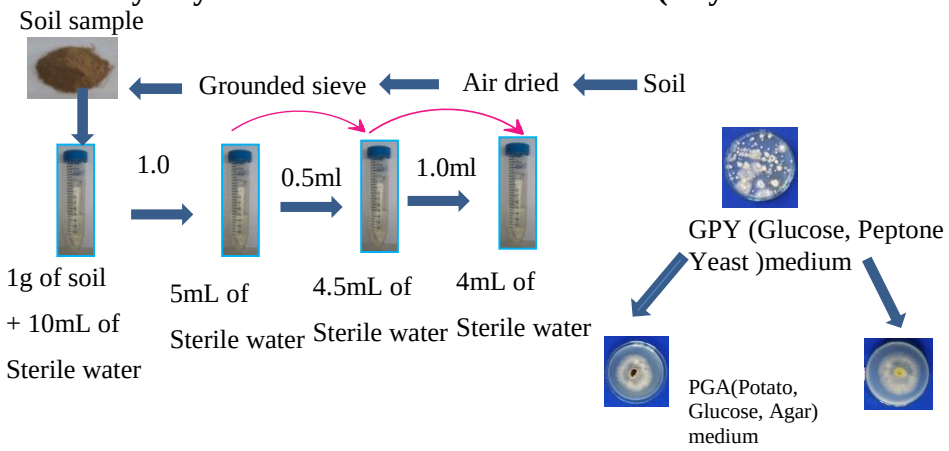


Figure 2 Procedure of physical treatment dilution method

Isolation By Direct Method (Ando, 2004)

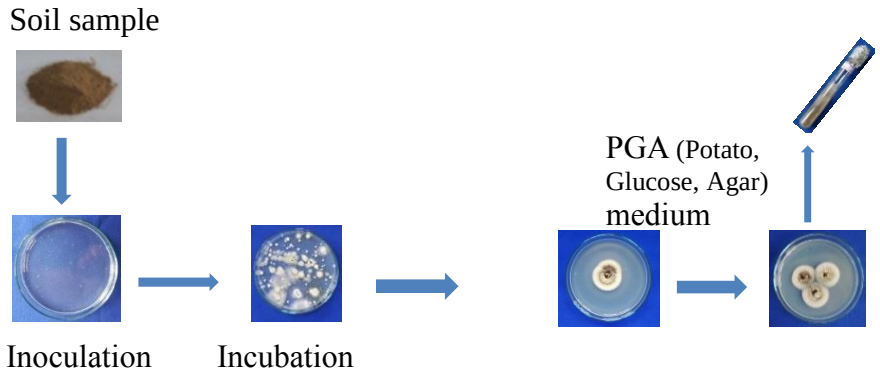


Figure 3 Procedure of direct method

Screening for antimicrobial activities by paper disc diffusion assay (Tomita,1988)

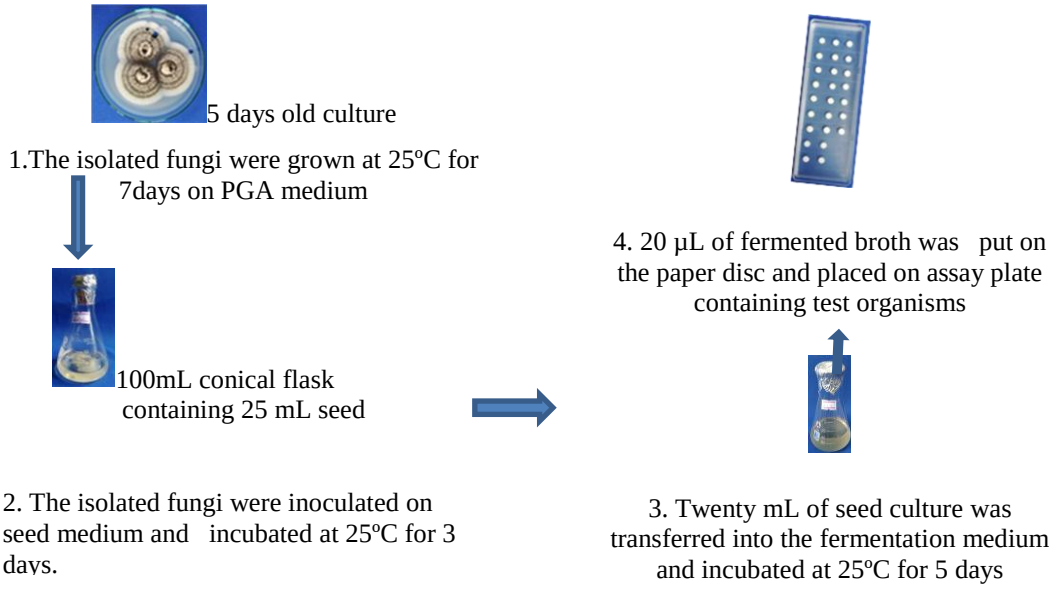


Figure 4 Screening for antimicrobial activities by paper disc diffusion assay

Procedure of fermentation for the effect of ages of inoculum (Omura 1985, Crueger and Crueger 1989)

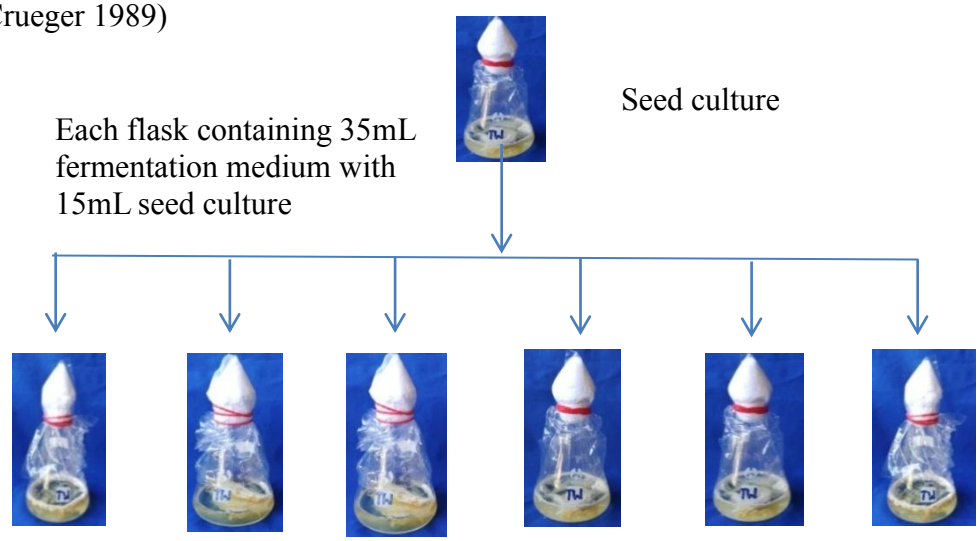


Figure 5 Procedure for the study on the effect of ages of inoculum for fermentation

Procedure of fermentation for the effect of sizes of inoculation (Omura 1985, Crueger and Crueger 1989)

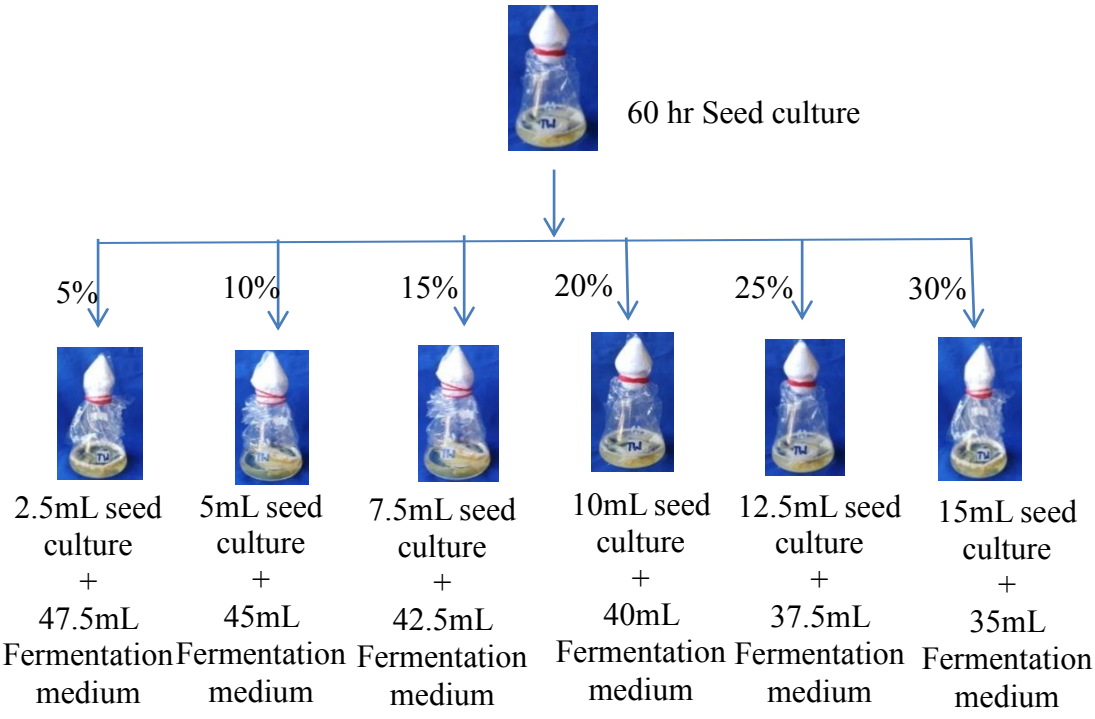


Figure 6 Procedure for the study on the effect of sizes of inoculation for fermentation

Studied on different carbon and nitrogen utilization for the fermentation

Optimal fermentation are very important for maximal productivity metabolites. In this study, carbon and nitrogen sources were employed in the fermentation for the production of antimicrobial metabolites. Carbon sources such as molasses, mannitol, soluble starch, rice powder and corn powder were used. Nitrogen sources such as soybean, Gelatin, NH_4NO_3, $(NH_4)_2SO_3$ and NH_4 CL were also used. In the investigation of carbon and nitrogen sources, each

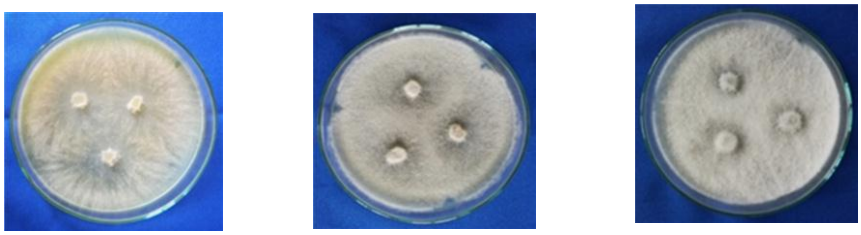
carbon sources 1.0g and nitrogen sources 1.0g were used in the fermentation as antibacterial activity on ***Staphylococcus aureus***.

Results

In the present research work, fungi were isolated from six different soil samples. The total fifteen fungi were isolated which belong to different methods as shown in (Table-2). Two isolated strains were tested for antibacterial activities with *Staphylococcus aureus* . In the present study, TW-3 (22.57 mm) and TW-14 (34.81 mm) were shown in Figure 9.

Table 2 Isolation of Soil Fungi from Six Different Soil Sample

Soil No.	Collected Area	Isolated Soil Fungi		
		Physical treatment dilution method	**Direct method**	**No: of fungi**
1	Hitaw Mu Pagoda	-	TW-01,TW-02 and TW-03	3
2	Ma Gyi Oak village	TW-05	TW-04	2
3	Myin Kya Kan village	TW-06 and TW-09	TW-07 and TW-08	4
4	Gyone Yar village	TW-10	TW-11	2
5	Nyaung Oak Phee village	-	TW-12 and TW-13	2
6	Quarter(3),Wundwin	TW-14	TW-15	2
Total isolated of soil fungi		5	10	15



Fungus TW-01 Fungus TW-02 Fungus TW-03

Figure 7 Morphology of soil fungi (5 days old culture on PGA medium)

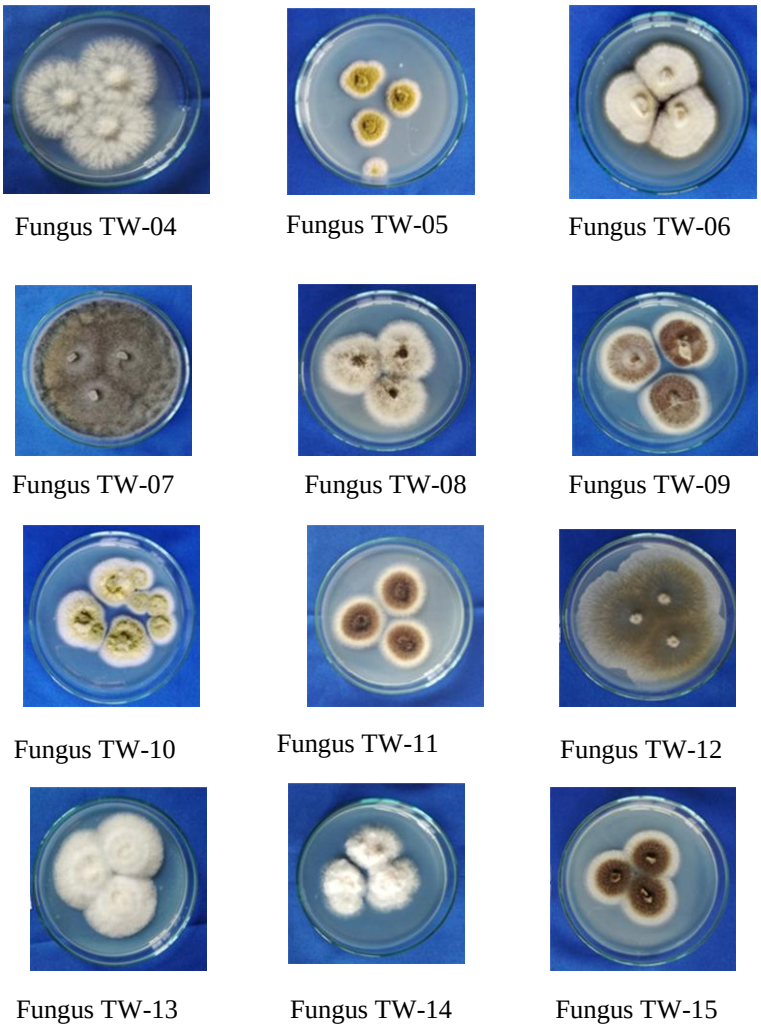


Figure 8 Morphology of soil fungi (5 days old culture on PGA medium)

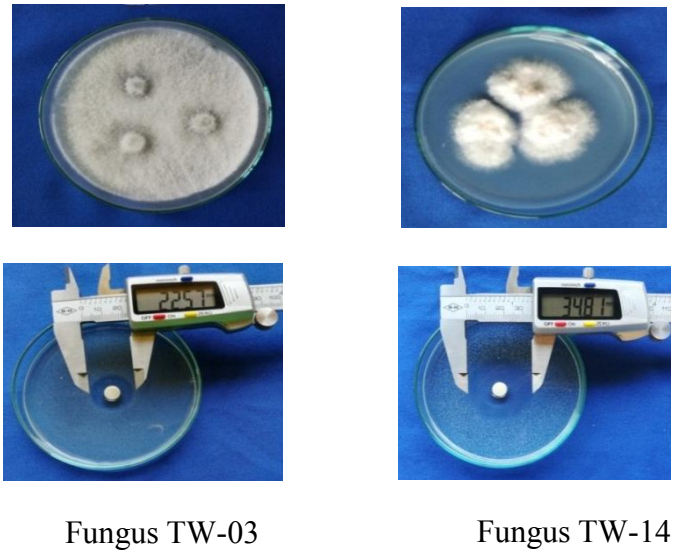
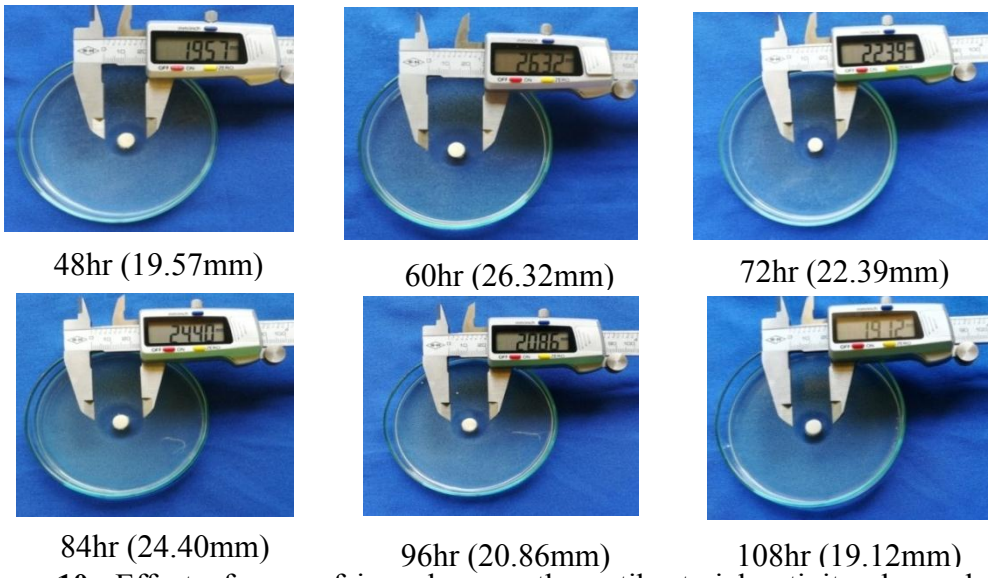


Figure 9 Effect of inoculation on the antibacterial activity shown by *Staphylococcus aureus*

The effect of ages of inoculation on the fermentation

In the effect of age of inoculum, TW-14(*Fusarium* sp.*)* was investigated by using 48, 60, 72, 84, 96, 108hr old culture age of inoculums. The results showed that 60hr age of inoculum gave the highest activities (26.16 mm) followed (24.40 mm) at 84hr and (22.39 mm) at 72hr age of inoculum. The results were shown in Table 3 and Figure 10.



48hr (19.57mm) 60hr (26.32mm) 72hr (22.39mm)

84hr (24.40mm) 96hr (20.86mm) 108hr (19.12mm)

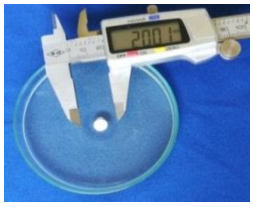
Figure 10 Effect of ages of inoculum on the antibacterial activity shown by *Staphylococcus aureus*

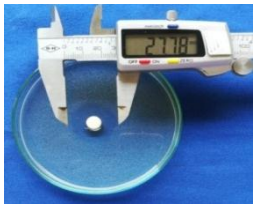
Table 3 The effect of ages of inoculums on the fermentation for TW-14 against *Staphylococcus aureus*

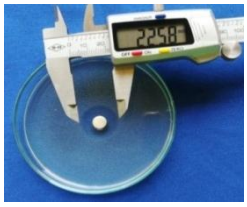
Sr. No	Age of inoculum	Activity (clear zones, mm)
1	48 hr	19.57 mm
2	60 hr	26.16 mm
3	72 hr	22.39 mm
4	84 hr	24.40 mm
5	96 hr	20.86 mm
6	108 hr	19.12 mm

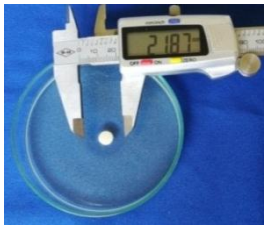
The effect of sizes of inoculums on the fermentation

In this research work, the effect of size of inoculums was studied using 5%, 10%, 15%, 20%, 25% and 30% inoculums. Using 10% showed significantly higher (27.78mm) than others, followed by 25% and 30% (24.04mm and 26.12mm) respectively in Table 4 and Figure 11.


5% (20.02mm)


10% (27.78mm)


15% (22.58mm)


20% (21.78mm)


25% (24.04mm)

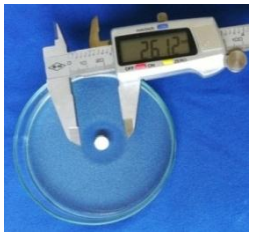

30% (26.12mm)

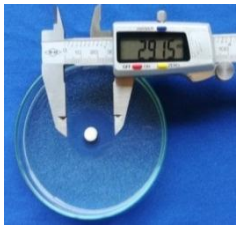
Figure 11 Effect of sizes of inoculum on the antibacterial activity shown by *Staphylococcus aureus*

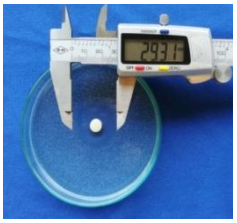
Table 4 The effect of sizes of inoculation on the fermentation for TW-14 against *Staphylococcus aureus*

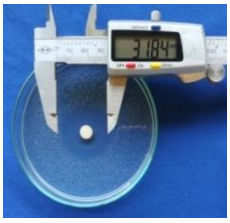
Sr. No.	Sizes of inoculum	Activity (clear zones, mm)
1	5%	12.58 mm
2	10%	27.78 mm
3	15%	20.01 mm
4	20%	21.87 mm
5	25%	24.04 mm
6	30%	26.12 mm

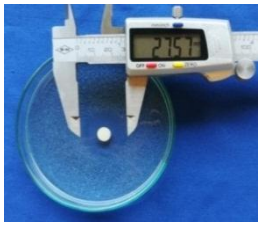
Effects of Carbon Utilization on Fermentation

There were variations in the level of antimicrobial activity when the different carbon sources were tested in the fermentation medium. The addition of different carbon sources displayed the highest antibacterial activities on soluble starch (31.84mm) followed by mannitol (29.31mm), molasses (29.15 mm), rice powder(27.57 mm) and corn powder(16.96 mm) were found as activity (Table 5 and Figure 12)


Molasses


Mannitol


Soluble starch


Rice powder


Corn powder

Figure 12 Effect on the carbon sources of selected fungi TW–14 against *Staphylococcus aureus*

Table 5 Effect on the carbon sources of selected fungi TW–14 against *Staphylococcus aureus*

Sr. No	Carbon Sources	Activity (Clear zones, mm) TW-14
1	Molasses	29.15mm
2	Mannitol	29.31mm
3	Soluble starch	31.84mm
4	Rice powder	27.57mm
5	Corn powder	16.96mm

Effects of Nitrogen Utilization on Fermentation

There were variations in the level of antimicrobial activity when the different nitrogen sources were tested in the fermentation medium. When the addition of various nitrogen sources, the significant inhibition zones on soybean (31.68mm) followed by NH_4NO_3(30.60mm), KNO_3 (24.34mm), gelatin (24.12mm) and $(NH_4)_2SO_4$(23.98mm), were found on activity (Table 6 and Figure 13)

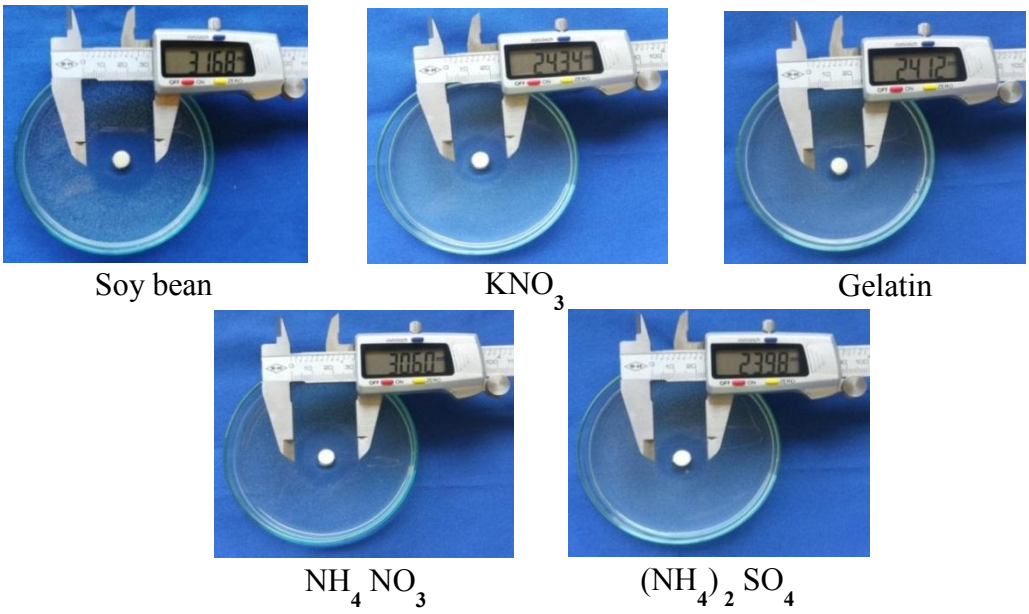


Figure 13 Effect of the nitrogen on the antibacterial activity of TW–14 against *Staphylococcus aureus*

Table 6 Nitrogen Utilization for the Fermentation on *Staphylococcus aureus*

Sr. No	Nitrogen Sources	Activity (Clear zones, mm) TW-14
1	Soybean	31.68mm
2	KNO_3	24.34mm
3	Gelatin	24.12mm
4	NH_4NO_3	30.60mm
5	$(NH_4)_2SO_4$	23.98mm

Discussion and Conclusion

The environmental factors such as pH, temperature, moisture, organic carbon and nitrogen play an important role in distribution of mycophora.(Adams *et al*., 1999).

Soil samples were collected from six different places at Wundwin Township in Mandalay Region. The isolation of fungi were isolated by using physical treatment dilution method (Hayakawa and Kobayashi, 2005) and direct method (Ando, 2004). According to the result, three fungi TW-01, TW-02 and TW-03 were isolated from the soil no-1.Two fungi TW-04 and TW-05 were isolated from the soil no-2.Four fungi TW-06, TW-07, TW-08 and TW-09 were isolated from the soil no-3.Two fungi TW-10 and TW-11 were isolated from the soil no-4. Two fungi TW-12 and TW-13 were isolated from the soil no-5. Two fungi TW-14 and TW-15 were isolated from the soil no.6.

Then, antimicrobial activities of soil fungi were tested by paper disc diffusion assay method. The fungal extracts were tested for antimicrobial activity against test organisms *Staphylococcus aureus.* Among them TW-14(*Fusarium* sp.*)* showed highest antibacterial against *Staphylococcus aureus*. In this investigation six different hours of 48hr, 60hr, 72hr, 84hr, 96hr and 108hr were consumed. For the sizes of inoculation 5%, 10%, 15%, 20%, 25% and 30% were used respectively. According to the results from this study, it is considered that the optimum ages of inoculum is 60hr (26.16 mm) and optimization size is 10% (27.78 mm).

In carbon and nitrogen sources utilization, carbon sources such as glucose, sucrose, lactose, glycerol, and soluble starch were used. Nitrogen sources such as yeast extract, $NaNO_3$, urea, soybean and peanut cake were also used. The results obtained in carbon sources study indicated that soluble starch (31.84 mm) is highest activity and corn powder(16.96 mm) is lower activity(Table 4 and Figure 12). In nitrogen sources study, soybean (31.68mm) is highest activity and $(NH_4)_2 SO_4$ (23.98 mm) is lower activity (Table 5 and Figure 13).

In the present study, the isolated fungus (*Fusarium* sp.) was then screened for the production of antimicrobial compound and it is an effort to understand the soil fungal diversity in Wundwin Township, Mandalay Region.

References

Adams, C.P., Bamford, K. M. and Early, M. P. (1999). **Principles of Horticulture** (3rd Ed.), utterworth Heineman, p. 25.

Aina VO, Adewuni AAJ, Hauwa Haruna and Amina Zaraki. Isolation and identification of fungi associated with the deterioration of painted wall surfaces within Kaduna polytechnic. Asian Journal of Medical Sciences,(2011); 3 (6): 250-253.

Ando, K. (2004). **Isolation and identification of fungi**. Workshop at Pathein University.

B.I. Duerden, T.M.S. Reid, J.M. Jewsbury, **Microbial and parasitic infection**, 7th ed., Great Britain: Edward Arnold, (1993).

Gutcho, Sydney,(1973). **Proteins from Hydrocarbons**, Noyes Data Corporation, Park Ridge, NJ

Hayakawa , A. and M. Kobayashi. (2005). **Screening for rare actinomycetes from soil.** J Microbial., 76, 240-242

Omalu, I.C.J., Ayanwale, V.A., Ajalaruru, A.B., Mohammed, A..Z., Bala, J. and Chukwuemeka V. (2011) Isolation Of Fungi And Bacteria From Housefly (Musca Domestica L.) Larvae. The Internet Journal of Microbiology. 9(1).

Smith, Biotechnology, Cambridge, New York, USA, (1996).

Stanbury, P.F., Whitaker, A. and Hall, S.J. (1995). **Media for industrial fermentations**. In Principles of Fermentation Technology pp. 93–121. Oxford: Pergamon Press

Vogel, Henry C. (ed.), (1983). **Fermentation and Biochemical Engineering Handbook**, Noyes Publications, Park Ridge, NJ

Y. Kulkarni and Y. Aynihojri, **Isolation and characterization of actinomycetes and optimization of its production**, M.Sc thesis, Department of Microbiology, University of Pune, (1995).

Zabriski, D. W., *et al*.,(1980). **Trader's Guide to Fermentation Media Formulation**, Trader's Oil Mill Co., Ft. Worth, TX

QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF *CORDIA DICHOTOMA* G. FORST.

Khin Than Oo[1], Swe Swe Aye[2]

Abstract

There is a very few scientific information on *Cordia dichotoma* G.Forst., so the qualitative and quantitative analysis of leaves, barks, fruits, seeds and roots was performed by using the methods given in WHO (1998) and Trease and Evans (2002). The plant specimens were collected from Loilem Township,Southern Shan State. Identification of the plant was done by standard procedure. In preliminary phytochemical study, the three different extracts such as petroleum ether, chloroform, and ethanol extracts of leaves, barks, fruits , seeds and roots were found to contain tannins, flavonoids, steroids and terpenoids whereas alkaloids were present in 1%hydrochloric acid extract of leaves, barks and roots. However, reducing sugar, glycoside and cyanogenic glycoside were absent in the whole plant parts. Moreover, the watery extract of leaves, barks, fruits and roots contain amino acids, carbohydrates, starch, saponins, and phenolic compounds where as the seeds has no starch, saponins, phenolic compounds and flavonoids. The extractive values with different solvents and ash values were also analyzed and recorded. The Energy Dispers ive X-Rays Fluorescence Spectrophotometer (EDXRF) analysis was used to investigate the elements present in different plants parts. According to the results, the barks of *Cordia dichotoma* G.Forst. has more calcium element (73.109 %) than other parts of the plants whereas potassium in fruits was (76.266 %) and iron in seeds was (44.082 %).The Atomic Absorption Spectrometer (AAS) analysis was performed to investigate the heavy metal contents in powdered of different parts of the plants. In nutritional study, it was significantly found that 40.25 % of carbohydrates in barks, 30.78 % of crude fat in fruits and 48.71 % of crude fibers in seeds. The obtain data from this research could be use for crude development.

Keywords: Phytochemical, Physicochemical , *Cordia dichotoma* G.Forst.

Introduction

Cordia is a genus of flowering plants, belong to the family Boraginaceae, subfamily Cordioideae. The family Boraginaceae composed of about 130 genera and six subfamilies: Boraginoideae, Cordioideae, Ehretioideae, Heliotropioideae, Hydrophylloideae and Lennooideae. The subfamily Cordioideae contains the genus *Cordia*, which is comprised of evergreen trees and shrubs (Thirupathi *et al*., 2008); about 300 species of *Cordia* have been identified worldwide. *Cordia dichotoma* G. Forst., is a perennial trees, growing mostly in tropical and sub-tropical region in India. In Myanmar, it is mostly found in Loilem Township in the Southern Shan State. The leaves have been used for wrap cheroot.

The chemical screening of both leaves and fruits revealed the presence of pyrrolizidine alkaloids, coumarins, flavonoids, saponins, terpenes and sterols. (Alarcon, 1994). Aalkaloid, saponin, quercetin and coumarin are present in stem bark of Cordia dichotoma (Moheboob *et al*.,2018). The seeds of this plant reported to contain fatty acids and flavonoids (Awadi, 2001). *Cordia dichotoma* G.Forst. are used as anti-ulcer (Parmar, 1998; Nazim & Kakoti, 2013) anti-inflammatory (Rapisarda *et al*., 1992; Ficarra *et al*.,1995; Kuppast & Nayak, 2006) analgesic

[1] Lecturer, Department of Botany, University of Mawlamyine
[2] Lecturer, Department of Botany, University of Yangon

(Rapisarda *et al*., 1992; Ficarra *et al*.,1995) anticancer (Rahman, 2015) antimicrobial (Nariya *et al*., 2011), hepatoprotective and diuretic purposes (Parmar,1998; Nazim, 2013).

Even though many medicinal uses are granted with documented research, there is no pharmacognosy research in Myanmar. Therefore, the aims of the present research is to investigate so the qualitative and quantitative analysis on *Cordia dichotoma* G. Forst.

Materials and Methods

Collection and preparation of samples

The sample plant *Cordia dichotoma* G. Forst. was collected from Loilem Township, Southern Shan State during the months of April to July, 2018. The leaves, barks, fruits, seeds and roots were washed and cut into small pieces and air dried in room temperature for several days. After being completely dried, they were pulverized by grinder machine to get powder and stored in airtight containers to prevent it from moisture and air-borne contamination.

Preliminary phytochemical test of leaves, barks, fruits, seeds and roots

Preliminary phytochemical test were carried out according to British Pharmacopoeia, 1968; Central Council for Research in Unani Medicine, 1987; Harbone, 1984 and Trease and Evans, 2002.

Physicochemical properties of leaves, barks, fruits, seeds and roots

Physicochemical properties were carried out according to quality control method of WHO, 1998 at the Department of Botany, University of Yangon.

Determination of elemental analysis of leaves, barks, fruits, seeds and roots

The concentrations of elements in *Cordia dichotoma* G.Forst. powdered leaves, barks, fruits, seeds and roots were analyzed by using Energy Dispersive X-ray Florescence (EDXRF) spectrometer at University of Research Center in Yangon University. The elements in leaves, barks, fruits, seeds and roots were analyzed by Atomic Absorption Spectroscopy (AAS) at University of Research Center in Yangon University.

Determination of nutritional value of leaves, barks, fruits, seeds and roots

Nutritional values of the leaves were determined by Association of Official Analytical Chemist (AOAC) method, (AOAC, 2002).

Results

Scientific Name - *Cordia dichotoma* G. Forst.

Local Name - Thanatphet or Thanat
Family - Boraginaceae

Outstanding characters

Perennial trees. Leaves simple, alternate. Inflorescence terminal and axillary cymes. Flowers white coloured, pentamerous, hypogynous. Stamen 5, petalostemonous, base of filament hairy anther dithecous, dorsifixed. Ovary monocarpellary, tetralocular, style twice bifid. Fruits drupe, pink. Seeds 1, globose, pale brown, wrinkled (Figure 1)

A . A plant in natural habit. B. Inflorescence. C. A bunch of ripen fruit

Figure 1 Habit of *Cordia dichotoma* G.Forst.

Preliminary phytochemical investigation

In preliminary phytochemical test, the present or absence of alkaloid, α amino acid, carbohydrate, starch, reducing sugar, cyanogenic glycoside, glycoside, saponin, phenolic compound, tannin, flavonoid, steroid and terpenoid were observed in leaves, barks, fruits, seeds and roots. The results were shown in Table (1) and Figures (4-14).

Table 1 Preliminary phytochemical results of *Cordia dichotoma* G.Forst.

No.	Test	Extract	Test Reagents	Observation	Results				
					leave	bark	fruit	seed	root
1	Alkaloid	1% HCl	Wagner′s reagent	Deep purple ppt	+	+	_	_	+
			Dradendroff's reagent	Deep purple ppt	+	+	_	_	+
2	α –amino acid	H_2O	Ninhydrin solution	purple color	+	+	+	+	+
3	Carbohydrate	H_2O	10% α Naphthol+ H_2SO_4	Red ring	+	+	+	+	+
4	Starch	H_2O	Iodine	Blue color	+	+	+	_	+
5	Reducing sugar	H_2O	Benedict solution	No change in color	_	_	_	_	_
6	Cyanogenic glycoside	H_2O	conc: H_2SO_4 + sodium picrate solution	No change in color	_	_	_	_	_
7	Glycoside	H_2O	10% Lead acetate solution	No change in color	_	_	_	_	_
8	Saponin	H_2O	Distilled water	Forthing	+	+	+	_	+
9	Phenolic compound	H_2O	1% Ferric chloride	Bluewish green	+	+	+	_	+
10	Tannin	EtOH	3 Drop of 1% gelatin solution	White ppt	+	+	+	+	+
11	Flavonoid	EtOH	Mg & conc: HCl	Brown colour	+	+	+	_	+
12	Steroid	P.E	Acetic anhydride & H_2SO_4	Blue color	+	+	+	+	+
13	Terpenoid	$CHCl_3$	Acetic anhydride & H_2SO_4	Green color	+	+	+	+	+

(+) = present (-) = absent ppt = precipitate

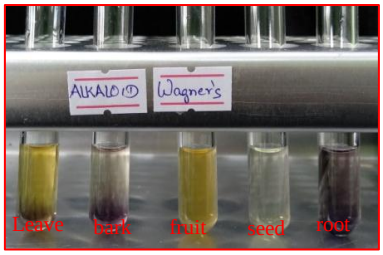


Figure 2 Alkaloid test

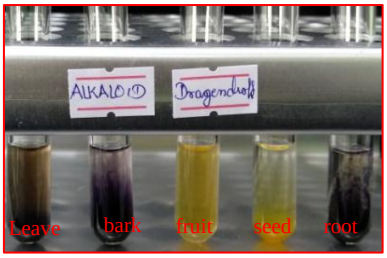


Figure 3 Alkaloid test

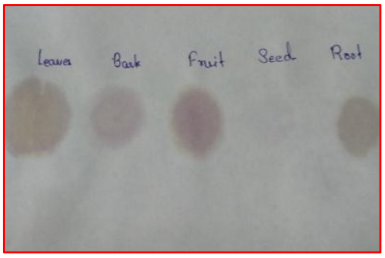


Figure 4 Amino acid test

Figure 5 Carbohydrate test

Figure 6 Starch test

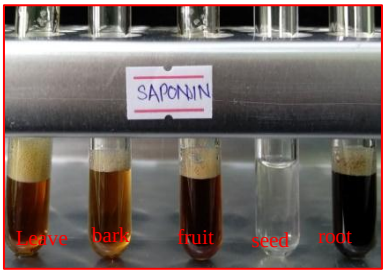


Figure 7 Saponin test

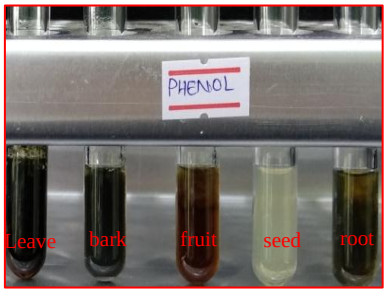


Figure 8 Phenol test

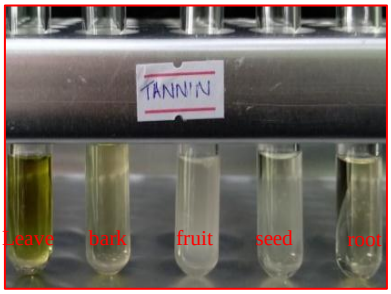


Figure 9 Tannin test

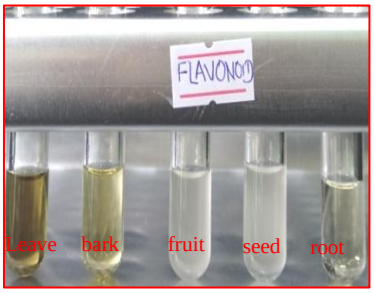


Figure 10 Flavonoid test

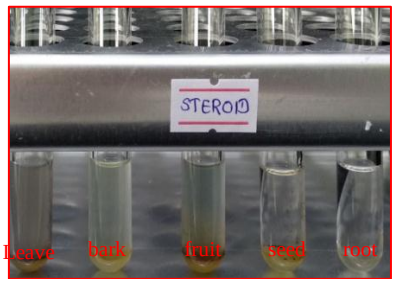


Figure 11 Steroid test

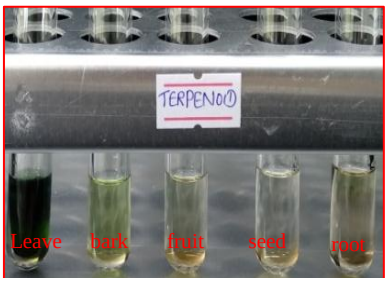


Figure 12 Terpenoid test

Physico-chemical investigation

Table 2 Physico-chemical examination of *Cordia dichotoma* G. Forst.

No	Physico-chemical Character	Leaves verage (%	Barks verage(%	Fruits Average (%)	Seeds Average (%)	Roots Average (%)
1.	Moisture content	11.45	8.18	13.87	10.42	10.46
2.	Total ash content	8.38	7.81	13.12	7.04	4.36
3.	Acid insoluble ash content	10.78	17.11	11.97	8.04	17.33
4.	Water soluble ash content	37.77	24.76	35.00	35.60	22.24
5.	Hexane soluble content	2.32	0.26	13.78	4.48	0.2
6.	Petroleum ether soluble content	2.42	0.3	12.62	4.78	0.1
7.	Chloroform soluble content	3.5	0.46	20.12	4.48	0.28
8.	Acetone soluble content	2.42	0.94	24.00	5.14	0.46
9.	Ethyl acetate soluble content	5.32	1.8	30.36	5.76	1.2
10	Ethanol soluble content	11.14	6.2	10.18	5.5	2.88
11	Methanol soluble content	13.22	8.86	9.26	5.22	5.32
12	Distilled water soluble content	22.42	7.08	18.26	1.14	4.72

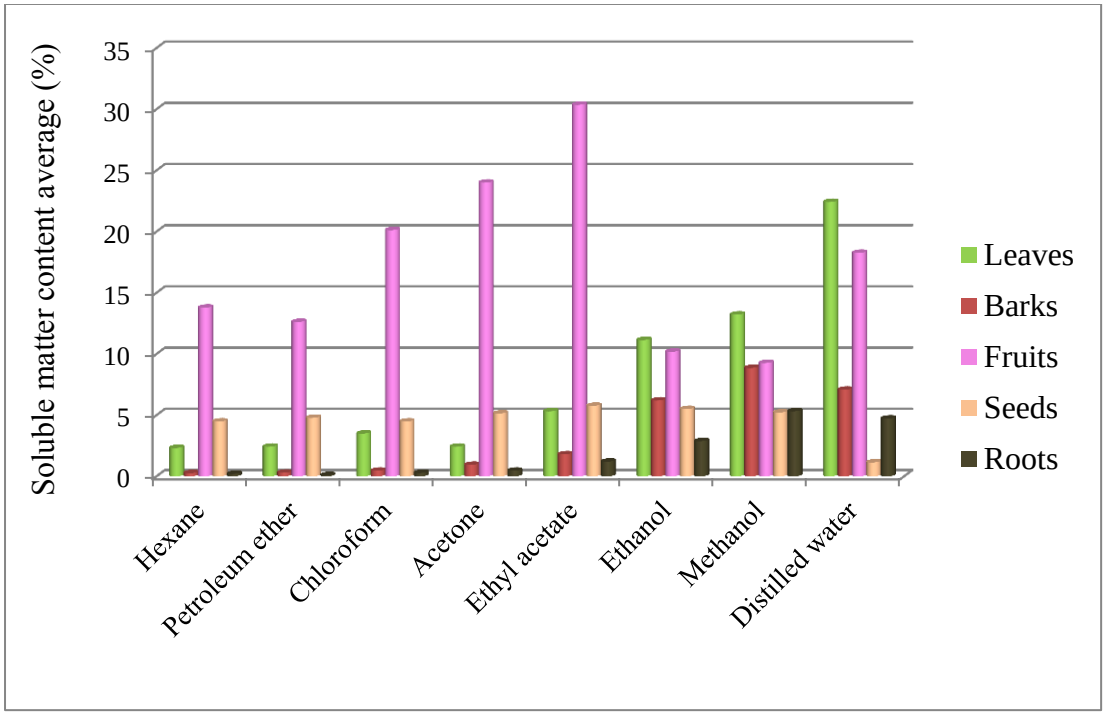


Figure 13 Solubility tests from the leaves, barks, fruits, seeds and roots of *Cordia dichotoma* G.Forst.

Determination of some elements (EDXRF)

The content of elements in leaves, barks, fruits, seeds and roots of *Cordia dichotoma* were determined by using EDXRF analysis . It was found that calcium and potassium, iron were significantly present in barks, fruits and seeds. The spectrum and spectral data were shown in Table (3) and Figures. (14-19).

Table 3 Elemental analysis of *Cordia dichotoma* G. Forst. by using EDXRF

No.	Elements	Leaves Content (%)	Barks Content (%)	Fruits Content (%)	Seeds Content (%)	Roots Content (%)
1	Calcium (Ca)	46.521	73.109	17.814	42.97	42.854
2	Potassium (K)	34.934	18.297	76.266	12.948	37.75
3	Iron (Fe)	15.466	7.625	2.942	44.082	16.812
4	Sulphur (S)	1.346	-	1.821		-
5	Titanium (Ti)	0.955	-	-		1.449
6	Manganese (Mn)	0.301	0.312	-		0.447
7	Zinc (Zn)	0.189	0.156	0.248	-	-
8	Copper (Cu)	0.185	0.26	0.279	-	0.393
9	Rubidium(Rb)	0.103	-	0.63	-	0.168
10	Strontium (Sr)	-	0.241	-	-	0.127

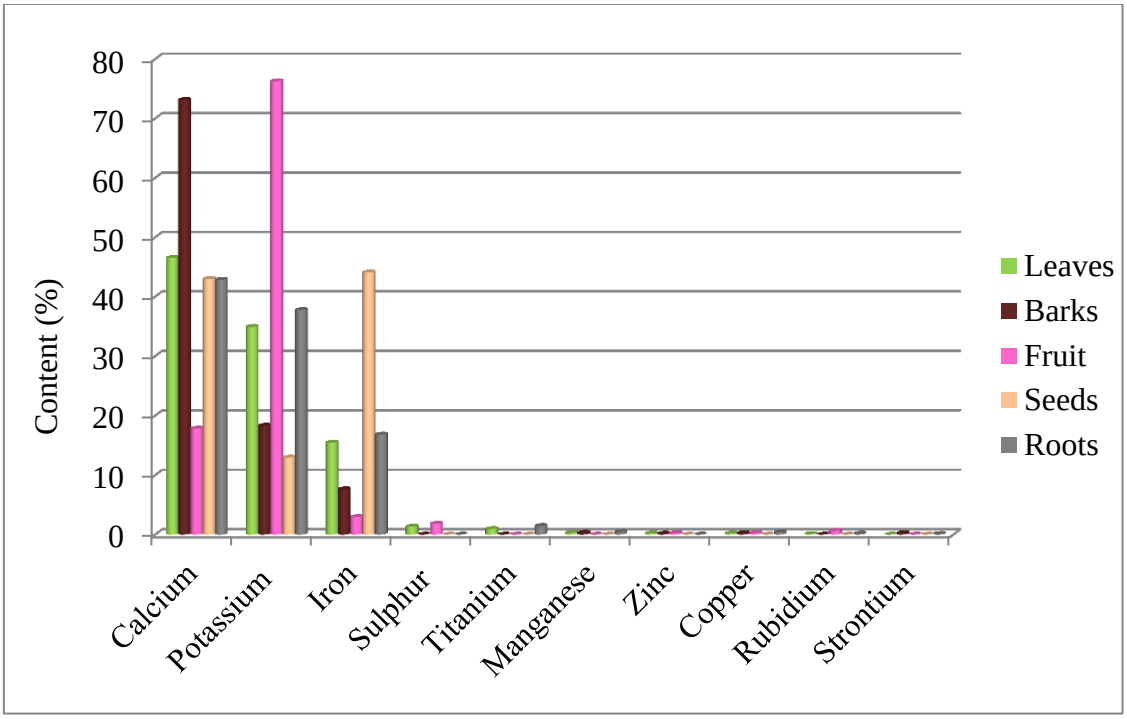


Figure 14 The elemental analysis of leaves barks, fruits, seeds and roots of *Cordia dichotoma* G. Forst.

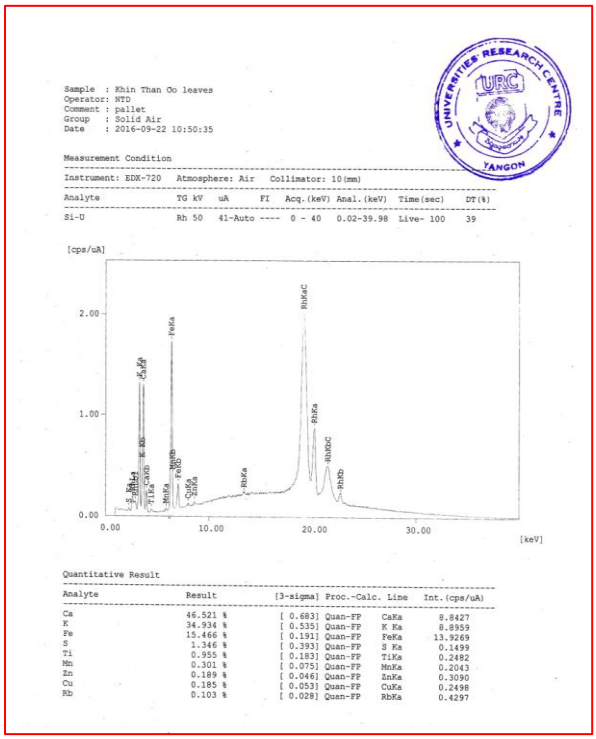


Figure 15 EDXRF spectrum of Leaves

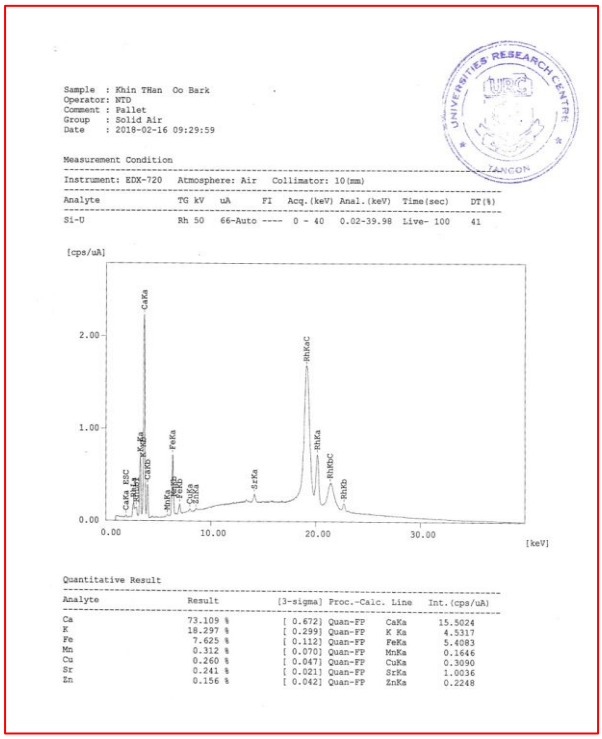


Figure 16 EDXRF spectrum of Barks

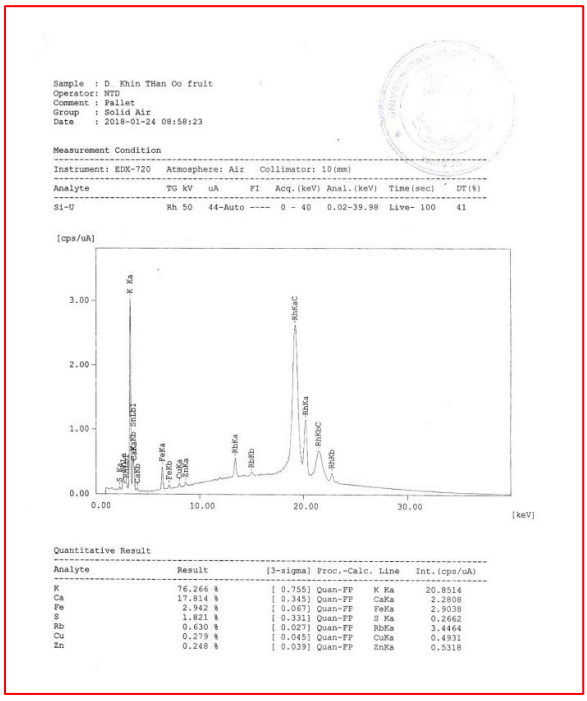


Figure 17 EDXRF spectrum of Fruits

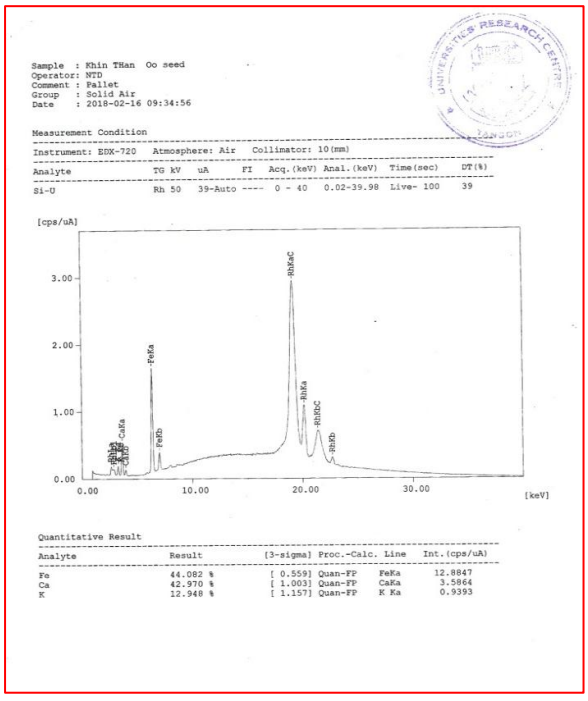


Figure 18 EDXRF spectrum of Seeds.

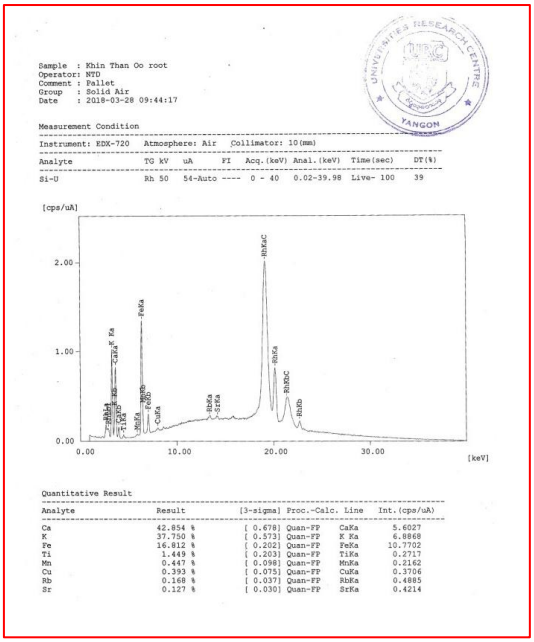


Figure 19 EDXRF spectrum of Roots

Quantitative determination of some elements (AAS)

The heavy metals such as Arsenic (As) , Lead (Pb) and Cadimum (Cd) contents in different plant parts were detected. According to the results, the roots of *Cordia dichotoma* G.Forst. has more lead elements than other parts of the plants. The results were shown in table (4) figure (20).

Table 4 Results of heavy metals analysis of *Cordia dichotoma* G. Forst.

No.	Type of Element	Leaves (mg/L)	Barks (mg/L)	Fruits (mg/L)	Seeds (mg/L)	Roots (mg/L)
1.	Lead (Pb)	0.814	0.627	0.415	0.198	4.499
2.	Cadmium (Cd)	0.047	0.041	0.092	0.098	0.042
3.	Arsenic (As)	0.002	0.002	0.001	0.001	0.121

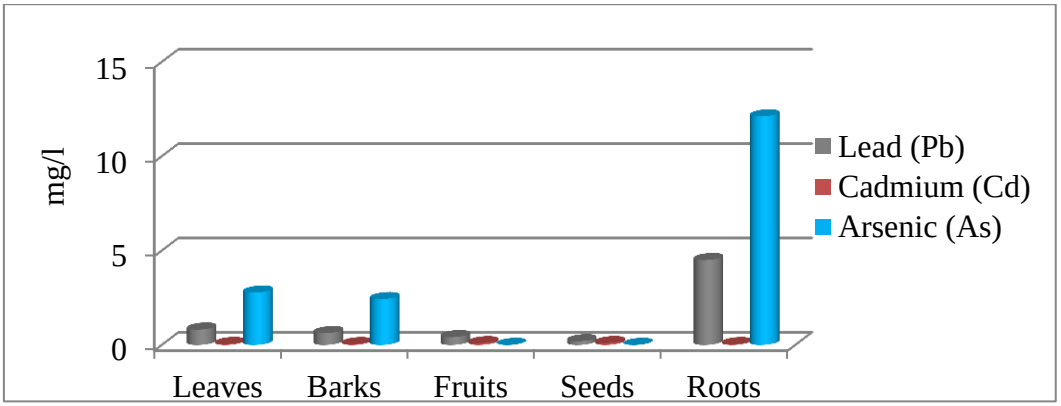


Figure 20 Heavy metals analysis of leaves, barks, fruits, seeds and roots of *Cordia dichotoma* G. Forst.

Nutritional values of *Cordia dichotoma* G. Forst.

The nutritional value such as crude fiber, crude protein, crude fat and carbohydrate values of the leaves, barks, fruits, seeds and roots of *Cordia dichotoma* G.Forst. were observed. The result were shown in Table (5) and Figure (21).

Table 5 Nutritional values of *Cordia dichotoma* G. Forst.

No	Type of Nutrients	Leaves content %	Barks content %	Fruits content %	Seeds content %	Roots content %
1	Ash	10.4	8.98	12.78	1.07	3.13
2	Crude protein	15.03	5.8	11.29	5.59	-
3	Crude fiber	20.11	38.29	14.4	48.71	38.83
4	Crude fat	1.55	0.8	30.78	5.11	0.09
5	Carbohydrate	37.73	40.25	20.44	28.71	-
6	Energy value(Kcal/100g)	230	197	403	185	-

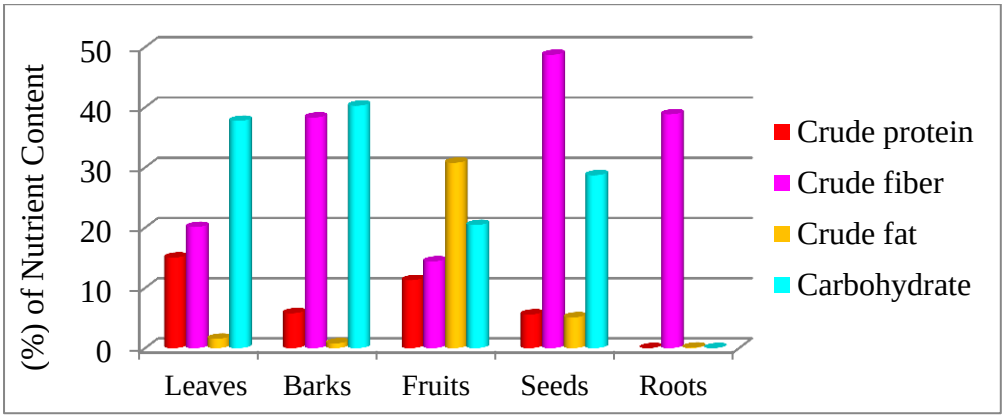


Figure 21 The nutritional values of leaves barks, fruits, seeds and roots of *Cordia dichotoma* G. Forst


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 - 06-2771/16
Page 1/1

1	Company's Name	: Daw Khin Than Oo
2	Address	: Mawlamyaing University
3	Phone No.	: 09-5058854
4	Date Received	: 17.10.2016
5	Sample Number	: 2398/16
6	Product Name	: Cordia dichotoma G. Frost Leave
7	Type of Test	: Nutrition Package
8	Date of Issue	: 28.10.2016
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)	15.18%
2	Ash	AOAC-2000(930.05)	10.40%
3	Crude Protein	AOAC-2000(920.152) (Kjeldahl Method)	15.03%
4	Crude Fiber	AOAC-2000 (978.10) Fiber Cap Method	20.11%
5	Crude Fat (Ether Extract)	AOAC(Buchi Soxhlet Method)	1.55%
6	Carbohydrate	By Difference	37.73%
7	Energy Value (Kcal / 100 g)		230

Remarks

Nutrition Facts (100 gm)

Energy	230	Kcal
Protein	15	gm
Fat	2	gm
Carbohydrate	38	gm

Tin Naing Win
Manager
FIDSL

(This laboratory analysis report shall not be reproduced except in full, without written approval of the laboratory.)


Myanmar Food Processors and Exporters Association (MFPEA)
Food Industries Development Supporting Laboratory (FIDSL)
LABORATORY ANALYSIS REPORT
FIDSL-Ad-06-01- 04338 /18

1	Company's Name	: Daw Khin Than Oo
2	Address	: Botany Department, Mawlamyaing University
3	Phone No.	: 09-796277837
4	Date Received	: 17.10.2018
5	Sample Number	: 3121/18
6	Product Name	: Cordia dichotoma G.Forst (Bark)
7	Test Performed date	: 12.11.2018
8	Type of Test	: Nutrition Package
9	Date of Issue	: 28.11.2018
10	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)	5.61%
2	Ash	AOAC-2000(930.05)	8.98%
3	Crude Protein	AOAC-2000(920.152) (Kjeldahl Method)	5.80%
4	Crude Fiber	AOAC-2000 (978.10) Fiber Cap Method	38.29%
5	Crude Fat (Ether Extract)	AOAC(Buchi Soxhlet Method)	0.80%
6	Carbohydrate	By Difference	40.52%
7	Energy Value (kcal / 100 g)		197

Nutrition Facts 1 package (100 g)

Energy	197	kcal
Protein	6	g
Fat	1	g
Carbohydrate	41	g

Tin Naing Win
Manager
FIDSL

(This laboratory analysis report shall not be reproduced except in full, without written approval of the laboratory.)


Myanmar Food Processors and Exporters Association (MFPEA)
Food Industries Development Supporting Laboratory (FIDSL)
UMFCCI Tower, 7th Floor, Room No.(4), No.(29), Min Ye Kyaw Swar Road, Lanmadaw Township, Yangon, Myanmar.
FIDSL

LABORATORY ANALYSIS REPORT

FIDSL-Ad-06-01- 04340 /18

1	Company's Name	: Daw Khin Than Oo
2	Address	: Botany Department, Mawlamyaing University
3	Phone No.	: 09-796277837
4	Date Received	: 9.11.2018
5	Sample Number	: 3249/18
6	Product Name	: Cordia dichotoma G.Forst (Fruit)
7	Test Performed date	: 12.11.2018
8	Type of Test	: Nutrition Package
9	Date of Issue	: 28.11.2018
10	Results	

(This Laboratory analysis report is based solely on the sample(s) submitted by the customer.)

Sr. No	Test Parameter	Test Method	Result
1	Moisture	AOAC-2000(934.06)	10.34%
2	Ash	AOAC-2000(930.05)	12.78%
3	Crude Protein	AOAC-2000(920.152) (Kjeldahl Method)	11.29%
4	Crude Fiber	AOAC-2000 (978.10) Fiber Cap Method	14.40%
5	Crude Fat (Ether Extract)	AOAC(Buchi Soxhlet Method)	30.75%
6	Carbohydrate	By Difference	20.44%
7	Energy Value (kcal / 100 g)		403

Nutrition Facts 1 package (100 g)		
Energy	403	kcal
Protein	11	g
Fat	31	g
Carbohydrate	20	g

(for) Tin Naing Win
Manager
FIDSL

(This laboratory analysis report shall not be reproduced except in full, without written approval of the laboratory.)


Myanmar Food Processors and Exporters Association (MFPEA)
Food Industries Development Supporting Laboratory (FIDSL)
UMFCCI Tower, 7th Floor, Room No.(4), No.(29), Min Ye Kyaw Swar Road, Lanmadaw Township, Yangon, Myanmar.
FIDSL

LABORATORY ANALYSIS REPORT

FIDSL-Ad-06-01- 04339 /18

1	Company's Name	: Daw Khin Than Oo
2	Address	: Botany Department, Mawlamyaing University
3	Phone No.	: 09-796277837
4	Date Received	: 17.10.2018
5	Sample Number	: 3122/18
6	Product Name	: Cordia dichotoma G.Forst (Seeds)
7	Test Performed date	: 12.11.2018
8	Type of Test	: Nutrition Package
9	Date of Issue	: 28.11.2018
10	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)	10.81%
2	Ash	AOAC-2000(930.05)	1.07%
3	Crude Protein	AOAC-2000(920.152) (Kjeldahl Method)	5.59%
4	Crude Fiber	AOAC-2000 (978.10) Fiber Cap Method	48.71%
5	Crude Fat (Ether Extract)	AOAC(Buchi Soxhlet Method)	5.11%
6	Carbohydrate	By Difference	28.71%
7	Energy Value (kcal / 100 g)		185

Nutrition Facts 1 package (100 g)		
Energy	185	kcal
Protein	6	g
Fat	5	g
Carbohydrate	29	g

(for) Tin Naing Win
Manager
FIDSL

(This laboratory analysis report shall not be reproduced except in full, without written approval of the laboratory.)


Myanmar Food Processors and Exporters Association (MFPEA)
Food Industries Development Supporting Laboratory (FIDSL)
UMFCCI Tower, 7th Floor, Room No.(4), No.(29), Min Ye Kyaw Swar Road, Lanmadaw Township, Yangon, Myanmar.
FIDSL

LABORATORY ANALYSIS REPORT

FIDSL-Ad-06-01- 04341 /18

1	Company's Name	: Daw Khin Than Oo
2	Address	: Botany Department, Mawlamyaing University
3	Phone No.	: 09-796277837
4	Date Received	: 9.11.2018
5	Sample Number	: 3250/18
6	Product Name	: Cordia dichotoma G.Forst (Root)
7	Test Performed date	: 12.11.2018
8	Type of Test	: Nutrition Package
9	Date of Issue	: 28.11.2018
10	Results	

(This Laboratory analysis report is based solely on the sample(s) submitted by the customer.)

Sr. No	Test Parameter	Test Method	Result
1	Moisture	AOAC-2000(934.01)	12.30%
2	Ash	AOAC-2000(930.05)	3.13%
3	Total Nitrogen	AOAC-2000(920.152) (Kjeldahl Method)	0.50%
4	Crude Fiber	AOAC-2000 (978.10) Fiber Cap Method	38.83%
5	Crude Fat (Ether Extract)	AOAC(Buchi Soxhlet Method)	0.09%

(for) Tin Naing Win
Manager
FIDSL

(This laboratory analysis report shall not be reproduced except in full, without written approval of the laboratory.)

Discussion and Conclusion

In this investigation, the preliminary phytochemical tests, physico-chemical properties, elemental analysis and nutritional values of *Cordia dichotoma* G Forst. had been studied.

In preliminary phytochemical study, alkaloids, saponins, phenolic compounds, tannins, steroids and terpenoids were present in leaves, barks, fruits, seeds and roots of *Cordia dichotoma* G.Forst. but reducing sugar, glycoside and cyanogenic glycoside were absent.The physico-chemical properties the most significantly soluble matter content of leave *Cordia dichotoma* G.Forst. sample was in water, followed by methanol, ethanol and ethyl acetate at least soluble in hexane. Powdered of barks and roots were the most soluble in methanol whereas the powdered fruits and seeds were the most soluble in ethyl acetate solvent. These results were agreement with those described by Parmar, 1998; Mahour 2008; Jamkhande *et al*., 2013; Nazim *α* Kakoti, 2013.

According to the EDXRF results, Calcium (Ca), Potassium (K) and Iron were found as principal elements in leaves, barks, fruits, seeds and roots. Among them, the bark of *Cordia dichotoma* G.Forst. was found to contain the highest amount of Calcium 73.10%. Calcium is an essential element that plays a vital role in metabolic function (WHO guide line, 2013). The iron in the selected plant ranges from 7.62% - 76.26%. Among them, the seeds of *Cordia dichotoma* G. Forst. was found to contain the highest amount of iron 44.082% (WHO guide line, 2006).

According to the results of (AAS), the heavy metals such as lead (Pb), Cadmium (Cd) and Arsenic (As) in this plant are found to be below permissible levels of WHO, 2005.

In quantitative determination of nutritional value, the result of present study showed that 15.03% crude protein, 20.11% of crude fibers, 37.73% of carbohydrate are present in leaves. Hussain, 2013 documented that the leaves contain 12.15% of crude protein, 16-27% crude fibres.

Thus, it can be concluded that the results obtained from the present study will be useful for the potential drugs investigations.

Acknowledgements

I would like to express appreciation to Dr. Aung Myat Kyaw Sein, Rector, Dr. Mie Mie Sein, Dr. San San Aye, Pro-Rector of Mawlamyine University for permission to carry out this research work. I wish to express my special thanks to Dr. Mar Lar Aung, Head of Department of Botany, Mawlamyine University for her advice and encouragement.

References

Alarcon, D. L., M. J. Martin, and V. Motilva., (1994), "Antiulcerogenic activity of flavonoids and gastric protection"**.** Journal of Ethnopharmacol. 42, p.161-170

Awadi, Al. FM, TS. Srikumar, JT. Anim and I.Khan, (2001), "Antiinflammatory Effects of *Cordia myxa* Fruit on experimentally induced colitis in rats". Nutrition, 17 (5), p.391-396

AOAC (2000), *Official Method of Analysis of the association of Official Analytical Chemists*.17th ed., Washington D.C.

Ayurvedic Pharmacopoeia of India, (2016), Government of India Ministry of AYUSH.

British Pharmacopoeia, (1968), Published Under the direction of the General Medicinal Council. Medicinal Act: London. Willian Clowes and Sons Limited.

Central Council for Research in Unani Medicine, (1987), *Phytochemical standards of Unani formulation*, New Delhi.

Ficarra, R., P. Ficarra, S. Tommasini, M.L. Calabro, S. Ragusa, R. Barbera and A. Rapisarda, (1995), "Leaf extracts of some *Cordia* species: analgesis and anti-inflammatory activities as well as their chromatographic analysis". Farmaco 50(4): 245-256

Harbone, J.D. (1984), *Phytochemical Methods*. A Guide to Modern Techniques of Plant Analysis. Chapman and Hall Ltd., New York

Kuppast, I J & V P Nayak (2005), "Wound healing activity of Cordia dichotoma Forst. f. fruit". National collages of Pharmacy, Karnataka, India.

Maheboob, Shaikh & Dhotre. Revansiddha (2018), ″ Phytochemical analysis of stem bark of some medicinal plants". International Journal Research in Pharmacy and Pharmaceutical Sciences, vol.3, p.86-89

Nariya PB, NR Bhalodia, VJ Shukla, RN Acharya, (2011), "Antimicrobial and antifungal activities of *Cordia dichotoma* (Forester F.) bark extracts". Ayu. ; 32: p.585–589.

Nazim, H & BB Kakoti, (2013),"Review on ethno botany and psychopharmacology of *Cordia dichotoma"*. Journal of drug delivery and therapeutics; 3(1), 110-113

Parmar NS & Shikha parmar, (1998), "Anti-ulcer potential of flavonoids". Indian J. Physiol.Pharmacol. 48, p.343-351

Rahman Azizur Md & Arshad Hussain, (2015), "Phytochemical and analytical evaluation of *Cordia dichotoma* Linn. leaves″ Pharmacognosy Journal, Vol 7.

Rapisarda A., R Berbera, A De Pasquale, P Ficarra, S.Tommasin, ML Caldbro, and S. Hungsa (1992), "*Cordia francisci, C. martinicensis, C. myxa, C. serratifolia* and Culumfolia leaves as new source of routine; Analgesis and anti-inflammatory activity". Plant Medica; 58(S 1), p. 643-649

Trease G.E. & W.C. Evans., (2002), *Pharmacognosy*. 15th ed. Edinburgh London, New York.

Thirupathi K, SS Kumar, VS Raju, B Ravikumar, DR Krishna and GK Mohan. (2008), "A review of medicinal plants of the genus *Cordia*; their chemistry and Pharmacological uses"**.** J Natn Rem; 8 (1): p.1-10

WHO (1998), *Quality control Methods for medicinal plant materials*, World Health Organization, Geneva.

WHO (2005), WHO Quality Control Methods for Medicinal nonessential trace element having function neither in Plant Materials, Geneva.

WHO Guidelines (2006), Guidelines on food fortification with micronutrients, World health Organization, Geneva, p. 57-131

WHO Guidelines (2013), Calcium supplementation in pregnant woman, World health Organization, Geneva, p.31

INVESTIGATION OF *CURCUMA PETIOLATA* ROXB. RHIZOMES FOR THE PRESENCE OF CURCUMIN AND ITS ANTIMICROBIAL ACTIVITY

Htay Htay Lwin[1]

Abstract

The plant *Curcuma petiolata* Roxb., Myanmar name "Marlar" belongs to the family Zingiberaceae. The plants are widely distributed in Myanmar. It was collected from Dawei University Campus, Tanintharyi Region from July to October (2018). In this study, phytochemical properties, curcumin percentage and antimicrobial activity of *Curcuma petiolata* Roxb. rhizomes were investigated. Phytochemical tests of *Curcuma petiolata* Roxb. showed the presence of alkaloid, α-amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid. The percentage of curcumin content from the rhizomes of *Curcuma petiolata* Roxb. was done at Kayin State Medicinal Plants Resource Center by using HPLC. The yield percentage of curcumin content was 4.3%. Antimicrobial activity of rhizomes of *Curcuma petiolata* Roxb. was carried out at Botany Department, University of Yangon by using different solvent extracts (petroleum ether, chloroform, ethyl acetate, acetone, ethanol, methanol and water). Antimicrobial activity was also investigated on six microorganisms such as *Aspergillus flavus*, *Bacillus subtilis*, *Candida albicans*, *Echerichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. The extracts of *Curcuma petiolata* Roxb. rhizomes indicated antimicrobial activity against *Aspergillus flavus*, *Bacillus subtilis*, *Candida albicans*, *Echerichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. Among them, chloroform extract showed the most significant antimicrobial activity against *Aspergillus flavus* and acetone and aqueous extracts on *Echerichia coli*. Aqueous extract showed the most prominent antimicrobial activity against *Bacillus subtilis* and *Pseudomonas fluorescens*. Only methanolic extract showed antimicrobial activity against *Candida albicans.* Ethyl acetate extract showed the most powerful antimicrobial activity against *Xanthomonas oryzae*.

Keyword: *Curcuma petiolata* Roxb., curcumin percentage and antimicrobial activity.

Introduction

The plant *Curcuma petiolata* Roxb. belongs to the family Zingiberaceae. This family consists of 50 genera and 1300 species (Heywood, *et al*., 2007 and Trease and Evans, 2009). The member of this family distributed in South and South East Asia, some species in America and subtropical and warm-temperate Asia (Te-lin and Larsen, 2000).

In Myanmar, this family consists of about 18 genera and 125 species (Hundley and Chit ko ko, 1961). According to the Kress, *et al*., (2003), genus *Curcuma* contains 24 species of Myanmar are listed in Zingiberaceae. Some members of the Zingiberaceae yield dyes, spices, perfumes, and medicines. Various species are cultivated for their showy flowers (Te-lin and Larsen, 2000 and Heywood, *et al*., 2007).

The genus *Curcuma* is one of the largest genera in the Zingiberaceae, with about 80 species, and distributed throughout tropical Asia from India to South China, Southeast Asia, Papua New Guinea and Northern Australia. They are grown in wide range of altitudes from 100 –1300m on limestone hills. Generally, most *Curcuma* grows well in loose and sandy soil in shaded areas (Sirirugsa, *et al*., 2007).

The rhizomes of these species are used in traditional medicines (Perry, 1980). *Curcuma* species possess antioxidant activity and the pharmacological effects. Bioactive components such

[1] Dr, Associate Professor, Department of Botany, Hpa-an University

as curcuminoids are responsible for anti-oxidative and anti-inflammatory properties, wound healing, hypoglycemia and antimicrobial activities (Beghel, *et al*., 2013).

Curcuma petiolata Roxb. is one of *Curcuma* species which widely cultivated as an ornamental plant and has long been used as a folk botanical in Asia (Perry, 1980). The *Curcuma petiolata* Roxb. rhizomes extract contain high amount of curcumins with potent DPPH radical scavenging, ferrous reducing power and inhibition of lipid peroxidation activities (Thakam, *et al.,* 2012). Curcumin has a wide range of biological functions, especially the anticancer activity including bladder cancer (Gao, *et al*., 2012), pancreatic cancer (Plengsuriyakarn, *et al*., 2012), prostate cancer (Zhou, *et al*., 2014). Curcumin has also been found to greatly inhibit the metastasis of breast cancer cells. Previous reports have revealed that curcumin can inhibit cell proliferation of chronic granulocytic leukemia (CGL), glioblastoma, and oesophageal cancer through inducing autophagy (Chen, *et al*., 2014). In this paper, phytochemical test, curcumin percentage and antimicrobial activity of *Curcuma petiolata* Roxb. rhizomes were carried out.

The aim and objectives are to determine the preliminary phytochemical tests and to examine the curcumin percentage and to examine the antimicrobial activities from the different solvent extracts by using on six types of microorganisms.

Materials and Methods

Collection, Drying and Pulverization

The plant *Curcuma petiolata* Roxb. was collected from Dawei University Campus, Tanintharyi Region from July to October (2018). The sample of *Curcuma petiolata* Roxb. rhizomes were thoroughly washed and cut into small pieces and air-dried in room temperature for several days. When constant weight was obtained, the dried samples were pulverized by grinding machine to get powder and stored in airtight containers to prevent from moisture and air-borne contamination.

Phytochemical investigation of *Curcuma petiolata* Roxb. rhizomes

In this investigation, the powdered *Curcuma petiolata* Roxb. rhizomes were tested to find out the presence or absence of chemical constituents such as alkaloid, α-amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid compounds. Preliminary phytochemical tests were carried out at the Hpa-an University according to the methods of Marini Bettolo, *et al*., (1981), Central Council for Research in Unani Medicine (1987) and Sasikala and Sundaraganapathy (2017).

Determination of total curcumin content

The percentage of curcumin content from the rhizomes of *Curcuma petiolata* Roxb. were done at Kayin State Medicinal Plants Resource Center. The curcumin content of extract was determined by using HPLC model-L 7300.

Antimicrobial activities of different solvent extracts from *Curcuma petiolata* Roxb. rhizomes

Antimicrobial activities of different solvent extracts of *Curcuma petiolata* Roxb. rhizomes were tested on six pathogenic microorganisms by using paper disc diffusion method at the Department of botany, University of Yangon.

Preparation of crude extracts

The powdered of *Curcuma petiolata* Roxb. rhizomes were extracted with various solvents such as petroleum-ether, chloroform, ethyl-acetate, acetone, ethanol, methanol and water. The filtrates were evaporated by using water bath.

Preparation of sample for testing antimicrobial activity

Screening of Antimicrobial activity of crude extracts had been done by paper disc diffusion method. Paper disc having six millimeter diameter were utilized for antimicrobial test. Assay medium was prepared according to the method described by Cruickshank (1975). Assay medium was boiled and 20- 25 ml of the medium was poured into each conical flask, plugged with cotton wool and autoclaved at 121°C for 15 minutes. Then the conical flasks were cooled down to 40- 45°C and each of 0.1- 0.2 ml of test organisms were also added into the flask and then, poured into sterilized petridishes. After solidification, paper disc impregnated with sample were applied on the agar plates and incubated at 37°C for 24 hours. Then the diameter of inhibitory zone was measured with the help of a transparent ruler.

Results

Phytochemical investigation of *Curcuma petiolata* Roxb. rhizomes

Preliminary phytochemical tests indicated the presence of alkaloid, α-amino acids, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid of *Curcuma petiolata* Roxb. rhizomes. The experimental results were shown in Table (1).

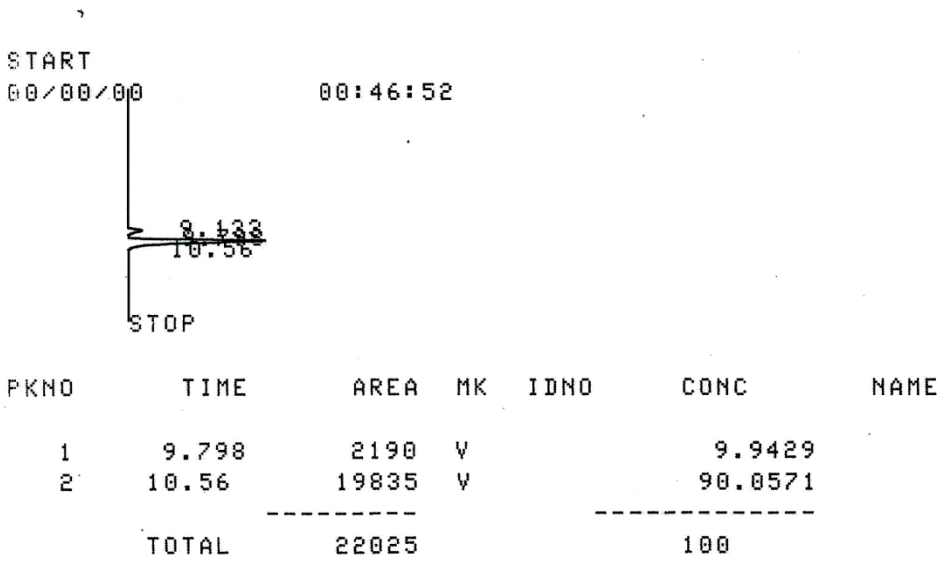
Table 1 Phytochemical test of *Curcuma petiolata* Roxb. rhizomes

No	Test	Extract	Test reagents	Observation	Results
1.	Alkaloid	EtOH	1.Dragendorff's reagent 2. Mayer's reagent 3.Wagner's reagent 4. Hager's reagent	Orange brown ppt White ppt Reddish brown ppt Yellow ppt	+ + + +
2.	α-amino acids	H_2O	Ninhydrin reagent	Pink spot	+
3.	Carbohydrate	H_2O	Benedict's solution	Brick red ppt	+
4.	Flavonoid	EtOH	HCl / Mg	Pink color	+
5.	Glycoside	EtOH	H_2O + NaOH	Yellow color	+
6.	Phenolic compound	EtOH	H_2O + 10% $FeCl_3$	Green color	+
7.	Protein	H_2O	Millon's reagent	White ppt turns red on heating	+
8.	Reducing sugar	H_2O	Fehling's solution A and B	Brick red ppt	+
9.	Saponin	H_2O	H_2O	Frothing	+
10.	Starch	H_2O	Iodine solution	Blue black	+
11.	Steroid	EtOH	$CHCl_3$ + conc:H_2SO_4	Green color	+
12.	Tannin	H_2O	5% $FeCl_3$ + H_2SO_4	Yellow brown ppt	+
13.	Terpenoid	EtOH	$CHCl_3$ + conc:H_2SO_4	Pink color	+

(+) = Present

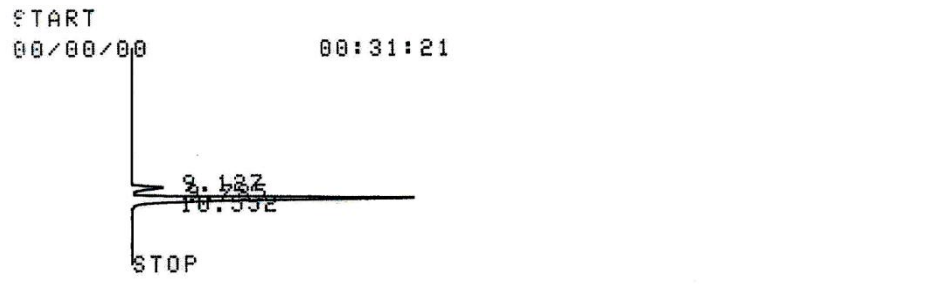
Determination of total curcumin content

The curcumin content of extract was determined by using HPLC model-L 7300. The yield percentage of total curcumin was 4.3 %. The experimental results were shown in Figure (1 to 4).



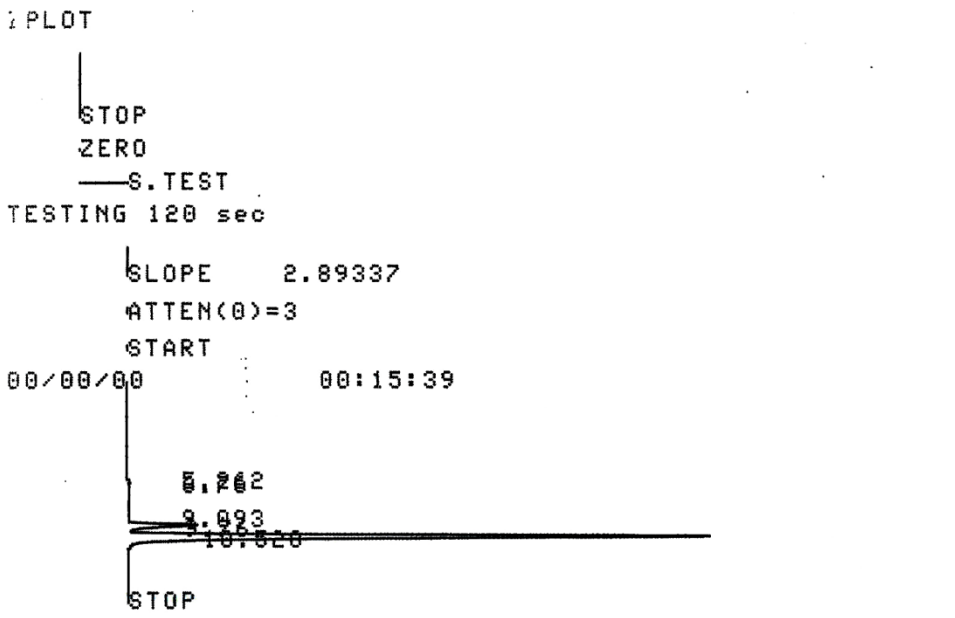
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	9.798	2190	V		9.9429	
2	10.56	19835	V		90.0571	
	TOTAL	22025			100	

Figure 1 HPLC chromatogram of cur cumin standard 25 ppm



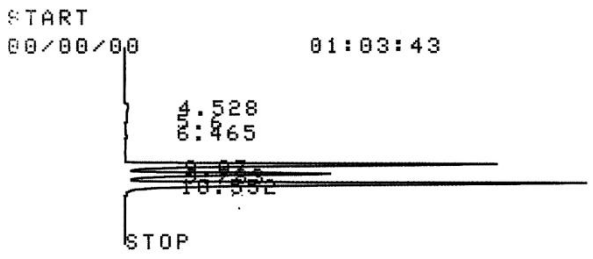
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	9.127	159			0.3505	
2	9.785	4538	V		10	
3	10.552	40683	V		89.6494	
	TOTAL	45380			100	

Figure 2 HPLC chromatogram of cur cumin standard 50 ppm



PKNO'	TIME	AREA	MK	IDNO	CONC	NAME
1	5.262	179			0.1935	
2	6.76	524	V		0.5665	
3	9.093	390			0.4213	
4	9.76	9390	V		10.148	
5	10.528	82044	V		88.6708	
	TOTAL	92527			100	

Figure 3 HPLC chromatogram of cur cumin standard 100 ppm



PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	4.528	1805			1.2464	
2	5.6	666	V		0.4598	
3	6.465	590	V		0.4074	
4	9.07	46944			32.4128	
5	9.783	27900	V		19.2637	
6	10.552	66927	V		46.2099	
	TOTAL	144832			100	

Figure 4 HPLC chromatogram of cur cumin from rhizomes extract of *curcuma petiolata* Roxb.

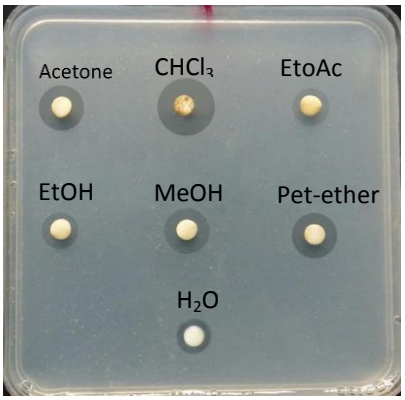
Antimicrobial activities of different solvent extracts of *Curcuma petiolata* Roxb. rhizomes by using paper disc diffusion method

The powdered of *Curcuma petiolata* Roxb. rhizomes were extracted with petroleum ether, chloroform, acetone, ethyl-acetate, ethanol, methanol and distilled water. The different extracts were tested on six pathogenic microorganisms by using paper disc diffusion method. According to this experiment, all extracts showed antimicrobial activity on *Aspergillus flavus* and *Echerichia coli*. Among them, chloroform extracts showed most significant antimicrobial activity against *Aspergillus flavus* (16mm) and acetone and aqueous extracts on *Echerichia coli* (14mm). Chloroform, ethyl acetate, acetone and aqueous extracts showed antimicrobial activity against *Bacillus subtilis.* Among them, aqueous extract showed the most significant antimicrobial activity against *Bacillus subtilis* (14mm). Petroleum ether, ethanol and methanol extracts did not show on *Bacillus subtilis.* Only methanol extracts showed antimicrobial activity against *Candida albicans* (14mm). Petroleum ether, chloroform, ethyl acetate, acetone ethanol and aqueous extracts did not show on *Candida albicans.* Chloroform, ethanol and aqueous showed antimicrobial activity on *Pseudomonas fluorescens.* Among them, aqueous extract showed the most significant antimicrobial activity against *Pseudomonas fluorescens* (16mm). Petroleum ether, ethyl acetate, acetone and methanol extracts did not show on *Pseudomonas fluorescens.* Chloroform and ethyl acetate extracts showed antimicrobial activity on *Xanthomonas oryzae.* Among them, ethyl acetate extract showed antimicrobial activity on *Xanthomonas oryzae* (18mm). Petroleum ether, acetone, ethanol, methanol and aqueous extracts did not show on *Xanthomonas oryzae*. The results were shown in Table (2) and Figure (5).

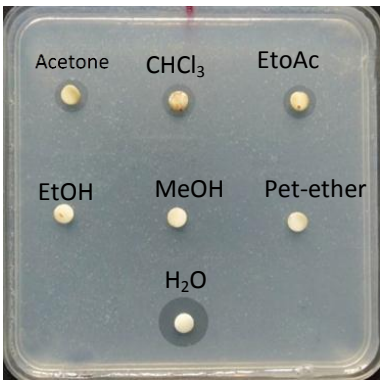
Table 2 Antimicrobial activities of different solvent extracts from *Curcuma petiolata* Roxb. rhizomes against (6) tested organism

No	Solvents	*A. flavus*	*B. subtilis*	*C. albicans*	*E. coli*	*P.fluorescen*	*X. oryzae*
1.	Acetone	14 mm	10 mm	-	14mm	-	-
2.	Chloroform	16mm	10 mm	-	12mm	10mm	12 mm
3.	Ethyl acetate	10 mm	10mm	-	10 mm	-	18 mm
4.	Ethanol	10mm	-	-	10 mm	12mm	-
5.	Methanol	14 mm	-	14mm	10mm	-	-
6.	Pet- ether	12mm	-	-	10 mm	-	-
7.	Aqueous	10mm	14 mm	-	14 mm	16 mm	-

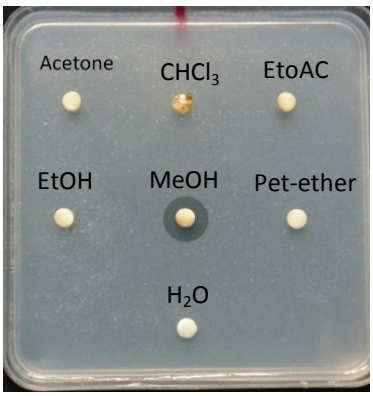
Paper disc size = 6 mm



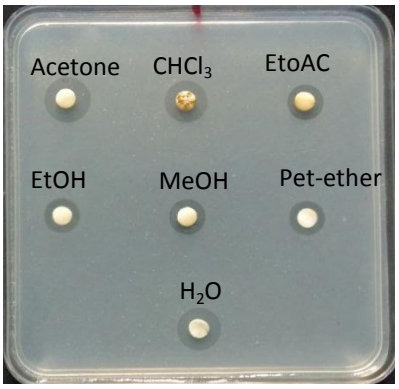
Aspergillus flavus



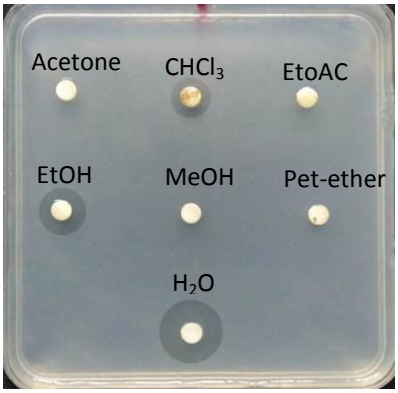
Bacillus subtilis



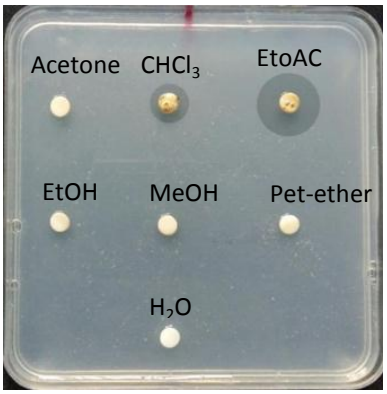
Candida albicans



Escherichia coli



Pseudomonas fluorescens



Xanthomonas oryzae

Figure 5 Antimicrobial activities of *Curcuma petiolata* Roxb. rhizomes

Discussion

In this investigation, phytochemical test, curcumin percentage and antimicrobial avtivity of *Curcuma petiolata* Roxb. rhizomes were carried out.

Khin Tar yar Myint, *et al*., (2018) stated that preliminary phytochemical test of *Curcuma petiolata* Roxb. rhizomes showed the presence of alkaloids, α–amino acid, carbohydrate, flavonoids, phenolic compound, reducing sugar, steroid and terpenoid.

In this research, the powdered sample of *Curcuma petiolata* Roxb. rhizomes contained alkaloid, α- amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid.

Thakam, *et al*., (2012) reported that the curcumin content of rhizomes extract of *Curcuma petiolata* Roxb. was determined by high performance liquid chromatography. The yield percentage of total curcumins content per weight of plant was 13.34 %.

In this research, the curcumin content of *Curcuma petiolata* Roxb. rhizomes extract was determined by using HPLC. The powdered sample of *Curcuma petiolata* Roxb. rhizomes contained 4.3% of curcumin.

The different extracts of *Curcuma petiolata* Roxb. rhizome were tested on six pathogenic microorganisms by using paper disc diffusion method. According to this experiment, all extracts showed antimicrobial activity on *Aspergillus flavus* and *Echerichia coli*. Among them, chloroform extracts showed most significant antimicrobial activity against *Aspergillus flavus* (16mm) and acetone and aqueous extract on *Echerichia coli* (14mm). Chloroform, ethyl acetate, acetone and aqueous extracts showed antimicrobial activity against *Bacillus subtilis.* Among them, aqueous extract showed the most significant antimicrobial activity against *Bacillus subtilis* (14mm). Only methanol extracts showed antimicrobial activity against *Candida albicans* (14mm). Chloroform, ethanol and aqueous extracts showed antimicrobial activity on *Pseudomonas fluorescens.* Among them, aqueous extract showed the most significant antimicrobial activity against *Pseudomonas fluorescens* (16mm). Chloroform and ethyl acetate extracts showed antimicrobial activity on *Xanthomonas oryzae.* Among them, ethyl acetate extracts showed antimicrobial activity on *Xanthomonas oryzae* (18mm).

Conclusion

The plant *Curcuma petiolata* Roxb. belongs to family Zingiberaceae. *Curcuma petiolata* Roxb. rhizomes contains alkaloid, α-amino acid, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, steroid, tannin and terpenoid.

The yield percentage of total curcumin content *Curcuma petiolata* Roxb. rhizomes was 4.3 %. Curcumin has a wide range of biological functions, especially the anticancer activity including bladder cancer, glioblastoma, and esophageal cancer, pancreatic cancer prostate cancer. Therefore, *Curcuma petiolata* Roxb. rhizomes possess anticancer activity.

The extracts of *Curcuma petiolata* Roxb. rhizomes indicated antimicrobial activity against *Aspergillus flavus, Bacillus subtilis*, *Candida albicans, Echerichia coli, Pseudomonas fluorescens* and *Xanthomonas oryzae*. Among them, chloroform extracts showed most significant antimicrobial activity against *Aspergillus flavus* and acetone and aqueous extracts on *Echerichia coli*. Aqueous extract showed the most significant antimicrobial activity against *Bacillus subtilis* and *Pseudomonas fluorescens*. Only methanol extracts showed antimicrobial activity against *Candida albicans.* Ethyl acetate extracts showed the most powerful antimicrobial activity against *Xanthomonas oryzae*.

Therefore, extracts of *Curcuma petiolata* Roxb. rhizomes is effective in protecting against bronchitis caused by *Aspergillus flavus*, alimentary tract infection, cardiac infection, sores and inflammation by *Candida albicans*, diarrhoea, dysentery by *Echerichia coli*. Fever, nausea and vomiting and rapid heart rate in human and leaf blight caused by *Pseudomonas fluorescens*. Extracts of *Curcuma petiolata* Roxb. rhizomes can prevent rice blight caused by *Xanthomonas oryzae.* So, *Curcuma petiolata* Roxb. rhizomes is effective on protection of diseases which caused by microorganisms.

Acknowledgement

I would like to express my special thanks to Dr Mya Mya Aye, Rector, Dr Than Than Myint, Pro-Rector, Hpa-an University for their allowing me to do this research paper. I also wish to extend my gratitude to Dr Khin San Nu, Professor and Head, Department of Botany, Hpa-an University for their advice and encouragement in preparing this paper.

References

Baghel, S. S., R. S. Baghel., K. Sharma and I. Sikarwar. (2013). **Pharmacological activities of Curcuma caesia**. International Journal of Green Pharmacy, January-March 2013.

Chen, N., S. F. Zhang., X. L. Wang and X. Q. Yang. (2014). **Autophagy-associated Targeting Pathways of Natural Products during Cancer Treatment**. Asian Pacific Journal of Cancer Prevention, Vol 15.

Cruickshank, R. J. P. (1975). **Medicinal Microbiology**. Living Stone Ltd., London.

Gao, S. M., J. J. Yang., C. Q. Chen., J. J. Chen., L. Ye., L. Wang., J. Wu., C. Xing and K. Yu. (2012). **Pure curcumin decreases the expression of WT1 by up regulation of miR- 15a and miR-16-1 in leukemic cells**. *J Exp Clin Cancer Res*, **31**, 27.

Heywood, V. H., R. K. Brumitt., A. Culhan and O. Seberg. (2007). **Flowering Plants of The World.** London: Oxford University Press.

Hundley, H.G and Chit Ko Ko. (1961). **List of Trees, Shrubs, Herbs and Principle Climbers of Burma**. (3rd ed.), Government printing press, Burma. Rangoon.

Khin Tar Yar Myint, Tin Sein Mar, Mu Mu Sein Myint, Mar Mar Myint, Phyu Phyu Win, Myint Myint Khaing, Mi Aye Aye Mon, Khine Khine Lwin and Khin Phyu Phyu. (2018). **Comperative Study of Active Curcumin content from Four Curcuma Species in Myanmar**. Myanmar Health Science Research Journal, Vol.30 No. 2.1**5**, 223-9.

Kress, W. J., R. A. D. E. Farr and Yin Yin Kyi. (2003). **A Checklist of the Trees, Shrubs, Herbs and Climbers of Myanmar.** Department of Systematic Biology, Botany, National Museum of Natural History, Washington, DC.

Marini Bettolo, G. B., M. Nicolet tic, and M. Patmia. (1981). **Plant Screening by Chemical Chromatographic Procedure Under Field Conditions.** Journal of Chromatogram.

Perry, L.M. (1980). **Attributed properties and Uses. Medicinal Plants of East and South East Asia**. MIT Press, Cambridge, Massachusetts, USA : 240-241.

Plengsuriyakarn, T., V. Viyanant., V. Eursitthichai., P. Picha., P. Kupradinun and A. Itharat. (2012). **Anticancer activities against cholangiocarcinoma, toxicity and pharmacological activities of Thai medicinal plants in animal models**. *BMC Complement Altern Med*, 12, 23.

Sasikala, M. and R. Sundaraganapathy. (2017). **Qualitative analysis of Alkaloids exist in the hydroalcoholic extract of Ipomoea aquatica for SSk**.in Tamil Nadu.International Journal of Chem Tech ResearchVol.10,No.7 PP 446-454.

Sirirugsa, P., K. Larsen and C. Maknoi. (2007). **Distribution and Species Diversity of Curcuma in Thailand**. Garden's Bulletin Singapore 59 (1&2):203-220.

Te-lin, W and K. Larsen.(2000). **Flora of China**. Vol 24:322-377.

Thakam, A., N. Saewanm., K. Kittigowittana and A. Jimtaisong. (2012). **Antioxidant and antityrosinase activities of metal complexes of *Curcuma petiolata* extract for cosmetic applications**. 1st Mae Fah Luang University International Conference.

Trease, G. E. and W. C. Evans. (2009). **A Text Book of Pharmacognosy**. 16th ed. Billiere Tindall. London.

Zhou, D.Y., N. Ding., Z.Y. Du., X. X. Cui., H. Wang., X. C. Wei., A. H. Conney., K. Zhang., X. Zheng. (2014). **Curcumin analogues with high activity for inhibiting human prostate cancer cell growth and androgen receptor activation**. *Mol Med Rep*, 10, 1315-22.

Central Council for Research in Unani Medicine. (1987). **Phytochemical Standards of Unani Formulation.** Ministry of Health, New Delhi.

STUDY ON GROWTH AND YIELD OF *PSOPHOCARPUS TETRAGONOLOBUS* (L.) DC. ON DIFFERENT TREATMENTS OF CHICKEN COMPOST

Zin Moe Moe[1], May May Aung[2], Soe Min Min Aye[3]

Abstract

The experiment was conducted in Pyay University Campus, Pyay Township, Bago Region. In this experiment, the compost was prepared by the mix of the chicken dung, rice straw and rice bran at 4:2:1. The analyzed results of the compost were moisture percentage of 16.797, the total nitrogen percentage of 0.565, the total phosphorous of 21.43, and the total potassium of 0.792, organic matter of 20.26, organic carbon of 17.54 and the C:N ratio of 6.62. The effectiveness of compost was studied by growing of *Psophocarpus tetragonolobus* (L.) DC., Pe-zaungya, set up in Completely Randomized Design (CRD) contained 4 treatments such as T_1 (Control), T_2 (15g compost plant^{-1}), T_3 (30g compost plant^{-1}), T_4 (45g compost plant^{-1}), each with five replications. The results of vegetative growth showed that the number of leaves per plant, leaf width, leaf length and single leaf area were higher in T_2 (15g compost plant^{-1}). The reproductive growth also showed that the first flowering days was earlier in T_1 (control). The maximum pod width and pod length were observed in T_4 (45g compost plant^{-1}) but number of pods per plant, pods weight per plant, pod yield per treatment and pod yield were higher in T_2 (15g compost plant^{-1}). In this experiment, T_4 (45g compost plant^{-1}) produced the least yield and it was less yield than T_1 (Control). It is therefore concluded that, the lower dose of chicken compost was suitable for the growing of *Psophocarpus tetragonolobus* (L.) DC. as to maintain soil restoration and crop production and also for yield improvement of *Psophocarpus tetragonolobus* (L.) DC.

Keywords: chicken compost, winged bean, CRD

Introduction

The winged bean, *Psophocarpus tetragonolous* (L.) DC. (Pe-zaungya) is a tropical legume plant also known by other names such as asparagus pea, goa bean and manila bean. It belongs to the family Leguminosae. The family has 590 to 690 genera and 12,000 to 1[1]7,000 species. Beans are globally important leguminous vegetables that has been used for several centuries as food for humans and feed for animals. Furthermore beans contain high amounts of protein and vitamins (Mohammad *et al*., 2016). Winged bean seems to prefer sandy loam soils or humus. The plants can tolerate acidic soils up to pH 4.8. In Burma, particularly in the plains, ground is divided in ridges spaced 60 cm broad and 20 to 25 cm high. The Spacing showed that 90 cm spacing both ways, i.e., inter and intra row distance is quite suitable. In Burma, particularly in the plains, ground is divided in ridges spaced 60 cm broad and 20 to 25 cm high. Seeds may be sown 2-5 cm deep and a wide range of population densities have been recommended depending up on seed size (Chandel *et al*., 1984). The entire plant is fit for human consumption from flowers and leaves to tuberous roots and seeds. Green pods have been widely used as a vegetable in South-East-Asia. Tubers are also used in Burma. Winged flour can be used as protein supplement in bread- making. Seeds can also be utilized for making edible oil and milk. The whole plant as well as processed seeds offer excellent animal feed (Sunanda *et al*., 2014).

[1] Dr, Associate Professor, Department of Botany, Pyay University
[2] Assistant Lecturer, Department of Botany, Pyay University
[3] Assistant Lecturer, Department of Botany, Pyay University

Chicken farm wastes (such as chicken manure) were used directly as an organic fertilizer for crops farming. Direct application of chicken dung into agricultural soil may cause environmental problems and it may become the breeding ground of pests such as flies. The biological transformation of chicken dung into environmental friendly and easy to handle organic fertilizer is necessary. Chicken compost contains rich plant nutrients that are vital for their growth including nitrogen, phosphorus, and potassium. In addition to supply of nutrients, the application of chicken farm waste also improves the chemical, physical and biological properties of soil. The transformation of chicken compost into environmental friendly and easy to handle organic fertilizer is necessary (Arifin *et al*., 2006).

The present investigation was undertaken with the aim of this experiment was to observe the yield of *Psophocarpus tetragonolous* (L.) DC. using the chicken compost. The specific objectives were to assess the effect of chicken compost on growth and yield of *Psophocarpus tetragonolous* (L.) DC., to analyze the N,P,K content of chicken compost and to determine the rate of chicken compost on growth and yield of winged bean.

Materials and Methods

Experimental Site

The experiment was conducted at Pyay University campus, Pyay Township, Bago Region during January to March 2018.

Analysis of soil sample and raw materials using chicken compost

Soil sample was collected from the growing area of Pyay University campus before the soil preparation. The collected soil samples, chicken dung, rice straw and rice bran, and also the chicken compost were analyzed in the soil laboratory, Land Use Division, Department of Agriculture, Yangon Region.

Soil Preparation

The soil from the growing area was mixed with ash in the ratio of 5:1 and the soil mix was watered and left for a week for thorough homogenization. Then the soil mixture was put into the polypropylene woven bag (45cm x 38cm).

Raw materials of compost

The composting process was conducted at Lat-pan-aine village, Pyay Township, Bago Region. Chicken dung, rice straw and rice bran were collected near the village. The rice straw was also chopped into 2-3 cm small pieces. Chicken dung, rice straw and rice bran were mixed at 4:2:1 followed by the method of Leif *et al.* (2015).

Composting process

Seventy kilograms of the mixture of the chicken dung, rice straw and rice bran (4:2:1) were put into the Bamboo bin (36"x36"x 36"). Then the bin was covered with the plastic sheet for maintaining the temperature of compost. Temperature was monitored throughout the composting period. Manual turning up of compost was done in every four days throughout the composting period (Figure 1).

Chicken dung | Rice bran | Rice straw | Measuring the temperature | Turning the compost | Chicken compost

Figure 1 Chicken composting process

Planting material

Wing bean seeds which were used by the farmers in this area were used as the planting material for this research.

Germination Test

Germination test was carried out before sowing seeds in the field. Full cheek seeds were selected without shrinkage were selected for germination test. The selected seeds were germinated in the germinating tray containing sand medium. Four plots were divided in the tray. One plot contained three rows and one row had ten seeds. Therefore, the total numbers of one hundred and twenty seeds were used in the germination test. After one week, the numbers of germinating seedlings were recorded. The germination rate was calculated following the formula developed by Soupe (2009).

$$\text{Germination rate (\%)} = \frac{\text{Total No. of Germinated Seedlings}}{\text{Total No. of Cultivated Seeds}} \text{ x } 100$$

Planting of *Psophocarpus tetragonolobus* L. and Experimental Layout

Five seeds of *Psophocarpus tetragonolobus* (L.) DC. were germinated in a polypropylene woven bag. Two weeks after sowing seeds, different rates of chicken compost treatment: T_1 - control (without compost), T_2 (15g compost plant^{-1}), T_3 (30g compost plant^{-1}). and T_4 (45g compost plant^{-1}) were treated to the assigned plantlet. Each treatment had five replicates were laid out in a completely randomized design (CRD). The spacing between bags was 45 cm and between rows was 45 cm. Hence the total experimental area was 285 cm x 360 cm ((Figure 2). Watering was done every day. Spraying of the organic pesticide such as Tamar pesticide, the extract of *Capsicum annum* and *Alium fruitescens* were carried out when the infestation of plants. Weeding was also carried out whenever it was necessary. The plant height was maintained at a height of 150 cm.

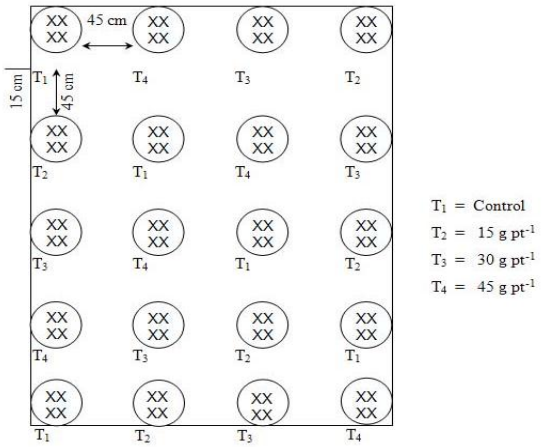


Figure 2 Experimental layout (CRD)

Determination of single Leaf Area

For measuring leaf area, length-width method was used in this experiment. The leaf sample was collected and measured the length and the maximum width and the area was computed as follow:

$$A = K\ L\ W$$

where, A = single leaf area, K =adjustment factor, L = leaf length, W = broadest width

K value varies with the shape of leaf and also it is affected by the variety, nutritional status, and growth stage of the leaf. K value for pulse is 0.70 (Myo Kywe, 1995).

Data Collection

Germination rate, vegetative growth such as petiole length, number of leaves per plant, leaf width, leaf length and single leaf area, reproductive growth like first flowering days, pod length, pod width, pods per plant, pods weight per plant, pods yield per treatment and pod yield were recorded. The collected data were analyzed using IRRISTAT software (6.0). Least significant differences (LSD) at 5% level of significant was used to compare mean differences.

Results

Analysis of soil, chicken dung, rice straw and rice bran and chicken compost

Physico-chemical analysis of the soil revealed that soil was neutral with pH of 7.22. The total nitrogen was 0.46%. It had an exchangeable cation K^+ content of 1.79 meq 100 g^{-1}, an available nutrients P, 66.94 ppm (Olsen), K_2O, 84.19 mg 100 g^{-1}, moisture content of 3.83%, organic carbon, 5.64% and humus content of 10.11% (Table 1).

Table 1 Analyzed results of the experimental soil

Parameters	**Composition**
Total N (%)	0.46
Exchangeable cation, K^+ (meq 100 g^{-1})	1.79
Available nutrients, P, ppm (Olsen)	66.94
Available nutrients, K_2O (mg 100 g^{-1})	84.19
pH	7.22
Moisture (%)	3.83
Organic Carbon (%)	5.64
Humus (%)	10.11

The analyzed characters of chicken dung (before composting) were the moisture percentage of 6.751, the total nitrogen percentage of 0.919, the total phosphorous of 2.716, and the total potassium of 0.845, organic matter of 10.195 and the C/N ratio of 6.434 (Table 2).

The physico-chemical analysis of the rice straw stated that the moisture percentage of 14.14, the total carbon percentage of 48.57, the nitrogen percentage of 0.70, phosphorous content of 0.253, the potassium percentage of 2.203 and the C/N ratio of 69.38 (Table 2).

The physico-chemical analysis of the rice bran showed that the moisture percentage of 14.98, the total carbon percentage of 52.21, the nitrogen percentage of 1.96, phosphorous content of 1.47, the potassium percentage of 2.08 and the C/N ratio of 26.64 (Table 2).

The analyzed characters of chicken compost revealed that the moisture percentage of 16.797, the total nitrogen percentage of 0.565, phosphorous percentage of 21.43, the potassium percentage of 0.792, organic matter of 20.26, Organic carbon of 17.54, the total carbon percentage of 17.54, the moisture percentage of 16.797 and the C/N ratio of 15.63:1 (Table 2).

Table 2 Nutrient contents of chicken dung, rice straw, rice bran and chicken compost

Parameters	Nutrient Contents			
	Chicken dung	**Rice bran**	**Rice straw**	**Chicken compost**
Total N %	0.919	1.96	0.70	0.565
Total P_2O_5 %	2.716	1.47	0.253	21.43
Total K_2O %	0.845	2.08	2.203	0.792
Total C %	-	52.21	48.57	
Organic matter (%)	10.195	-	-	20.26
Organic carbon (%)	-	-	-	17.54
Moisture (%)	6.751	14.98	14.14	16.797
C:N	6.434	26.64	69.38	15.63:1
pH (1:2.5)	-	-	-	6.62

Measurement of temperature, rainfall and humidity

Weather data was recorded daily from Department of Meteorology and Hydrology, Pyay Township, Bago Region (Table 3).

Table 3 Temperature, rainfall and humidity data of the experiment area from January to March 2018

Date	Mean temp (°C)	Mean Rain fall (mm)	Mean humidity (%)
January, 2018	23.40	0.01	71.80
February, 2018	26.55	-	57.50
March, 2018	29.85	-	54.00

Germination test

Among 30 seeds in each plot, plot 1 had 30 germinated plants (100% of germination), plot 2 had numbers of germinated plants 27 (% of germination), plot 3, 28 germinated plants (% of germination) and plot 4, 25 germinated plants (% of germination), respectively. Therefore average germination rate is 91.67 % (Table 4).

Table 4 Germination rate of *Psophocarpus tetragonolobus* (L.) DC.

Plot	No. of sown seeds	Germinated plants	Germination %
1	30	30	100.00
2	30	27	90.00
3	30	28	93.33
4	30	25	83.33
Average			91.67

Petiole length

The results of petiole length response to chicken compost treatments revealed that T_4 (45g compost plant^{-1}) had the longest length 21.97 cm followed by T_1 (control) 19.64 cm, then T_2 (15g compost plant^{-1}) 19.47 cm and T_3 (30g compost plant^{-1}) had 19.07 cm respectively. The growth in petiole length has increased weekly (Table 5 and Figure 3).

Table 5 Petiole length of *Psophocarpus tetragonolobus* (L.) DC. treated by chicken compost

Treatments	Petiole length (cm)				
	3 WAS	4 WAS	5 WAS	6 WAS	Mean
T_1 (Control)	4.58	4.78	4.89	5.39	19.64
T_2 (15g compost plant^{-1})	4.46	4.67	4.89	5.45	19.47
T_3 (30g compost plant^{-1})	3.92	4.48	5.03	5.64	19.07
T_4 (45g compost plant^{-1})	4.46	5.08	5.71	6.72	21.97
F-Test	0.95	0.66	0.84	1.36	-
5% LSD	ns	ns	ns	ns	-
CV %	15.80	10.10	11.90	17.00	-

WAS =Weeks after sowing CV% = coefficient variation (%) LSD = least significant difference

Number of leaves per plant

The results of number of leaves per plant response to chicken compost treatments showed that T_2 (15g compost plant^{-1}) had much leaves 13.15. The second highest leaf number was observed 11.78 in T_3 (30g compost plant^{-1}), the third highest leaf number T_1 (Control) and the least number was 11.11 T_4 (45g compost plant^{-1}). The growth in number of leaves has increased weekly (Table 6).

Table 6 Numbers of leaves per plant of *Psophocarpus tetragonolobus* (L.) DC. treated by chicken compost

Treatments	Numbers of leaves per plant				
	3 WAS	**4 WAS**	**5 WAS**	**6 WAS**	**Mean**
T_1 (Control)	4.10	6.54	9.60	26.30	11.64
T_2 (15g compost plant^{-1})	5.10	8.10	12.30	27.10	13.15
T_3 (30g compost plant^{-1})	4.20	6.60	11.60	24.70	11.78
T_4 (45g compost plant^{-1})	4.35	5.50	10.80	23.80	11.11
F-Test	ns	ns	*	*	-
5% LSD	1.17	2.82	3.76	10.03	-
CV %	`9.10	30.60	24.60	28.60	-

Leaf width

The mean value of leaf width among the chicken compost treatments gave that T_2 (15g compost plant^{-1}) was highest leaf width 6.62 cm. It was followed by T_4 (45g compost plant^{-1}) 6.26 cm, T_1 (control) 6.09 cm and T_3 (30g compost plant^{-1}) had least leaf width of 5.50 cm respectively. The growth in leaf width has increased weekly (Table 7 and Figure 3).

Table 7 Leaf width of *Psophocarpus tetragonolobus* (L.) DC. treated by chicken compost

Treatments	Leaf width (cm)				
	3 WAS	4 WAS	5 WAS	6 WAS	Mean
T_1 (Control)	5.71	5.92	6.09	6.63	6.09
T_2 (15g compost plant^{-1})	6.26	6.35	6.82	7.06	6.62
T_3 (30g compost plant^{-1})	5.21	5.37	5.50	5.92	5.50
T_4 (45g compost plant^{-1})	5.56	6.18	6.49	6.80	6.26
F-Test	ns	*	*	ns	-
5% LSD	0.90	0.84	0.91	0.68	-
CV %	11.5	10.1	10.6	7.4	-

Leaf length

The result of the mean leaf length among the chicken compost treatments showed that T_2 (15g compost plant^{-1}) had highest leaf length 5.13 cm. It was followed by T_4 (45g compost plant^{-1}) 4.83 cm, T_1 (Control) 4.75 cm and T_3 (30g compost plant^{-1}) had least leaf length of 4.58 cm respectively. The growth in leaf length has increased weekly (Table 8 and Figure 3).

Table 8 Leaf length of *Psophocarpus tetragonolobus* (L.) DC. treated by chicken compost

Treatments	Leaf length (cm)				
	3 WAS	4 WAS	5 WAS	6 WAS	Mean
T_1 (Control)	4.47	4.66	4.86	4.99	4.75
T_2 (15g compost plant^{-1})	4.84	5.00	5.21	5.46	5.13
T_3 (30g compost plant^{-1})	4.22	4.56	4.65	4.87	4.58
T_4 (45g compost plant^{-1})	4.41	4.64	4.93	5.33	4.83
F-Test	ns	ns	*	*	-
5% LSD	0.88	0.70	0.64	0.63	-
CV %	14.3	10.8	9.5	8.9	-

Single leaf area

The biggest leaf area were 24.06 cm^2 in T_2 (15g compost plant^{-1}), followed by T_4 (45g compost plant^{-1}) had 21.30 cm^2 and then T_1 (Control) had 20.34 cm^2, T_3 (30g compost plant^{-1}) had 17.87 cm^2 single leaf area. The growth in leaf area has increased weekly (Table 9).

Table 9 Single leaf area of *Psophocarpus tetragonolobus* (L.) DC. treated by chicken compost

Treatments	Leaf area (cm^2)				
	3 WAS	4 WAS	5 WAS	6 WAS	Mean
T_1 (Control)	17.92	19.40	20.81	23.23	20.34
T_2 (15g compost $plant^{-1}$)	21.24	22.89	24.97	27.12	24.06
T_3 (30g compost $plant^{-1}$)	15.68	17.32	18.10	20.39	17.87
T_4 (45g compost $plant^{-1}$)	17.19	20.16	22.47	25.39	21.30
F-Test	ns	ns	ns	ns	-
5% LSD	5.26	5.08	5.32	4.35	-
CV %	21.20	18.50	17.90	13.10	-

The summarized results of vegetative growth stated the effect of chicken compost on *Psophocarpus tetragonolobus* (L.) DC. that the highest petiole length was 21.97 cm in T_4 (45 g compost $plant^{-1}$), the maximum numbers of leaves per plant 13.15 cm, the greatest leaf length 5.13 cm, the largest leaf width 6.62 cm and the broadest single leaf area 24.06 cm^2 in T_2 (15g compost $plant^{-1}$), respectively (Table 10).

Table 10 Summarized vegetative growth on the *Psophocarpus tetragonolobus* (L.) DC. by the treatments of chicken compost

Treatments	Petiole length (cm)	Number of leaves per plant	Leaf width (cm)	Leaf length (cm)	Single leaf area (cm^2)
T_1 (Control)	19.64	11.64	6.09	4.75	20.34
T_2 (15g compost $plant^{-1}$)	19.47	13.15	6.62	5.13	24.06
T_3 (30g compost $plant^{-1}$)	19.07	11.78	5.50	4.58	17.87
T_4 (45g compost $plant^{-1}$)	21.97	11.11	6.26	4.83	21.30

Petiole length Leaf width Leaf length

Figure 3 Vegetative Growth of *Psophocarpus tetragonolobus* (L.) DC.

Reproductive Growth

First flowering days

The mean number of the earliest first flowering days is 58 days in T_1 (Control) followed by 59 days T_3 (30g compost $plant^{-1}$) and T_4 (45g compost $plant^{-1}$), 60 days in T_2 (15g compost $plant^{-1}$) respectively (Table 11 and Figure 4).

Table 11 First flowering days of *Psophocarpus tetragonolobus* (L.) DC.treated by chicken compost

Treatments	First Flowering Days
T_1 (Control)	58
T_2 (15g compost plant^{-1})	60
T_3 (30g compost plant^{-1})	59
T_4 (45g compost plant^{-1})	59
F- Test	ns
5% LSD	1.07
CV %	1.3

Pods per plant

The pods per plant of winged bean had the highest 8.60 T_2 (15g compost plant^{-1}) followed by 7.40 T_3 (30g compost plant^{-1}), 4.40 T_1 (control) and 3.60 T_4 (45g compost plant^{-1}) respectively. According to the statistical analysis showed that all data were significant (Table 12).

Table 12 Effect of chicken compost on pods per plant of *Psophocarpus tetragonolobus* (L.) DC.

Treatments	pods per plant
T_1 (Control)	4.40
T_2 (15g compost plant^{-1})	8.60
T_3 (30g compost plant^{-1})	7.40
T_4 (45g compost plant^{-1})	3.60
F. test	*
CV %	44.70
5%LSD	3.70

Pod Length

The pod length of winged bean had the highest pod length 12.42 cm T_4 (45g compost plant^{-1}) followed by 11.99 cm T_1 (Control), 10.73 cm T_2 (15g compost plant^{-1}) and 9.90 cm T_3 (30g compost plant^{-1}) respectively. According to the statistical analysis showed that all data were non-significant (Table 13 and Figure 4).

Table 13 Effect of chicken compost on single pod length per plant of *Psophocarpus tetragonolobus* (L.) DC.

Treatments	Pod Length (cm)
T_1 (Control)	11.99
T_2 (15g compost plant^{-1})	10.73
T_3 (30g compost plant^{-1})	9.90
T_4 (45g compost plant^{-1})	12.42
F. test	ns
CV %	19.00
5%LSD	2.94

Pod Width

The pod width of winged bean had the largest 5.95 cm T_4 (45g compost plant^{-1}) followed by 5.82 cm T_1 (Control), 5.17 cm T_3 (30g compost plant^{-1}) and 4.90 cm T_2 (15g compost plant^{-1}) respectively. According to the statistical analysis showed that all data were non-significant (Table 14 and Figure 4).

Table 14 Effect of chicken compost on single pod width of *Psophocarpus tetragonolobus* (L.) DC.

Treatments	pod width (cm)
T_1 (Control)	5.82
T_2 (15g compost plant^{-1})	4.90
T_3 (30g compost plant^{-1})	5.17
T_4 (45g compost plant^{-1})	5.95
F. test	ns
CV %	12.30

Pods Weight per plant

The pods weight per plant of winged bean had the highest 73.68 g T_2 (15g compost plant^{-1}) followed by 58.16 g T_3 (30g compost plant^{-1}), 37.46 g T_1 (Control) and 27.42 g T_4 (45g compost plant^{-1}) respectively. According to the statistical analysis showed that all data were non-significant (Table 15 and Figure 4).

Table 15 Effect of chicken compost on pods weight per plant of *Psophocarpus tragonolobus* (L.) DC.

Treatments	pods weight per plant (g)
T_1 (Control)	37.46
T_2 (15g compost plant^{-1})	73.68
T_3 (30g compost plant^{-1})	58.16
T_4 (45g compost plant^{-1})	27.42
F. test	ns
CV %	44.70
5%LSD	30.29

Pod yield per treatment (g)

T_2 (15g compost plant^{-1}) had highest pod yield per treatment 5894.40 g. T_3 (30 g plant^{-1}) had second highest yield 4652.80 g. T_1 (Control) produced 2996.80 g and it had the third yield followed by T_4 (45g compost plant^{-1}) had 2193.60 g (Table 16).

Table 16 Mean pod yield (g) per treatment of *Psophocarpus tetragonolobus* (L.) C. resulted from different rates of chicken compost

Treatments	Pod yield per treatment (g)
T_1 (Control)	2996.80
T_2 (15g compost plant^{-1})	5894.40
T_3 (30g compost plant^{-1})	4652.80
T_4 (45g compost plant^{-1})	2193.60

Pod yield

T_2 (15g compost plant^{-1}) had highest pod yield 3752.55 kg ha^{-1}. T_3 (30g compost plant^{-1}) had second highest yield 2961.28 kg ha^{-1}. T_1 (control) produced 1907.98 kg ha^{-1} and it had the third yield followed by T_4 (45g compost plant^{-1}) had 1170.31 kg ha^{-1} (Table 17).

Table 17 Mean pod yield (kg) of *Psophocarpus tetragonolobus* (L.) DC. resulted from different rates of chicken compost

Treatment	Pod yield (kg ha^{-1})
T_1 (Control)	1907.98
T_2 (15g compost plant^{-1})	3752.55
T_3 (30g compost plant^{-1})	2961.28
T_4 (45g compost plant^{-1})	1170.31

The summarized results of reproductive growth stated the effect of chicken compost on *Psophocarpus tetragonolobus* (L.) DC. The earliest first flowering days was in T_1 (Control). The greatest single pod width, 5.95 cm and pod length, 12.42 cm were investigated in T_4 (45g compost plant^{-1}). The maximum pods per plant 8.60, the broadest pods weight per plant 73.68 g, the best yield per treatment 5894.40 g and pod yield 3752.55 kg ha^{-1} respectively were observed in T_2 (15g compost plant^{-1}) (Table 18).

Table 18 Summarized reproductive growth on the *Psophocarpus tetragonolobus* (L.) DC. by the treatments of chicken compost

Treatments	First Flowering Days	pod width (cm)	pod length (cm)	pods per plant	pods weight per plant (g)	Pod yield per treatment (g)	Pod yield (kg ha^{-1})
T_1 (Control)	58	5.82	11.99	4.40	37.46	2996.80	1907.98
T_2 (15g compost plant^{-1})	60	4.90	10.73	8.60	73.68	5894.40	3752.55
T_3 (30g compost plant^{-1})	59	5.17	9.90	7.40	58.16	4652.80	2961.28
T_4 (45g compost plant^{-1})	59	5.95	12.42	3.60	27.42	2193.60	1170.31

Pod length Pod width Weighting the pods

Figure 4 Reproductive growth of *Psophocarpus tetragonolobus* (L.) DC.

Discussion and Conclusion

This research was conducted during January- March 2018. The growing of *Psophocarpus tetragonolobus* (L.) DC. using chicken compost on plant vegetative growth and reproductive growth were studied. In this experiment, the mixture of chicken dung, rice bran and rice straw in aerobic condition for 30 days was used as compost. This chicken compost was used in the growing of *Psophocarpus tetragonolobus* (L.) DC. The chemical properties of soil analysis stated that the total nitrogen of soil was 0.46%; exchangeable cation K^+, 1.79 meq $100g^{-1}$; available nutrients P, 66.94 ppm; available nutrients K_2O, 84.19 mg $100g^{-1}$; pH, 7.22; moisture, 3.83%; organic carbon, 5.64% and humus, 10.11% (Table 1). This compost was treated to the plants in 15 days intervals from three weeks after sowing to harvesting. The nutrient content of the chicken compost was 0.565, 21.43 and 0.792 percent N, P, K. The C:N ratio of chicken compost was 15.63:1 that should grow in the cultivation. Nutrient status of chicken compost used in the experiment. Manure pH of chicken compost was slightly acidic, 6.62 (Table 2). Arifin *et al*. (2006) revealed the biological transformation of chicken manure via composting technique has its disadvantage because the process may take several weeks to complete. Chicken compost contains rich plant nutrients that are vital for their growth including nitrogen, phosphorus, and potassium. Composted chicken manure provides a slow-release source of macro- and micronutrients and acts as a soil amendment. The germination test before experiment was observed that germination rate was 91.67% (Table 4). Chauhan *et al*. (2009) stated that the seed germination test was to measure the number of healthy well-development seedling. The monthly temperature, rainfall and relative humidity (RH) of the cultivation area were recorded from January to March 2018, the experimental period. The mean temperature 23.40-29.85℃, the rainfall of 0.01 mm and the relative humidity of 54.00- 71.80 % were recorded in this experiment (Table 3). Duke (1981) who reported *Psophocarpus tetragonolobus* (L.) DC. (winged bean) is grown from equator to 25˚ latitude in temperatures ranging from 15.4 - 27.5℃. The vegetative growth data in this experiment stated that the widest leaf width, 6.62 cm, the longest leaf length, 5.13 cm and maximum single leaf area, 24.06 cm^2 in T_2 (15g compost $plant^{-1}$) were recorded from this experiment (Table 10). The yield components data in this research showed that the earliest first flowering days, 58 DAS in T_1 (control), pod width, 5.95 cm and pod length, 12.42 cm were investigated in T_4 (45 g compost $plant^{-1}$) (Table 18). The earliest first flowering days in this experiment was observed in T_1 (control, without fertilizer treatment). While, the composts and organic fertilizer treatments showed the late flowering days. Tesfaw *et al*. (2013) who found the nutrient supply is also responsible for earliness or late start of blooming. In their result, the plots that received higher levels of fertilizers exhibited prolonged time to commence blooming. The maximum pods per plant 2.87, the broadest pods weight per plant 24.56 g, pods

yield per treatment 5894.40 g and the best yield 3752.55 kg ha^{-1} respectively were observed in T_2 (15g compost $plant^{-1}$) (Table 18). Thorne and Evans (1964) reported that the greatest potential for yield was achieved by the high density of leaves area per unit land area but photosynthetic rate of individually leaf will tend to be reduced due to mutual shading. Tesfaw *et al*. (2013) who found the nutrient supply is also responsible for earliness or late start of blooming. In their result, the plots that received higher levels of fertilizers exhibited prolonged time to commence blooming. The maximum pods per plant 2.87, the broadest pods weight per plant 24.56 g, pods yield per treatment 5894.40 g and the best yield 3752.55 kg ha^{-1} respectively were observed in T_2 (15g compost $plant^{-1}$). (Table18). Thorne and Evans (1964) reported that the greatest potential for yield was achieved by the high density of leaves area per unit land area but photosynthetic rate of individually leaf will tend to be reduced due to mutual shading. The chemical composition of chicken compost used in this study is presented in Table (2). This chicken compost was high in phosphorus. The properties of the chicken compost were in accordance with the reference value of chicken compost. These were phosphorous value and the slightly acidic of pH (6.62). The experimental data depended on the levels of chicken compost applied. Plants which had been fertilized by lower levels of chicken compost T_2 (15g compost $plant^{-1}$) produced highest yield than the higher levels application. The lowest level of chicken compost applied gave the highest yield. It was found that the amount of available phosphorous in the compost was high although the lowest level of chicken compost, T_2 (15g compost $plant^{-1}$) was used. It can be concluded that the lower dose of chicken compost (15 g $plant^{-1}$) would be suitable organic soil amendment for soil restoration and crop production and also for yield improvement of winged bean *Psophocarpus tetragonolobus* (L.) DC. according to the resulted data from this experiment. The utilization of chicken compost in lower rates resulted in benefits to farmer and it also enhanced soil fertility. It can be concluded that the lower dose of chicken compost was suitable for the growing of *Psophocarpus tetragonolobus* (L.) DC. as to maintain soil restoration and crop production and also for yield improvement of *Psophocarpus tetragonolobus* (L.) DC.

References

Arifin, B. A. Bono and J. Janaun. (2006). **The Transformation of Chicken Manure into Mineralized Organic Fertilizer**. Journal of Sustainability Science and Management Volume 1(1) : 58-63. Malaysia.

Chandel, K.S., K.C. Pant and R.K. Arora. (1984). **Winged Bean in India**. Nbpgr Sci. Mollogr:8. National Bureau of Plant Genetic Resources, New Dehli, India.

Chauhan, J. S.; Y. K. Tomar; A. Badoni; N. I. Singh; S. Ali; Debarati. (2009)**. Morphology, Germination and early Seedling Growth in *Phaseolus mungo* L. with Reference to the influence of Various Plant Growth Substances.** Journal of American Science. 5 (7); 34-41.

Duke, J. A. 1981. Handbook of legumes of world economic importance. Plenum press, New York, London, pp.345.

Leif, M. R. G., A. Ramonita and L. Maita. **(**2015). **Response of Pechay (Brassica napus L.) to Different Levels of Compost Fertilizer.** International Journal of Scientific and Research Publications, Volume 5, Issue 2, ISSN 2250-3153.

Mohammad, A. I., A. N. Boyce, M. M. Rahman, M. S. Azirun1 and M.A. Ashraf. (2016). **Effects of organic fertilizers on the growth and yield of bush bean, winged bean and yard long bean.** An International Journal, Volume 59, ISSN 1678-4324.

Myo Kywe. (1995). **Yield Components of Crops (General Principles)**. Agronomy Department, Institute of Agriculture, Yezin, Pyinmana.

Soupe, S. G. (2009). **Germination Rates and Percentages. Plant Physiol-Biology.** 327, 320, 363, 2782.

Sunanda R., A. Sinhababu and J. Tah. (2014). **Morphological and micromorphological studies of seeds of ten accessions of *Psophocarpus tetragonolobus* (L.) DC.** Environmental and Experimental Biology 12: 101–106. India.

Tesfaw A. Dechassa N. and W/T Sadik K., (2013). **Performance of hot pepper (*Capsicum annuum* L.) cultivars as influenced by nitrogen and phosphorus fertilizers at Bure, Upper Watershed of the Blue Nile in Northwestern Ethiopia.** Intern. Jour. of Agri. Sc.s 3 (8): 599-608.

Thorne, G.N. and A.S. Evans. (1964). **Influence of tops and roots on NAR of sugar beet and spinach beet and graft between them.** Ann. Bot., 28: 499-508.

HISTOLOGICAL CHARACTERS AND ITS ANTIMICROBIAL ACTIVITIES FROM LEAVES OF PLUMERIA *RUBRA* L.

Aye Aye Thant[1]

Abstract

The plant *Plumeria rubra* L.(Tayok-saga-ani) belongs to the Family Apocynaceae. They were collected from North Dagon Township in Yangon Region during the flowering periods. Then the collected plants were studied of classified, identified and histological characters were examined by the literature.In histological study of leaves, paracytic type of stomata were present on both surface.Multicellular trichomes, lacticiferous canals and calcium oxalate crystals were also present. Various solvent extracts of leaves were tested against the various microorganisms for antimicrobial activity by using agar-well diffusion method. It was found that acetone extracts of leaves showed more significant antimicrobial activity than different solvent extracts. This paper had recorded for standardization of drugs.

Keywords : *Plumeria rubra* L., antimicrobial activity, histological studies, leaves

Introduction

Plumeriarubra L. belongs to the family Apocynaceae, which is known as temple tree or pagoda tree in English and the plant is known as Tayok-saga-ani in Myanmar. These plants cultivated as an ornamental plant throughout in India and Myanmar. These plants are widely cultivated in the tropical and subtropical regions throughout the world. This family Apocynaceae included about 900 species (Hooker, 1882), 130 genera and 1000 species (Bailey, 1939) and 300 genera and 1300 species (Lawrence, 1969), 300 genera and 1400 species (Dassanayake, 1983).

In deciduous types, the leaves fall during winter-time and new leaves emerge during the spring flowering period. The flowers are tubular, expanding into a 'pinwheel' of five petals. Flowers are bloom from March to October. Maturation of the seed pods are usually in early spring from a previous season's pollination (Richard, 1998).

The Indian population has depended mainly upon plant based crude drugs for a variety of ailments *Plumeria* species are widely used as purgative, remedy for diarrhoea, cure of itch, bronchitis, cough, asthma, fever, bleeding piles, dysentery and tumors etc.

The medicinal plants have been used in traditional medicine for hundreds of years with reputation as efficacious remedies. These plants are rich sources of bioactive compounds and thus serve as therapeutic agents and raw materials for the manufacture of traditional and modern medicine.

This plant is famous for their attractiveness and fragrant flowers. The whole plants are used in cholera and indigestion. The flowers are used for perfumery and pectoral syrups. The latex are useful in rubefacient, drastic, purgative, cathartic, corrosive, abortifacient, itch, rheumatism and soothe irritation. The leaves are used to treat ulcers, leprorsy, inflammation, rubefacient, rheumatism, bronchitis, cholera, cough, antipyretic, antifungal, stimulant, asthma, fever, bleeding piles, dysentery, blood disorders and tumors. The fruits are reported to be used as abortifacient. The barks are used in asthma, ease constipation, menstruation and reduce fever, bitter, pungent heating, carminative, laxative, leprorsy, ulcers, gonorrhoea, purgative, emmenagogue, febrifuge, malaria, antiseptic, antiseptic, venereal sores, diarrhoea. The roots are

[1] Dr, Lecturer, Botany Department ,University of Yangon

used in carminative, thermogenic, laxative, leprorsy, astringent, ulcers and gastropath (Kirtikar, K.R and Basu, 1984, Khare, C.P, 2007).

In Myanmar, the leaves are used in abdominal tumors, inflammation, rheumatism and the latex are used in purgative, gastropath as traditional medicine. The flowers are used in carminative, diuretic, malaria, pruritis. It is also eaten as salad with other ingredients. The decoction of barks are used in leprosy, pruritis, heal boils, carbuncles, analgesic, febrifuge for prolong fevers, inflammation and ascites.(U San Hla, 1960; U Mya Win, 1966; Burmese Medicinal Plants, 1998 and KyawSoe& Tin MyoNgwe, 2002).

The antimicrobial activity was tested to know whether it possesses any medicinal values. Antimicrobial drugs must have a selective action against microorganisms. Today, antimicrobial drugs used as antibiotics specifics antimicrobial therapy may be instituted if the species of infecting microorganism is one whose drug-resistant variants are known not to assume clinical importance and antimicrobial drugs in general use is of advantage

Aims and Objectives

- To classify and identify the sample plant *Plumeria rubra* L.
- To study the histological characters from leaves of this plant
- To examine the diagnostic characters of powdered leaves for the standardization
- To investigate the antimicrobial activity

Material and Methods

The plant specimen of *Plumeria rubra* L. were collected from North Dagon Township, Yangon region during the flowering periods of March to October. The collected fresh specimen of both vegetative and reproductive parts of the plant were identified with the available literature of Hooker (1882), Bailey (1939), Backer (1963), Lawrence (1969), Hundley and Chit KoKo (1987) and Flora of Hong Kong (2009). Taxonomic description was recorded with the photograph of habit, leaves, inflorescences, flowers, L.S of flower and T.S of ovary and parts of the plant with measurement.

The histotological characters of leaves were also identified by the cut section according to the literature of Metcalf and Chalk (1950). The cut sections were used to clean with chlorohydrate solution and permanent slides were taken on the Olympus microscope. The specimens of leaves were washed with water and dried at room temperature for 2-3 weeks. The leaves were crushed by grinding with a blender to get the powder. Then, the powder sample was stored in the air tight container. Then the powder samples were examined for the diagnostic characters and used in standardization of the plant material for medicinal purpose

The test organisms in this study were obtained from the Developments centre for Pharmaceutical Technology for determination of antimicrobial activity. The study of antimicrobial activity was performed by agar well diffusion method described in Cruickshank.(1968)

Results

Trees with milky latex, perennials, 8m in high. Stem erect, branched. Bark rough, grey brown. Leaves simple, whorled, leaf blade oblong to lanceolate, laticiferous, 14-25 cm in length

and 5-9 cm in breadth, petiole, 1.6-3.6 cm in length and 04-0.7 cm in breadth, exstipulate.Inflorescence terminal, cymose, 5-9 flowers, 14-17 cm in length. Flowers whitish pink, bracteates, bracteolate, pedicellate, complete, bisexual, regular, actinomorphic, pentamerous, hypogynous.Sepals 5, aposepalous, imbricate, petaloid (dark red),0.1-0.4 cm in length and 0.1-0.3 cm in breadth, inferior. Petals (5), synpetalous, twisted, funnel form, petaloid, 3.4-3.9 cm in length and 2.1-2.7 cm in breadth, inferior. Stamens 5, apostamenous, epipetalous, filament very short, anther dithecous, dorsifixed, longitudinal dehiscence, introrse, inferior. Ovary bicarpellary, syncarpous, tetralocular(due to false septum present),stigma bifid, style slender, many ovules in each locule, axile placentation, superior. Fruits follicle, oblong, 11-25 cm in length and 2-3 cm in breadth.Seeds many, flat with a membranous wing, brown.

Habit

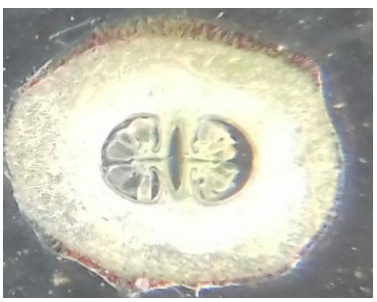
Ventral view of leaves

Dorsal view of leaves

Inflorescences

flowers

L.S of flower



T.S of ovary

Histological characters of leaves of *Plumeria rubra* L.

Lamina

In surface view, the epidermal cells of both surface were smooth, and thin wall. The cell wall of upper and lower surface were straight in lamina. Stomata were present on the upper surface and abundant in the lower surface. It was oval in shape and guard cell was crescent in shape. They were paracytic type.

In transverse section; multicellular trichomes, calcium oxalate crystals, thin-smooth cuticles were present on both surface. The upper and lower epidermal cells were rectangular in shaped. The mesophyll consisted of palisade and spongy parenchyma. The palisade was made up of two layers and spongy was irregular in shaped; which were loosely 7-9 layers and arranged with one another. They contained numerous chloroplast, oil cells and latex vessels. Vascular bundle embedded in the mesophyll cells, and bicollateral type.

Midrib

In surface view, the epidermal cells of both surface were rectangular in shaped. The lower surface cells were similar to upper surface cells. Stomata, unicellular trichomes and laticiferous canals were abundant on lower surface.

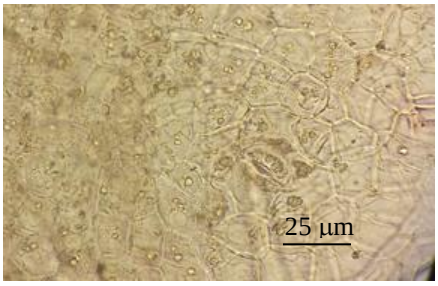
In transverse section, the cuticles were thin and smooth. The upper and lower epidermal cells were rectangular in shaped, single layer, thin-walled. Multicellular trichomes were present on adaxial side and collenchymatous cells were 20-24 layers and parenchymatous cells were 12-15 layers. At abaxial side, collenchymatous cells were 15-18 layers and parenchymatous cells were 17-20 layers. These cells were oval or polygonal in shaped and thin-walled, solitary crystals, oil cells and laticifierous canals were present on these layer. Vascular bundles were crescent in outline. These bundles were bicollateral type, xylem inner region and phloem peripheral and inner region, phloem cells 4-7 layers and xylem cells 3-6 per rows

Petiole

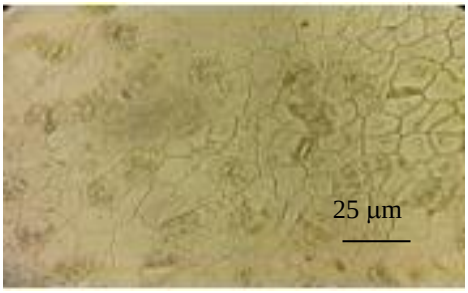
In surface view, the epidermal cells of both surface were rectangular to polygonal shaped and cell wall straight, stomata, oil cells, trichomes and calcium oxalate crystals were present on both surface.

In transverse section, the cuticles were thin and smooth, the upper and lower epidermal cells were angular or rounded, thin-walled, single layered. At adaxial side was heart-shaped, 21-24 layers of collenchymatous cells and 13-17 layers of parenchymatous cells. At abaxial side was convex, 16-20 layers of collenchymatous cells and 15-18 layers of parenchymatous cells. These cells were oval or polygonal in shaped and oil cells, calcium oxalate crystals and laticiferous canals, intercellular space were present. Vascular bundles were circular in outline. These bundles were bicollateral type, xylem inner region and phloem peripheral and inner region, phloem cells 2-4 layers and xylem cells 2-5 per rows.

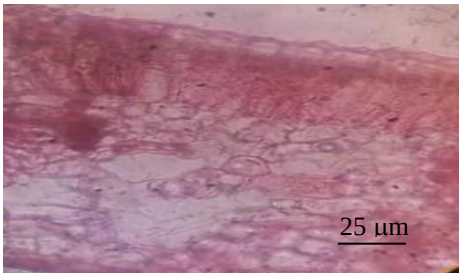
Histological characters of leaves of *Plumeria rubra* L.



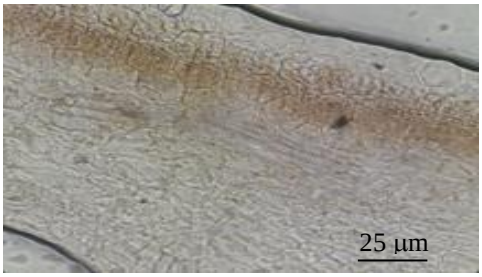
Upper surface of Lamina



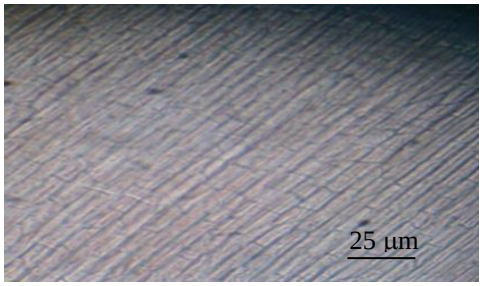
Lower surface of Lamina



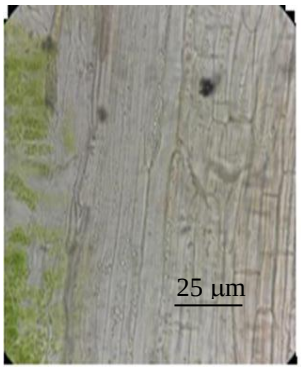
Transverse section of Lamina



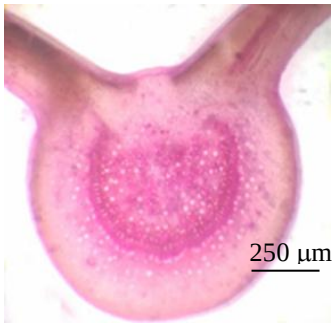
Transverse section of lamina with latex vessels



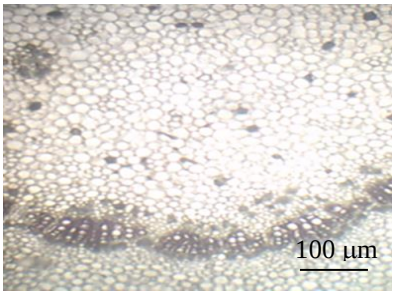
Upper surface of midrib



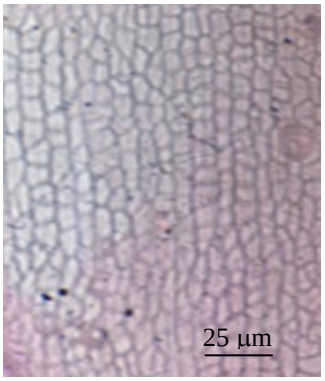
Lower surface of midrib showing trichome



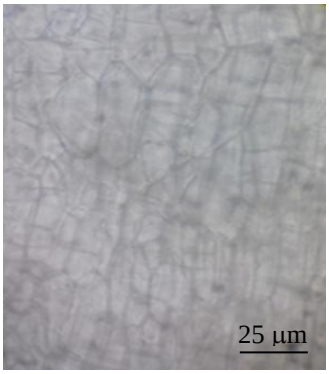
Transverse section of midrib



Close up view of vascular bundle of midr

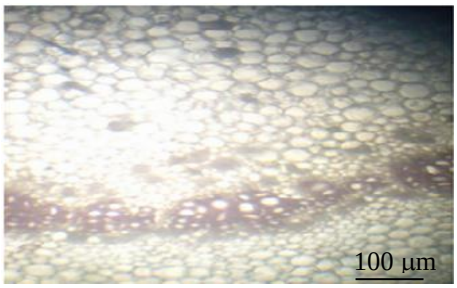


Upper surface of petiole



Lower surface of petiole

Transverse section of petiole

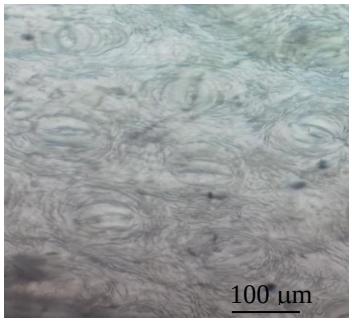


Close up view of vascular bundle of petiole

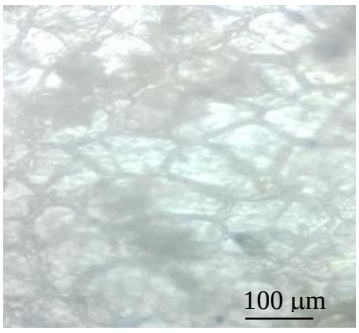
Sensory characters from powdered leaves of *Plumeria rubra* L.

Samples	Color	Odor	Taste	Texture
Leaves	Dark Green	Aromatic	Bitter	Granular

Histological characters of powdered leaves of *Plumeria rubra* L



Stomata

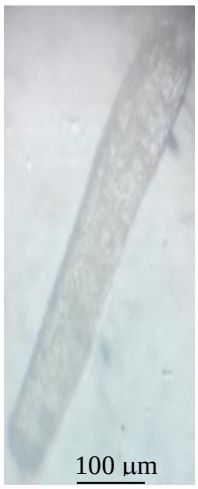


Epidermal cell

Unicellular trichome

Tracheid

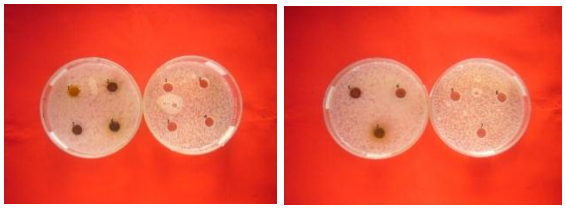
Fiber



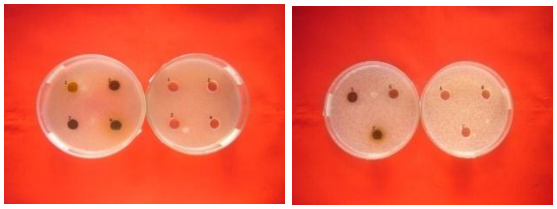
Pitted vessel

Scalariform vessel

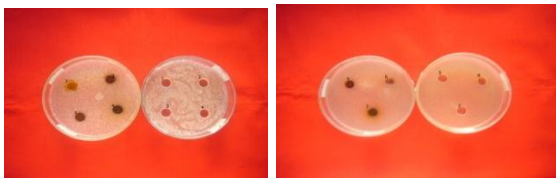
Comparative antimicrobial activities of control and different solvent extracts of leaves of *Plumeria rubra* L.



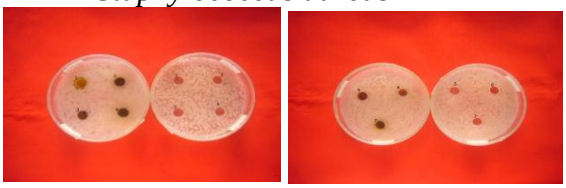
Bacillus subtilis



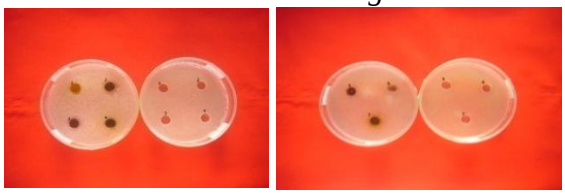
Staphylococcus aureus



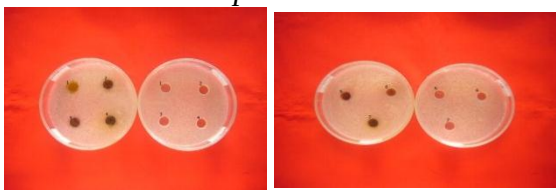
Pseudomonas aeruginosa



Bacillus pumalis



Candida albicans



Escherichia coli

Antimicrobial activity of different solvent extracts of leaves of *Plumeria rubra* L.

Solvents	**Organisms**					
	Bacillus subtilis	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Bacillus pumalis***	***Candida albican***	***Escherichia coli***
Pet-ether	11mm	-	-	11mm	11mm	11mm
$CHCL_3$	11mm	11mm	-	11mm	12mm	13mm
MeoH	12mm	12mm	-	12mm	13mm	12mm
Acetone	14mm	13mm	-	12mm	13mm	13mm
EtoAc	12mm	12mm	-	-	13mm	13mm
EtoH	14mm	13mm	-	11mm	12mm	12mm
Water	-	11mm	-	11mm	11mm	11mm

Agar well – 10 mm

Discussion and Conclusion

In this research, the plant of *Plumeria rubra* L. belonging to the family Apocynaceae have been studied. This plant was collected from North Dagon Township Yangon Region. The morphological characters of vegetative and reproductive parts of the plant, the histological characters and antimicrobial activity from leaves of *Plumeria rubra* L. were studied..

In morphological studies, *Plumeria rubra* L. was perennial tree, latex present. The leaves were simple, whorled, margin entire, leaf blade lanceolate. Inflorescences were cyme (corymb) terminal or axillary. Flowers were pink, fragrant, actinomorphic, pentamerous. Stamen epipetalous, anther sagittate or dithecous.Ovary bicarpellary, stigma bifid, style 1, axile placentation, disc present. Fruit follicles or berry. The seeds were membranous wings. These characters were in agreement with those mentioned by Hooker, 1882; Bailey, 1939; Backer, 1963; Lawrence, 1969 and Dassanayake, 1983.

According to the leaves of histological character studies; the stomata were present on both surface and paracytic type. Vascular bundles of midrib and petiole were circular shaped. These bundles arranged in the form of ring and bicollateral type. Multicellular trichomes, laticiferous canals, calcium oxalate crystals were present. Latex traces were large radial channels or strands. These characters were in agreement with those mentioned by Metcalf and Chalk, 1950.

In the antimicrobial activity of crude extracts from leaves of *Plumeria rubra* L.were tested on six microorganisms. Acetone extracts showed the best activity on *Bacillus subtilis,Staphylococcus aureus,Bacillus pumalis,Candida albicans*, *Escherichia coli* .From this finding, it may be inferred of leaves of *Plumeria rubra* L. can be effective in the formulation of medicine for the treatment of many diseases such as eye infection, urinary infection, respiratory system infection, boil, wound, food poison, pneumonia, intestinal diseases, bone and joint infections, gastrointestinal, chronic lung infections and skin infection.The effectiveness of leaves were carried out for clinical trial of their drugs.

Acknowledgements

I would like to express thanks to Dr Aye Pè,Professor and Head, Dr Myint Aung (Professor), Dr Baydar (Professor), Dr Thandar Aye (Professor), Department of Botany, University of Yangon for their encouragement and guidance in this research.

References

Bailey, L.H, (1939), **The Standard Cyclopedia of Horticulture Vol.I,** The Macmillan Company, New York

Backer, C.A., (1963), **Flora of Java Vol II,** N.V.P Noordhoff-Groningen, Netherland

Cruickshank, C.B.E., (1970). Medicinal Microbiology.London :hurchillLiyingstone.

Hundley, H.G and Chit KoKo, (1987), **List of Trees, Shrubs, Herbs and Principal of Climbers,** etc, Government Printing Press, Yangon.

Hooker, SIR J.D, (1882). **The Flora of British India Vol.III,** L. Reeve and Company, London

Kirtikar, K.R and Basu, B.D., (1984), **Indian Medicinal Plants Vol.III**, India

Kyaw Soe & Tin Myo Ngwe, (2002), **Medicinal Plants of Myanmar,** Identification and Uses of some 100 Commonly Used Species, Series I, Pyizone Publishing Hourser, Lanmadaw Township, Yangon

Lawrence, G.H.M, (1969), **Taxonomy of Vascular Plants,** the Macmillan Company, New York

Metcalf, C.R and Chalk, L, (1950), **Anatomy of the Dictyledons,** Leaves, Stems and Woods in Relation to Taxonomy with Notes on Economic Uses Vol.II, Oxford University Press, London

Richard, A., (1998), **Ornamental flowers;***Plumeria*, Department of Horticulture, College of Tropical Agriculture & Human Resources.

Trease, G.E and Evans, W.C, (1989), **Pharmacognosy 14thEdn;** ELBS, Baillre Tindal, London

U San Hla, (1960), **Handbooks of Natural Foods;** the Universal Printing Works, 76 A, Inya Road, Rangoon.

U Mya Win, (1966), **နွယ်မြက်သစ်ပင်ဆေးဖက်ဝင်ကြသည့် ရုပ်ပြဆေးဘယ အဘိဓာန် အမှတ်(၂),** Yangon

Wiart, C., (2002), **Medicinal Plants of Southeast Asia,** Kuala Lumpur, Pearson Malaysia

HYPOGLYCEMIC ACTIVITY OF WATERY EXTRACT OF THE LEAVES OF *THUNBERGIA LAURIFOLIA* LINDL. (PAN-YE-SUT-NWE)

Naw tharaphi aung[1], swe swe aye[2], khine khine lwin[3]

Abstract

Thunbergia laurifolia Lindl. is commonly known as Rang Chuet belongs to the family Acanthaceae and Myanmar name is pan-ye-sut-nwe. The plant samples were collected from Daik-U Township, Bago Region. The present work was done to investigate the acute toxicity and hypoglycemic activity. The acute toxicity study of watery extracts of *Thunbergia laurifolia* Lindl. leaves were carried on albino mice by using OECD guideline 423 method. There were no lethality and toxic effect of the albino mice observed up to the dose of 5 g/kg body weight during observation period of two weeks. Hypoglycemic activity of watery extract of the leaves of *Thunbergia laurifolia* Lindl. were tested on adrenaline-induced hyperglycemic rats by the method of Agrawal & Paridhavia, 2007. The rats with watery extract (0.75 g/kg and 1.5 g/kg) showed significant decrease in blood glucose concentration at 2 hr, 3 hr and 4 hr ($p<0.01$- $p<0.001$) whereas watery extract (3 g/kg) showed significant decrease in blood glucose concentration at 3 hr ($p<0.05$) and 4 hr ($p<0.01$). The results indicated that watery extracts of the leaves possessed significant hypoglycemic effect on adrenaline-induced hyperglycemic rats. From the experimental data, it can be concluded that *Thunbergia laurifolia* Lindl. could be used as potential herbal medicine for hypoglycemic activities. It also can be safe to eat the leaves of *Thunbergia laurifolia*Lindl. because the leaves are non-toxic.

Keywords *: Thunbergia laurifolia* Lindl., hypoglycemic activity, acute toxicity.

Introduction

Thunbergia laurifolia Lindl. belongs to the family Acanthaceae, under the order Lamiales. The generic name *Thunbergia* commemorates Carl Peter Thunberg, Swedish physician and Professor at Upsala. The specific epithet *laurifolia* refers to its laurel shaped leaves (Gledhill, 2008). Its English name is laurel-clock vine or blue trumpet vine, the common name in Myanmar is pan-ye-sut-nwe or kyikan-hnokthi or kyininwe.

Chuthaputti (2010) reported that the leaves of *Thunbergia laurifolia* Lindl. contains sterols such as β-sitosterol, stigmasterol, alphaspinasterol and lupeol.

Thunbergia laurifolia Lindl. contained phenolic acids such as caffeic acid, gallic acid, procateuchuic acid and chlorogenic acid (Oonsivilai *et al*., 2007).

The chromatographic separation of secondary compounds has been investigated such as grandifloric acid and 8-epi-grandifloric acid (Kanchanapoom *et al*., 2002).

Diabetes is an important public health problem. Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. Diabetes caused 1.5 million deaths in 2012. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs. It may present with characteristic symptoms such as thirst, polyuria, blurring of vision and weight loss (WHO, 2016).

Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and alpha glucosidase inhibitors which are used as monotherapy or in combination to achieve better glycemic regulation. Many of these oral antidiabetic agents have a number of serious adverse effects. Thus, managing diabetes without any side effects is still a challenge. Plant drugs and herbal formulations are considered to be less

toxic and free from side effects than synthetic ones. Based on the WHO recommendations, hypoglycemic agents of plant origin used in medicine which play an important role in future drug development programs. Easy availability, least side effects and low cost make the herbal preparations which are the main key player of all available therapies especially in rural areas. (www.diabete.qc.ca)

Thunbergia laurifolia Lindl. leaves have been reported to have antioxidant, antimicrobial, detoxifying, anti-diabetic activites and non-toxic effects (Chan *et al*., 2011). The biological activitiessuch as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective and antidiabetes activitieswere found in *Thunbergia laurifolia* Lindl. (Junsi & Siripongvertikorn, 2016).

Antioxidant supplementation derived from medicinal plants is more interested. Due to high antioxidant activity and low toxicity, *Thunbergia laurifolia* Lindl. is used as natural antioxidant supplementation to prevent oxidative stress-related pathology (Palipoch *et al*., 2013).

However, the scientific investigations for acute toxicity and hypoglycemic activity from *Thunbergia laurifolia* Lindl. are still lacking in Myanmar. Therefore, the aims of the present research were to investigate the acute toxicity and hypoglycemic activity of watery extract of the leaves of *Thunbergia laurifolia* Lindl.

Figure 1 Habit of *Thunbergia laurifolia* Lindl.

Materials and Methods

Acute toxicity test of watery extract of leaves of *Thunbergia laurifolia* Lindl. on albino mice

The acute toxicity test on albino mice was carried out according to the method of OECD Guideline 423 at Department of Medical Research (DMR).

Materials

Test animals	-	18 female albino mice (ddy strain, body weight 25– 30 g)
Test agents	-	Distilled water, watery extract of *Thunbergia laurifolia* Lindl.
Apparatus	-	Mice cages, animal balance, 18 gauge dosing cannula, disposal syringes (1 ml and 5 ml), rubber glove and mask
Dose Schedule	-	2 g/kg and 5 g/kg (body weight) of albino mice
Period of observation	-	14 days

Methods

Female albino mice, weighing between 25-30 g were randomly selected and kept in their cages for at least 5 days prior to dosing to allow for acclimatization for laboratory condition. The mice were kept fasting overnight for 18 hours but were allowed with free access to water. Following period of fasting, mice were weighed and test substance was administered. Group (1) served as control and only 10 ml/kg of distilled water was given orally by using intragastric needle. In this study, starting dose 2 g/kg was chosen. The extract was dissolved in distilled water and required doses were given orally with 6 animals (3 animals per step). After administration of the test agent orally, they were allowed to have food and water. The sign of toxicity such as changes in skin and fur, eyes and mucous membrane, respiratory, circulatory, central nervous system were observed on test animals. Mice were observed individually after dosing at one time during the first 30 minutes hourly up to 4 hours for first day. After that all mice were monitored daily and weighed weekly. They were found to survive at the dose of 2 g/kg during observation period of 14 days. So, another 3 albino mice were administered with of watery extract 5 g/kg. The mice were observed for toxic sign by using the method described above. No toxic signs and lethality were observed in these 3 mice at the dose of 5 g/kg. So, another 3 mice were administered with watery extract 5 g/kg. A total of 6 albino mice were used at the dose level of 5 g/kg of the watery extract.

Body weight analysis

Individual weights of animals were recorded before the administration of drug on 1st day of the study and on 14th days of the experiment. Weight changes were recorded and calculated.

Figure 2 Groups of mice in acute toxicity study

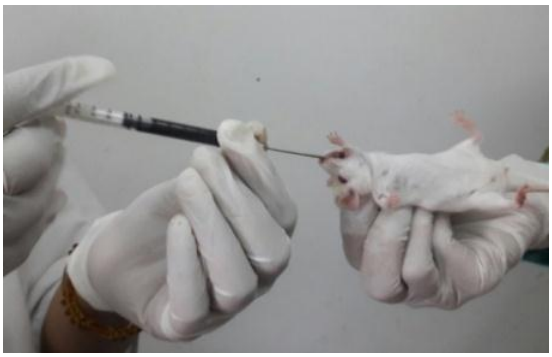


Figure 3 Administration of extract suspension to mice

Hypoglycemic activity of watery extract of leaves of *Thunbergia laurifolia* Lindl.

The hypoglycaemic activity of watery extract was studied on adrenaline induced hyperglycaemic rats model (Gupta *et al*., 1967).

Materials

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

Test agents - Distilled water, watery extract, Glibenclamide tablets (B. P 5 mg, India), Adrenaline injection (1 mg/ml) (Myanmar Pharmaceutical Factory)

Apparatus - Aluminium cages, Animal balance, Spirit cotton wools, disposable syringes with needle (1 ml and 5 ml), Glucometer, Test strips, 18 gauge dosing needles, rubber gloves and masks

Dose Schedule - Watery extract of *Thunbergia laurifolia* Lindl. 0.75 g/kg, 1.5 g/kg and 3g/kg body weight

Method

In this experiment, adult albino rats (Wistar strain) of both sexes (body weight 180 - 250 g) were used. Eight rats were kept without food for 18 hours before the experiment but water was orally allowed freely. They were served as control group and oral administration of distilled water (10 ml/kg) body weight was given. Before the oral administration of distilled water to the control group, blood sample was collected from tail vein and blood glucose level was determined by glucometer. After 30 minute, the rats were made hyperglycemic by injection with adrenaline (0.4 ml/kg) subcutaneously to nape of the neck. The sample of blood were collected from the tail veins by cutting the tail (1 mm) at 1 hr, 2 hr, 3 hr and 4 hr intervals after subcutaneous injection of adrenaline. After taking the blood samples from tail vein, the tail was rubbed with cotton wool soaked in absolute alcohol to protect the puncture against infection. The result was read on the glucometer which was expressed in (mg/dl) of blood glucose level on the glucometer screen. Then, washout period of 1 week was done.

In this study, three different doses of watery extract of *Thunbergia laurifolia* Lindl. leaves (0.75 g/kg, 1.5 g/kg and 3 g/kg) and standard drug, glibenclamide (4 mg/kg) were tested for hypoglycemic activity in the rats. One week wash out period was done in between the determination of hypoglycemic activity of the different doses of the extracts and glibenclamide.

The detail procedure for testing of hypoglycemic activity was the same as mentioned above by using the same eight rats.

Data Analysis

The results were shown in (Mean ± SE). Student “t” test (Paired “t” test) was used for statistical comparison between blood glucose concentrations of the control group and experimental groups. P-value <0.05 was considered to be significant. Percent reduction of hyperglycemia was calculated by following formula:

$$\text{Percent reduction} = \frac{C-T}{C} \times 100$$

C = Rise in blood glucose of control

T = Rise in blood glucose level of tested animals

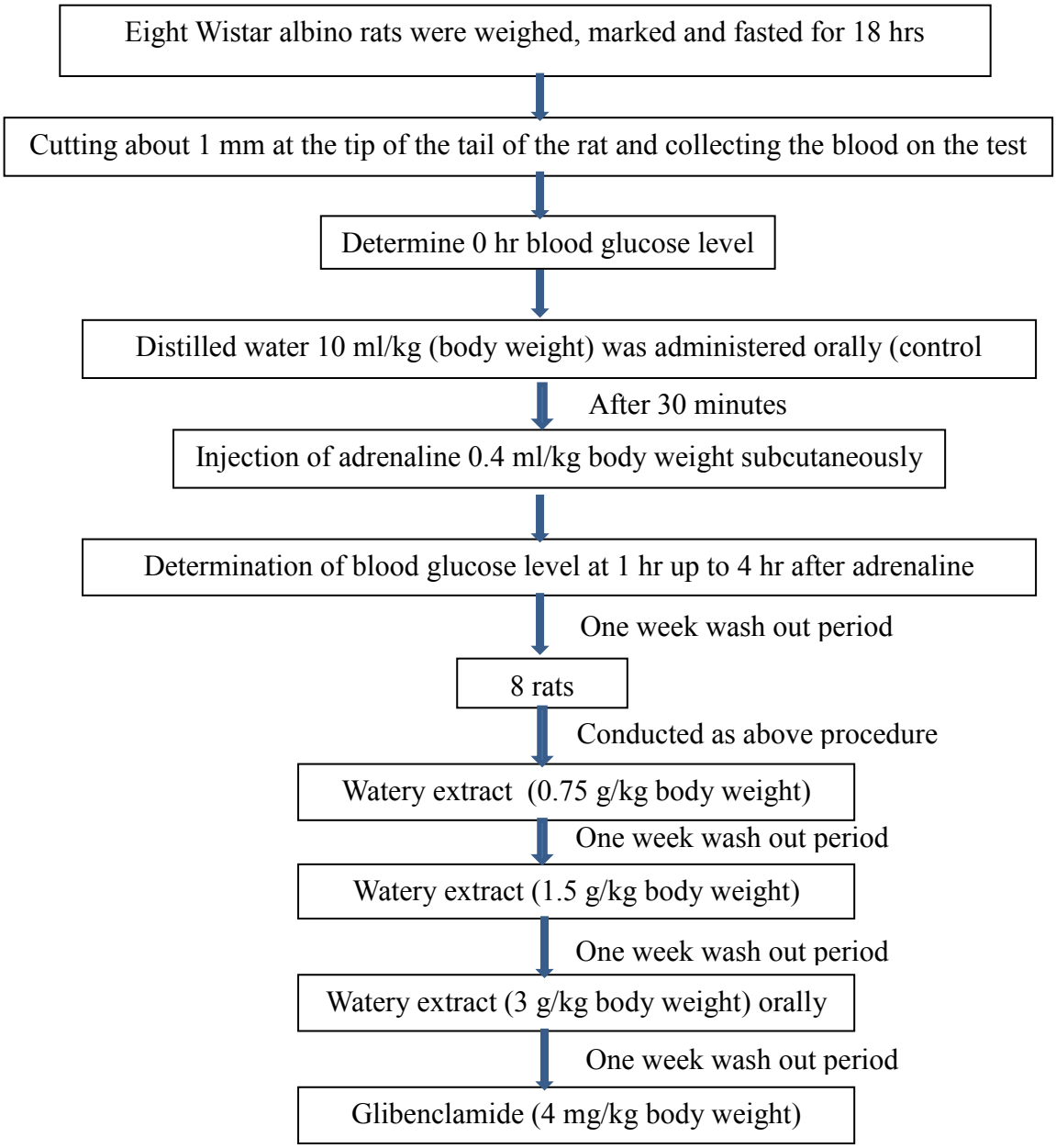


Figure 4 Flow chart for hypoglycemic activity testing in albino rats

Figure 5 Albino rats in cages and each contains 2 rats

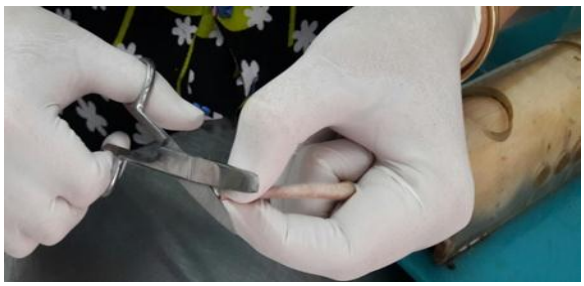


Figure 6 Cutting the tip of tail of the rats

Figure 7 Determining of blood glucose level in rats by using glucometer

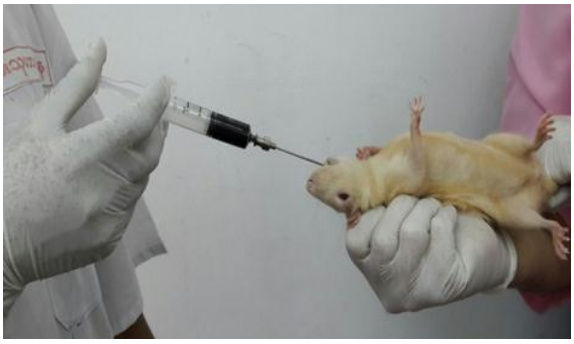


Figure 8 Administration of extract suspension to rat

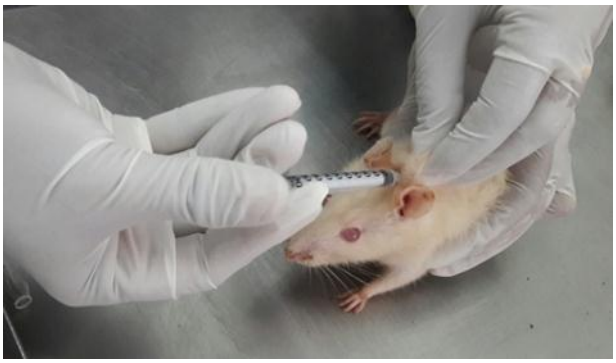


Figure 9 Adrenaline injection into nape of neck of albino rat

Results

Acute toxicity test of watery extract of the leaves of *Thunbergia laurifolia* Lindl. on albino mice

The acute toxicity test was carried out according to the guideline of OECD-423 method. In this experiment, the mice were administered with the does of 2 g/kg and 5 g/kg (body weight) of watery extract of the leaves of *Thunbergia laurifolia* Lindl. No lethality and toxic signs of the mice were observed up to 14 days observation period. There were no significant differences in body weight of the test groups when compared with control group. Therefore, the medium lethal dose (LD_{50}) was observed to be more than 5 g/kg (body weight). The results of acute toxicity were shown in Table 1.

There were no significant differences in body weights between the control group and watery extract of *Thunbergia laurifolia* Lindl. leaves (2 g/kg) receiving group at one week and two week after given the extracts. There was significant increase in body weight of watery extract (5 g/kg) receiving group at two week after extract administration when compared with control group. The results were shown in Table 1.

Table 1 Acute toxicity test of watery extract of *Thunbergia laurifolia* Lindl. in albino mice

No of Group	Type of drug administration	No of mice tested	Dosage	Observed period	No. of death
1	Control (distilled water)	6	10 ml/kg	14 days	0/6
2	Watery extracts	6	2 g/kg	14 days	0/6
3	Watery extracts	6	5 g/kg	14 days	0/6

Hypoglycemic activity of watery extract of the leaves of *Thunbergia laurifolia* Lindl. on the adrenaline induced hyperglycemic rats

Hypoglycemic activity of watery extract was tested by using adrenaline induced hyperglycemic rat model which was described in (Gupta *et al*., 1964 and Agrawal & Paridhavia, 2007). In this study, both sexes (body weight 180-250 g) of Wistar strain albino rats were used. Eight albino rats were served as control group with oral administration of distilled water (10 ml/kg) body weight. Then, washout period for a week was done. After the washout period of one week, the same eight rats were tested again with oral administration of each dose level of watery extract (0.75 g/kg, 1.5 g/kg and 3 g/kg) of the leaves of *Thunbergia laurifolia* Lindl. and standard drug (glibenclamide - 4 mg/kg). The results of mean blood glucose concentrations of 8 albino rats treated with watery extract of the leaves (0.75 g/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 mg/kg) were 59 ± 2.96 mg/dl, 157 ± 4.24 mg/dl, 175.5 ±6.01mg/dl, 160.38±6.44mg/dl and 126.13± 7.81 mg/dl respectively. Significant decreases in blood glucose were observed at 2 hr, 3 hr and 4 hr ($p<0.05$ - $p<0.001$).

The results of mean blood glucose concentrations of 8 albino rats treated with watery extract of the leaves (1.5 g/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 mg/kg) were 59.5 ± 1.94 mg/dl, 154 ± 7.35 mg/dl, 170.63 ± 6.22 mg/dl, 166.75 ± 12.49 mg/dl and 137.25 ±13.34 mg/dl respectively. Significant decrease in blood glucose were observed at 2 hr, 3 hr and 4 hr ($p<0.05$ - $p<0.001$). The results of mean blood glucose concentrations of 8 albino rats treated with watery extract of the leaves (3 g/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 mg/kg) were 62.88 ± 2.06 mg/dl, 164.88 ± 8.82 mg/dl, 187.25 ± 11.16 mg/dl, 169.88 ± 10.36 mg/dl and 132.88 ± 10.71 mg/dl respectively. Significant decreases in blood glucose were observed at 3 hr and 4 hr ($p<0.05$ - $p<0.01$).

The results of mean blood glucose concentrations of 8 albino rats treated with standard drug (glibenclamide - 4 mg/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 mg/kg) were 66.5 ± 4.5 mg/dl, 148.63 ± 6.21 mg/dl, 164.13 ± 6.62 mg/dl, 135.38 ± 8.01 mg/dl and 105.25 ± 7.47 mg/dl respectively. Significant decreases in blood glucose were observed at 3 hr and 4 hr ($p<0.01$- $p<0.001$). The results were shown in Table 2-3 and Figure 10-11.

Table 2 Mean blood glucose concentration (Mean ± SE) of watery extract and standard drug, glibenclamide to adrenaline-induced hyperglycemic rat model

	Blood glucose concentration (mg/dl)				
	0 hr	1 hr	2 hr	3 hr	4 hr
Control	64.88 ± 2.42	165.38 ± 7.43	205.75 ± 6.71	227.13 ± 10.43	204.5 ± 14.39
watery extract 0.75 g/kg	59 ± 2.96	157 ± 4.24	175.5 ± 6.01	160.38 ± 6.44	126.13 ± 7.81
watery extract 1.5 g/kg	59.5 ± 1.94	154 ± 7.35	170.63 ± 6.22	166.75 ± 12.49	137.25 ± 13.34
watery extract 3 g/kg	62.88 ± 2.06	164.88 ± 8.82	187.25 ± 11.16	169.88 ± 10.36	132.88 ± 10.71
Glibenclamide 4 mg/kg	66.5 ± 4.5	148.63 ± 6.21	164.13 ± 6.62	135.38 ± 8.01	105.25 ± 7.47

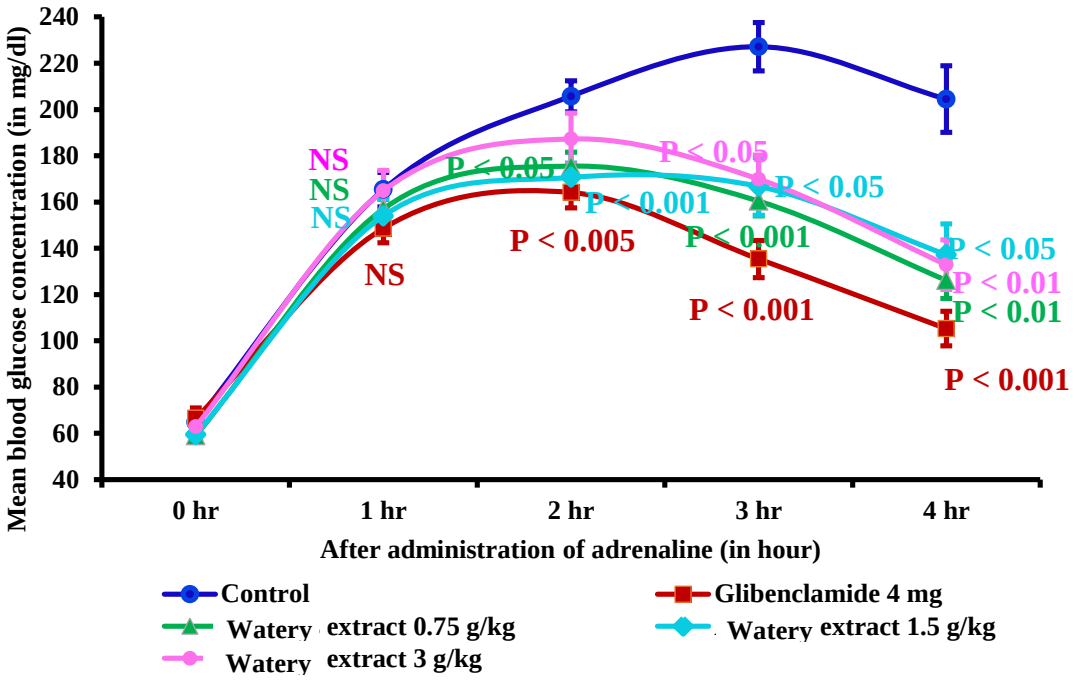


Figure 10 Time course of the effects of watery extract of *Thunbergia laurifolia* Lindl. leaves (0.75 g/kg, 1.5 g/kg and 3 g/kg) and glibenclamide (4 mg/kg) to adrenaline induced hyperglycemic rat model

Table 3 Percent reductions (Mean ± SE) of hyperglycemic with watery extract and glibenclamide (4 mg/kg) to adrenaline-induced hyperglycemic rat model

	Percent reduction of hyperglycemic			
	1 hr	**2 hr**	**3 hr**	**4 hr**
Glibenclamide 4 mg/kg	13.72 ± 10.8	30.09 ± 6.05	56.22 ± 5.8	71.36 ± 5.03
Watery extract 0.75 g/kg	-3.33 ± 11.72	15.83 ± 7.39	36.56 ± 4.69	49.64 ± 7.21
Watery extract 1.5 g/kg	0.98 ± 12.1	20.78 ± 4.21	30.93 ± 11.16	40.92 ± 11.71
Watery extract 3 g/kg	-7.23 ± 15.31	10.43 ± 9.72	31.1 ± 9.31	46.63 ± 10.54

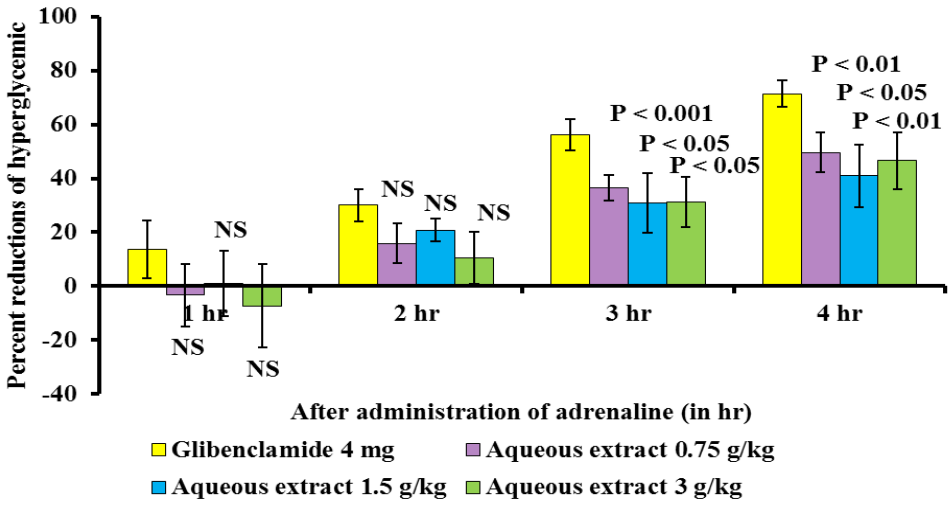


Figure 11 Percent reductions of hyperglycemic with watery extract of *Thunbergia laurifolia* Lindl. leaves (0.75 g/kg, 1.5 g/kg and 3 g/kg) and glibenclamide (4 mg/kg) to adrenaline induced hyperglycemic rats model (NS = not significant)

Discussion and Conclusion

In this study, the acute toxicity of watery extract of the leaves of *Thunbergia laurifolia* Lindl. were studied on albino mice by using OECD guideline 423. Oral route of administration was used because it is the route intended to be used in human. There were no toxic effect and lethality even with the maximum dose of 5 g/kg body weight. Therefore, the median lethal dose LD_{50} of the watery extract of *Thunbergia laurifolia* Lindl. leaves was found to be more than 5 g/kg body weight.

In an earlier acute toxicity study of watery leaf extract of *Thunbergia laurifolia* Lindl. in mice at 1, 2, 4 and 8 g/kg, it was reported that the extract is non toxicand safe for consumption (Usanawarong *et al.*, 2000). These observations are agreed with the results of the present study.

In this study, hypoglycemic activity of watery extract of the leaves of *Thunbergia laurifolia* Lindl. was investigated on adrenaline-induced hyperglycemic rats. The effect of the test extracts were compared to the standard drug, glibenclamide.

Aritajat *et al*. (2004) stated that the hypoglycemic activity of the watery extract from the leaves of *Thunbergia laurifolia* Lindl. was evaluated in normoglycemic and alloxon-induced diabetic rats. Watery extract of the leaves of *Thunbergia laurifolia* Lindl. caused significant decrease ($p<0.001$) levels of blood glucose in diabetic rats.

In the present results, the rats treated with watery extract (0.75 g/kg) showed significant decrease in the blood glucose concentration at 2 hr ($p< 0.05$), 3 hr ($p< 0.001$) and 4 hr ($p< 0.01$). The rats treated with watery extract (1.5 g/kg) showed significant decrease in the blood glucose concentration at 2 hr ($p< 0.001$), 3 hr($p< 0.05$) and 4 hr ($p< 0.05$) whereas at the dose of (3 g/kg) of watery extract, significant decreases in the blood glucose concentration were found at 3 hr ($p< 0.05$) and 4 hr ($p< 0.01$). These results are agreed with Aritajat *et al.*, 2004.

Mean percent reductions of hyperglycemia with watery extract of the leaves of *Thunbergia laurifolia* Lindl. ranged from (36.56 % to 49.64 %) at the dose of 0.75 g/kg, from (20.78 % to 40.92 %) at 1.5 g/kg and from (31.1 % to 46.63 %) at3 g/kg. Mean percent reductions of hyperglycemia with standard drug, glibenclamide ranged from (30.09 % to 71.36). Therefore, watery extract of the leaves of *Thunbergia laurifolia* Lindl. possessed significant hypoglycemic effect in hyperglycemic rats and the extract was less effective than glibenclamide.

The phytochemical constituents (β-Sitosterol, lupeol) were present in the leaves of *Thunbergia laurifolia* Lindl. (Chuthaputti, 2010). Hypoglycemic activity of the leaves of *Thunbergia laurifolia* Lindl. may be due to the presence of phytochemical constituents in the leaves. Therefore, the leaves of *Thunbergia laurifolia* Lindl. could be used as herbal medicine for hypoglycemic activities. It also can be safe to eat the leaves of *Thunbergia laurifolia* Lindl. because the leaves are non-toxic.

Acknowledgement

The authors would like to express their profound gratitude to the Department of Higher Education (Yangon Office), Ministry of Education, Yangon, Myanmar, for provision of opportunity to do this research and Myanmar Academy of Arts and Science for allowing to present this paper.

References

Agrawal, S.S. and M. Paridhavi, (2007), *Screening methods for anti-diabetic drugs*, Universities Press (India) Pivate Limited, p-515.

Aritajat, S., S. Wutteerapol and K. Saenphet, (2004) "Anti-diabetic effect of *Thunbergia laurifolia* Lindl, Aqueous extract", *Southeast Asian Journal Tropical Medicine Public Health*, Vol-35 (suppl 2): pp. 53-57.

Chan, E.W.C., S.Y. Eng, Y.P. Tan and Z.C. Wong, (2011) "Phytochemical and pharmacological properties of *Thunbergialaurifolia* Lindl. : A review", *Pharmacognosy journal*, Vol. 3 (24).

Chuthaputti, A., (2010) "Rang Jerd : Laurel Clock Vine (*Thunbergia laurifolia* Lindl.): a detoxifying herb", *Journal of Thai Traditional and Alternative Medicine*, Vol. 8(2-3), pp. 211-220.

Gledhill, D., (2008), *The name of plants*, Published in the United States of America by Cambridge University Press, New York.

Gupta, S., S.C.L. Verma, V.P. Gerg and M. Ri, (1967) "Antidiabetic effect of *Tinospora cordifolia*",*India journal of medical research*, Vol. 545(7), pp. 733-745.

Jaiboon, V., J. Boonyanuphap, S. Suwansri, P. Ratanatraiwong and C. Hansawasdi, (2011) "Alpha amylase inhibition and roasting time of local vegetables and herbs prepared for diabetes risk reducing chili paste", *Asian journal of food and agro-industry*, Vol. 4(02), pp. 103-113.

Junsi, M. and S. Siripongvertikorn, (2016) "*Thunbergia laurifolia*, a traditional herbal tea of Thailand: botanical, chemical composition, biological properties and processing influence", *International Food Research Journal*,Vol. 23(3), pp. 923-927.

Kanchanapoom, T., R. Kasai and K. Yamasaki, (2002) "Iridoidglucosides from *Thunberg laurifolia* Lindl." , *Phytochemistry*, Vol. 60, pp. 769-771.

Oonsivilai, R., C. Cheng, J. Bomser, M.G. Ferruzzi and S. Ningsanond, (2007) "Phytochemical profiling and phase II enzyme-inducing properties of *Thunbergialaurifolia*Lindl. Extracts", *Journal of Ethnopharmacology*, Vol. 114(3), pp. 300-306.

Organization for Economic Cooperation and Development – 423, (2001). *Guidelines for the testing of chemical acute oral toxicity. Acute toxicity class method 423*, pp. 1-14.

Palipoch, S., C. Punsawad and P. Suwannalert, (2013) "*Thunbergia laurifolia*, a new choice of natural antioxidant to prevent oxidative stress-related pathology: A review", *Journal of medicinal plants research*, Vol. 7(12), pp. 698-701.

Usanawarong, S., T. Thesiri, T. Mahakunakorn and S. Parasupattana, (2000) "Effect of *Thunbergia laurifolia* Lindl. on detoxication of paraquat", *Khon Kaen Univ Res J*, Vol. 5, pp. 11-17.

World Health Organization, (2016). *Global report on diabetes*. WHO press, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

(http://www.diabete.qc. Ca)

HYPOGLYCEMIC ACTIVITY OF WATERY EXTRACT OF THE LEAVES OF *THUNBERGIA LAURIFOLIA* LINDL. (PAN-YE-SUT-NWE)

Naw Tharaphi Aung[1], Swe Swe Aye[2], Khine Khine Lwin[3]

Abstract

Thunbergia laurifolia Lindl. is commonly known as Rang Chuet belongs to the family Acanthaceae and Myanmar name is pan-ye-sut-nwe. The plant samples were collected from Daik-U Township, Bago Region. The present work was done to investigate the acute toxicity and hypoglycemic activity. The acute toxicity study of watery extracts of *Thunbergia laurifolia* Lindl. leaves were carried on albino mice by using OECD guideline 423 method. There were no lethality and toxic effect of the albino mice observed up to the dose of 5 g/kg body weight during observation period of two weeks. Hypoglycemic activity of watery extract of the leaves of *Thunbergia laurifolia* Lindl. were tested on adrenaline-induced hyperglycemic rats by the method of Agrawal & Paridhavia, 2007. The rats with watery extract (0.75 g/kg and 1.5 g/kg) showed significant decrease in blood glucose concentration at 2 hr, 3 hr and 4 hr ($p<0.01$- $p<0.001$) whereas watery extract (3 g/kg) showed significant decrease in blood glucose concentration at 3 hr ($p<0.05$) and 4 hr ($p<0.01$). The results indicated that watery extracts of the leaves possessed significant hypoglycemic effect on adrenaline-induced hyperglycemic rats. From the experimental data, it can be concluded that *Thunbergia laurifolia* Lindl. could be used as potential herbal medicine for hypoglycemic activities. It also can be safe to eat the leaves of *Thunbergia laurifolia* Lindl. because the leaves are non-toxic.

Keywords *: Thunbergia laurifolia* Lindl., hypoglycemic activity, acute toxicity.

Introduction

Thunbergia laurifolia Lindl. belongs to the family Acanthaceae, under the order Lamiales. The generic name *Thunbergia* commemorates Carl Peter Thunberg, Swedish physician and Professor at Upsala. The specific epithet *laurifolia* refers to its laurel shaped leaves (Gledhill, 2008). Its English name is laurel-clock vine or blue trumpet vine, the common name in Myanmar is pan-ye-sut-nwe or kyikan-hnokthi or kyininwe.

Chuthaputti (2010) reported that the leaves of *Thunbergia laurifolia* Lindl. contains sterols such as β-sitosterol, stigmasterol, alphaspinasterol and lupeol.

Thunbergia laurifolia Lindl. contained phenolic acids such as caffeic acid, gallic acid, procateuchuic acid and chlorogenic acid (Oonsivilai *et al*., 2007).

The chromatographic separation of secondary compounds has been investigated such as grandifloric acid and 8-epi-grandifloric acid (Kanchanapoom *et al*., 2002).

Diabetes is an important public health problem. Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. Diabetes caused 1.5 million deaths in 2012. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs. It may present with characteristic symptoms such as thirst, polyuria, blurring of vision and weight loss (WHO, 2016).

Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and alpha glucosidase inhibitors which are used as

[1] Dr, Assistant Lecturer, Department of Botany, University of Yangon
[2] Dr, Lecturer, Department of Botany, University of Yangon
[3] Dr, Deputy Director, Pharmacology Research Division, Department of Medical Research

monotherapy or in combination to achieve better glycemic regulation. Many of these oral antidiabetic agents have a number of serious adverse effects. Thus, managing diabetes without any side effects is still a challenge. Plant drugs and herbal formulations are considered to be less toxic and free from side effects than synthetic ones. Based on the WHO recommendations, hypoglycemic agents of plant origin used in medicine which play an important role in future drug development programs. Easy availability, least side effects and low cost make the herbal preparations which are the main key player of all available therapies especially in rural areas. (www.diabete.qc.ca)

Thunbergia laurifolia Lindl. leaves have been reported to have antioxidant, antimicrobial, detoxifying, anti-diabetic activites and non-toxic effects (Chan *et al*., 2011). The biological activitiessuch as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective and antidiabetes activitieswere found in *Thunbergia laurifolia* Lindl. (Junsi & Siripongvertikorn, 2016).

Antioxidant supplementation derived from medicinal plants is more interested. Due to high antioxidant activity and low toxicity, *Thunbergia laurifolia* Lindl. is used as natural antioxidant supplementation to prevent oxidative stress-related pathology (Palipoch *et al*., 2013).

However, the scientific investigations for acute toxicity and hypoglycemic activity from *Thunbergia laurifolia* Lindl. are still lacking in Myanmar. Therefore, the aims of the present research were to investigate the acute toxicity and hypoglycemic activity of watery extract of the leaves of *Thunbergia laurifolia* Lindl.

Figure 1 Habit of *Thunbergia laurifolia* Lindl.

Materials and Methods

Acute toxicity test of watery extract of leaves of *Thunbergia laurifolia* Lindl. on albino mice

The acute toxicity test on albino mice was carried out according to the method of OECD Guideline 423 at Department of Medical Research (DMR).

Materials

Test animals	-	18 female albino mice (ddy strain, body weight 25– 30 g)
Test agents	-	Distilled water, watery extract of *Thunbergia laurifolia* Lindl.
Apparatus	-	Mice cages, animal balance, 18 gauge dosing cannula, disposal syringes (1 ml and 5 ml), rubber glove and mask
Dose Schedule	-	2 g/kg and 5 g/kg (body weight) of albino mice
Period of observation	-	14 days

Methods

Female albino mice, weighing between 25-30 g were randomly selected and kept in their cages for at least 5 days prior to dosing to allow for acclimatization for laboratory condition. The mice were kept fasting overnight for 18 hours but were allowed with free access to water. Following period of fasting, mice were weighed and test substance was administered. Group (1) served as control and only 10 ml/kg of distilled water was given orally by using intragastric needle. In this study, starting dose 2 g/kg was chosen. The extract was dissolved in distilled water and required doses were given orally with 6 animals (3 animals per step). After administration of the test agent orally, they were allowed to have food and water. The sign of toxicity such as changes in skin and fur, eyes and mucous membrane, respiratory, circulatory, central nervous system were observed on test animals. Mice were observed individually after dosing at one time during the first 30 minutes hourly up to 4 hours for first day. After that all mice were monitored daily and weighed weekly. They were found to survive at the dose of 2 g/kg during observation period of 14 days. So, another 3 albino mice were administered with of watery extract 5 g/kg. The mice were observed for toxic sign by using the method described above. No toxic signs and lethality were observed in these 3 mice at the dose of 5 g/kg. So, another 3 mice were administered with watery extract 5 g/kg. A total of 6 albino mice were used at the dose level of 5 g/kg of the watery extract.

Body weight analysis

Individual weights of animals were recorded before the administration of drug on 1st day of the study and on 14th days of the experiment. Weight changes were recorded and calculated.

Figure 2 Groups of mice in acute toxicity study

Figure 3 Administration of extract suspension to mice

Hypoglycemic activity of watery extract of leaves of *Thunbergia laurifolia* Lindl.

The hypoglycaemic activity of watery extract was studied on adrenaline induced hyperglycaemic rats model (Gupta *et al*., 1967).

Materials

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

Test agents - Distilled water, watery extract, Glibenclamide tablets (B. P 5 mg, India), Adrenaline injection (1 mg/ml) (Myanmar Pharmaceutical Factory)

Apparatus - Aluminium cages, Animal balance, Spirit cotton wools, disposable syringes with needle (1 ml and 5 ml), Glucometer, Test strips, 18 gauge dosing needles, rubber gloves and masks

Dose Schedule - Watery extract of *Thunbergia laurifolia* Lindl. 0.75 g/kg, 1.5 g/kg and 3g/kg body weight

Method

In this experiment, adult albino rats (Wistar strain) of both sexes (body weight 180 - 250 g) were used. Eight rats were kept without food for 18 hours before the experiment but water was orally allowed freely. They were served as control group and oral administration of distilled water (10 ml/kg) body weight was given. Before the oral administration of distilled water to the control group, blood sample was collected from tail vein and blood glucose level was determined by glucometer. After 30 minute, the rats were made hyperglycemic by injection with adrenaline (0.4 ml/kg) subcutaneously to nape of the neck. The sample of blood were collected from the tail veins by cutting the tail (1 mm) at 1 hr, 2 hr, 3 hr and 4 hr intervals after subcutaneous injection of adrenaline. After taking the blood samples from tail vein, the tail was rubbed with cotton wool soaked in absolute alcohol to protect the puncture against infection. The result was read on the glucometer which was expressed in (mg/dl) of blood glucose level on the glucometer screen. Then, washout period of 1 week was done.

In this study, three different doses of watery extract of *Thunbergia laurifolia* Lindl. leaves (0.75 g/kg, 1.5 g/kg and 3 g/kg) and standard drug, glibenclamide (4 mg/kg) were tested for hypoglycemic activity in the rats. One week wash out period was done in between the determination of hypoglycemic activity of the different doses of the extracts and glibenclamide.

The detail procedure for testing of hypoglycemic activity was the same as mentioned above by using the same eight rats.

Data Analysis

The results were shown in (Mean ± SE). Student “t” test (Paired “t” test) was used for statistical comparison between blood glucose concentrations of the control group and experimental groups. P-value <0.05 was considered to be significant. Percent reduction of hyperglycemia was calculated by following formula:

$$\text{Percent reduction} = \frac{C-T}{C} \times 100$$

C = Rise in blood glucose of control

T = Rise in blood glucose level of tested animals

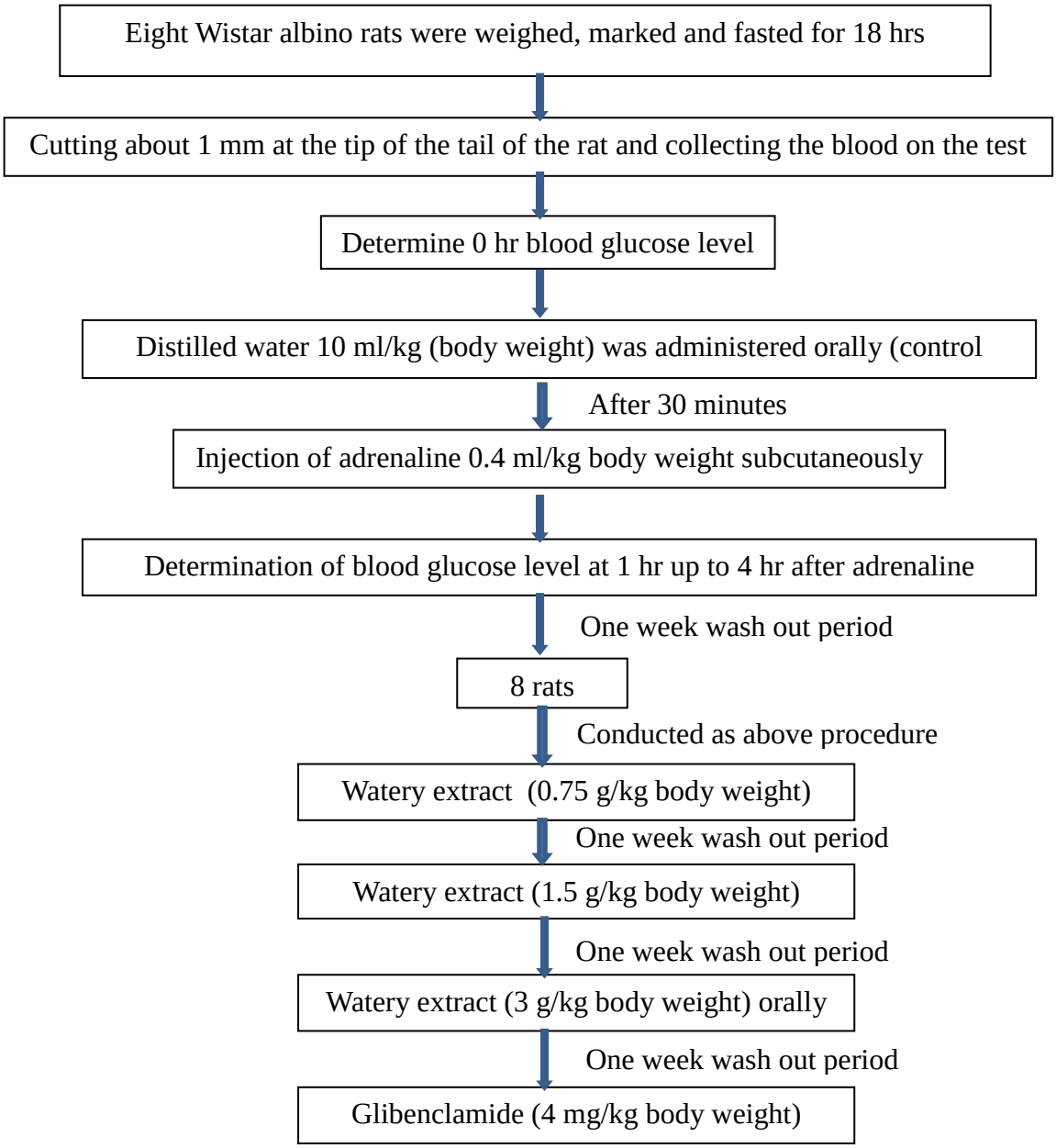


Figure 4 Flow chart for hypoglycemic activity testing in albino rats

Figure 5 Albino rats in cages and each contains 2 rats

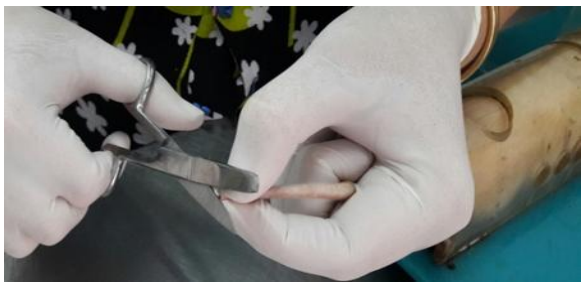


Figure 6 Cutting the tip of tail of the rats

Figure 7 Determining of blood glucose level in rats by using glucometer

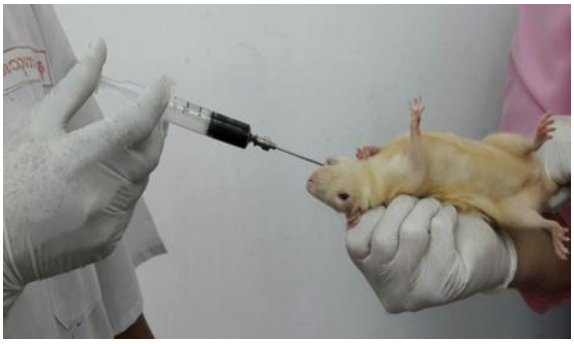


Figure 8 Administration of extract suspension to rat

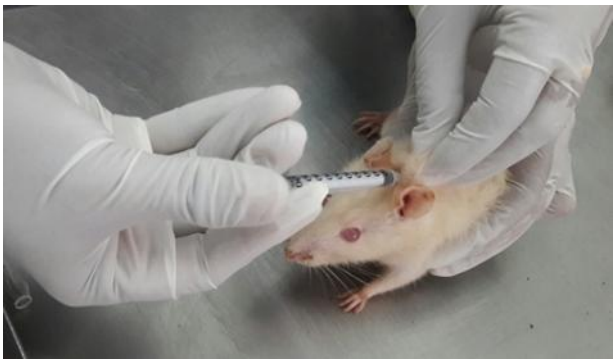


Figure 9 Adrenaline injection into nape of neck of albino rat

Results

Acute toxicity test of watery extract of the leaves of *Thunbergia laurifolia* Lindl. on albino mice

The acute toxicity test was carried out according to the guideline of OECD-423 method. In this experiment, the mice were administered with the does of 2 g/kg and 5 g/kg (body weight) of watery extract of the leaves of *Thunbergia laurifolia* Lindl. No lethality and toxic signs of the mice were observed up to 14 days observation period. There were no significant differences in body weight of the test groups when compared with control group. Therefore, the medium lethal dose (LD_{50}) was observed to be more than 5 g/kg (body weight). The results of acute toxicity were shown in Table 1.

There were no significant differences in body weights between the control group and watery extract of *Thunbergia laurifolia* Lindl. leaves (2 g/kg) receiving group at one week and two week after given the extracts. There was significant increase in body weight of watery extract (5 g/kg) receiving group at two week after extract administration when compared with control group. The results were shown in Table 1.

Table 1 Acute toxicity test of watery extract of *Thunbergia laurifolia* Lindl. in albino mice

No of Group	Type of drug administration	No of mice tested	Dosage	Observed period	No. of death
1	Control (distilled water)	6	10 ml/kg	14 days	0/6
2	Watery extracts	6	2 g/kg	14 days	0/6
3	Watery extracts	6	5 g/kg	14 days	0/6

Hypoglycemic activity of watery extract of the leaves of *Thunbergia laurifolia* Lindl. on the adrenaline induced hyperglycemic rats

Hypoglycemic activity of watery extract was tested by using adrenaline induced hyperglycemic rat model which was described in (Gupta *et al*., 1964 and Agrawal & Paridhavia, 2007). In this study, both sexes (body weight 180-250 g) of Wistar strain albino rats were used. Eight albino rats were served as control group with oral administration of distilled water (10 ml/kg) body weight. Then, washout period for a week was done. After the washout period of one week, the same eight rats were tested again with oral administration of each dose level of watery extract (0.75 g/kg, 1.5 g/kg and 3 g/kg) of the leaves of *Thunbergia laurifolia* Lindl. and standard drug (glibenclamide - 4 mg/kg). The results of mean blood glucose concentrations of 8 albino rats treated with watery extract of the leaves (0.75 g/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 mg/kg) were 59 ± 2.96 mg/dl, 157 ± 4.24 mg/dl, 175.5 ±6.01mg/dl, 160.38±6.44mg/dl and 126.13± 7.81 mg/dl respectively. Significant decreases in blood glucose were observed at 2 hr, 3 hr and 4 hr ($p<0.05$ - $p<0.001$).

The results of mean blood glucose concentrations of 8 albino rats treated with watery extract of the leaves (1.5 g/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 mg/kg) were 59.5 ± 1.94 mg/dl, 154 ± 7.35 mg/dl, 170.63 ± 6.22 mg/dl, 166.75 ± 12.49 mg/dl and 137.25 ±13.34 mg/dl respectively. Significant decrease in blood glucose were observed at 2 hr, 3 hr and 4 hr ($p<0.05$ - $p<0.001$). The results of mean blood glucose concentrations of 8 albino rats treated with watery extract of the leaves (3 g/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 mg/kg) were 62.88 ± 2.06 mg/dl, 164.88 ± 8.82 mg/dl, 187.25 ± 11.16 mg/dl, 169.88 ± 10.36 mg/dl and 132.88 ± 10.71 mg/dl respectively. Significant decreases in blood glucose were observed at 3 hr and 4 hr ($p<0.05$ - $p<0.01$).

The results of mean blood glucose concentrations of 8 albino rats treated with standard drug (glibenclamide - 4 mg/kg) at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after subcutaneous injection of adrenaline (0.4 mg/kg) were 66.5 ± 4.5 mg/dl, 148.63 ± 6.21 mg/dl, 164.13 ± 6.62 mg/dl, 135.38 ± 8.01 mg/dl and 105.25 ± 7.47 mg/dl respectively. Significant decreases in blood glucose were observed at 3 hr and 4 hr ($p<0.01$- $p<0.001$). The results were shown in Table 2-3 and Figure 10-11.

Table 2 Mean blood glucose concentration (Mean ± SE) of watery extract and standard drug, glibenclamide to adrenaline-induced hyperglycemic rat model

	Blood glucose concentration (mg/dl)				
	0 hr	1 hr	2 hr	3 hr	4 hr
Control	64.88 ± 2.42	165.38 ± 7.43	205.75 ± 6.71	227.13 ± 10.43	204.5 ± 14.39
watery extract 0.75 g/kg	59 ± 2.96	157 ± 4.24	175.5 ± 6.01	160.38 ± 6.44	126.13 ± 7.81
watery extract 1.5 g/kg	59.5 ± 1.94	154 ± 7.35	170.63 ± 6.22	166.75 ± 12.49	137.25 ± 13.34
watery extract 3 g/kg	62.88 ± 2.06	164.88 ± 8.82	187.25 ± 11.16	169.88 ± 10.36	132.88 ± 10.71
Glibenclamide 4 mg/kg	66.5 ± 4.5	148.63 ± 6.21	164.13 ± 6.62	135.38 ± 8.01	105.25 ± 7.47

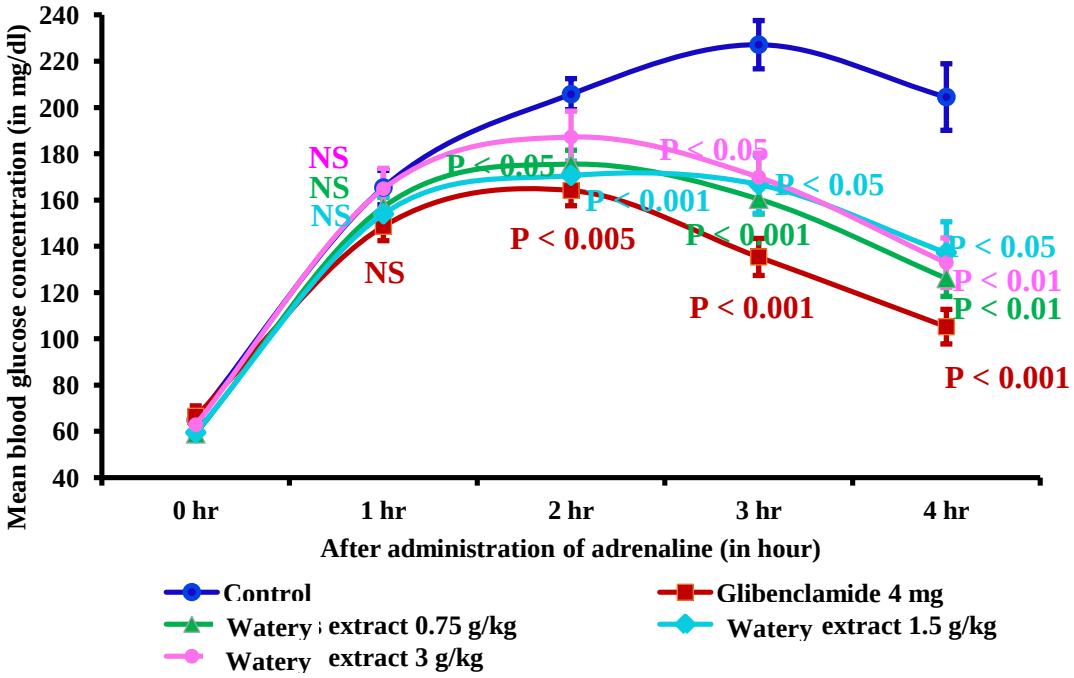


Figure 10 Time course of the effects of watery extract of *Thunbergia laurifolia* Lindl. leaves (0.75 g/kg, 1.5 g/kg and 3 g/kg) and glibenclamide (4 mg/kg) to adrenaline induced hyperglycemic rat model

Table 3 Percent reductions (Mean ± SE) of hyperglycemic with watery extract and glibenclamide (4 mg/kg) to adrenaline-induced hyperglycemic rat model

	Percent reduction of hyperglycemic			
	1 hr	**2 hr**	**3 hr**	**4 hr**
Glibenclamide 4 mg/kg	13.72 ± 10.8	30.09 ± 6.05	56.22 ± 5.8	71.36 ± 5.03
Watery extract 0.75 g/kg	-3.33 ± 11.72	15.83 ± 7.39	36.56 ± 4.69	49.64 ± 7.21
Watery extract 1.5 g/kg	0.98 ± 12.1	20.78 ± 4.21	30.93 ± 11.16	40.92 ± 11.71
Watery extract 3 g/kg	-7.23 ± 15.31	10.43 ± 9.72	31.1 ± 9.31	46.63 ± 10.54

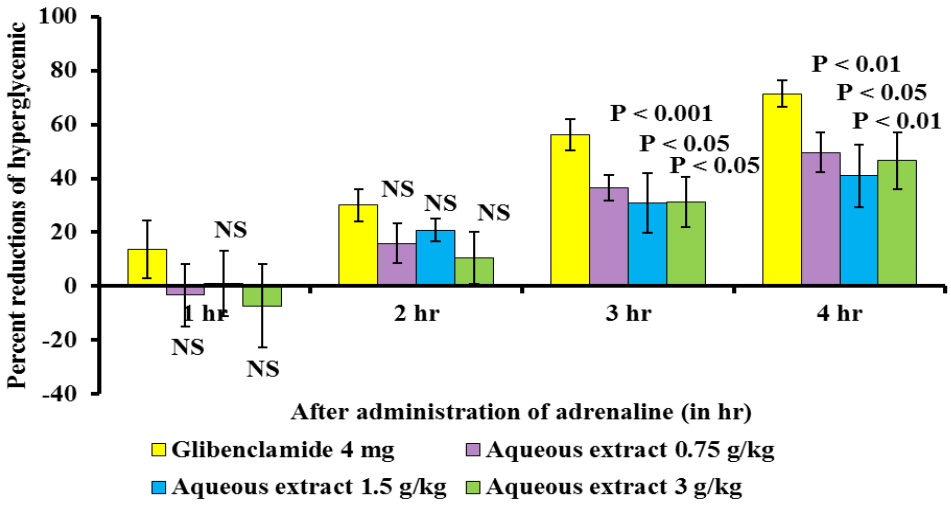


Figure 11 Percent reductions of hyperglycemic with watery extract of *Thunbergia laurifolia* Lindl. leaves (0.75 g/kg, 1.5 g/kg and 3 g/kg) and glibenclamide (4 mg/kg) to adrenaline induced hyperglycemic rats model (NS = not significant)

Discussion and Conclusion

In this study, the acute toxicity of watery extract of the leaves of *Thunbergia laurifolia* Lindl. were studied on albino mice by using OECD guideline 423. Oral route of administration was used because it is the route intended to be used in human. There were no toxic effect and lethality even with the maximum dose of 5 g/kg body weight. Therefore, the median lethal dose LD_{50} of the watery extract of *Thunbergia laurifolia* Lindl. leaves was found to be more than 5 g/kg body weight.

In an earlier acute toxicity study of watery leaf extract of *Thunbergia laurifolia* Lindl. in mice at 1, 2, 4 and 8 g/kg, it was reported that the extract is non toxicand safe for consumption (Usanawarong *et al.*, 2000). These observations are agreed with the results of the present study.

In this study, hypoglycemic activity of watery extract of the leaves of *Thunbergia laurifolia* Lindl. was investigated on adrenaline-induced hyperglycemic rats. The effect of the test extracts were compared to the standard drug, glibenclamide.

Aritajat *et al*. (2004) stated that the hypoglycemic activity of the watery extract from the leaves of *Thunbergia laurifolia* Lindl. was evaluated in normoglycemic and alloxon-induced diabetic rats. Watery extract of the leaves of *Thunbergia laurifolia* Lindl. caused significant decrease ($p<0.001$) levels of blood glucose in diabetic rats.

In the present results, the rats treated with watery extract (0.75 g/kg) showed significant decrease in the blood glucose concentration at 2 hr ($p< 0.05$), 3 hr ($p< 0.001$) and 4 hr ($p< 0.01$). The rats treated with watery extract (1.5 g/kg) showed significant decrease in the blood glucose concentration at 2 hr ($p< 0.001$), 3 hr($p< 0.05$) and 4 hr ($p< 0.05$) whereas at the dose of (3 g/kg) of watery extract, significant decreases in the blood glucose concentration were found at 3 hr ($p< 0.05$) and 4 hr ($p< 0.01$). These results are agreed with Aritajat *et al.,* 2004.

Mean percent reductions of hyperglycemia with watery extract of the leaves of *Thunbergia laurifolia* Lindl. ranged from (36.56 % to 49.64 %) at the dose of 0.75 g/kg, from (20.78 % to 40.92 %) at 1.5 g/kg and from (31.1 % to 46.63 %) at3 g/kg. Mean percent reductions of hyperglycemia with standard drug, glibenclamide ranged from (30.09 % to 71.36). Therefore, watery extract of the leaves of *Thunbergia laurifolia* Lindl. possessed significant hypoglycemic effect in hyperglycemic rats and the extract was less effective than glibenclamide.

The phytochemical constituents (β-Sitosterol, lupeol) were present in the leaves of *Thunbergia laurifolia* Lindl. (Chuthaputti, 2010). Hypoglycemic activity of the leaves of *Thunbergia laurifolia* Lindl. may be due to the presence of phytochemical constituents in the leaves. Therefore, the leaves of *Thunbergia laurifolia* Lindl. could be used as herbal medicine for hypoglycemic activities. It also can be safe to eat the leaves of *Thunbergia laurifolia* Lindl. because the leaves are non-toxic.

Acknowledgement

The authors would like to express their profound gratitude to the Department of Higher Education (Yangon Office), Ministry of Education, Yangon, Myanmar, for provision of opportunity to do this research and Myanmar Academy of Arts and Science for allowing to present this paper.

References

Agrawal, S.S. and M. Paridhavi, (2007), *Screening methods for anti-diabetic drugs*, Universities Press (India) Pivate Limited, p-515.

Aritajat, S., S. Wutteerapol and K. Saenphet, (2004) "Anti-diabetic effect of *Thunbergia laurifolia* Lindl, Aqueous extract", *Southeast Asian Journal Tropical Medicine Public Health*, Vol-35 (suppl 2): pp. 53-57.

Chan, E.W.C., S.Y. Eng, Y.P. Tan and Z.C. Wong, (2011) "Phytochemical and pharmacological properties of *Thunbergialaurifolia* Lindl. : A review", *Pharmacognosy journal*, Vol. 3 (24).

Chuthaputti, A., (2010) "Rang Jerd : Laurel Clock Vine (*Thunbergia laurifolia* Lindl.): a detoxifying herb", *Journal of Thai Traditional and Alternative Medicine*, Vol. 8(2-3), pp. 211-220.

Gledhill, D., (2008), *The name of plants*, Published in the United States of America by Cambridge University Press, New York.

Gupta, S., S.C.L. Verma, V.P. Gerg and M. Ri, (1967) "Antidiabetic effect of *Tinospora cordifolia*", *India journal of medical research*, Vol. 545(7), pp. 733-745.

Jaiboon, V., J. Boonyanuphap, S. Suwansri, P. Ratanatraiwong and C. Hansawasdi, (2011) "Alpha amylase inhibition and roasting time of local vegetables and herbs prepared for diabetes risk reducing chili paste", *Asian journal of food and agro-industry*, Vol. 4(02), pp. 103-113.

Junsi, M. and S. Siripongvertikorn, (2016) "*Thunbergia laurifolia*, a traditional herbal tea of Thailand: botanical, chemical composition, biological properties and processing influence", *International Food Research Journal*,Vol. 23(3), pp. 923-927.

Kanchanapoom, T., R. Kasai and K. Yamasaki, (2002) "Iridoidglucosides from *Thunberg laurifolia* Lindl." , *Phytochemistry*, Vol. 60, pp. 769-771.

Oonsivilai, R., C. Cheng, J. Bomser, M.G. Ferruzzi and S. Ningsanond, (2007) "Phytochemical profiling and phase II enzyme-inducing properties of *Thunbergialaurifolia*Lindl. Extracts", *Journal of Ethnopharmacology*, Vol. 114(3), pp. 300-306.

Organization for Economic Cooperation and Development – 423, (2001). *Guidelines for the testing of chemical acute oral toxicity. Acute toxicity class method 423*, pp. 1-14.

Palipoch, S., C. Punsawad and P. Suwannalert, (2013) "*Thunbergia laurifolia*, a new choice of natural antioxidant to prevent oxidative stress-related pathology: A review", *Journal of medicinal plants research*, Vol. 7(12), pp. 698-701.

Usanawarong, S., T. Thesiri, T. Mahakunakorn and S. Parasupattana, (2000) "Effect of *Thunbergia laurifolia* Lindl. on detoxication of paraquat", *Khon Kaen Univ Res J*, Vol. 5, pp. 11-17.

World Health Organization, (2016). *Global report on diabetes*. WHO press, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

(http://www.diabete.qc. Ca)

YIELD AND YIELD COMPONENTS OF FIVE SELECTED VARIETIES OF *ZEA MAYS* L.

Rose May Yi[1], Mya Zarli[2], Wunna Htoon[3]

Abstract

This study was conducted to investigate yield and yield components of five different maize varieties such as V1: Waxy corn (Sticky Big) indicated as ***Zea mays* L.var. *ceratina*** Kulesh., V2: Sweet corn (Angel 131) as ***Zea mays* L. var. *saccharata*** (Sturter) L. H. Bailey., V3: Flint corn (Padamyar) as ***Zea mays*. L. var. *indurata*** (Sturter) L.H. Bailey., V4: Peruvian maize (Nga Cheik) as ***Zea mays*. L. var. *microspermae*** (Sturter)L.H. Bailey., and V5: Flour corn (Meilan) as ***Zea mays.* L. var. *amylacea*** (Sturter) L.H. Bailey.was carried out at Vegetables, Fruits Research and Development Center (VFRDC), Yemon, Hlegu Township, Yangon Region from September to January, 2018. The experiment was laid out in randomized complete block design with four replications. The data recorded on various parameters were analyzed using computer software Statistics 8.0. The results showed that the plant height of Angel 131 (Sweet corn) (V2) (215.15cm) which was the tallest and the shortest (143.70) by Nga Cheik (V4) at 56 DAS (day after sowing). The maximum number of leaf (12.8) was found in Angel 131 (V2) and the minimum number of leaf (8.94) in Nga Cheik (V4). The maximum kernel weight (326.60 g) and the maximum grains yield (103.38 g) in Angel 131 (V2) and the minimum kernel weight (137.60 g) and the minimum grains yield (39.51g) in Nga Cheik (V4) were also recorded. It is concluded that among five varieties, Angel 131 (V2), Sticky Big (V1) and Meilan (V5) varieties were observed the highest value of yield and yield production.

Keywords – *Zea mays* L., five maize varieties, yield and yield components

Introduction

Maize (*Zea mays* L.) is a member of the grass family, Poaceae. It is believed that maize was originated in Mexico and introduced to West Africa in the early 1500s by the Portuguese traders (Salunkhe, D.K., and B.B. Desai. 2000).

Maize (*Zea mays* L.) is the world's widely grown highland cereal and primary staple food crop in many developing countries (Kandil, 2013). It was originated in America and first cultivated in the area of Mexico more than 7,000 years ago, and spread throughout North and South America (Hailare, 2000). Maize is the third most important cereal after wheat and rice all over the world serving as staple food for many countries (Frova *et al*., 1999). In 2014, the United States topped the list of ten maize producing countries which includes China, Brazil, EU-27, Ukraine, Argentina, India, Mexico, South Africa and Canada with an amount of about 351 million metric tons. It is the short duration crop, capable of producing large quantity of food grain. It can be grown twice a year, both for grain and fodder (AGBIOS, 2005 a).

In Myanmar, maize crop was produced regularly in Northern Shan State, Mandalay Region and Ayeyarwady Region. Nowadays, quality seeds have been changed in production. The cultivar of yield maize has been increased annually after the period of 2009, local maize production has been tried to have more year-round production. The harvest period of maize in Myanmar is commencing from August or September and in full swing during October or November (MPBSA, 2013).

Maize is tall, annual plant with an extensive fibrous root system. It is cross pollinating species with the female (ear) and male (tassel) flower in separate places on the plant and versatile

[1] Assistant Lecturer, Department of Botany, University of Yangon
[2] Lecturer, Department of Botany, University of Yangon
[3] Staff Office, Fruits Research and Development Center (VFRDC), Yemon, Hlegu Township, Yangon Division

crop and everything on acorn plant is useable. The female flowers are arranged in a spike on short branch and are characterized by long and feathery style, which emerge out of the cob. The grain is typically a single seeded dry fruit, caryopsis, having two kinds of endosperm, the outer yellow and hard while the inner white and soft (AGBIOS, 2005 a).

Seed germination is an essential process in any plant development in order to obtain an optimal number of seedling that results in higher seed yield (Yusuf. C.S., Makate N. and Jacob. R., 2014).Maize maintains its growing at high temperatures. Maize plant needs 10-11 ºC temperature to start germination. It needs a temperature parameter above 15 ºC (17-18 ºC) and 30 ºC for optimum and maximum temperature respectively.

Maize is classified as sweet, pop, flour, silage or feed corn, depending on the type of carbohydrate stored in the ear. The average chemical composition of the grain is starch 68% - 70%, protein 5-11% and oil 3.5 - 5% respectively. Every part of the maize plant has economic value which the grain, leaves, stalk, tassel and cob can all be used to produce a large variety of food and non-food production. The husk of the maize is traditionally used in making tamales. The kernels are ground into food. The stalks become animal food and the silks are used for medicinal teas (Sleper, 2006).Therefore, aims and objectives of this study were to investigate the growth of five selected varieties and to evaluate the effect of yield and yield components of five selected varieties of *Zea mays* L.

Materials and Methods

Experimental site and plant materials

The field experiment was conducted at Vegetables and Fruits Research and Development Center (VFRDC), Hlegu Township, Yangon Region during September to December, 2018. In this experiment, a total of five maize varieties were collected from (VFRDC). These maize varieties were *Zea mays* L. var. Sticky Big (V1), *Zea mays* L. var. Angel 131 (V2), *Zea mays* L. var. Padamyar (V3), *Zea mays* L. var. NgaCheik (V4) and *Zea mays* L. var. Meilan (V5). Sticky Big (V1) is a commercial sticky maize and V5: Meilan is a glutinous maize. *Zea mays* L. var. Angel 131 (V2) is a commercial sweet maize and both V3 and V4 are local sticky maize varieties. Randomized complete block design (RCBD) with four replications were used in this experiment.

Soil Preparation and Fertilization

The soil was disk-ploughed thrice, leveled and harrowed. A basal dose of 15:15:15: (N:P:K) compound fertilizer and well-decomposed chicken manure were incorporated into the top soil at the rate of 50 kg ac^{-1} and 2 ton ac^{-1}, respectively shortly prior to planting. Lime at the rate of 100 kg ac^{-1} was incorporated into the soil during soil preparation. Seeds were treated with captan (3a,4,7,7a-tetrahydro-2- [(trichloromethyl)thio]- 1H-isoindole-1,3 (2H)-dione) at the rate of 5 g kg^{-1} seeds to protect from fungal diseases before planting. Plot size was 4 m x 4.5 m with 90 cm spacing of between rows and 30 cm between plants. Seeds were firstly germinated in the germination tray and the plantlets were transplanted into the field at 10 days after emergence. N:P:K compound fertilizer (15:15:15) was incorporated again into the soil twice at the rate of 50 kg ac^{-1} at one month and two months after transplanting. Irrigation was done daily until one month after transplanting and it continued with 2 days intervals. Hand weeding was done weekly until two weeks before harvest.

Data collection

The data for plant height, leaf number, leaf area and node length, stem girth, were collected at weekly intervals. The number of kernel row per ear, number of kernel per row, 100% grains weight, total dry matter per plant (dry weight, shoot weight and root weight) and grains yield were also collected for each variety.

Methods

Leaf Area (LA)

Leaf area was calculated according to (Montgomery, 1911).

Leaf area (LA) cm^2 = K x length (cm) x width (cm)

K value varies with the shape of the leaf which in turn is affected by cultivar, nutritional status, and growth stage of the leaf. K value for maize is (0.75).

Experimental Design and Statistical Analysis

The experiment was carried out using Randomized Completely Block Design (RCBD) in field. The totals of five maize varieties were carried out in the study. Each treatment consisted of four replications. Each replication included 5 samples plants. The data were subjected for analysis of variance according to a RCBD design and all calculation was performed using Statistic-8 package and Least Significance Differences (LSD) was used to compare treatment means (Gomez and Gomez, 1984).

Results

Plant height

Plant height had statistically significant differences ($P \leq 0.01$) among the maize varieties at 56 DAS (day after sowing). At 56 DAS, the tallest plant height was observed in Angel 131 (V2) 215.15 cm pt^{-1} followed by Meilan (V5) 191.60 cm pt^{-1} and Sticky big (V1) 188.75 cm pt^{-1} among the varieties. In contrast, the tallest means of plant height was found in Angel 131 (V2) 122.74 cm pt^{-1} and the shortest means in Nga Cheik (V4) 85.72 cm pt^{-1} among varieties throughout the growing period (Table 1. and Figure.1).

Number of leaves

The total number of maize leaves was gradually increased overtime among varieties. At 56DAS, the maximum number of leaves was observed in Angel 131 (V2) which was 12.80 pt^{-1} followed by Meilan (V5) which was 12.20 pt^{-1}, Sticky big (V1) which was 11.40 pt^{-1} and Padamyar (V3) which was 11.00 pt^{-1}. The minimum number of leaves was found in Nga Cheik (V4) which was 10.35 pt^{-1}. However, the mean differences in number of leaves were highly significant among the treatments during cultivation (Table 1. and Figure 1.).

Leaf area

Among the varieties at 56 DAS, the maximum leaf area was observed in Angel 131 (V2) which was 688.48 cm^2 pt^{-1}followed by Meilan (V5) which was 664.33 cm^2 pt^{-1}, Sticky big (V1) which was 626.35 cm^2pt^{-1} and Padamyar (V3) which was 603.13 cm^2 pt^{-1}. The minimum leaf area was found in Nga Cheik (V4) which was 583.77 cm^2 pt^{-1}. However, the mean differences in leaf area were highly significant among the treatments during cultivation (Table 1. and Figure 1.).

Node length

Among the varieties, the maximum node length was observed in Angel 131 (V2) which was 18.8 cm pt^{-1} followed by Meilan (V5) which was 18.47 cm pt^{-1}, V1 (Sticky big) which was 18.45 cm $pt^{-1}$and V3 (Padamyar) which was 14.7 cm pt^{-1}. The minimum node length was found in V4 (Nga Cheik) which was 14.45 cm pt^{-1}. The mean differences in stem numbers were not significance among the treatments during cultivation (Table 1. and Figure 1.).

Stem girth

Among the varieties at 56 DAS, the maximum stem girth was observed in Angel 131 (V2) which was 8.35 cm pt^{-1} followed by Meilan (V5) which was 8.12 cm pt^{-1}, Sticky big (V1) which was 7.85 cm pt^{-1} and Padamyar (V3) which was 7.77 cm pt^{-1}. The minimum stem girth was found in Nga Cheik (V4) which was 7.4 cm pt^{-1}. However, the mean differences in stem numbers were not significance among the treatments during cultivation (Table 1. and Figure 1.).

Table 1 Selected maize varieties of plant height, leaf numbers, leaf area, node length, stem girth at 56 DAS

Maize Varieties	Plant height ($cmpt^{-1}$)	Leaf numbers (pt^{-1})	Leaf area (cm^2pt^{-1})	Node length (cm)	Stem girth (cm)
Sticky Big (V1)	188.75	11.40	626.35	18.45	7.85
Angel 131(V2)	215.15	12.80	688.48	18.8	8.35
Padamyar (V3)	182.10	11.00	603.13	14.7	7.77
Nga Cheik (V4)	143.70	10.35	583.77	14.45	7.4
Melian (V5)	191.60	12.20	664.33	18.47	8.12
F-test	**	**	**	ns	ns
5%LSD	8.92	0.71	35.87	1.80	0.63
CV%	6.85	8.70	8.06	15.14	11.39

ns = non significance, ** = 1% level of highly significance

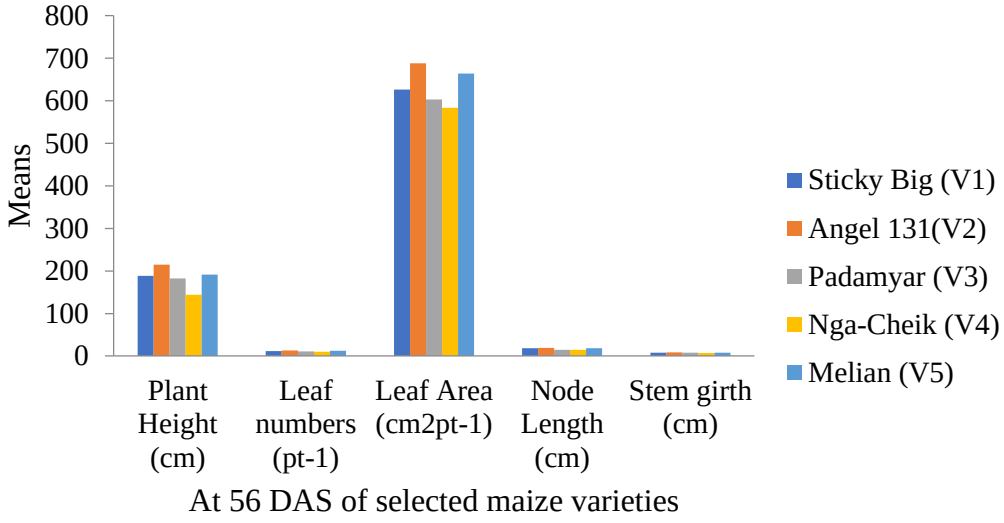


Figure1 Selected maize varieties of plant height, number of leaves, Leaf Area, Node length and Stem girth

Yield and Yield Components of selected maize varieties of *Zea mays* L.

1. **Number of kernel rows ear^{-1}**
   Among the maize varieties, Angel 131 (V2) has obtained the highest value of (16.65 ear^{-1}) followed by (Meilan) V5 (15.10 ear^{-1}), V1 (Sticky big) which was (14.40 ear^{-1}), V3 (Padamyar) which was (12.50 ear^{-1}) and the lowest number of kernel rows ear^{-1} was found (11.75 ear^{-1}) in Nga Cheik (V4).

2. **Number of kernel per rows**
   The highest mean number of kernel per rows was found in Angel 131 (V2) (36.95 row^{-1}) followed by (Meilan) V5 (32.10 row^{-1}), V1 (Sticky big) which was (31.40 row^{-1}), V3 (Padamyar) which was (24.60 row^{-1}) and the lowest number of kernel per rows was found (24.42 row^{-1}) in Nga Cheik (V4).

3. **100 grains dry weight (g)**
   The maximum number of 100 grains dry weight was found in Angel 131 (V2) which was (21.80 g) followed by (Meilan) V5 (17.47 g), V1 (Sticky big) which was (13.90 g), V3 (Padamyar) which was (13.47 g) and the minimum number of 100 grains dry weight was found (13.07 g) in Nga Cheik (V4).

4. **Total dry matter (pt^{-1})**
   The highest mean total plant dry matter per plant was found in Angel 131 (V2) which was (124.34 g) followed by (Meilan) V5 (114.63 g), V1 (Sticky big) which was (105.22 g), V3 (Padamyar) which was (99.11g) and the lowest mean total dry matter per plant was found (86.06 g) in Nga Cheik (V4).

5. **Grains Yield (gpt^{-1})**
   It was observed that the maximum grains yield in Angel 131(V2) (103.38 gpt^{-1}), followed by (Meilan) V5 (70.13 gplant^{-1}), followed by V1 (Sticky big) (62.55 gpt^{-1}), followed by V3 (Padamyar) (41.42 gpt^{-1}) and the minimum grains yield was found (39.51 gpt^{-1}) in Nga Cheik (V4).

Table 2 Yield and Yield Components of selected maize varieties of *Zea mays* L.

Varieties	V1	V2	V3	V4	V5	F-test	LSD%	CV%
No.of kernel rows ear^{-1}	14.40	16.65	12.50	11.75	15.10	**	0.46	4.70
No. of kernel per rows	31.40	36.95	24.60	24.42	32.10	**	3.39	16.0
100 grains dry weight (g)	13.90	21.80	13.47	13.07	17.47	**	0.55	7.01
Total dry matter (pt^{-1})	105.22	124.34	99.11	86.06	114.63	**	1.65	2.21
Grain yield (gpt^{-1})	62.55	103.38	41.42	39.51	70.13	**	1.43	3.34

F-test = significance, LSD 0.05% = Least significant difference of 5% level, CV% = Coefficient variation

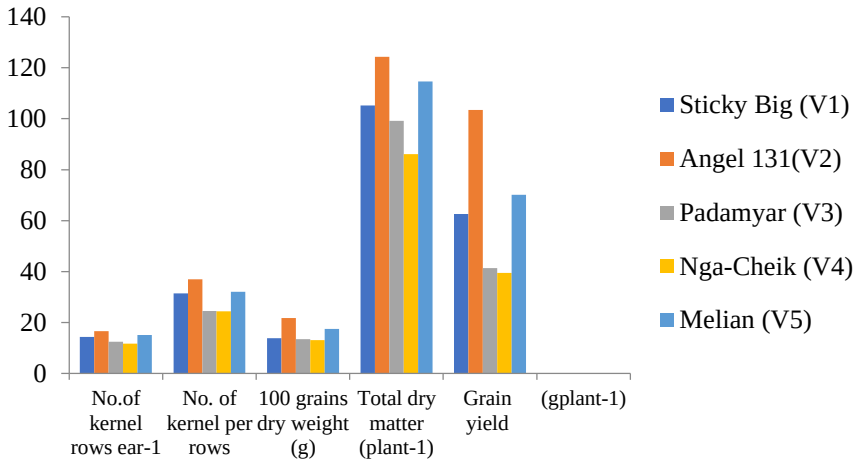


Figure 2 Yield and Yield Components of selected maize varieties of *Zea mays* L.

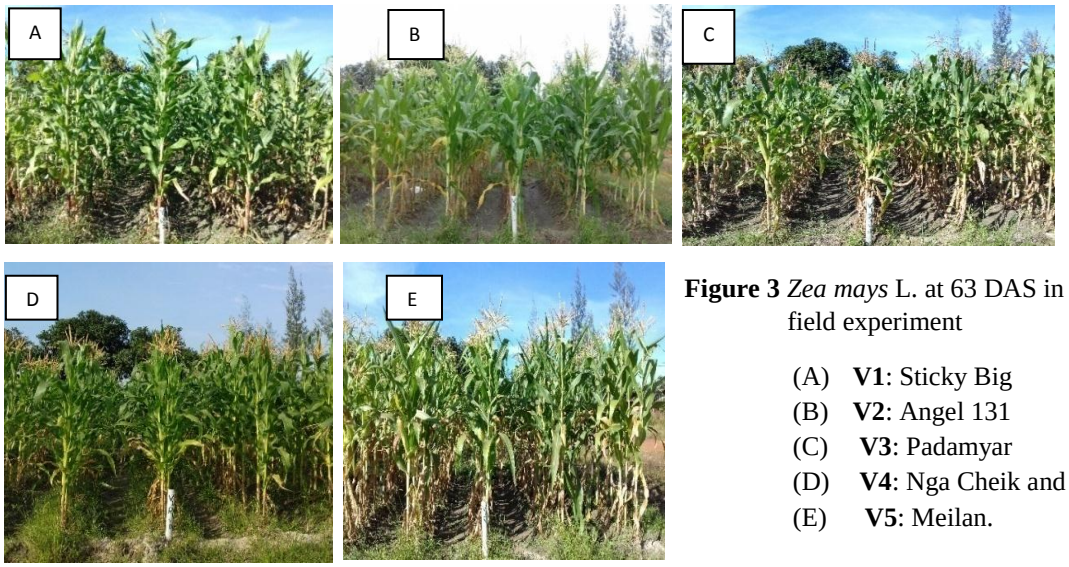


Figure 3 *Zea mays* L. at 63 DAS in the field experiment

(A) **V1**: Sticky Big
(B) **V2**: Angel 131
(C) **V3**: Padamyar
(D) **V4**: Nga Cheik and
(E) **V5**: Meilan.

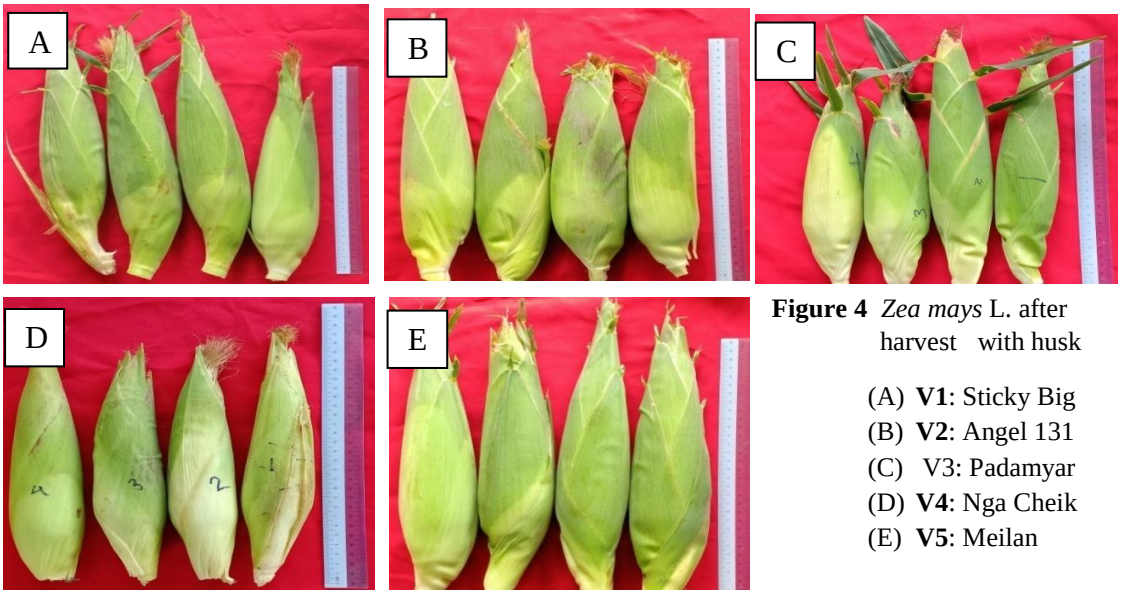


Figure 4 *Zea mays* L. after harvest with husk

(A) **V1**: Sticky Big
(B) **V2**: Angel 131
(C) V3: Padamyar
(D) **V4**: Nga Cheik
(E) **V5**: Meilan

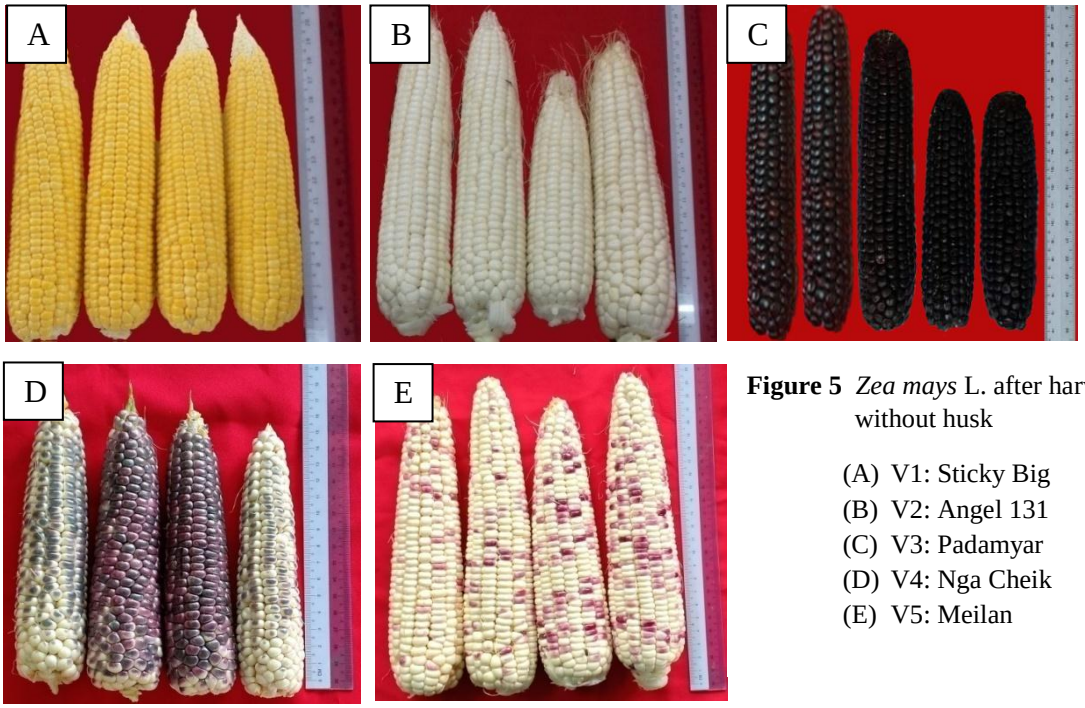


Figure 5 *Zea mays* L. after harvest without husk

(A) V1: Sticky Big
(B) V2: Angel 131
(C) V3: Padamyar
(D) V4: Nga Cheik
(E) V5: Meilan

Discussion and Conclusion

In this experiment, the soil of the cultivation area was the sandy loam, pH 6.74 and temperature of 27ċ. The plant height, leaf number, number of kernel row ear^{-1}, number of kernel row^{-1}, 100 grain weight and grain yield of the sweet corn was 215.15 cm plant^{-1}, 12.80 cm^2 plant^{-1}, 10 ear^{-1}, 16.65 row^{-1}, 21.80 g and 103.38 gplant^{-1}. However, the sweet corn grown in Iran was the temperature of 21ċ, pH of 7.8 and silty clay soil. The plant height, leaf number, number of kernel row ear^{-1}, number of kernel row^{-1}, 100 grain weight and grain yield of the Iran (sweet corn) was 142 cm plant^{-1}, 14.3 cm^2 plant^{-1}, 17.13 ear^{-1}, 37.07 row^{-1}, 40.5 g and 89.6 gplant^{-1}. The plant height, number of leaves, number of kernel row ear^{-1} and 100 grains weight of the plants in Iran (sweet corn) was higher than in this experiment (Milandan, 2015). However, the yield of Iran (sweet corn) was lesser. It may be due to the less cob number was produced in Iran (sweet corn).

Moreover, the difference in yield may be due to the different in soil type, soil pH and temperature. The preferable soil pH of sweet corn was 5-7 and the temperature was 30 ċ (www. homeguide.sfgate.com).The soil pH and temperature of this experiment was in accordance with the preferable range of reference. Therefore, the plant in this experiment produced the higher yield than the plants in Iran (sweet corn).

In terms of waxy corns, four different varieties of waxy corn were grown in sandy-loam soil of this experiment under soil ph of 6.74, temperature of 27 ċ. The plant height, leaf number, number of kernel row ear^{-1}, number of kernel row^{-1}, 100 grains weight and grain yield of these waxy corn were 143.7-191.60 cm plant^{-1}, 10.35-12.20 cm^2 plant^{-1}, 11.75-15.10 ear^{-1}, 24.42-32.10 row^{-1}, 13.07-17.47 g and grain yield 39.51-70.13 g plant^{-1}. In Vietnam, the waxy corns were grown in silt-loam soil with the pH of 5-9 and the temperature of 16.56 ċ. The plant height, leaf number, number of kernel row ear^{-1}, number of kernel row^{-1}, 100 grains weight and grain yield of these waxy corn were 90-104.3 cm plant^{-1}, 16.3-16.6 cm^2 plant^{-1}, 10.13.6 ear^{-1}, 18.2-28 row^{-1}, 15.2-18.8 g and grain yield 40-72g plant^{-1} (Pham, 2018). It was found that the yield were not as

much different the plants in this experiment and the plants in Vietnam experiment, although the soil type, soil pH and temperature were different. It was also different in the plant height and the number of leaves. Therefore, it can be assumed that waxy corns are resistant to the different soil type, soil pH and temperature.

In conclusion, the sweet corn can be grown in Hlegu area to obtain the high yield. However, the waxy corns can be grown in any areas and it may be the resistant varieties.

Acknowledgements

We would like to thanks Professor Dr Aye Pe, Head of Botany, Department, University of Yangon, for allowing us to under-taken this research by providing the department facilities throughout the study period. I also indebted to Dr Thanda Aye, Professor, Department of Botany, University of Yangon, for her invaluable suggestions and guidance to our research work.

References

AGBIOS (2005) a.**Biology of Maize**. AGBIOS Canada, available online at hppt://www. Agbios.com? estudies. php? book = ESA &ev = MON810 & Chapter = Appdx 1&1 agn.

Frova C, Krajewski P, Fonzo ND, Villa M, Sari-Gorla M. (1999). Genetic analysis of drought tolerance in maize by molecular markers. I. Yield components. Theoretical and Applied Genetics, 99: 280-288.

Gomez, K.A., and A.A. Gomez. (1984). **Statistical Procedures for Agricultural Research 2nd Edition.** John Wiley & Sons, NY.

Hailare,L., (2000). **Corn: An American Native**. Spanning the Gap.The newsletter of Delaware Water Gap National Recreation Area Vol. 22 No. 1 Spring 2000. U.S. Dept. of the Interior National Park Service.

Kandil, E.E., (2013). **Response of Some Maize Hybrids (*Zea mays* L.) to Different Levels of Nitrogenous Fertilization.** Journal of Applied Sciences Research, 9(3): 1902-1908.

Milandan, J.J.; S.C.Mason, G.k;Kruger, T.DGalusha, Z. Kmail. (2015). **Waxy maize yield and components as influenced by environment, water regime, and hybrid.** Maydica electronic publication. www.researchgate.net.

Montgeomery E. G. (1911). **Correlation Studies in Corn.Nebraska Agronomy**.Exp. Sta. Annual Report.

MPBSA, (2013). **All Rights Reserved**. Powered by Trade Myanmar Co.Ltd.

Pham Q.T., Trung N.Q., and Anh N.T.N. (2018). **Evaluation selections of purple waxy corn lines for new hybrids variety development.**

Salunkhe, D.K., and B.B. Desai. (2000)**. Post-harvest biotechnology of vegetables. Vol II, CRC press, Inc. Boca Raton, Florida.**

Sleper DA, JM Poehlman (2006). **Breeding Field Crops.**The Blackwell Publishing Inc Ames Lowa, Columbus, USA.

Yusuf. C.S., Makate N. and Jacob. R., (2014). **Effect of seed size on germination and early growth of maize (*Zea mays* L.)**

www. homeguide sf.gate.com.

DISEASE SYMPTOMS ON RICE PLANTS AND IDENTIFICATION OF THE PATHOGENS CAUSING DISEASES

Nway Nandar Aung[1], Kathy Myint[2], Bay Dar[3]

Abstract

The sample of diseased rice plants were collected from Tamartakaw village, Twan-tay Township. The pathogenic fungi were identified by their pure colony morphology and spores formation. In the present work, two different types of disease symptoms and two different kinds of pathogenic fungi such as *Fusarium* sp. (1) which causes Narrow Brown Leaf Spot and *Mucor* sp. (1) which causes Blight were studied on *Oryzasativa* L. (That-gyi Saba). In *Oryzasativa* L. Thatt-lat Saba, *Gloeosporium*sp. (1) that caused White Spot, *Mucor*sp. (2) that caused Blight, *Gloeosporium*sp. (2) that caused Brown Spot, *Rhizoctonia* sp. (1) that caused Leaf Scald , *Rhizoctonia* sp. (2) that caused Sheath Blight and *Fusarium* sp. (2) that caused Grain Discoloration, In the case of *Oryza sativa* var. *glutinosa* (Kaung-nyin, That-Nge Saba), 4 different types of disease symptoms and 4 different kinds of pathogenic fungi were investigated these include Leaf Stripe caused by *Rhizoctonia* sp. (3), Leaf Streak caused by *Rhizoctonia* sp. (4), Blight caused by *Mucor* sp. (3) and Black Kernel caused by *Gloeosporium* sp. (3), respectively.

Keywords: Rice plant, *Oryza sativa*, *Mucor* sp., *Fusarium* sp., *Gloeosporium*sp., *Rhizoctonia* sp.

Introduction

Rice is the important staple food for Myanmar 48 million peoples of which 75% directly depend on farming (Soe Soe Thein *et al*., 2002). On the average each person in Myanmar eats 195 kg of rice annually (Soe Soe Thein *et al*., 2002).

From East Asia, rice was spread to Southeast and South Asia. Rice (*Oryzasativa*) is a major staple food for approximately two-third of the world's population. More than 90% of the world's rice is both grown and consumed in developing countries. Rice cultivation can be grown practically anywhere and is well-suited to countries and regions (Vauhgn and Stich, 1991).

More than 27 diseases caused by microbes are common in rice plants. Widespread diseases, which cause severe yield losses, include Blight (caused by *Xanthomonasoryzae or Rhizoctoniasolani*), Blast (caused by *Magnaporthegrise*) and Sheath Blight (caused by *Rhizoctoniasolani*) (web 1).

Most damaging pests are rice stink bug (*Oebaluspugnax*), flea beetle and aphids. Other important diseases (Miscellaneous diseases) are alkalinity or salt damange (caused by excessive salt concentration in soil or water), bronzing (Zinc deficiency) and cold injury (low temperature) (web 1).

Methods

Collection of disease symptoms from rice plants

The fungal pathogens were collected from diseased rice plants from the field of Tamartakaw village, Twan-Tay Township.

[1] PhD Candidate, Department of Botany, University of Yangon
[2] Lecturer, Department of Botany, University of Yangon
[3] Professor, Department of Botany, University of Yangon

Figure 1 The study site of Tamartakaw village, Twan-Tay Township

Isolation of fungal pathogen

Pathogens were isolated and cultured from diseased specimens before they can be identified. For surface lesions on leaves and stems small pieces of diseased tissue of a few cubic millimeters excised from the lesion margin were sterilized in 70% alcohol for 3 mins. And then it was placed on to the agar growth medium in sterile petridishes. When fungi were growing on the surface of the agar medium a pure culture can be obtained by direct transfer to a growth medium.

The developing fungal colony was examined after 5-7 days growing and then isolated into slant test tube with Potatoes Dextrose Agar medium. Subcultures of fungal isolates were carried out several times successfully with Potatoes Dextrose Agar slants medium until the pure culture was obtained. Pure culture was maintained at room temperature and subculture was prepared every twice a month. Pure colonies were cultured in the petridishes with Potatoes Dextrose Agar medium to examine color of colony and spore formation.

Identification and classification of disease symptoms

The disease symptoms were classified and identified according to Roberts (1984).

Identification and classification of fungal pathogens

Isolated medium

It was cultured on PDA medium according to Atlas, 1993.

PDA medium

Mash Potato	- 200 g
Peptone	- 3 g
Dextrose	- 20 g
Agar	- 20 g
Distilled water	- 1000ml

(Add Chloramphenicol (0.001/1) for antibacterial activity)

Preparation of PDA medium

The potato was peel off and weigh 200g and cut into small pieces and put in beaker with 1000 ml distilled water. It was boiled the contents for about 30 minutes and collected the extract. Transfer dextrose 20g and agar 20 g were added into extract and gently heat and shake to dissolve the ingredients and adjusted to 1000 ml with distilled water pour the medium was

poured into two or more Elenmyer flasks, put cotton plug, covered with aluminum foil and autoclave at 121°C for 20 minutes.

Culture and stock medium

It was cultured on Czapek (Dox) Agar medium according to Ronald, 1993.

Czapek (Dox) Agar medium

$NaNO_3$ - 0.2 g

K_2HPO_4 - 0.1 g

$MgSO_4$ - 0.05 g

KCl - 0.05 g

$FeSO_4$-$7H_2O$ - 0.001 g

Sucrose - 3.0 g

Agar - 2.0 g

Distilled water - 100 ml

pH 7.3 at 25°C

Identification and classification of pathogens

The pathogens were identified and classified according to Barnett (1960) and Dube (1983).

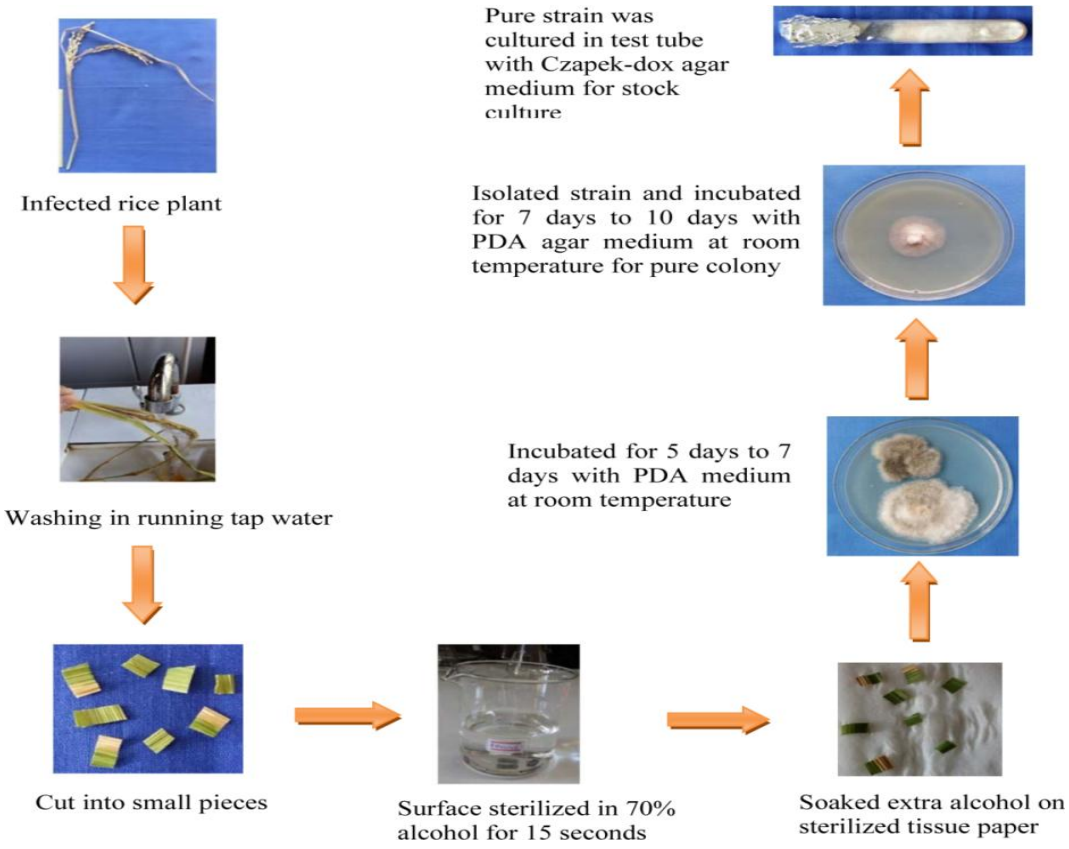


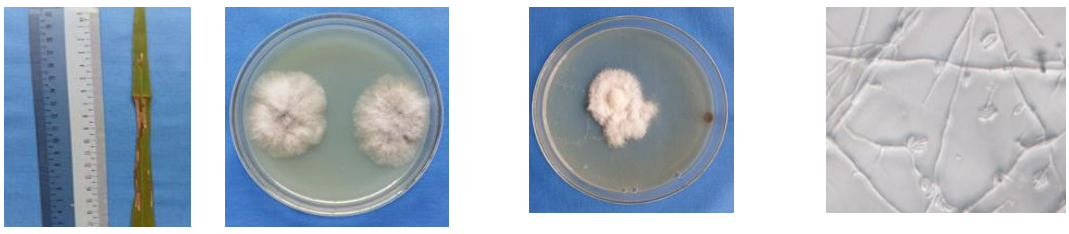
Figure 2 Isolation procedure of fungal pathogen from disease symptom (George, 1978)

Results

In this study, total disease symptoms are seven in two different varieties of rice. Two different types of disease symptoms and two different kinds of pathogenic fungi were occurred in *Oryzasativa* L. (That-gyi Saba) such as Narrow Brown Leaf Spot caused by *Fusarium* sp. (1) and Blight caused by *Mucor* sp. (1). Five different types of disease symptoms and 5 different kinds of pathogenic fungi such as Blight caused by *Mucor*sp. (2), Brown Spot caused by *Gloeosporium*sp., Leaf Scald caused by *Rhizoctonia* sp. (1), Sheath Blight caused by *Rhizoctonia* sp. (2) and Grain Discoloration caused by *Fusarium* sp. (2) were examined from diseased rice plants *Oryzasativa* L. (That-latt Saba).

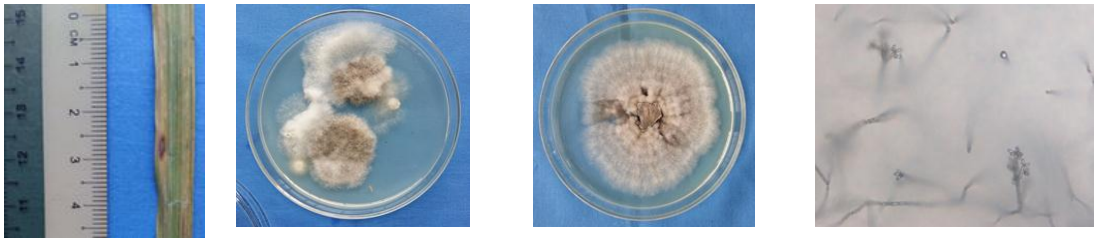
Table 1 Characters of pathogenic fungi on diseased rice plants from cultivated field of Tarmartakaw village, Twan-Tay Township

No	Variety of Rice	Disease Symptoms	Species
1.	*Oryza sativa* L. (ThatGyi Saba)	Narrow Brown Leaf Spot	*Fusarium* sp.(1)
		Leaf Blight	*Mucor* sp. (1)
2.	*Oryza sativa* L. (That-latt Saba)	Leaf Blight	*Mucor* sp.(2)
		Brown Spot	*Gleosporium* sp.
		Leaf Scald	*Rhizoctonia* sp.(1)
		Sheath Blight	*Rhizoctonia* sp.(2)
		Grain Discoloration	*Fusarium* sp.(2)
3.	*Oryza sativa ver. glutinosa*. (Kaung Nyin Saba) (Thet Nge Saba)	Leaf Streak	*Rhizoctonia* sp. (3)
		Leaf Stripe	*Rhizoctonia* sp. (4)
		Blight	*Mucor* sp.(3)
		Black Kernel	*Gleosporium* sp.(3)



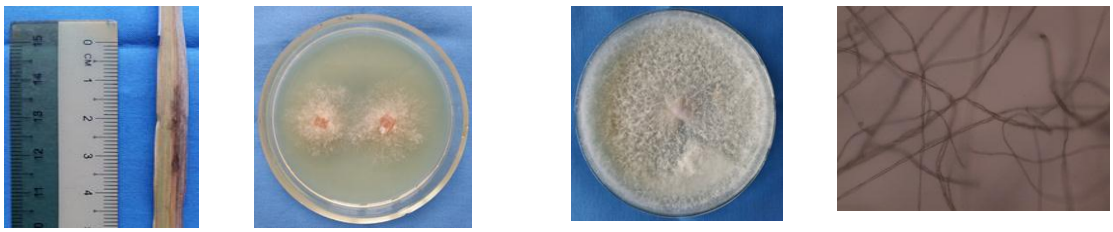
a b c d

Figure 3 Narrow Brown Leaf Spot on ***Oryza sativa*L**. (TaungPyanGyi); a.Disease symptom of Narrow Brown Leaf Spot, b. Fungal isolated from Narrow Brown Leaf Spot (5-7 days old culture) on PDA, c.Pure fungal colony from Narrow Brown Leaf Spot disease (white color) (5-7 days old culture) on CzapekDox, d. Micrograph of *Fusarium*sp. (1)



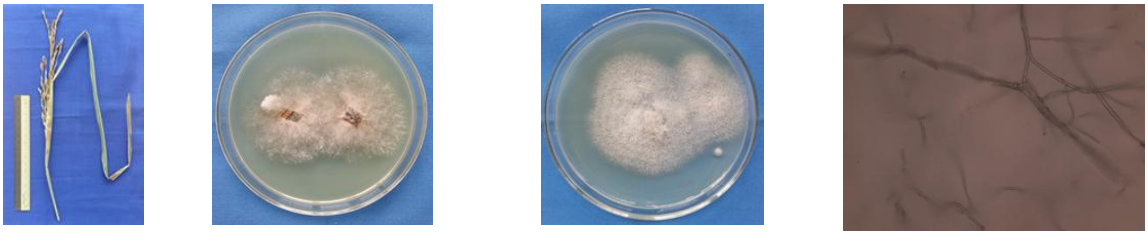
a b c d

Figure 4 Brown Leaf Spot on ***Oryza sativa*** **L**. (TaungPyan yin); a.Disease symptom of Brown Leaf Spot, b. Fungal isolated from Brown Leaf Spot (5-7 days old culture) on PDA, c. Pure fungal colony from Brown Leaf Spot disease white color) (5-7 days old culture) on CzapekDox, d. Micrograph of *Gloeosporium* sp.



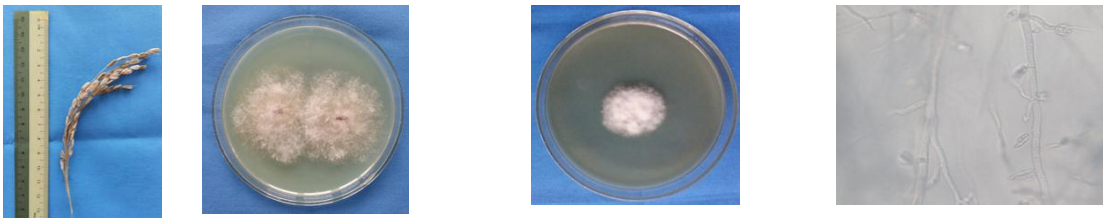
a b c d

Figure 5 Leaf Scald on ***Oryza sativa*** **L**. (Taung Pyan yin); a. Disease symptom of Leaf scald, b. Fungal isolated from Narrow Brown Leaf Spot (5-7 days old culture) on PDA, c. Pure fungal colony from Narrow Brown Leaf Spot disease (white color) (5-7 days old culture) on CzapekDox, d. Micrograph of *Gloeosporium* sp.



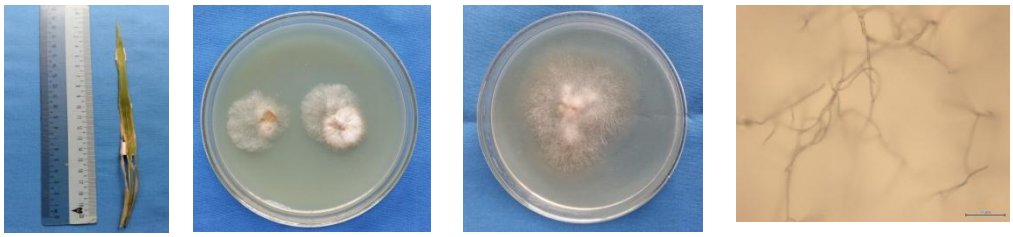
a b c d

Figure 6 Sheath Blight on ***Oryza sativa*** **L**. (Taung Pyan yin); a. Disease symptom of Sheath Blight b. Fungal isolated from Sheath Blight (5-7 days old culture) on PDA, c. Pure fungal colony from Sheath Blight disease (white color) (5-7 days old culture) on CzapekDox, d. Micrograph of *Gloeosporium* sp.



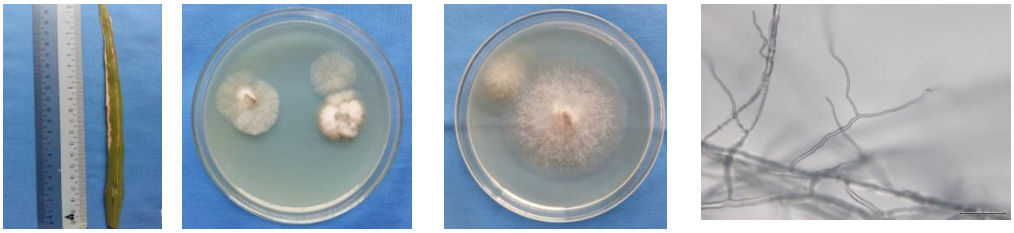
a b c d

Figure 7 Grain Discoloration on ***Oryza sativa*** **L**. (TaungPyan yin); a.Disease symptom of Grain Discoloration, b. Fungal isolated from Grain Discoloration (5-7 days old culture) on PDA, c. Pure fungal colony from Grain Discoloration disease (white color) (5-7 days old culture) on CzapekDox, d. Micrograph of *Fusarium* sp. (2)



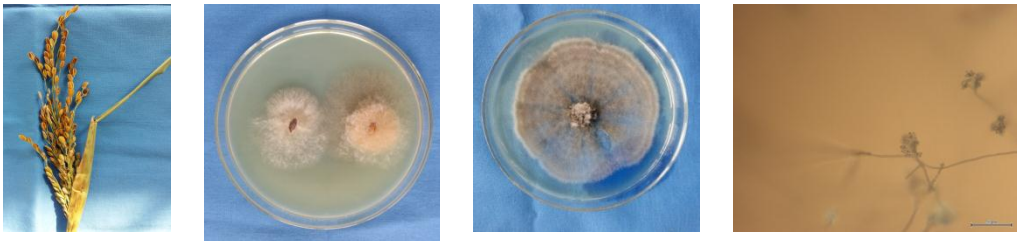
a b c d

Figure 8 Leaf streak on ***Oryza sativa* ver. *glutinosa*** (Kaung nyin); a. Disease symptom of Leaf streak, b. Fungal isolated from Leaf streak (5-7 days old culture) on PDA, c. Pure fungal colony from Leaf streak disease (white color) (5-7 days old culture) on CzapekDox, d. Micrograph of *Rhizotonia* sp. (3)



a b c d

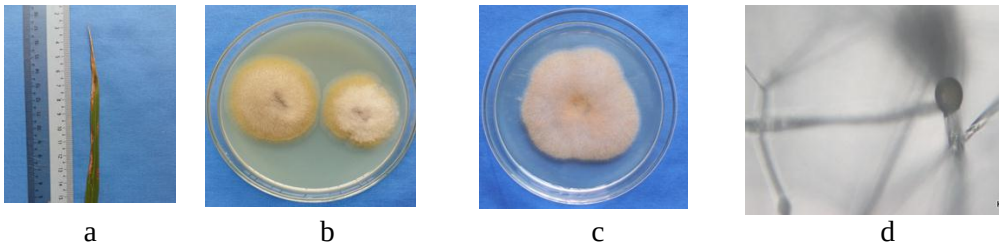
Figure 9 Leaf stripe on ***Oryza sativa* ver. *glutinosa*** (Kaung nyin); a. Disease symptom of Leaf stripe, b. Fungal isolated from Leaf stripe (5-7 days old culture) on PDA, c. Pure fungal colony from Leaf stripe disease (white color) (5-7 days old culture) on CzapekDox, d. Micrograph of *Rhizotonia* sp. (4)



a b c d

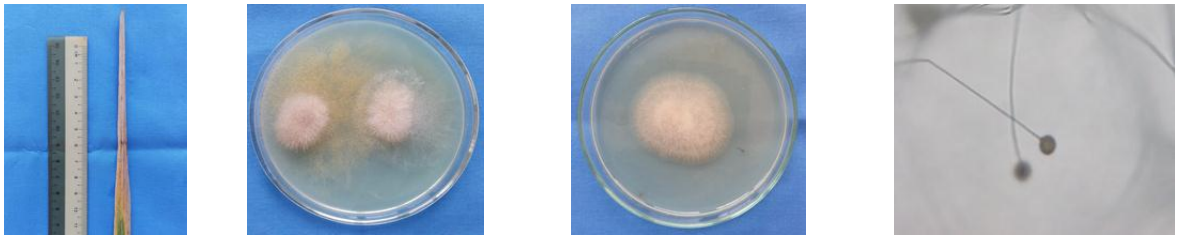
Figure 10 Black kernel on ***Oryza sativa* ver. *glutinosa*** (Kaung nyin); a. Disease symptom of Black kernel, b. Fungal isolated from Black kernel (5-7 days old culture) on PDA, c. Pure fungal colony from Black kernel disease (white color) (5-7 days old culture) on CzapekDox, d. Micrograph of *Gleosporium sp. (2)*

According to the result the disease symptoms of Leaf Blight caused by *Mucor* sp. was isolated from three varieties of rice.



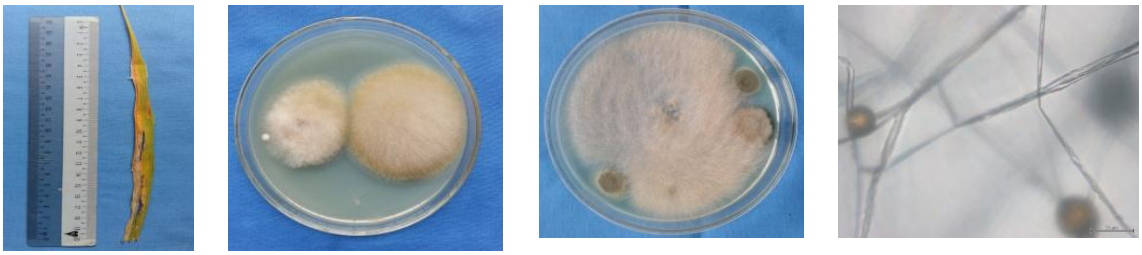
a b c d

Figure 11 Leaf Blight on ***Oryza sativa* L.** (TaungPyanGyi); a.Disease symptom of Leaf Blight, b. Fungal isolated from Leaf Blight (5-7 days old culture) on PDA, c. Pure fungal colony from Leaf Blight disease (white color) (5-7 days old culture) on CzapekDox, d. Micrograph of *Mucor*sp. (1)



A b c d

Figure 12 Leaf Blight on ***Oryza sativa* L.** (Taung Pyan yin); a. Disease symptom of Leaf Blight, b. Fungal isolated from Leaf Blight (5-7 days old culture) on PDA, c. Pure fungal colony from Leaf Blight disease (white color) (5-7 days old culture) on CzapekDox, d. Micrograph of *Mucor*sp. (2)



a b C d

Figure 13 Leaf Blight on ***Oryza sativa* ver *glutinosa***. (Kaug nyin); a. Disease symptom of Leaf Blight, b. Fungal isolated from Leaf Blight (5-7 days old culture) on PDA, c. Pure fungal colony from Leaf Blight disease (white color) (5-7 days old culture) on CzapekDox, d. Micrograph of *Mucor*sp. (3)

Discussion and Conclusions

In this study, the diseased rice plants were examined according to the disease symptoms which were found in field of Tamartakaw village, Twan-Tay Township, Yangon Region, in Myanmar. The disease symptoms rice plants were identified according to Roberts, 1984.

The fungi were isolated and cultured on potato dextrose agar (PDA) medium and CzapekDox agar medium for pure and stock culture. And then the pathogens were classified and identified by morphological characters of pure colony and spore formation by Barnett, 1960.

In the present study, two different types of disease symptoms and two different kinds of pathogenic fungi such as Narrow Brown Leaf Spot caused by *Fusarium* sp. (1) and Blight caused by *Mucor* sp. (2) on *Oryza Sativa* L. (Thet-gyi). Six different types of disease symptoms and 6 different kinds of pathogenic fungi such as, disease symptom of Blight caused by *Mucor*sp. (2), Brown Spot caused by *Gloeosporium* sp., Leaf Scald caused by *Rhizoctonia* sp. (1), Sheath Blight caused by *Rhizoctonia* sp. (2) and Grain Discoloration caused by *Fusarium* sp. (2), respectively, were examined from diseased rice plants (*Oryzasativa* L., That-latt Saba, Four different kinds of disease symptoms and 4 different kinds of pathogenic fungi such as Leaf Stripe caused by *Rhizoctonia* sp. (3), Leaf Streak caused by *Rhizoctonia* sp. (4), Blight caused by *Mucor* sp. (3) and Black Kernal caused by *Gloeosporium* sp. (3), respectively, were examined from diseased rice plants (*Oryza sativa ver glutinosa*), respectively.

The fungal disease symptoms may be occurred about 25-35 days after planting of rice seedling. Leaf Blight disease was most important diseases that produce heavy losses to the farmer.

Acknowledgements

I am greatly indebted to Dr. Aye Pe, Professor and Head, Department of Botany, University of Yangon, for his guidance and suggestion to carry out this research. I also wish to thank Dr. Thanda Aye, Professor, Department of Botany, University of Yangon for her valuables suggestions. I also indebted to supervisor Dr. Bay Dar, Professor, Department of Botany, University of Yangon for her kind guidance and supervision. And I also wish to thank to Dr. Yay Chan, Professor and Head, Universities' Research Center, University of Yangon for his valuable advice.

References

Atlas, R.M. (1993) **Handbook of Microbiological Media** CRC Press, London.

Barneth, H. L (1960) **Illustrated Genera of Imperfect Fungi. Second Edition. Department of Plant Pathology, Bacteriology and Entomology**. West Virginia University Morgantown, West Virginia.

Bonman, J. M, B. A. Estrada, J. M. Banding (1989) **Leaf and neck Blast resistance in tropical lowland rice cultivars**. *Plant Dis., 73:388-390*

George,N. A. 1978. **Plant Pathology, Second Edition**. Academic press. INC. Printed in the United States of America. Page_ 186.

Jia Y, S. A. M. Adams, G. T. Bryan, H.P. Hershay and B. Valent, 2000.**Direct interaction of resistance genes products confers rice blast resistance**. *Embo*. J., **19**: 4004-4014.

Roberts, D. A and Boothryd, C. W. 1984. **Fundamentals of Plant Pathology**. Second Edition, Printed in the United States of America.

Ronald, M. (1993) **Handbook of Microbiological Media**. International Standard Book Number 0-8493-2944-2, United States of America.

SoeSoeThein, Tin Aye AyeNaing, M. Finckh and A. Bnerkert, 2002. Effects of Increasing Cropping Iintensity on Rice Production in Myanmar-International Symposium.

Tessa, R.S. (2002) **Piony rice savants win int'l agri*prize***.*Inquirer News Survice*.

Tirmali, A. M, S. B. Latake, N. J. Bendra (2001**) Evaluation of new fungicides for control of Blast disease of rice**. *J.Maharashra Agri. Univ*., **26**: 197-198

Vaughn, D.A and L. A. stitch (1991) **Gene flow from the jungle to farmers**. Bio.Science 41:22-28

FERMENTATION STUDIES OF *CEPHALOSPORIUM* SP. ISOLATED FROM *HESPERETHUSA CRENULATA* (ROXB.) ROEM.

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

Abstract

Endophytic fungal strain *Cephalosporium* sp. was isolated from the wood of *Hesperethusa crenulata* (Roxb.) Roem. For the fermentation conditions such as various carbon and nitrogen sources, culture media, age of inoculum, size of inloculum and pH utilization of this strain *Cephalosporium* sp. were conducted at Microbiology Lab, Department of Botany, University of Yangon. In utilization of carbon sources, honey and sucrose were good whereas yeast extract, meat extract and malt extract were the best nitrogen sources. In antimicrobial activity of various carbon sources, sucrose medium showed very high activity whereas various nitrogen sources, yeast extract medium indicated very high activity against eight test organisms. In the investigation of the morphological characters on various media, the seven cultural media were good media. According to the result of antimicrobial activity on various media, sucrose/yeast extract medium was the best for fermentation. 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 from this strain. In the study of pH utilization, pH 7 was the best for extraction of the bioactive compounds.

Keywords: *Cephalosporium* sp., Fermentation studies, *Hesperethusa crenulata*

Introduction

Fermentation procedures have to be developed for the cultivation of microorganisms under optimal conditions and the production of desired metabolites or enzymes by microorganisms. A clear understanding of microbial growth kinetics is necessary if a large-scale process is to be properly managed. The physiological condition of the inoculum is crucial to the length of the lag phase. The proper cultivation and transfer of inoculums are essential for the production of both primary and secondary metabolites. The pure culture (seed culture) media and culture conditions often have to be designed for optimal yields. However, the kinetics of product formation is not necessarily correlated with the length of the lag phase (Yamane, 1984).

Seed culture must be made in order to have enough inoculum for a large fermenter. If a production fermenter is starter with too little inoculum, growth is delayed and the product formation rate can be unsatisfactory. The optimal inoculum concentration for the production determines the number of stages of seed culture that are required (Gaden, 1959).Optimal fermentation conditions such as proper kinetic growth (ages and sizes of inoculum) are very important for the production of metabolites (Omura, 1985).

Several media which must be optimized are composition of ingredients, quality, carbon and nitrogen relationship, impurities, variability from batch to batch, order of solution or suspension of ingredients, pH value before and after sterilization on the entire nutrient solution or on individual components, and changes in the sterilized nutrient solution before inoculation due to increase in temperature and aeration (Malek, 1984).

The objectives of this study are to study the utilization of carbon and nitrogen sources, to evaluate antimicrobial activity of *Cephalosporium* sp. on eight test organisms by using various carbon and nitrogen sources, to conduct the morphological characters and antimicrobial activity

[1] 2PhD MB-1, Department of Botany, University of Yangon
[2] Associate Professor, Department of Botany, University of Yangon

of *Cephalosporium* sp. on various media, to investigate the optimal fermentation studies such as age of inoculum, size of inoculum and pH utilization of *Cephalosporium* sp..

Materials and Methods

Utilization of carbon and nitrogen sources

In this study the morphological characters of *Cephalosporium* sp. were studied by using various carbon and nitrogen sources. Carbon sources are glycerol, glucose, sucrose, mannitol, honey and starch, whereas nitrogen sources are peptone, yeast extract, meat extract, malt extract, oat meal and soy bean. Basal media for 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 nitrogen sources are glycerol 1.0%, K_2HPO_4 0.01%, $MgSO_4$ 0.01% and $CaCO_3$ 0.01% (Monaghan, 1999).

Antimicrobial activity of *Cephalosporium* sp. by using various carbon and nitrogen sources

Twelve 50mL flasks containing 25mL of various carbon and nitrogen sources were utilized. A small piece of fungal strain grown on slant culture was transferred into each flask. Twelve flasks were incubated on shaker at 180rpm for fourteen days. The fermented broth in each flask was used to check antimicrobial activity by paper disc diffusion assay (Monaghan, 1999; Phay, 1997).

Morphological characters of *Cephalosporium* sp. on various media

In this study various media were employed for media optimization. A piece of fungus from plate culture of strain (*Cephalosporium* sp.) was inoculated in each of various media plates and incubated for 5-7 days. 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), medium 11 (Sucrose, Malt extract, Soluble starch medium) and medium 12 (Honey medium) (Dubey & Maheshwari, 2009).

Antimicrobial activity of *Cephalosporium* sp. by using various media

A fungal piece from plate culture of strain (*Cephalosporium* sp.) was inoculated into each of twelve (50mL) conical flasks containing 25mL of various fermentation media. These flasks were incubated at 180rpm 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).

Age of inoculum for *Cephalosporium* sp.

One day old, two days old and three days old of seed culture were transferred into 250ml fermentation flasks containing 100mL of sucrose/yeast extract medium respectively. They were incubated for eight days. Then, these fermented broths were checked for their inhibitory activity by paper disc diffusion assay (Monaghan, 1999).

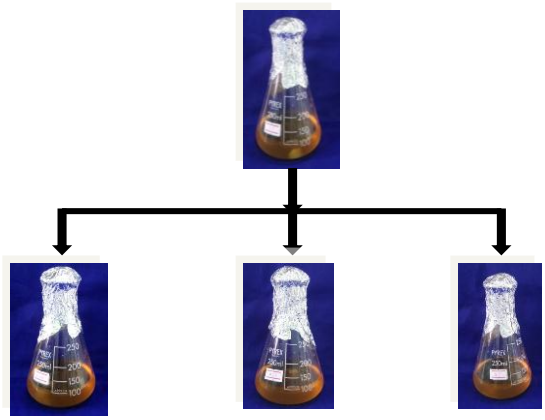


Figure 1 Fermentation flasks for age of inoculum

Size of inoculum for *Cephalosporium* sp.

The proper cultivation and transfer (size of inoculum) are essential for the production of bioactive metabolites. A piece from fungal plate culture of strain *Cephalosporium* sp. was inoculated into 250mL of conical flasks containing 100mL of SY seed medium. The flasks were incubated at 30^{o}C 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 the seven conical flasks (250mL) containing 100mL of fermentation medium. The fermentation was carried out for ten days (Monaghan, 1999).

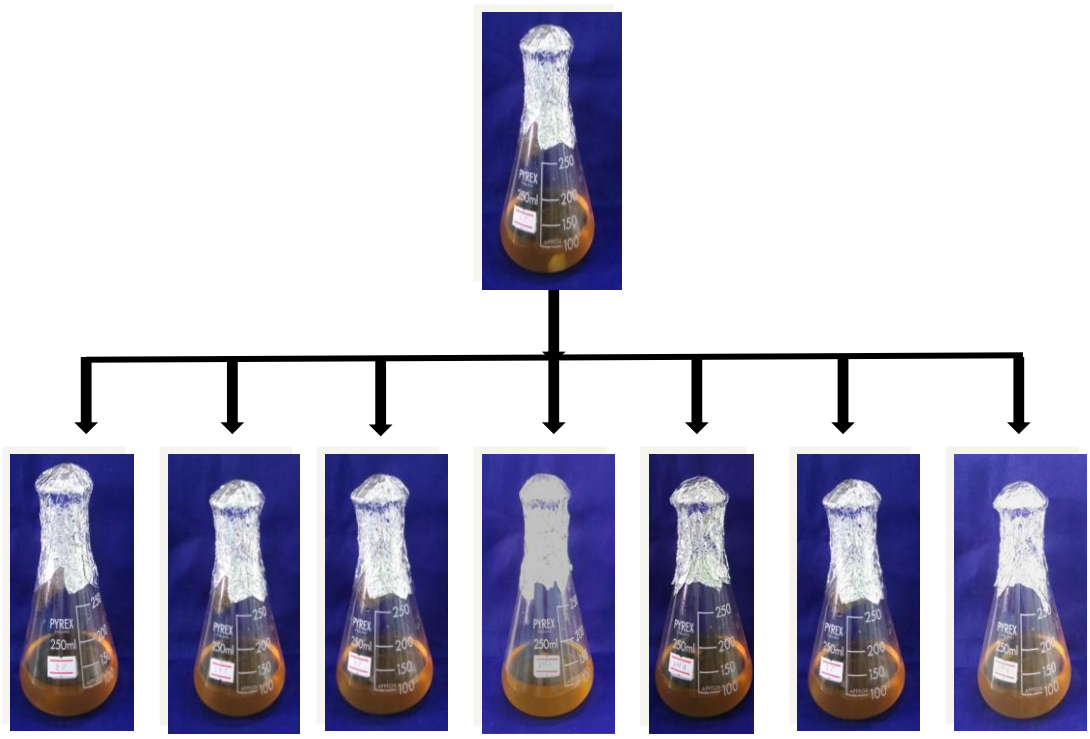


Figure 2 Fermentation flasks for size of inoculum

pH utilization for *Cephalosporium* sp.

For the seed culture, a piece from fungal plate culture of strain (*Cephalosporium* sp.) was inoculated into 250mL of conical flask containing 100mL of SY medium and then the flasks were incubated at room temperature for two days. The seven 300mL conical flasks containing 100mL fermentation medium in each 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 2 days. After 2 days, these flasks were checked antimicrobial activity (Monaghan, 1999).

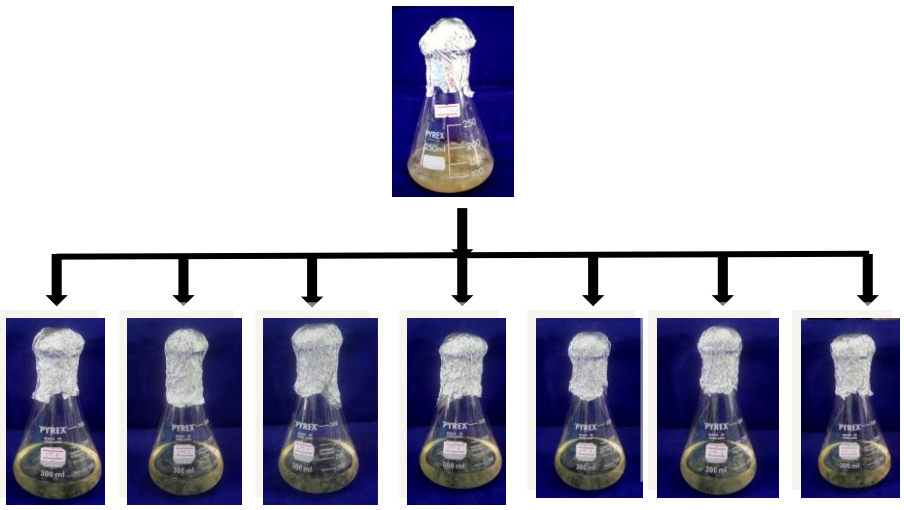
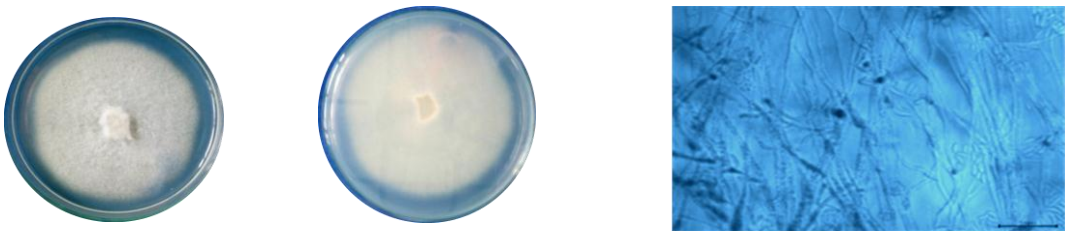


Figure 3 Fermentation flasks for pH utilization

Results

Morphological and microscopic characters of isolated strain *Cephalosporium* sp.

The surface color and reverse color of *Cephalosporium* sp. was the same color white. It shows conidia (phialospores) hyaline, 1 celled, septate hyphae which are typically very fine and narrow. They usually appear in clusters, in balls or rarely as fragile chains. It is identified as *Cephalosporium* sp. as shown in Figure 4.



Surface view Reverse view

Figure 4 Morphological and microscopic characters of *Cephalosporium* sp.

Carbon utilization

Among carbon sources, honey medium was the best carbon sources whereas soluble starch medium was also suitable for fermentation as shown in Table 1 and Figures 5.

Table 1 Morphological characters of *Cephalosporium* sp. on various carbon sources

No.	Carbon sources	Growth	Surface color	Reverse color
C1	Glucose	Poor	White	White
C2	Sucrose	Poor	White	White
C3	Soluble starch	Moderate	White	White
C4	Glycerol	Poor	White	White
C5	Mannitol	Poor	White	White
C6	Honey	Good	White	White

Figure 5 Morphological characters of *Cephalosporium* sp. on various carbon sources

Nitrogen utilization

In nitrogen sources, meat extract, malt extract and yeast extract media were the best nitrogen sources as shown in Table 2 and Figure 6.

Table 2 Morphological characters of *Cephalosporium* sp. on various nitrogen sources

No.	Nitrogen sources	Growth	Surface color	Reverse color
N1	Peptone	Poor	White	White
N2	Oat meal	Poor	White	White
N3	Meat extract	Good	White	White
N4	Malt extract	Good	White	White
N5	Yeast extract	Good	White	White
N6	Soybean	Poor	White	White

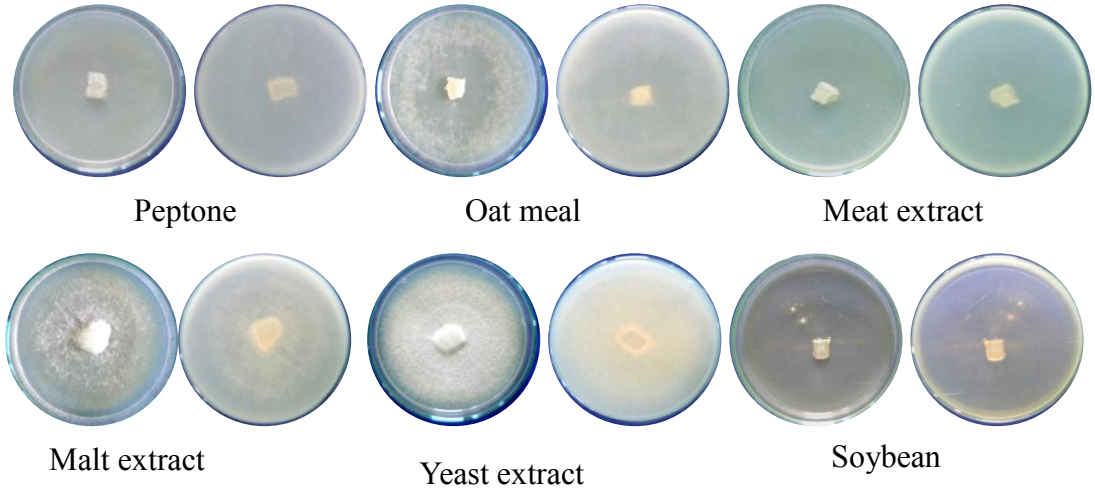


Figure 6 Morphological characters of *Cephalosporium* sp. on various nitrogen sources

Antimicrobial activity of *Cephalosporium* sp. on carbon sources and nitrogen sources

Fermented broths of *Cephalosporium* sp. in four carbon media C1, 2, 3 and 6 showed very high antimicrobial activity on eight test organisms from 2 to 7 days. Fermented broth of *Cephalosporium* sp. in mannitol medium exhibited weak activity on some test organisms from 2 to 5 days while fermented broth of *Cephalosporium* sp. in glycerol medium was also indicated weak activity on *Agrobacterium tumefaciens* and *Aspergillus flavus* on 4th and 5th days. Fermented broths of *Cephalosporium* sp. in four nitrogen media N1, 2, 4 and 5 exhibited very high activity on eight test organisms from 2 to 8 days. Fermented broth of *Cephalosporium* sp. in nitrogen medium (meat extract) indicated weak activity on eight test organisms from 2 to 6 days, but soybean medium did not show any activity as shown in Table 4 & 5 and Figure 7.

Table 3 Inhibitory zones of fermented broths of *Cephalosporium* sp. on various carbon sources at 7th day fermentation

Carbon sources T.O	C 1	C 2	C 3	C 6
Agrobacterium tumefaciens	18	**18**	12	17
Aspergillus flavus	16	**20**	12	19
Bacillus subtilis	17	**13**	13	18
Candida albicans	22	**25**	14	20
Malassezia furfur	18	**23**	13	23
Micrococcus luteus	17	**23**	13	18
Salmonella typhi	14	**22**	-	17
Xanthomonas oryzae	19	**23**	13	25

Table 4 Inhibitory zones of fermented broths of *Cephalosporium* sp. on various nitrogen sources at 7th day fermentation

Nitrogen sources T.O	N 1	N 2	N 4	N 5
Agrobacterium tumefaciens	13	20	17	**21**
Aspergillus flavus	11	19	19	**21**
Bacillus subtilis	15	19	20	**21**
Candida albicans	11	19	22	**25**
Malassezia furfur	11	21	15	**21**
Micrococcus luteus	10	17	17	**21**
Salmonella typhi	12	21	16	**25**
Xanthomonas oryzae	-	20	21	**23**

10-12 mm = weak activity, 13-17 mm = high activity, >18 mm = very high activity, Disc size = 6 mm, T.O = Test organism

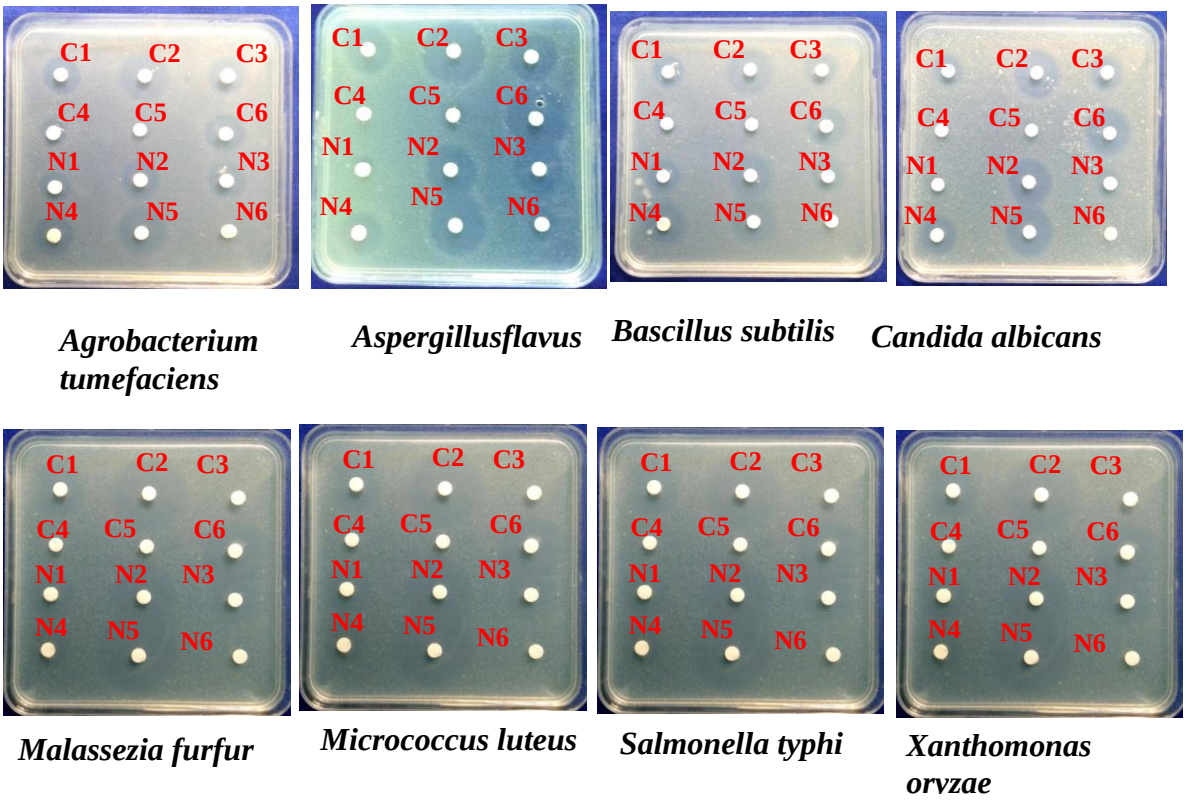


Figure7 Inhibitory zones of *Cephalosporium* sp. on various carbon and nitrogen sources against eight test organisms

Morphological characters of *Cephalosporium* sp. on various media

In the investigation of morphological characters of *Cephalosporium* sp. on various media, seven media (1, 3, 5, 7, 9, 10 and 11) were good whereas *Cephalosporium* sp. showed moderate growth on medium 2 and also its showed poor growth on media 4, 6, 8 and 12. Surface and reverse colors of *Cephalosporium* sp. on various media were white, but reverse color of medium 3 was pale yellow and that of medium 10 was yellow as shown in Table 5 and Figure 8.

Table 5 Morphological characters of *Cephalosporium* sp. on various media

Media	Various media	Growth	Surface color	Reverse color
M 1	Peptone/Yeast	Good	White	White
M 2	Meat/Polypeptone/NaCl	Moderate	White	White
M 3	Yeast/Malt/Glucose	Good	White	Pale yellow
M 4	Glycerol/K_2HPO_4/$MgSO_4$/NaCl	Poor	White	White
M 5	Oat Meal	Good	White	White
M 6	Glycerol/K_2HPO_4	Poor	White	White
M 7	Soybean/Mannitol	Good	White	White
M 8	K_2HPO_4/$MgSO_4$/NaCl	Poor	White	White
M 9	Sucrose/Yeast/NaCl/$CaCO_3$	Good	White	White
M 10	Malt/Meat	Good	White	Yellow
M 11	Sucrose/Malt/Starch	Good	White	White
M 12	Honey	Poor	White	White

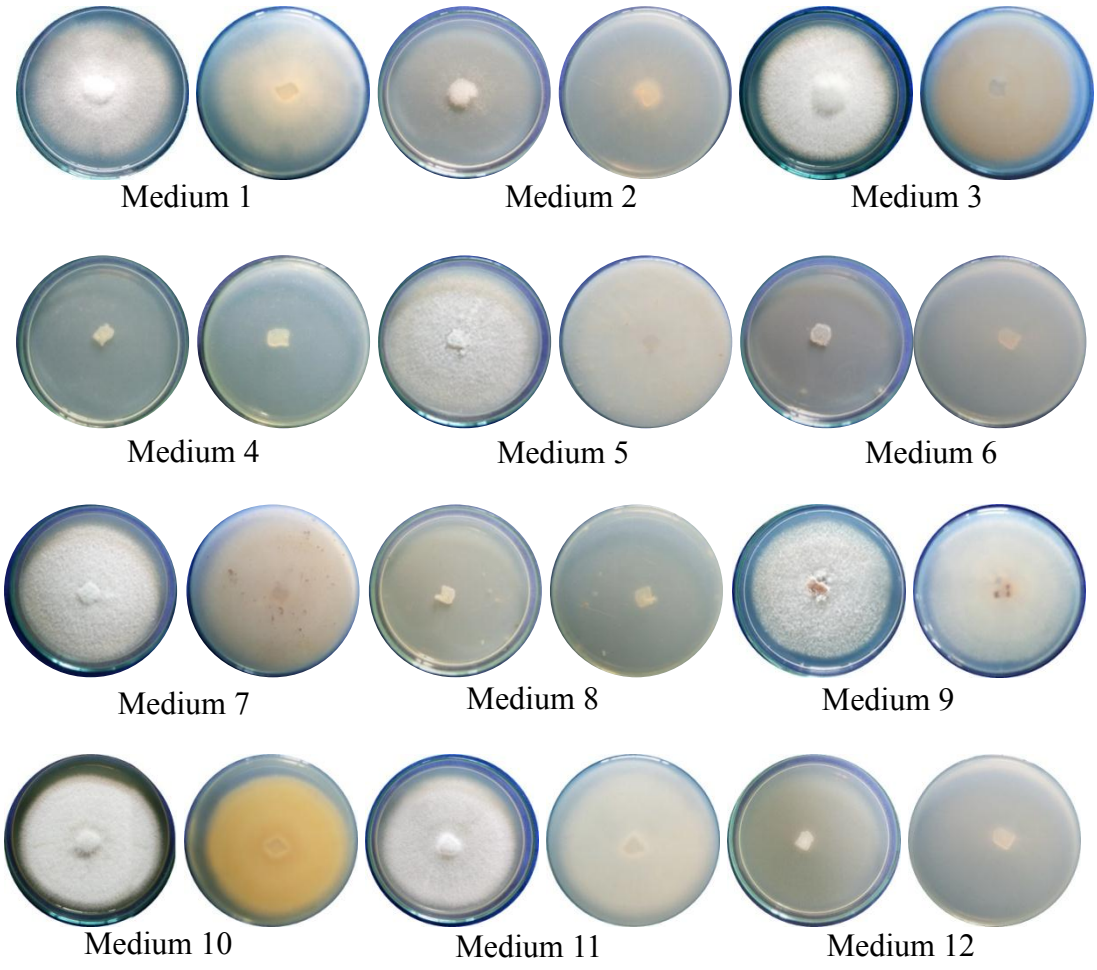


Figure 8 Morphological characters of *Cephalosporium* sp. on various media

Antimicrobial activity of *Cephalosporium* sp. on various media

Strain (*Cephalosporium* sp.) in medium 11 (sucrose/malt/soluble starch) and medium 9 (sucrose/yeast extract medium) showed highest antimicrobial activity on eight test organisms. The fermented broths of *Cephalosporium* sp. in media 2, 10 and 12 indicated high activity on eight test organisms while the fermented broths on media 3, 5, 7 and 8 were also exhibited weak activity on eight test organisms. The fermented broths of *Cephalosporium* sp. in medium 4 showed antimicrobial activity on some test organisms, but medium 6 did not show any activity as shown in Table 6 and Figure 9.

Table 6 Inhibitory zones (mm) of fermented broths of *Cephalosporium* sp. on various media at 7th day

Various media / T.O	M 1	M 2	M 3	M 5	M 7	M 8	M 9	M 10	M 11	M 12
Agrobacterium tumefaciens	22	17	13	12	15	**27**	16	19	14	22
Aspergillus flavus	25	21	11	10	17	**26**	18	22	17	25
Bacillus subtilis	24	21	14	10	15	**26**	17	21	13	24
Candida albicans	21	20	14	11	13	**27**	16	18	14	21
Malassezia furfur	20	20	11	-	14	**28**	13	20	16	20
Micrococcus luteus	23	21	13	10	16	**23**	14	20	15	23
Salmonella typhi	26	22	11	-	15	**33**	19	19	13	26
Xanthomonas oryzae	25	19	11	-	17	**32**	21	22	17	25

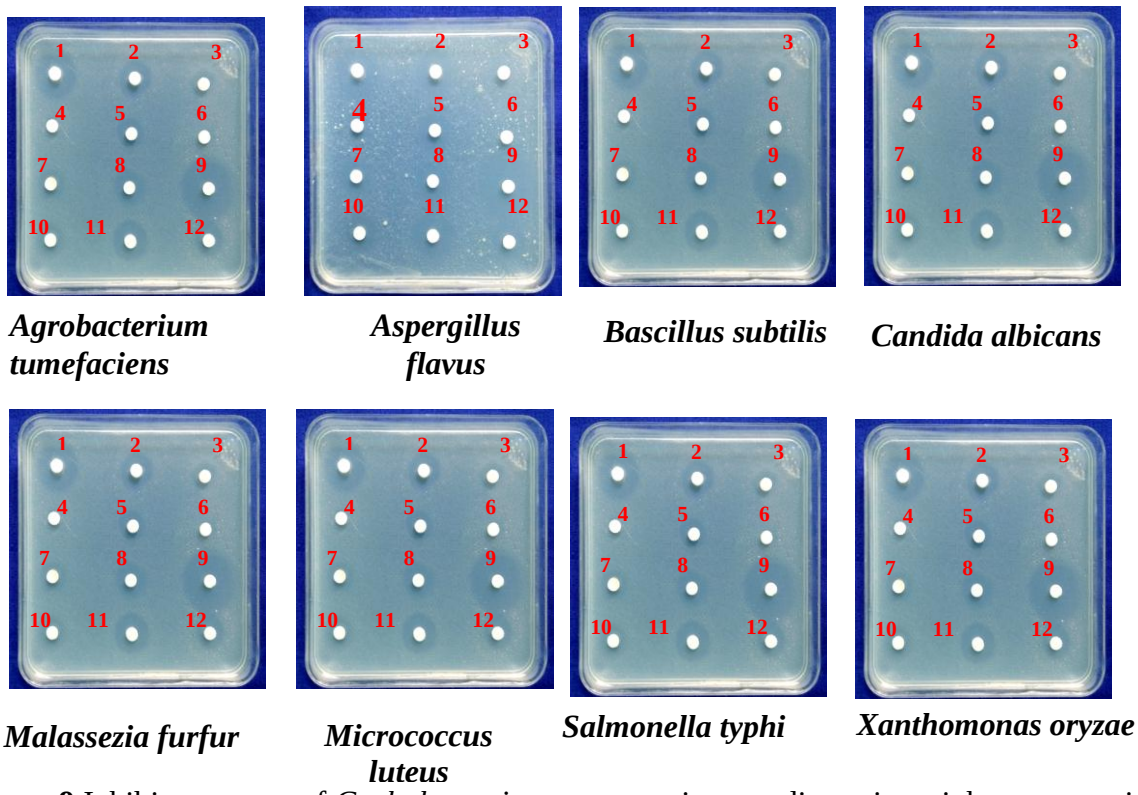


Figure 9 Inhibitory zones of *Cephalosporium* sp. on various media against eight test organisms

Fermentation studies of *Cephalosporium* sp.

Age of inoculum

In the study of age of inoculum, fermented broth of two days old seed culture showed the highest activity against eight test organisms: (*Agrobacterium tumefaciens, Aspergillus flavus, Bacillus subtilis, Candida albicans, Malassezia furfur, Micrococcus luteus, Salmonella typhus* and *Xanthomonas oryzae*) as shown in Table 7 and Figure 10.

Table 7. Age of inoculum (mm) for *Cephalosporium* sp. on *Salmonella typhi*

Cephalosporium sp.	2nd day	3rd day	4th day	5th day	6th day	7th day
Fermentation 1 (one day old)	25	26	25	25	24	16
Fermentation 2 (two days old)	**35**	**36**	**36**	**35**	**27**	**25**
Fermentation 3 (three days old)	21	24	24	19	19	18

10-12 mm = weak activity, 13-17 mm = high activity, >18 mm = very high activity, Disc size = 6 mm, T.O = Test organisms

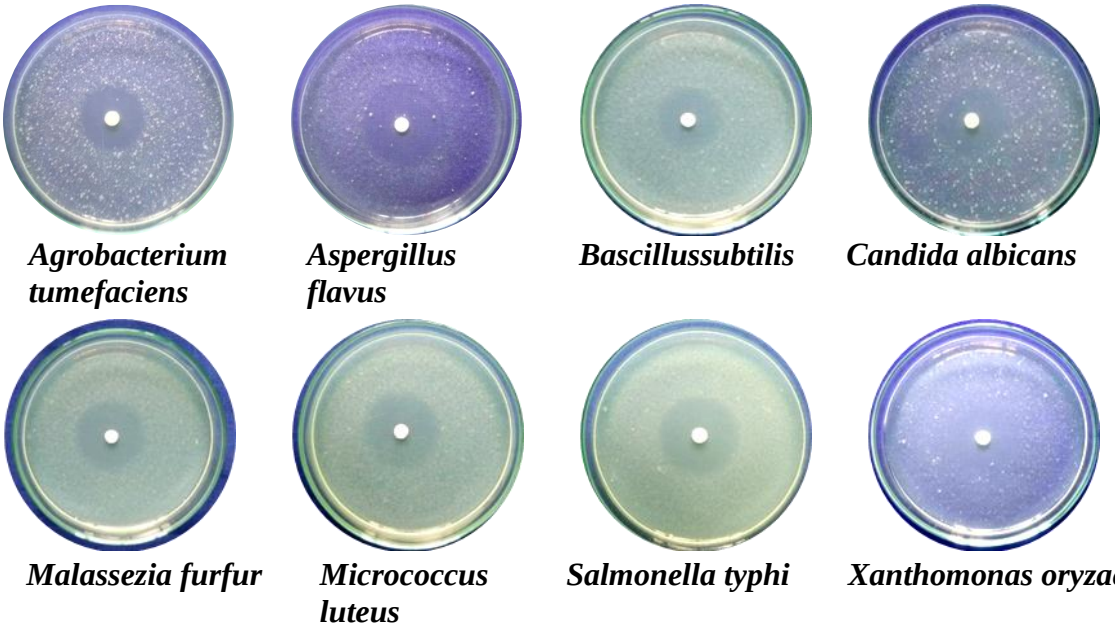


Figure 10 Inhibitory zones of fermented broth (*Cephalosporium* sp.) of two days old against eight test organisms

Size of inoculum

In the study of size of inoculum optimization for *Cephalosporium* sp., among the seed cultures (0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0% and 3.5%) 1.5% of seed culture at fifth day fermentation was suitable for the production of the bioactive compounds as shown in Table 8 and Figure 11.

Table 8 Age of inoculum (mm) for *Cephalosporium* sp. on *Salmonella typhi*

Cephalosporium sp.	2nd day	3rd day	4th day	5th day	6th day	7th day
Fermentation 1 (one day old)	25	26	25	25	24	16
Fermentation 2 (two days old)	**35**	**36**	**36**	**35**	**27**	**25**
Fermentation 3 (three days old)	21	24	24	19	19	18

10-12 mm = weak activity, 13-17 mm = high activity, >18 mm = very high activity, Disc size = 6 mm, T.O = Test organism

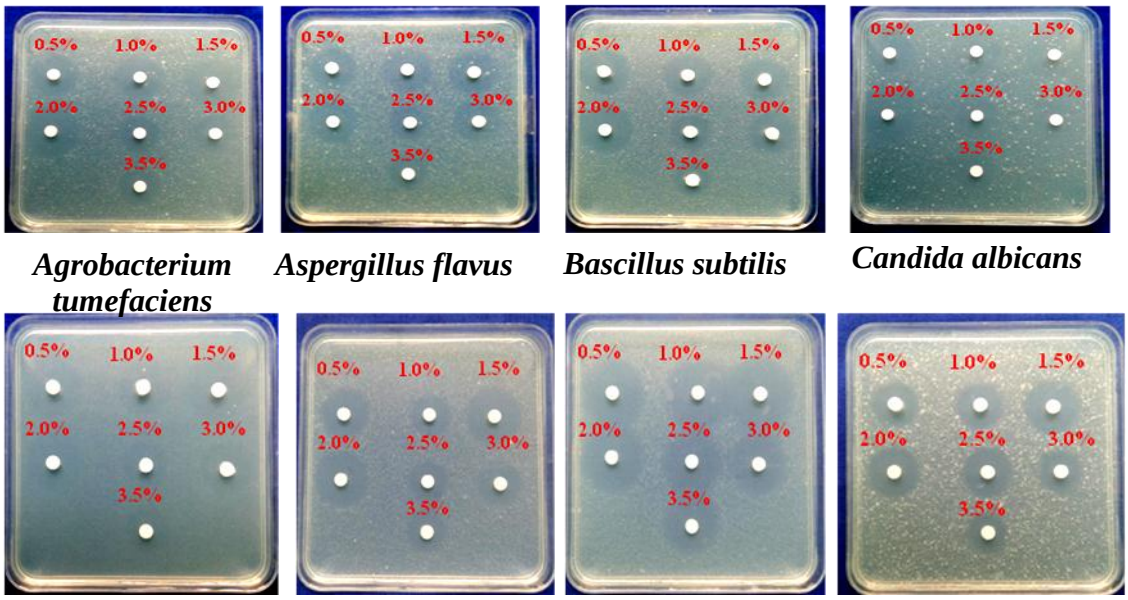


Figure 11 Inhibitory zones (mm) for sizes of inoculum (*Cephalosporium* sp.)

Effect of various pH for *Cephalosporium* sp.

Among pH 4, 5, 6, 7, 8, 9 and 10 of fermented broths of strain *Cephalosporium* sp., pH 7 was the best for extraction of the bioactive compounds from fermented broth according to the result of inhibitory zones against eight test organisms as shown in Table 9 and Figure 12.

Table 9 Inhibitory zones (mm) of *Cephalosporium* sp. with various pH on *Salmonella typhi*

Day / pH	2 days	3 days	4 days	5 days	6 days	7 days
pH-4	18	20	25	16	22	23
pH-5	17	14	19	18	23	22
pH-6	17	16	22	19	21	19
pH-7	**21**	**23**	**28**	**28**	**26**	**25**
pH-8	12	12	17	15	10	15
pH-9	18	18	26	20	22	21
pH-10	13	12	17	15	12	15

10-12 mm = weak activity, 13-17 mm = high activity, >18 mm = very high activity, Disc size = 6 mm, T.O = Test organism

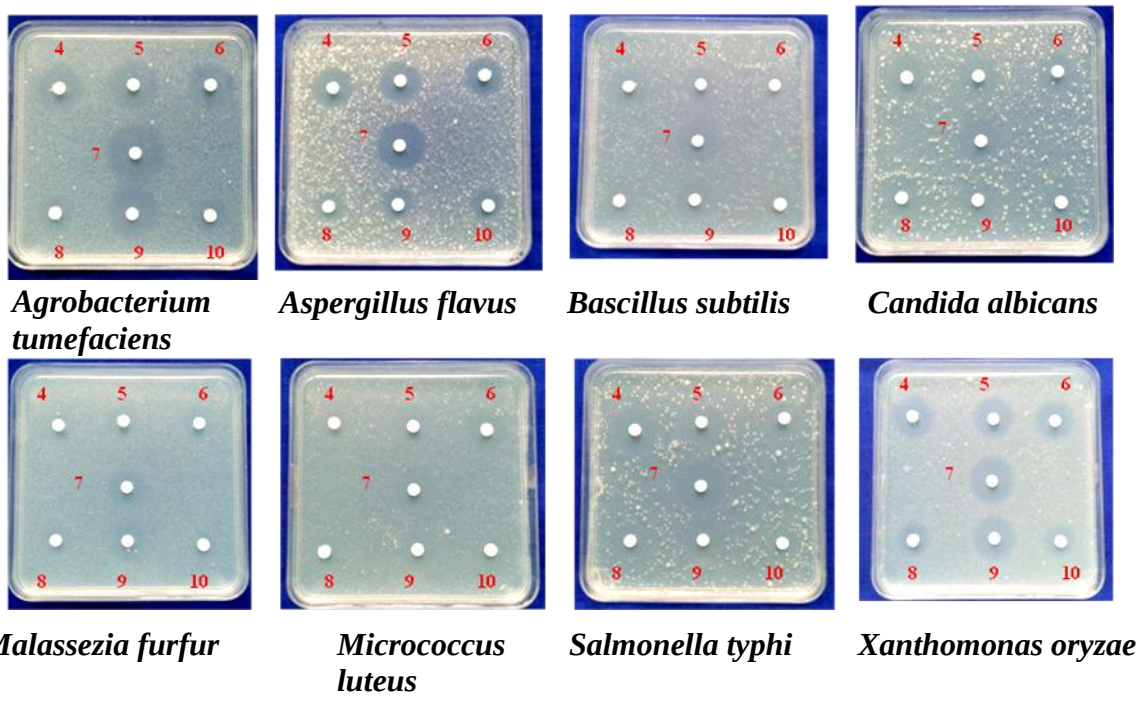


Figure 12 Inhibitory zones (mm) of pH utilization for *Cephalosporium* sp.

Discussion and Conclusion

Endophytic fungal strain *Cephalosporium* sp. isolated from the wood of *Hesperethusa crenulata* (Roxb.) Roem. was used to investigate the optimal fermentation conditions in order to produce its bioactive secondary metabolites. In the morphological character of carbon sources, honey was the best whereas in nitrogen sources, yeast extract, meat extract and malt extract were the best for *Cephalosporium* sp.. Kyawt Kyawt Aung (2014) also reported that starch was the best whereas in nitrogen sources, yeast extract and soybean were the best for endophytic fungal strain.

Klaic *et al.*, (2014) carried out optimization of various media, carbon and nitrogen sources for fungal growth and they also reported that peptone and yeast extract were the best nitrogen sources for the fungal growth. In 2015, Na Yu and Lu He investigated the optimal fermentation of endophytic fungus BS002 which was isolated from *Sophora flavescens*. They also reported that glucose, potato starch as carbon sources and peptone as nitrogen sources were the best for fermentation.

In antimicrobial activity of carbon and nitrogen sources, four carbon sources (glucose, sucrose, soluble starch and honey) and four nitrogen sources (peptone, yeast extract, malt extract and oat meal) showed very high activity on eight test organisms. Yee Yee Thu (2006) reported that glucose and yeast extract media indicated high activity against *Candida albicans.*

In the morphological characters of various media, seven media were good for fermentation to produce antimicrobial metabolites from *Cephalosporium* sp.. As a result of antimicrobial activity on various media, sucrose/yeast extract medium and sucrose/malt extract/soluble starch medium were the best for fermentation medium. For age of inoculum, two days old seed culture of this strain showed the highest activity against eight test organisms. Hnin

Wit Mhone (2018) and Soe Soe Yu Hnin (2018) also reported that two days old seed culture was the best for fermentation.

In the study for size of inoculum optimization, 1.5% of seed culture was suitable for the production of bioactive metabolites. Hnin Wit Mhone (2018) and Soe Soe Yu Hnin (2018) also stated that 1.5% of seed culture was suitable for the production of bioactive metabolites. In screening of optimal pH for fermentation of *Cephalosporium* sp., pH 7 was the best for extraction of the bioactive compounds from fermented broth. Kavish *et al.,* (2016) investigated the effects of pH, carbon sources and nitrogen sources on activity and they found the highest activity at pH 7. Shweta *et al.,* (2015) reported that the optimal pH of many endophytic fungi was pH 7 and they also carried out carbon and nitrogen sources for fermentation.

In conclusion, the best fermentation medium for strain *Cephalosporium* sp. consists of either sucrose/yeast extract medium or sucrose/malt/soluble starch medium. The best fermentation condition was 1.5 % of two days old seed culture at pH7 to produce bioactive metabolites from *Cephalosporium* sp..

Acknowledgements

We would like to express our sincere thanks to all Chairpersons and Professors from Department of Botany for their valuable advice and kind encouragements to present this paper at the Myanmar Academy of Arts and Sciences.

References

Dubey R.C. and Maheshwari D.K. (2009). **Culture Media in Practical Microbiology.** ISBN: 81-219-2153-8 RAM NAGAR, NEW DELHI-110055 p.365-376.

Gaden, E. L. (1959). **Fermentation process kinetics**. J. Biochem. Microbio. Techn. Eng. 1, 413-429.

Hnin Wit Mhon, (2018). **Bioactive compound produced by endophytic fungal strain isolated from *Psidiumg uajava* L.** Ph.D Thesis; Department of Botany, University of Yangon.

Kyawt Kyawt Aung, (2014). **Investigation of Bioactive Compound Produced by Endophytic Fungal Strain Isolated from *Coccinia indica* Wight. & ARN** Ph. D Thesis; Department of Botany University of Yangon.

Kavish Rajput, Sheetal Chanyal and Pavan Kumar Agrawal, (2016). **Optimization Of Protease Production By Endophytic Fungus, Alternaria Alternata Isolated From Gymnosperm Tree – Cupressus Torulosa D. Don** World journal of pharmacy and pharmaceutical sciences Volume 5, Issue 7, 1034-1054, ISSN 2278-4357.

Kajal Wani, Dr. Shweta Saboo, Pooja Solanke, Pooja Tidke, (2016). **Production Of Novel Secondary Metabolites From Endophytic Fungi By Using Fermentation Process.** Indo American Journal of Pharmaceutical Research, ISSN NO: 2231- 6876.

Klaic R., D. Sallet, R. C. Kuhn, P. Salbego, G.S. Centenaro1, R. J. S. Jacques, V. C. Guedes, S. L. Jahn, M. A. Mazutti, (2014). **Optimization Of Fermentation Media For The Growth Of A Fungus Used As Bioherbicide.** XX Congresso Brasileiro de Engenharia Quimico 19 a 22 de outubro de.

Malek, I., J. Ricia and J. Votruba, (1984)**. Continuous cultivation of microorganisms-ecological significance of physiological state studies, Biotechnology, Medicine, and Environment.**8326-334.

Monaghan R.L.., M.M Galiardi., and S.L Streicher, (1999). **Culture preservation and inoculum development, Manual of Industrial Microbiology and Biotechnology.**2nd edition, p 29-48.

Na Yu and Lu He, (2015). **Fermentation Condition Optimization for Endophytic Fungus BS002 Isolated from *Sophora Flavescens* by Response Surface Methodology.** 7th International Conference on CCTA, Beijing, china. Singer, IFIP Advances in Information and Communication Technology, AICT-420 (Part II), pp.342-350.

Omura, S. (1984). **Microbial growth kinetics and secondary metabolites, J. Fermentation Technology, 46: 134-140**

Phay N. (1997). **Studies on Selective Antibiotics.** Doctoral Thesis. Faculty of Agriculture, Hokkaido University, Japan.

Shweta Sao, Amit Sharma and Shukla R. V. (2015). **Physiological And Nutritional Studies Of Some Endophytic Fungi Of Achanakmar Amarkantak Biosphere Reserve, Chhattisgarh** World journal of pharmacy and pharmaceutical sciences Volume 4, Issue 9, 1188 1198, ISSN 2277– 7105.

Soe Soe Yu Hnin, (2018). **Investigation of the Antimicrobial Compounds Isolated from Bioactive Fungal Strains Produced from *Zingiber cassumunar* Roxb.** Ph.D Thesis; Department of Botany, University of Yangon.

Yamme, T., and S. Shimizu., (1984).**Fed-batch techniques in microbial processes.** Advances in Biochem. Eng. Biotechnology 30, 147-184.

Yee Yee Thu, (2006). **Novel Antimicrobial Metabolites Produced by *Trichoderma* sp., *Streptomyces* sp. and *Chaetomium* sp. Isolated from *Mimusop selengi* L., Soil and *Tamarix cananriensis* Willd.**, Ph.D Thesis; Department of Botany, University of Yangon.

EFFECTS OF FERMENTATION PARAMETERS ON THE PRODUCTION OF OPTIMUM PACKED CELL VOLUMES OF ISOLATED *LACTOBACILLUS* SPECIES (H-1) AND (N-2)

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

Abstract

Lactobacillus spp. (H-1) and (N-2) were isolated from the samples bought from Kamayut and North Dagon markets using tomato juice agar medium. These lactic acid bacteria were applied in the study of the effects of fermentation parameters on the optimum packed cell volumes of lactic acid bacteria. The experiments were conducted during May 2018 to December 2018 in the Fermentation Department, Pharmaceutical Research Department, Ministry of Industry - 1, Yangon Region. After the isolation and identification of *Lactobacillus* species, the effects of various fermentation conditions were investigated. This study was carried out by preparing the fermentation medium, and then measuring the pH (4 to 7), ages of culture (1 to 5 days) and sizes of inoculum (5%, 10% and 15%) with fermentation period (1 to 7 days). Packed cell volume (PCV) and pH resulted in fermented broth were recorded. According to experimental results, H - 1 and N - 2 strains were found that 3 days of cultured age, 10% size of inoculum and pH - 6.00 with the fermentation period of 3 days showed maximum packed cell volume.

Keyword: *Lactobacillus*, fermentation, broth.

Introduction

Fermentation is a mean by which cells grow anaerobically dispose of excess hydrogen atoms generated during the breakdown of sugar, known as glycolysis in which the terminal hydrogen acceptor is an organic molecule. In lactic acid bacteria, they dump excess hydrogen on to pyruvic acid, the breakdown product of glucose and this produces lactic acid.

The fermentation conditions, such as temperature, pH medium composition, dissolved oxygen tension (DOT) and types of neutralizer greatly influence the growth of *Lactobacilli* (Lim *et al.,* 2007). Inoculum sizes may have effect on pH, acidity, viable counts and kind of fermented milk. The growth of starter culture is affected by many factors such as milk chemical composition, the amount of inoculum, temperature and time of incubation and the cooling time. Addition of 3, 5 and 10% inoculums resulted in the significantly increase population of lactic acid bacteria during milk fermentation (Wardani *et al.,* 2017).

The optimum inoculum concentration in goat milk was 3% with the incubation temperature at 43°C using *L. bulgaricus* and *S. thermophilus* as starter cultures. After 24 h fermentation the pH of fermented milk with inoculum size of 3%, 5%, and 10% drop the pH to 4.41; 3.94, 4.05 respectively (Shu *et al.,* 2014). The present research is to examine optimum pH, age of culture, size of inoculum and fermentation period for collection of cell sediments.

Materials and Methods

In this study, microbiological work was conducted from May 2018 to December 2018 in the Fermentation Department, Pharmaceutical Research Department, Ministry of Industry 1, Yangon Region.

[1] PhD, Candidate, Department of Botany, University of Yangon

[2] Associate Professor, Department of Botany, University of Yangon

Instruments

The following instruments are used in this experiment. Autoclave is used to sterilize equipment and supplies by subjecting them to pressurized saturated steam at 121 °C (249 °F) for around 15–20 minutes depending on the size of the load and the contents. Incubator is a device used to grow and maintain microbiological cultures or cell cultures. Hot air ovens are electrical devices which use dry heat to sterilize. Centrifuge is used to separate supernatant and pellet.

Figure 1 Autoclave

Figure 2 Incubator

Figure 3 Hot air oven

Figure 4 Centrifuge

Preparation of fermentation medium

Tomato juice agar medium (tomato juice - 20.00 g, yeast extract - 10.00 g, dextrose - 10.00 g, dipotassium phosphate - 0.50 g, monopotassium phosphate - 0.50 g, magnesium sulphate - 0.20 g, manganese sulphate - 0.01 g, ferrous sulphate - 0.01 g, sodium chloride - 0.01 g, agar - 20.00 g, pH - 6.7) was used both for inoculum and fermentation medium.

Preparation of age of culture and size of inoculums

Lactobacillus spp. (H-1) and (N-2) strains from isolated pure culture in tomato juice agar slant were transferred into another slants and incubated at 37°C for 1 to 5 days. Different ages of culture of *Lactobacillus* spp. (H-1) and (N-2) were used as inoculums. The size of inoculum were 5%, 10% and 15% respectively of fermentation medium.

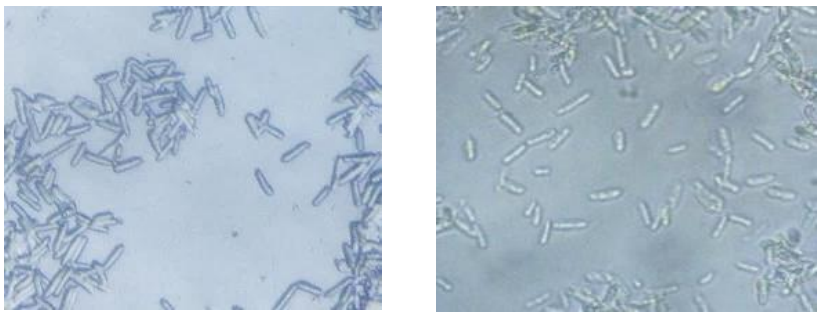
Culture for seed inoculums and fermentation culture of *Lactobacillus* spp. (H-1) and (N-2)

One loopful of 1 to 5 days old culture were aseptically inoculated into a prepared inoculum broth medium (such as pH – 4 to 7) by a sterilized loop and shake gently and inoculums at 37°C for 1 day. Bacteria suspension (5%) was taken from 1 day inoculum of age culture medium and transferred to the 95 ml fermentation medium. At the same time, 10% of inoculums were transferred into their respective flasks containing the 90 ml of fermentation medium. Similarly, 15% of inoculums were transferred into their respective flasks containing the 85 ml of fermentation medium. The experiment procedure was repeated by using inoculums of different age (1 to 5 days).

Determination of packed cell volume and pH

After incubation at 1 to 7 days, 10 ml each of fermentation broth were poured into centrifuge tubes. Then, centrifuged at 2000 rpm for 20 min and the bacterial cell sediments at bottom of the tubes were used for packed cell volume determination. The pH values of fermented broth medium was measured daily by portable pH meter up to 7 days.

Results



H-1 (1000x) N-2 (1000x)

Figure 5 Microscopic characters of *Lactobacillus* species

Determination of optimum conditions of *Lactobacillus* spp. (H - 1) and (N - 2) for fermentation with respect to age of culture, inoculums size and pH

Bacterial growth condition was optimized based on ages of culture, sizes of inoculum and pH of medium. The effects of different ages of culture (1, 2, 3, 4 and 5 days) and sizes of inoculums (5%, 10% and 15%) on the production of packed cell volume of H - 1 and N - 2 (*Lactobacillus* species) were studied. The medium of pH (4, 5, 6 and 7) and fermentation period during 1 to 7 days were also analyzed in this experiment.

In H - 1 strain, the minimum and maximum pH were 4.33 - 6.11 (at pH 4), 5.43- 6.92 (at pH 5), 4.68 - 6.26 (at pH 6) and 5.23 - 6.93 (at pH 7) in 1 day culture aged at 1 to 7 days fermentation period. Among the 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume were 0.11 ml (at pH 4), 0.12 ml (at pH 5), 0.15 ml (at pH 6) and 0.18 ml (at pH 7) in 10% size of inoculum. These results were exhibited in Table - 1. At fermentation period of 1 to 7 days, the minimum and maximum pH were 4.27 - 6.10 (at pH 4), 5.55 - 6.80 (at pH 5), 5.40 - 6.29 (at pH 6) and 5.70 - 7.14 (at pH 7) in 2 days culture aged. Among the 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume were 0.12 ml (at pH 4), 0.15 ml (at pH 5),

0.20 ml (at pH 6) and 0.20 ml (at pH 7) in 10% size of inoculum. These results were exhibited in Table - 2.

In H - 1 strain, the minimum and maximum pH were 4.20 - 6.17 (at pH 4), 5.31- 6.84 (at pH 5), 5.50 - 6.28 (at pH 6) and 5.43 - 6.85 (at pH 7) in 3 days culture aged at 1 to 7 days fermentation period. Among the 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume were 0.11 ml (at pH 4), 0.20 ml (at pH 5), 0.24 ml (at pH 6) and 0.21 ml (at pH 7) in 10% size of inoculum. These results were showed in Table - 3. At fermentation period of 1 to 7 days, the minimum and maximum pH were 4.38 - 5.70 (at pH 4), 5.41 - 6.69 (at pH 5), 5.50 - 6.42 (at pH 6) and 5.47 - 6.54 (at pH 7) in 4 days culture aged. At pH 5 in 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume was 0.12 ml. Among the 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume were 0.12 ml (at pH 4), 0.20 ml (at pH 6 and 7) in 10% size of inoculum. These results were showed in Table - 4. In H - 1 strain, the minimum and maximum pH were 4.32 - 5.72 (at pH 4), 4.97 - 6.72 (at pH 5), 5.39 - 6.25 (at pH 6) and 5.43 - 6.57 (at pH 7) in 5 days culture aged at 1 to 7 days fermentation period. In 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume was 0.10 ml. Among the 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume were 0.12 ml (at pH 5), 0.18 ml (at pH 6 and 7) in 10% size of inoculum. These results were showed in Table - 5.

Table 1 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 1 day old culture and 5%, 10% and 15% sizes of inoculum (H – 1)

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
1	5	4.00	4.50	4.82	5.55	5.79	5.93	6.00	**6.11**	0.01	0.08	0.08	0.10	0.10	0.10	0.08	0.08
	10	4.00	**4.33**	4.87	5.07	5.28	5.51	5.65	5.65	0.02	0.10	0.10	**0.11**	0.10	0.10	0.10	0.10
	15	4.00	4.67	4.88	5.13	5.40	5.63	5.78	5.80	0.02	0.08	0.08	0.10	0.10	0.08	0.08	0.05
	5	5.00	5.50	5.81	6.04	6.15	5.97	5.80	5.72	0.01	0.08	0.10	0.10	0.10	0.10	0.10	0.08
	10	5.00	**5.43**	5.87	6.17	6.38	6.31	6.35	6.35	0.02	0.10	**0.12**	**0.12**	**0.12**	**0.12**	0.10	0.10
	15	5.00	5.67	5.98	6.23	6.30	6.53	**6.92**	6.84	0.02	0.10	0.10	0.10	0.10	0.08	0.08	0.05
	5	6.00	6.04	6.10	6.26	6.21	6.13	5.82	5.70	0.01	0.08	0.12	**0.15**	**0.15**	0.12	0.10	0.10
	10	6.00	5.99	6.17	6.19	5.98	5.74	5.40	5.55	0.02	0.10	0.12	**0.15**	0.13	0.13	0.10	0.10
	15	6.00	6.10	6.17	**6.26**	5.93	5.49	5.60	**4.68**	0.02	0.10	0.13	0.13	0.13	0.13	0.10	0.10
	5	7.00	6.71	6.40	6.30	6.02	5.91	5.90	5.96	0.01	0.08	0.10	0.12	0.10	0.10	0.10	0.10
	10	7.00	6.83	6.50	6.29	6.09	5.60	5.55	**5.23**	0.02	0.10	0.15	**0.18**	0.15	0.15	0.12	0.10
	15	7.00	**6.93**	6.45	6.32	6.29	6.33	5.94	5.55	0.02	0.12	0.12	0.13	0.13	0.10	0.10	0.09

Table 2 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 2 day old culture and 5%, 10% and 15% sizes of inoculum (H – 1)

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
2	5	4.00	4.55	4.71	5.19	5.44	5.67	5.80	**6.10**	0.01	0.08	0.10	0.10	0.10	0.10	0.10	0.09
	10	4.00	4.55	4.87	5.00	5.31	5.60	5.73	5.95	0.02	0.10	0.10	**0.12**	**0.12**	0.10	0.10	0.10
	15	4.00	**4.27**	4.76	4.96	5.01	5.25	5.58	5.80	0.02	0.10	0.10	0.11	0.11	0.10	0.10	0.10
	5	5.00	**5.55**	5.81	6.19	6.21	6.36	6.08	5.82	0.01	0.08	0.10	0.12	0.12	0.12	0.10	0.09
	10	5.00	5.65	6.00	6.27	6.41	6.50	6.53	6.45	0.02	0.12	0.12	**0.15**	0.12	0.12	0.10	0.10
	15	5.00	5.77	6.06	6.32	6.51	6.65	6.78	**6.80**	0.02	0.10	0.10	0.12	0.12	0.12	0.10	0.10
	5	6.00	5.91	6.13	**6.29**	5.95	5.72	5.53	**5.40**	0.01	0.08	0.11	0.16	0.15	0.13	0.11	0.09
	10	6.00	6.12	6.18	6.23	6.17	6.09	5.85	5.81	0.02	0.12	0.12	**0.20**	0.17	0.15	0.15	0.11
	15	6.00	6.19	6.17	6.23	6.07	5.95	5.88	5.70	0.02	0.08	0.12	0.12	0.12	0.12	0.11	0.10
	5	7.00	6.55	6.32	6.27	6.00	6.12	5.93	5.80	0.01	0.10	0.11	0.15	0.12	0.12	0.11	0.10
	10	7.00	**7.14**	6.69	6.32	6.22	6.05	5.84	**5.70**	0.02	0.12	0.18	**0.20**	0.17	0.15	0.15	0.15
	15	7.00	6.84	6.57	6.39	6.32	6.14	5.96	5.71	0.02	0.08	0.10	0.12	0.12	0.10	0.10	0.10

Table 3 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 3 day old culture and 5%, 10% and 15% sizes of inoculum (H – 1)

Age of culture (day)	Size of noculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
3	5	4.00	4.31	4.78	5.20	5.87	6.09	6.13	**6.17**	0.01	0.10	0.10	0.12	0.10	0.10	0.10	0.10
	10	4.00	**4.20**	4.68	4.85	5.02	5.36	5.52	5.73	0.02	0.10	0.11	**0.13**	0.11	0.10	0.10	0.10
	15	4.00	4.40	4.75	4.89	5.04	5.15	5.29	5.40	0.02	0.10	0.11	0.11	0.11	0.10	0.09	0.09
	5	5.00	**5.31**	5.80	6.19	5.73	5.59	5.40	5.38	0.01	0.10	0.10	0.15	0.13	0.10	0.10	0.10
	10	5.00	5.80	6.08	6.25	6.32	6.36	6.30	6.23	0.02	0.12	0.15	**0.20**	**0.20**	0.17	0.15	0.12
	15	5.00	5.60	6.15	6.24	6.69	6.75	6.79	**6.84**	0.02	0.10	0.12	0.13	0.10	0.10	0.09	0.09
	5	6.00	6.14	6.21	6.26	6.15	5.84	5.85	**5.50**	0.01	0.10	0.15	0.17	0.13	0.11	0.10	0.10
	10	6.00	6.10	6.17	6.20	6.11	5.80	5.74	5.55	0.02	0.10	0.20	**0.24**	0.20	0.17	0.17	0.15
	15	6.00	6.06	6.12	**6.28**	6.15	5.92	5.80	5.77	0.02	0.10	0.15	0.20	0.15	0.10	0.09	0.09
	5	7.00	6.79	6.53	6.24	6.20	5.81	5.82	5.75	0.01	0.10	0.12	0.15	0.13	0.12	0.12	0.12
	10	7.00	**6.85**	6.51	6.20	6.11	5.80	5.65	5.55	0.02	0.15	0.18	**0.21**	0.20	0.17	0.15	0.15
	15	7.00	6.80	6.59	6.24	5.92	5.70	**5.43**	5.48	0.02	0.10	0.12	0.20	0.15	0.10	0.10	0.09

Table 4 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 4 day old culture and 5%, 10% and 15% sizes of inoculum (H – 1)

Age of culture (day)	Size of noculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
4	5	4.00	4.39	4.60	4.98	5.15	5.30	5.53	5.60	0.01	0.10	0.10	0.10	0.10	0.10	0.10	0.08
	10	4.00	4.41	4.76	4.98	5.02	5.22	5.45	**5.70**	0.02	0.10	0.10	**0.12**	0.12	0.10	0.10	0.10
	15	4.00	**4.38**	4.82	4.95	5.04	5.49	5.65	5.60	0.02	0.09	0.10	0.10	0.10	0.10	0.08	0.08
	5	5.00	5.59	5.85	6.28	6.20	6.06	5.73	5.80	0.01	0.10	0.10	**0.12**	**0.12**	0.10	0.10	0.08
	10	5.00	**5.41**	5.76	6.08	6.19	6.20	6.35	6.25	0.02	0.10	0.10	**0.12**	**0.12**	0.10	0.10	0.10
	15	5.00	5.88	6.22	6.32	6.54	**6.69**	6.65	6.60	0.02	0.10	0.10	**0.12**	0.10	0.10	0.08	0.08
	5	6.00	6.18	6.30	**6.42**	6.37	6.25	5.90	5.69	0.01	0.08	0.10	0.12	0.12	0.12	0.10	0.08
	10	6.00	6.11	6.15	6.28	6.23	6.24	5.92	**5.50**	0.02	0.12	0.19	**0.20**	0.18	0.15	0.15	0.15
	15	6.00	6.17	6.26	6.25	5.98	5.75	5.70	5.71	0.02	0.12	0.15	0.15	0.15	0.08	0.08	0.08
	5	7.00	6.52	6.39	6.19	6.04	5.70	5.67	**5.47**	0.01	0.08	0.12	0.15	0.15	0.15	0.10	0.08
	10	7.00	6.44	6.24	6.25	5.96	5.81	5.80	5.67	0.02	0.12	0.15	**0.20**	0.18	0.12	0.12	0.10
	15	7.00	**6.54**	6.38	6.25	6.12	5.83	5.71	5.59	0.02	0.12	0.15	0.15	0.15	0.10	0.08	0.08

Table 5 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 5 day old culture and 5%, 10% and 15% sizes of inoculum (H – 1)

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
5	5	4.00	**4.32**	4.50	4.95	5.28	5.40	5.57	5.50	0.01	0.08	0.08	**0.10**	**0.10**	0.08	0.08	0.05
	10	4.00	4.56	4.76	4.93	5.19	5.30	5.47	5.65	0.02	0.08	**0.10**	**0.10**	**0.10**	**0.10**	**0.10**	0.08
	15	4.00	4.50	4.89	5.24	5.57	5.64	5.70	**5.72**	0.02	**0.10**	**0.10**	**0.10**	**0.10**	0.05	0.05	0.05
	5	5.00	**4.97**	5.40	5.95	6.28	6.41	6.23	6.29	0.01	0.08	0.08	0.10	0.10	0.08	0.08	0.05
	10	5.00	5.36	5.55	5.83	6.09	6.30	6.47	6.65	0.02	0.08	0.10	**0.12**	**0.12**	0.10	0.10	0.08
	15	5.00	5.50	6.19	6.24	6.67	6.70	6.70	**6.72**	0.02	0.10	0.10	0.10	0.10	0.05	0.05	0.05
	5	6.00	6.02	6.13	6.16	5.70	5.40	5.52	**5.39**	0.01	0.08	0.08	0.10	0.10	0.10	0.10	0.05
	10	6.00	5.97	6.08	6.20	6.14	5.50	5.45	5.57	0.02	0.12	0.12	**0.18**	**0.18**	0.15	0.10	0.10
	15	6.00	6.04	6.24	**6.25**	5.62	5.49	5.67	5.63	0.02	0.10	0.10	0.12	0.10	0.05	0.05	0.05
	5	7.00	6.52	6.20	6.25	6.28	6.13	5.95	5.84	0.01	0.08	0.08	0.10	0.10	0.10	0.08	0.05
	10	7.00	**6.57**	6.48	6.29	6.07	5.77	5.57	5.51	0.02	0.10	0.12	**0.18**	**0.18**	0.15	0.10	0.10
	15	7.00	6.50	6.43	6.25	6.05	5.70	5.61	**5.43**	0.02	0.08	0.10	0.12	0.10	0.08	0.05	0.05

The blue color square indicate the minimum value and the green color square indicate the maximum value.

In N - 2 strain, the minimum and maximum pH were 4.37 - 6.07 (at pH 4), 5.31-6.92 (at pH 5), 5.59 - 6.29 (at pH 6) and 5.65 - 6.93 (at pH 7) in 1 day culture aged at 1 to 7 days

fermentation period. At pH 6 in 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume was 0.15 ml. Among the 5%, 10% and 15% inoculum sizes, maximum amount of packed cell volume were 0.11 ml (at pH 4), 0.12 ml (at pH 5) and 0.18 ml (at pH 7) in 10% size of inoculum. These results were showed in Table - 6. At fermentation period of 1 to 7 days, the minimum and maximum pH were 4.27 - 5.97 (at pH 4), 5.52 - 6.84 (at pH 5), 5.40 - 6.27 (at pH 6) and 5.80 – 6.91 (at pH 7) in 2 days culture aged. At pH 4 in 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume was 0.12 ml. Among the 5%, 10% and 15% inoculum sizes, maximum amount of packed cell volume were 0.15 ml (at pH 5), 0.20 ml (at pH 6 and 7) in 10% size of inoculum. These results were presented in Table - 7. At fermentation period of 1 to 7 days, the minimum and maximum pH were 4.26 - 6.07 (at pH 4), 5.21 - 6.83 (at pH 5), 5.70 - 6.29 (at pH 6) and 5.45 - 6.80 (at pH 7) in 3 days culture aged. Among the 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume were 0.13 ml (at pH 4), 0.20 ml (at pH 5), 0.25 ml (at pH 6) and 0.22 ml (at pH 7) in 10% size of inoculum. These results were presented in Table 8. At fermentation period of 1 to 7 days, the minimum and maximum pH were 4.26 – 6.07 (at pH 4), 5.21 - 6.83 (at pH 5), 5.70 - 6.29 (at pH 6) and 5.45 - 6.80 (at pH 7) in 4 days culture aged. In 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume were 0.11 ml (At pH 4), 0.12 ml (At pH 5). Among the 5%, 10% and 15% inoculum sizes, maximum amount of packed cell volume was 0.20 ml (at pH 6 and 7) in 10% size of inoculum. These results were exhibited in Table - 9. At fermentation period of 1 to 7 days, the minimum and maximum pH were 4.32 - 5.80 (at pH 4), 5.07- 6.77 (at pH 5), 5.45 - 6.25 (at pH 6) and 5.51 - 6.87 (at pH 7) in 5 days culture aged. At pH 4 in 5%, 10% and 15% inoculum sizes, maximum value of packed cell volume was 0.10 ml. Among the 5%, 10% and 15% inoculum sizes, maximum amount of packed cell volume were 0.12 ml (at pH 5), 0.18 ml (at pH 6 and 7) in 10% size of inoculum. These results were exhibited in Table - 10.

Table 6 **Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 1 day old culture and 5%, 10% and 15% sizes of inoculum (N – 2)**

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	5	4.00	4.42	4.72	5.14	5.59	5.81	6.00	**6.07**	0.01	0.08	0.08	0.10	0.10	0.08	0.08	0.08
	10	4.00	**4.37**	4.87	5.11	5.48	5.51	5.58	5.62	0.02	0.10	0.10	**0.11**	**0.11**	0.10	0.10	0.10
	15	4.00	4.51	4.80	5.13	5.44	5.67	5.79	5.83	0.02	0.08	0.10	0.10	0.10	0.08	0.08	0.06
	5	5.00	**5.31**	5.73	6.07	6.25	6.07	6.10	6.32	0.01	0.08	0.10	0.10	0.10	0.10	0.08	0.08
	10	5.00	5.43	5.87	6.17	6.38	6.31	6.35	6.33	0.02	0.10	**0.12**	**0.12**	**0.12**	0.10	0.10	0.10
	15	5.00	5.47	5.88	6.03	6.30	6.52	**6.92**	6.84	0.02	0.09	0.10	0.10	0.10	0.08	0.08	0.05
	5	6.00	6.07	6.13	6.26	6.21	6.15	5.92	5.80	0.02	0.08	0.12	**0.15**	**0.15**	0.10	0.10	0.10
	10	6.00	6.09	6.17	**6.29**	6.08	5.84	5.80	5.85	0.02	0.10	0.12	**0.15**	0.13	0.12	0.10	0.10
	15	6.00	6.20	6.17	6.26	5.93	**5.59**	5.60	5.68	0.02	0.10	0.10	0.12	0.12	0.10	0.10	0.10
	5	7.00	6.77	6.42	6.30	6.02	5.88	5.90	5.93	0.01	0.08	0.08	0.10	0.10	0.10	0.10	0.10
	10	7.00	6.90	6.50	6.29	6.07	5.90	5.85	5.83	0.02	0.10	0.15	**0.18**	**0.18**	0.15	0.12	0.10

Table 7 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 2 day old culture and 5%, 10% and 15% sizes of inoculum

(N – 2)

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
2	5	4.00	4.45	4.71	5.15	5.47	5.60	5.84	**5.97**	0.01	0.08	0.10	0.10	0.10	0.10	0.09	0.09
	10	4.00	4.58	4.77	5.01	5.35	5.60	5.73	5.85	0.02	0.10	0.10	**0.12**	**0.12**	**0.12**	**0.12**	0.10
	15	4.00	**4.27**	4.76	5.11	5.48	5.51	5.63	5.80	0.02	0.10	0.10	**0.12**	0.11	0.10	0.10	0.10
	5	5.00	**5.52**	5.78	6.15	6.41	6.36	6.28	6.32	0.01	0.08	0.10	0.12	0.12	0.10	0.10	0.09
	10	5.00	5.65	6.00	6.27	6.41	6.50	6.53	6.45	0.02	0.12	0.12	**0.15**	**0.15**	0.12	0.10	0.10
	15	5.00	5.53	6.00	6.20	6.51	6.66	6.78	**6.84**	0.02	0.10	0.10	0.12	0.12	0.10	0.10	0.10
	5	6.00	6.16	6.13	**6.27**	5.95	5.81	5.53	**5.40**	0.02	0.10	0.12	0.16	0.15	0.13	0.10	0.10
	10	6.00	6.10	6.18	6.23	6.25	6.09	5.83	5.85	0.02	0.12	0.12	**0.20**	0.15	0.15	0.15	0.11
	15	6.00	6.19	6.17	6.26	6.09	5.95	5.80	5.70	0.02	0.10	0.12	0.12	0.12	0.12	0.11	0.10
	5	7.00	6.59	6.31	6.27	6.00	6.16	5.99	5.80	0.01	0.10	0.11	0.13	0.13	0.12	0.11	0.10
	10	7.00	**6.91**	6.65	6.30	6.22	6.25	5.94	**5.80**	0.02	0.12	0.18	**0.20**	**0.20**	0.15	0.15	0.15
	15	7.00	6.85	6.57	6.36	6.32	6.18	5.96	6.01	0.02	0.10	0.10	0.12	0.12	0.12	0.10	0.10

Table 8 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 3 day old culture and 5%, 10% and 15% sizes of inoculum (N – 2)

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
3	5	4.00	4.43	4.68	5.10	5.54	5.79	5.93	**6.07**	0.01	0.10	0.10	0.12	0.12	0.12	0.10	0.10
	10	4.00	**4.26**	4.58	4.85	5.11	5.46	5.62	5.73	0.02	0.10	0.11	**0.13**	0.11	0.11	0.11	0.10
	15	4.00	4.30	4.65	4.89	5.02	5.19	5.39	5.47	0.02	0.10	0.11	**0.13**	0.12	0.10	0.10	0.10
3	5	5.00	**5.21**	5.60	5.99	5.73	5.59	5.30	5.28	0.01	0.10	0.10	0.14	0.13	0.11	0.10	0.10
	10	5.00	5.50	6.08	6.23	6.32	6.46	6.40	6.31	0.02	0.12	0.15	**0.20**	0.17	0.17	0.15	0.12
	15	5.00	5.38	5.85	6.20	6.59	6.75	6.79	**6.83**	0.02	0.10	0.12	0.13	0.10	0.10	0.10	0.10
	5	6.00	6.13	6.20	**6.29**	6.15	6.04	5.95	**5.70**	0.02	0.10	0.15	0.18	0.13	0.11	0.10	0.10
	10	6.00	6.16	6.20	**6.29**	6.10	5.88	5.74	5.79	0.02	0.12	0.20	**0.25**	0.20	0.20	0.17	0.15
	15	6.00	6.06	6.12	6.28	6.15	5.92	5.80	5.77	0.02	0.10	0.15	0.20	0.15	0.12	0.10	0.10
	5	7.00	6.70	6.53	6.20	6.18	5.85	5.82	5.75	0.01	0.10	0.12	0.15	0.13	0.13	0.12	0.12
	10	7.00	**6.80**	6.51	6.29	6.11	5.89	5.85	5.87	0.02	0.15	0.20	**0.22**	0.20	0.18	0.15	0.15
	15	7.00	6.76	6.59	6.25	5.99	5.70	**5.45**	5.47	0.02	0.10	0.12	0.20	0.15	0.15	0.12	0.10

Table 9 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 4 day old culture and 5%, 10% and 15% sizes of inoculum (N – 2)

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
4	5	4.00	4.35	4.65	4.74	5.00	5.34	5.53	5.62	0.01	0.10	**0.11**	**0.11**	0.10	0.10	0.09	0.08
	10	4.00	4.41	4.76	4.98	5.02	5.22	5.45	5.70	0.02	0.10	0.10	**0.11**	**0.11**	0.10	0.10	0.10
	15	4.00	**4.28**	4.72	4.99	5.04	5.49	5.68	**5.79**	0.02	0.08	0.10	0.10	0.10	0.08	0.08	0.08
	5	5.00	5.59	5.85	6.28	6.20	6.05	5.75	5.80	0.01	0.10	**0.12**	**0.12**	**0.12**	0.10	0.10	0.08
	10	5.00	**5.44**	5.76	6.00	6.19	6.27	6.35	6.35	0.02	0.10	**0.12**	**0.12**	**0.12**	0.10	0.10	0.08
	15	5.00	5.88	6.14	6.32	6.50	6.69	6.65	**6.70**	0.02	0.10	**0.12**	**0.12**	0.10	0.10	0.08	0.08
	5	6.00	6.28	**6.37**	6.29	6.33	6.25	5.90	5.78	0.02	0.08	0.10	0.12	0.12	0.10	0.10	0.08
	10	6.00	6.16	6.20	6.28	6.23	6.25	5.99	**5.70**	0.02	0.12	0.19	**0.20**	0.18	0.18	0.15	0.15
	15	6.00	6.15	6.26	6.25	6.18	5.85	5.80	5.81	0.02	0.12	0.12	0.15	0.15	0.10	0.10	0.09
	5	7.00	**6.82**	6.49	6.29	6.06	5.90	5.67	**5.60**	0.01	0.08	0.12	0.13	0.10	0.10	0.10	0.08
	10	7.00	6.74	6.24	6.25	5.98	5.81	5.86	5.77	0.02	0.12	0.15	**0.20**	**0.20**	0.15	0.12	0.10
	15	7.00	6.74	6.68	6.35	6.12	5.88	5.71	**5.60**	0.02	0.12	0.15	0.15	0.15	0.10	0.10	0.08

Table 10 Effects of different age and size of inoculum on the production of packed cell volume (pH 4, 5, 6, 7); 5 day old culture and 5%, 10% and 15% sizes of inoculum (N – 2)

Age of culture (day)	Size of inoculum (%)	pH								Packed cell volume (ml)							
		Fermentation period (day)								Fermentation period (day)							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
5	5	4.00	**4.32**	4.60	4.95	5.38	5.50	5.67	**5.80**	0.01	0.08	**0.10**	**0.10**	**0.10**	**0.10**	0.05	0.05
	10	4.00	4.46	4.87	5.03	5.29	5.40	5.47	5.65	0.02	0.08	**0.10**	**0.10**	**0.10**	0.08	0.08	0.08
	15	4.00	4.50	4.69	5.04	5.57	5.65	5.70	5.72	0.02	0.08	0.08	**0.10**	**0.10**	0.08	0.05	0.05
	5	5.00	**5.07**	5.40	5.91	6.26	6.41	6.23	6.21	0.01	0.08	0.08	0.10	0.10	0.08	0.08	0.08
	10	5.00	5.46	5.58	5.83	6.07	6.30	6.57	6.65	0.02	0.08	0.10	**0.12**	**0.12**	0.10	0.08	0.08
	15	5.00	5.50	6.02	6.24	6.57	6.70	**6.77**	6.75	0.02	0.08	0.10	0.10	0.08	0.05	0.05	0.05
	5	6.00	6.07	6.13	6.20	5.98	5.60	5.52	**5.45**	0.02	0.08	0.10	0.10	0.10	0.10	0.10	0.08
	10	6.00	5.97	6.08	6.20	6.14	5.70	5.75	5.69	0.02	0.12	0.15	**0.18**	0.15	0.15	0.10	0.10
	15	6.00	6.04	**6.26**	6.25	5.62	5.79	5.67	5.63	0.02	0.10	0.10	0.12	0.12	0.10	0.10	0.06
	5	7.00	6.52	6.32	6.25	6.28	6.20	5.95	5.89	0.01	0.08	0.08	0.11	0.10	0.10	0.08	0.08
	10	7.00	**6.87**	6.50	6.30	6.07	5.97	5.74	**5.51**	0.02	0.10	0.12	**0.18**	**0.18**	0.14	0.12	0.10
	15	7.00	6.80	6.53	6.25	6.07	5.80	5.61	5.63	0.02	0.10	0.10	0.12	0.10	0.08	0.08	0.05

The blue color square indicate the minimum value and the green color square indicate the maximum value.

Discussion and Conclusion

In H-1 strain, packed cell volume (0.24 ml) was the maximum amount in 3 day cultured age and 10% size of inoculum at pH-6. In N-2 strain, packed cell volume (0.25 ml) was the maximum amount in 3 day cultured age and 10% size of inoculum at pH-6. The maximum yield (0.24 ml in H-1 strain) and (0.25 ml in N-2 strain) were recorded in the fermentation period of 3 day.

In 2007, Mar stated that *Lactobacillus* species in 3, 4 and 5 days of age of cultures with the fermentation period of 72 and 96 hours showed the best packed cell volume. The present result agreed with those reported by Mar. Win (1981) reported that 24 to 48 hours old *Lactobacillus casei* culture might be suitable for the effective growth of bacterial cells accompanied by the higher yield of lactic acid in M.R.S. medium. Shu *et al.,* 2016 showed that the optimum incubation temperature was 37°C and the optimum inoculum size was 5%, for growth of *Bifidobacterium bifidum*, *Lactobacillus acidophilus*. The optimum inoculum size of *L. acidophilus* and *L. casei* were all 7% on fermentation of goat milk (Chen *et al.,* 2015). According to these literatures, the results of present work were somewhat different.

Wang *et al.,* 2015 reported that the optimal inoculum size for *L. casei* was 10%. In 2017, Wardani *et al.,* stated that inoculum size may has effect on pH, acidity, viable counts and flavor of fermented milk. Addition of 3, 5 and 10% inoculums resulted in the significantly increase population of lactic acid bacteria during milk fermentation. The data of present research were found to be in agreement with above authors.

The fermented broth medium pH were between 4.20 and 6.17 (at initial pH-4),4.97 and 6.92 (at initial pH-5), 4.68 and 6.42 (at initial pH-6), 5.23 and 7.14 (at initial pH-7) during the fermentation period in H-1 strain. In N-2 strain, the fermented broth medium pH were between 4.26 and 6.07 (at initial pH-4), 5.07 and 6.92 (at initial pH- 5), 5.40 and 6.37 (at initial pH-6), 5.45 and 6.93 (at initial pH-7) during the fermentation period. According to these results, the pH values for maximum packed cell volume was assumed to be in acid side.

Tomas *et al.,* 2002 described that the MRS broth with a pH of 6.5 and a temperature of 37°C yield the highest growth and are the optimal conditions. Yang *et al.,* 2018 stated that the optimal condition for lactic acid bacteria (LAB) was determined in MRS broth, pH 6.2 at 37 °C. Cachon and Divie`s (1994) found that for growth of *L. lactis*, the optimal pH was 6.5. This reported data was in agreement with those mentions.

In the investigation of optimizing the fermentation, H-1 and N-2 strains were found that 3 day of cultured age, 10% size of inoculum and pH-6 with the fermentation period of 3 day showed maximum packed cell volume. It could be concluded that the maximum yield of packed cell volume was produced by *Lactobacillus* species; age of culture (3 days), size of inoculum (10%) at 3 days of fermentation periods and the optimum pH of medium was 6. It is aimed to conduct further study which deal with the application of obtained packed cell volume of lactic acid bacteria in the feed of chicken as probiotic activity.

Acknowledgements

I would like to express my deepest thank to Professor Dr. Aye Pe, Head of Botany Department, University of Yangon, for his valuable guidance and suggestions during this research. I also thank to Dr. Thandar Aye, Professor, Department of Botany Department, University of Yangon, for her helpful advice and suggestions. I sincerely and gratefully express my appreciation to Dr. Kyaw Than Htay, Director, Pharmaceutical Research Department, Ministry of Industry 1, for providing all the research facilities. Thanks are also due to Daw Myint Myint Lwin, Daw Cherry Aung and all staff members of the Fermentation Department, Pharmaceutical Research Department, Ministry of Industry 1, for their cooperation and help during the present research.

References

Cachon, R. and Divie`s, C. (1994). "**Generalized model of the effect of pH on lactate fermentation and citrate bioconversion in *Lactococcus lactis*.**" Applied Microbiology and Biotechnology. Vol. 41, pp. 694–699.

Chen, H., Zhang, Q., Wan, H., Shu, G. and Li, H. (2015). "**Effect of Total Inoculum Size Containing *Lactobacillus acidophilus* or *Lactobacillus casei* on Fermentation of Goat Milk.**" Advance Journal of Food Science and Technology. Vol. 7(3), pp. 183-186.

Dubey, R. C. and Maheshwari, D. K. (2002). "**Practical microbiology.**" S Chand and Co.Ltd., New Delhi.

Lim, C. H., Rahim, R. A., Ho, Y. W. and Arbakariya, B. A. (2007). "**Optimization of Growth medium for Efficient Cultivation of *Lactobacillus salivarius* i 24 using Response Surface Method.**" Malaysian journal of microbiology. Vol. 3(2), pp. 41-47.

Mar, T. T. (2007). "**Isolation and identification of *Lactobacillus acidophilus* as probiotics in chicken.**" PhD, Thesis; Department of Zoology, University of Yangon.

Shu, G., Li, C., Chen, H. and Wang, C. (2014). "**Effect of Inoculum and Temperature on the Fermentation of Goat Yogurt.**" Advance Journal of Food Science and Technology. Vol. 6(1), pp. 68-71.

Shu, G., Lei, N., Wan, H., Wang, C., Chen, H. and Li, H. (2016). "**Effect of Temperature and Inoculum Size on Fermentation of Goat Yogurt Containing *Lactobacillus acidophilus* and *Bifidobactrium bifidum*.**" Advance Journal of Food Science and Technology. Vol. 10(8), pp. 616-620.

Tomas M. S. J., Bru, E., Wiese, B., Holgado, D. R. and Macias, M. E. N. (2002). **"Influence of pH, temperature and culture media on the growth and bacteriocin production by vaginal *Lactobacillus salivarius* CRL 1328.**" Journal of Applied Microbiology. Vol. 93, pp. 714–724.

Wang, J., Wang, Q., Xu, Z., Zhang, W. and Xiang, J. (2015). "**Effect of Fermentation Conditions on *L*-Lactic Acid Production from Soybean Straw Hydrolysate".** Journal of Microbiology and Biotechnology. Vol. 25(1), pp. 26-32.

Wardani, S. K., Cahyanto, M. N., Rahayu, E. S. and Utami, T. (2017). "**The effect of inoculum size and incubation temperature on cell growth, acid production and curd formation during milk fermentation by *Lactobacillus plantarum* Dad 13.**" International Food Research Journal. Vol. 24(3), pp. 921-926.

Win, Mg. (1981). "**Production of lactic acid by *Lactobacillus casei* isolated from fermented milk."**

M.Sc, Thesis. Department of Botany. Arts and Science University, Rangoon.

Yang, E., Fan, L., Yan, J., Jiang, Y., Doucette, C., Fillmore, S. and Walker, B. (2018). "**Influence of culture media, pH and temperature on growth and bacteriocin."**

SCREENING AND ISOLATION OF LIPOLYTIC FUNGI FROM DIFFERENT SOURCES

May Barani Shu Shu Tan[1], Bay Dar[2]

Abstract

Fungi were isolated from seven different sources. Soil sample was collected from the car workshop as fuel oil contaminated soil, Thuwana Township, Yangon Region, Myanmar. Other samples were collected from pork sausage, cheese, margarine, tea leaves in bean oil with salt, scraped coconut shell and scraped coconut peel. Fungal strains were directly isolated from 6 different sources. Diluted soil (concentration - 10^{-3}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}) was used to culture the fungi. Fungal strains were cultured on Potato Dextrose Agar (PDA) medium. Lipolytic fungi were screened using Tributyrin Agar (TBA) medium. Total twenty-five fungi were observed from seven different sources. Two fungal strains from pork sausage, five fungal strains from cheese, one fungal strain from margarine, two fungal strains from tea leaves in bean oil with salt, seven fungal strains from scraped coconut and eight fungal strains from fuel oil contaminated soil were observed. Among them, ten lipolytic fungi showed clear zone of hydrolysis around fungal colony on TBA medium that indicated lipase enzyme was produced. The isolated fungi were identified by their pure colony morphology and spore formation according to the references. In the present study, ten different types of lipolytic fungi were observed from six different sources.

Keywords: Lipolytic fungi, Different sources, Spore formation

Introduction

Lipase enzymes (Triacylglycerol acyl-hydrolase; EC 3.1.1.3) hydrolyze triacylglycerols which are the major constituents of fats and oils. Lipases catalyze the hydrolysis of long chain triacylglycerols to diacylglycerols, monoacylglycerols, fatty acids and glycerol. Lipase is a subclass of the esterases. Lipase enzyme has important roles in different biotechnological and industrial processes due to their diverse catalytic properties and substrate specificity. Their activities are used in the food-, pharmaceutical-, leather- and detergent industries as well as in the production of fine chemicals and biodiesel. Most of the current commercial enzymes are derived from microbial sources e.g. bacteria or filamentous fungi. The main advantage of enzyme production by microorganisms is that microorganisms can produce large amounts of enzyme economically (Alexandra, 2017).

Lipase producing microorganisms are in a wide range of environments such as industrial wastes, compost heaps, oilseeds, deteriorated food, vegetable oils processing factories and dairy products. Soil contaminated with oils also possesses a huge variety of enzymes producing microorganisms. Microorganisms are being used as lipase producers. Although plants, animals, fungi and bacteria widely produce lipase enzymes, fungal lipases are being used for various biotechnological purposes. Among those, filamentous fungi are considered as an ideal source of lipase production because they produce extracellular enzymes. *Aspergillus, Penicillium*, *Mucor*, *Rhizopus* and *Geotrichum* are the most luxuriant sources of lipase enzyme (Pandey *et al.*, 2016). Filamentous fungi are known as good lipase producers and numerous fungal enzymes are used in various food industrial processes. Since lipases produced by filamentous fungi are mainly extracellular, extraction and purification are relatively easy. This reason mentions to the fact that fungal lipases belong to the most important groups of commercial enzymes (Kotogan *et al.*,

[1] PhD student, Department of Botany, University of Yangon
[2] Professor, Department of Botany, University of Yangon

2014). In view of current and potential applications, lipases are considered to be a promising class of industrial enzymes (Kumar *et al.*, 2012).

This study was undertaken to isolate and identify the lipase producing fungi because of the numerous potential uses of lipase enzyme. The aim and objectives of this study were to screen lipolytic fungi from different sources and to identify lipolytic fungi into genus level according to their phenotypic characters and spore formation.

Materials and Methods

Sample preparation from different sources

1. Collection and isolation of lipolytic fungi from pork sausage (PSR)

Small pieces of pork sausage sample were kept in a plastic container. Pork sausage in the plastic container was incubated for two weeks until fungal growth was observed.

2. Collection and isolation of lipolytic fungi from cheese (CH)

Cheese was incubated in the plastic cup for eight days until fungal growth was observed.

3. Collection and isolation of lipolytic fungi from margarine (BM)

Small amount of margarine sample was kept in the plastic container. Margarine in the container was incubated for a month until fungal growth was observed.

4. Collection and isolation of lipolytic fungi from scraped coconut shell (CB)

Fungal growth was occurred on scraped coconut shell and taken from the coconut shop in the market.

5. Collection and isolation of lipolytic fungi from scraped coconut peel (SCP)

Scraped coconut peel in a petridish was incubated for thirteen days until fungal growth was observed.

6. Collection and isolation of lipolytic fungi from tea leaves in bean oil with salt (TOS)

Tea leaves in bean oil with salt were incubated in a container for five days until fungal growth was observed.

7. Collection and isolation of lipolytic fungi from fuel oil contaminated soil (OS)

Fuel oil contaminated soil sample was taken from the car workshop, Thuwana Township, Yangon Region, Myanmar. Soil sample was dried in the air. A ten-fold dilution series of soil was prepared according to Alexander and Strete (2001). The mixture of soil sample 1 g and 9 ml distilled water was carried out serial dilutions from 10^{-1} to 10^{-9}. After preparation of serial dilutions, 0.1 ml (100 uL) from selected dilutions was cultivated on Potato Dextrose Agar (PDA) medium plates and incubated at room temperature.

Fuel oil storage place at car workshop | Sample collected place | Collected soil sample | Soil sample was dried in the air

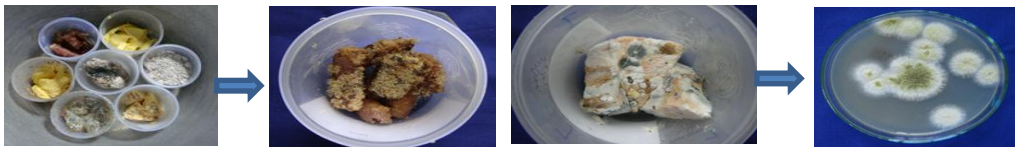
Figure 1 Soil sample collection from car workshop as fuel oil contaminated soil

Cultivation of Fungi

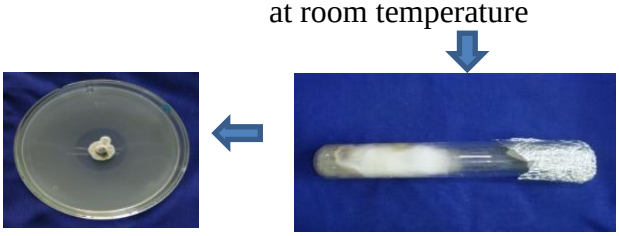
Fungal strains were directly collected from pork sausage, cheese, margarine, tea leaves in bean oil with salt, scraped coconut shell, scraped coconut peel and diluted soil contaminated with fuel oil. Fungi were cultivated and isolated on Potatoes Dextrose Agar (PDA) medium at room temperature for 5 - 7 days old. The pure fungal strains were maintained in test tubes with PDA medium. PDA medium was also used as stock culture medium or sub-culture medium for maintenance of fungus according to Atlas, 1993. All stock cultures were stored at 4 ºC. Potato Dextrose Agar (PDA) medium constituents: Mash Potato: 200 g, Peptone: 3 g, Dextrose: 20 g, Agar: 20 g, Distilled water: 1000 mL, pH: 6.5 ± 2 (Atlas, 1993). Chloramphenicol was added for antibacterial activity.

Screening of lipolytic fungi using Tributyrin Agar (TBA) medium

Screening of lipase producing fungi was done using tributyrin as a substrate on agar plates. Two different percentages (0.1 % and 1 %) of Tributyrin were used in this study. Lipolytic fungi were screened using Tributyrin Agar medium with 0.1 % tributyrin (Composition %/mL: Peptone: 0.5 g, Yeast extract: 0.3 g, Tributyrin (HiMedia): 0.1 mL, Agar: 2.0 g, pH: 6.0) according to Kotogan *et al.*, (2014) and Griebeler *et al.*, (2011). In addition, Tributyrin Agar (TBA) medium with 1 % tributyrin (composition %/mL: Peptone: 0.5 g, Yeast extract: 0.3 g, Agar: 2.0 g, Tributyrin (HiMedia): 1.0 mL, pH: 7.5 ± 0.2) was also used for screening of lipolytic fungi according to Wadia and Jain (2017). Clear hydrolytic halo regions occurred around colonies, it indicated that lipase enzyme was produced. All the isolated fungal cultures were inoculated on TBA plates and incubated at room temperatue for 2 - 17 days.



Different sources | Fungal growth on sources | Incubated for 5 days to 7 days with PDA medium at room temperature



Lipolytic fungi were screen on TBA medium | Pure strains were maintained in test tubes with PDA medium

Figure 2 Procedure of screening and isolation of lipolytic fungi from different sources

Identification of lipolytic fungi

Fungi were identified according to Barnett (1960) and Dube (1983).

Yeast Extract Agar medium

Yeast Extract Agar medium (Yeast extract: 3 g, Agar: 20 g, Distilled water: 1000 mL, pH: 7.0 ± 0.2. Chloramphenicol was added for antibacterial activity.) was used for direct microscopic examination of colony and spore formation according to Atlas, 1993. Lipolytic fungal strains were inoculated on Yeast Extract Agar medium plates and incubated at room temperature for 2 to 3 days before direct examination under microscope.

Results

Isolation of lipolytic fungi form different sources

Lipolytic fungi were isolated from seven different sources. In this study, ten different types of lipolytic fungi as shown in Table 1 and Figure 3 to 12 such as one *Aspergillus* sp. from pork sausage (Figure 3), one *Penicillium* sp. and one *Aspergillus* sp. from cheese(Figure 4 and 5), one *Aspergillus* sp. from tea leaves in bean oil with salt (Figure 6), one *Aspergillus* sp. from scraped coconut shell (Figure 7), one *Aspergillus* sp. from scraped coconut peel (Figure 8) and one *Monilia* sp. and three *Aspergillus* sp. from fuel oil contaminated soil (Figure 9, 10, 11 and 12) were observed. Each lipolytic fungus was identified based on their characters of pure colony morphology and spore formation according to Barnett (1960) and Dube (1983). The results were shown in Table 1 and Figure 3 to 12.

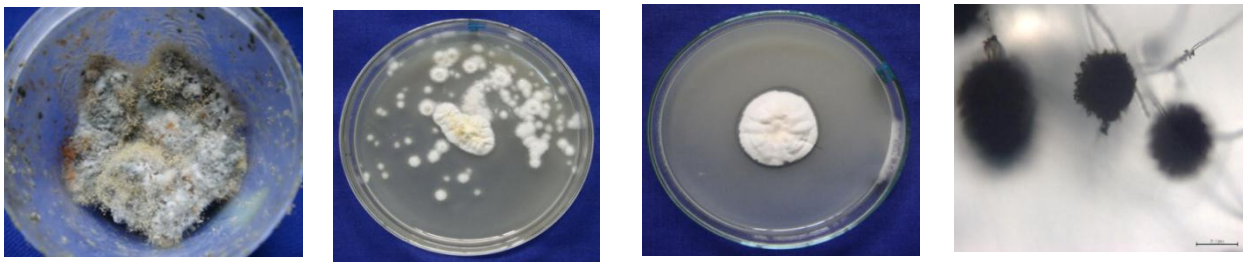
Table 1 Lipolytic fungi from different sources

No.	Fungal sources	Lipase producing fungi	Code of isolated strains	Clear zone/Halo region	
				0.1%TBA	1% TBA
1.	Pork sausage	*Aspergillus* sp. (1)	PSR-1	After 2-5 days	After 5- 17 Days
2.	Cheese	*Penicillium* sp.	CH-1	‖	‖
		Aspergillus sp. (2)	CH-3	‖	‖
3.	Tea leaves in bean oil with salt	*Aspergillus* sp. (3)	TOS-1	‖	‖
4.	Scraped coconut shell	*Aspergillus* sp. (4)	CB-5	‖	‖
5.	Scraped coconut peel	*Aspergillus* sp. (5)	SCP-4	‖	‖
6.	Fuel oil contaminated soil	*Monilia* sp.	OS-6	‖	‖
		Aspergillus sp. (6)	OS-3	‖	‖
		Aspergillus sp. (7)	OS-8	‖	‖
		Aspergillus sp. (8)	OS-13	‖	‖

Identification of isolated lipolytic fungi

Characteristics of mycelium and spore formation of *Aspergillus* sp. isolated from Pork Sausage (PSR)

Aspergillus sp. (1) colony was yellow color inside and white color periphery. Mycelia were scattered in culture.

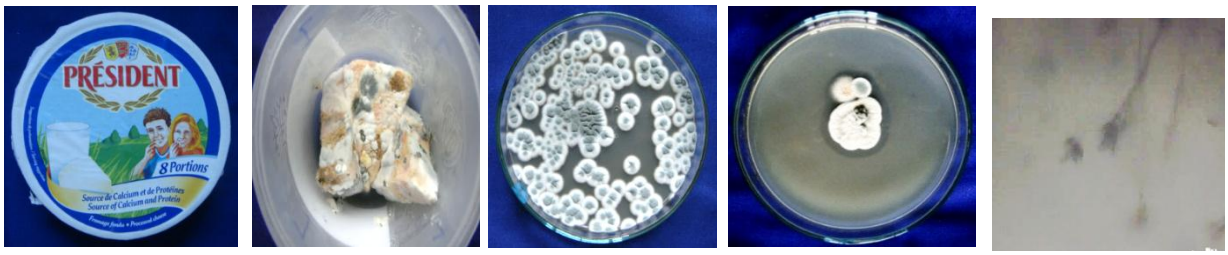


(A) **(B)** **(C)** **(D)**

Figure 3 Lipolytic fungus *Aspergillus* sp. (1) from pork sausage
(A) Fungal growth on pork sausage **(B)** Pure fungal colony (5 - 7 days old) (yellow color inside and white color periphery) (PSR - 1) **(C)** Clear zone (halo) around fungal colony (2 days old) on 1 % TBA medium **(D)** Micrograph of *Aspergillus* sp. (1) (X 200)

Characteristics of mycelium and spore formation of *Penicillium* sp. isolated from cheese (CH)

Penicillium sp. colony was green color inside and white color periphery. Mycelia were scattered in culture.

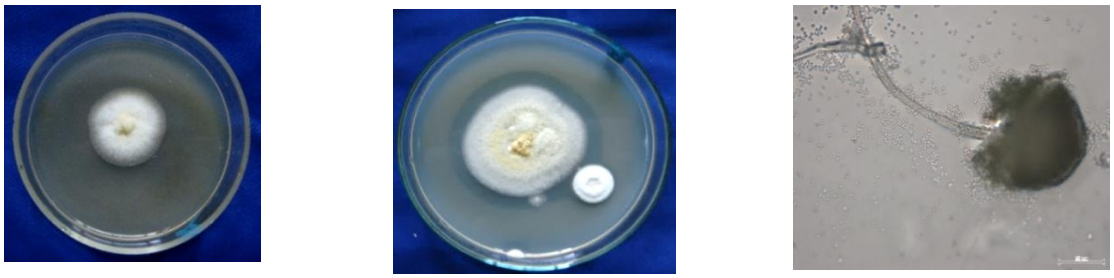


(A) **(B)** **(C)** **(D)** **(E)**

Figure 4 Lipolytic fungus *Penicillium* sp. (1) from cheese
(A) Collected cheese source **(B)** Fungal growth on cheese **(C)** Pure fungal colony (5-7 days old) (green color inside and white color periphery) (CH - 1) **(D)** Clear zone (halo) around fungal colony (5 days old) on 0.1 % TBA medium **(E)** Micrograph of *Penicillium* sp. (X 400)

Characteristics of mycelium and spore formation of *Aspergillus* sp. isolated from cheese (CH)

Aspergillus sp. (2) colony was yellow color inside and white color periphery. Mycelia were scattered in culture.

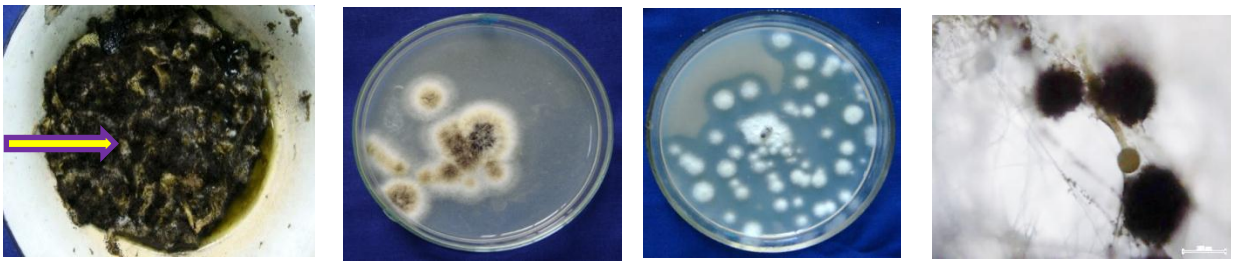


(A) **(B)** **(C)**

Figure 5 Lipolytic fungus *Aspergillus* sp. (2) from cheese **(A)** Pure fungal colony (5-7 days old) (yellow color inside and white color periphery) from cheese (CH.3) **(B)** Clear zone (halo) around fungal colony (5 days old) on 0.1% TBA medium **(C)** Micrograph of *Aspergillus* sp. (3) (X 400)

Characteristics of mycelium and spore formation of *Aspergillus* sp. isolated from tea leaves in bean oil with salt (TOS)

Aspergillus sp. (3) colony was black color inside yellow and white color periphery. Mycelia were scattered in culture.

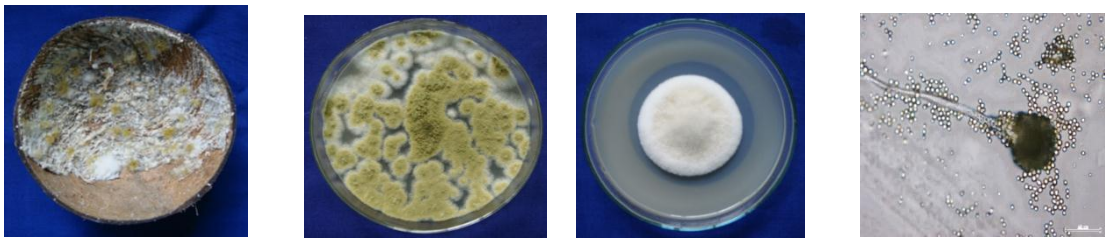


(A) **(B)** **(C)** **(D)**

Figure 6 Lipolytic fungus *Aspergillus* sp. (3) from tea leaves in bean oil with salt **(A)** Fungal growth on tea leaves in bean oil with salt **(B)** Pure fungal colony (5- 7 days old) (black color inside and yellow white color periphery) (TOS- 1) **(C)** Clear zone (halo) around fungal colony (7 days old) on 1% TBA medium **(D)** Micrograph of *Aspergillus* sp. (3) (X 200)

Characteristics of mycelium and spore formation of *Aspergillus* sp. isolated from scraped coconut shell (CB.)

Aspergillus sp. (4) colony was green color inside and white color periphery. Mycelia were scattered in culture.

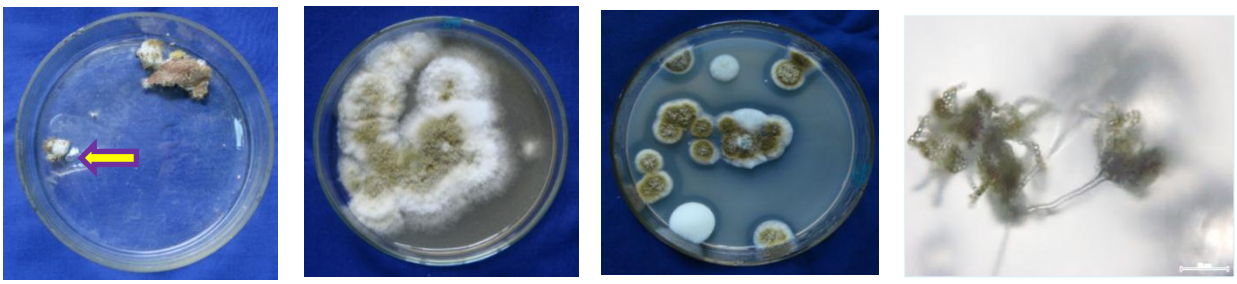


(A) **(B)** **(C)** **(D)**

Figure 7 Lipolytic fungus *Aspergillus* sp. (4) from scraped coconut shell
(A) Collected scraped coconut shell **(B)** Pure fungal colony (5 - 7 days old) (green color inside and white color periphery) (CB-5) **(C)** Clear zone (halo) around fungal colony (17 days old) on 1% TBA medium **(D)** Micrograph of *Aspergillus* sp. (4) (X 400)

Characteristics of mycelium and spore formation of *Aspergillus* sp. isolated from scraped coconut peel (SCP)

Aspergillus sp. (5) colony was green color inside white color periphery. Mycelia were scattered in culture.



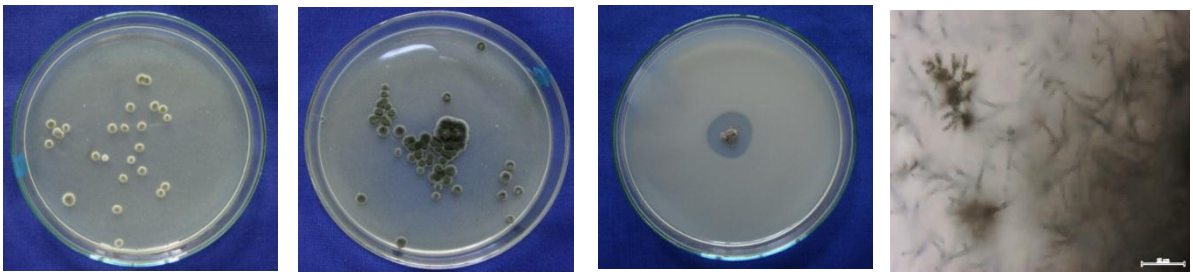
(A) **(B)** **(C)** **(D)**

Figure 8 Lipolytic fungus *Aspergillus* sp. (5) from scraped coconut peel

(A) Fungal growth on collected scraped coconut peel **(B)** Pure fungal colony (5 - 7 days old) (green color inside and white color periphery) (SCP.4) **(C)** Clear zone (halo) around fungal colony (7 days old) on 1% TBA medium **(D)** Micrograph of *Aspergillus* sp. (5) (X 400)

Characteristics of mycelium and spore formation of *Monilia* sp. isolated from fuel oil contaminated soil (OS)

Monilia sp. colony was black green color inside and white color periphery.



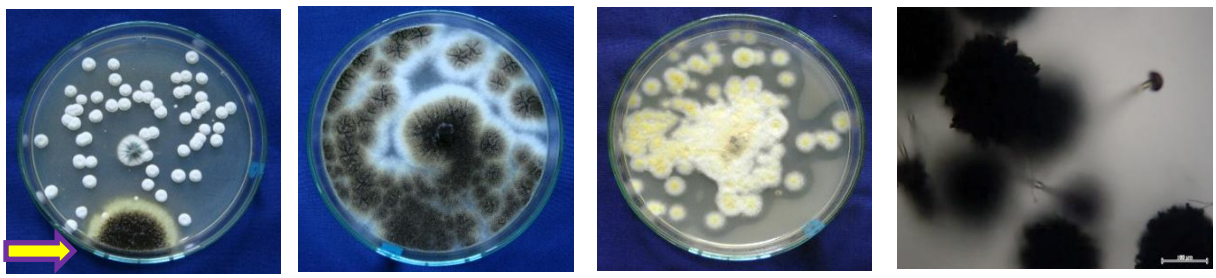
(A) **(B)** **(C)** **(D)**

Figure 9 Lipolytic fungus *Monilia* sp. from fuel oil contaminated soil

(A) Fungus isolated from soil sample (10^{-6}) **(B)** Pure fungal colony (5-7 days old) (black green color inside and white color periphery) from soil sample (10^{-6}) (OS.6) **(C)** Clear zone (halo) around fungal colony (17days old) on 1% TBA medium **(D)** Micrograph of *Monilia* sp. (X 400)

Characteristics of mycelium and spore formation of *Aspergillus* sp. isolated from fuel oil contaminated soil (OS)

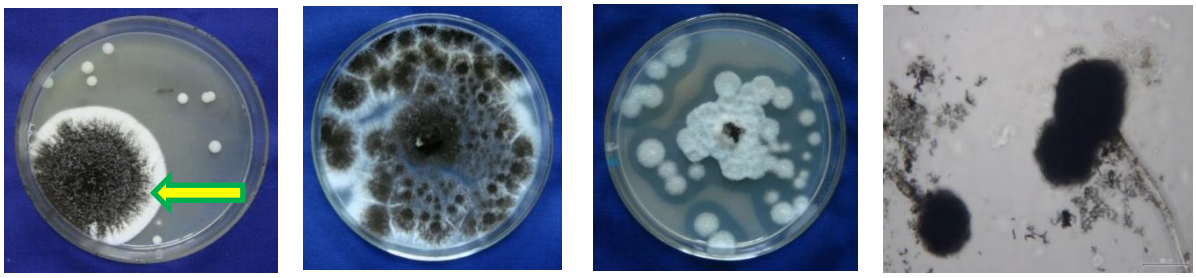
Aspergillus sp. (6) colony was black color inside and yellow white color periphery. Mycelia were scattered in culture. *Aspergillus* sp. (7) colony was black color inside and white color periphery. Mycelia were scattered in culture. *Aspergillus* sp. (8) colony was yellow color inside and white colony periphery. Mycelia were scattered in culture.



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

Figure 10 Lipolytic fungus *Aspergillus* sp. (6) from fuel oil contaminated soil

(A) Fungus isolated from soil sample (10^{-3}) **(B)** Pure fungal colony (5-7 days old) (black color inside and yellow white color periphery) from soil sample (10^{-3}) (OS.3) **(C)** Clear zone (halo) around fungal colony (6 days old) on 1% TBA medium **(D)** Micrograph of *Aspergillus* sp. (6) (X 200)



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

Figure 11 Lipolytic fungus *Aspergillus* sp. (7) from fuel oil contaminated soil
(A) Fungus isolated from soil sample (10^{-8}) **(B)** Pure fungal colony (5-7 days old) (black color inside and white color periphery) from soil sample (10^{-8}) (OS.8) **(C)** Clear zone (halo) around fungal colony (6 days old) on 1% TBA medium **(D)** Micrograph of *Aspergillus* sp. (7) (X 200)

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

Figure 12 Lipolytic fungus *Aspergillus* sp. (8) from fuel oil contaminated soil

(A) Fungus isolated from soil sample (II. 10^{-7}) **(B)** Pure fungal colony (5-7 days old) (yellow color inside and white color periphery) from soil sample (II. $10^{-7)}$ (OS.13) **(C)** Clear zone (halo) around fungal colony (5 days old) on 0.1% TBA medium **(D)** Micrograph of *Aspergillus* sp. (8) (X 200)

Table 2 Characters of lipolytic fungi isolated from different sources

Sr. No.	Fungal sources	Code of isolated strains	Macroscopic characters of lipolytic fungi	Microscopic characters of lipolytic fungi	Species
1.	Pork sausage	PSR-1	Mycelium was yellow color inside and whit color periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose, radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (1)
2	Cheese	CH-1	Mycelium was green color inside and white color periphery. Mycelia were scattered in culture.	Conidiophores arising from the mycelium singly, branched near the apex to form a brush-like, conidia-bearing apparatus, conidia brightly colored in mass, 1-celled, mostly globose or ovoid, produced basipetally.	*Penicillium* sp.

Sr. No.	Fungal sources	Code of isolated strains	Macroscopic characters of lipolytic fungi	Microscopic characters of lipolytic fungi	Species
3.		CH-3	Mycelium was yellow color inside and white color periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose, radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (2)
4.	Tea leaves in bean oil with salt	TOS-1	Mycelium was black color inside yellow and white color periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose, radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (3)
5.	Scraped coconut shell	CB-5	Mycelium was green color inside and white color periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose, radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (4)
6.	Scraped coconut peel	SCP-4	Mycelium was green color inside white color periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose, radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (5)
7.	Fuel oil contaminated soil	OS-6	Mycelium was black green color inside and white color periphery.	Mycelium white or gray, abundant in culture; conidia gray or tan in mass, 1-celled, short cylindric to rounded, catenulate, formed acropetally, conidiophore branched.	*Monilia* sp.
8.		OS-3	Mycelium was black color inside and yellow white color periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (6)
9.		OS-8	Mycelium was black color inside and white color periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (7)
10.		OS-13	Mycelium was yellow color inside and white colony periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (8)

Discussion and Conclusion

In this research work, screening, isolation and identification of lipase producing fungi were studied from different sources. Total twenty-five fungi were observed from seven different sources. Two fungal strains from pork sausage, five fungal strains from cheese, one fungal strain from margarine, two fungal strains from tea leaves in bean oil with salt, seven fungal strains from scraped coconut and eight fungal strains from fuel oil contaminated soil were observed. Among them, ten different types of lipolytic fungi were observed from six different sources.

In this study, lipolytic fungi such as different species of *Aspergillus* from pork sausage, scraped coconut shell, scraped coconut peel, tea leaves in bean oil were observed. *Penicillium* sp. and *Aspergillus* sp. were observed from cheese. Zohri *et al.* (2014) also reported that *Aspergillus niger*, *A. terreus*, *A. flavus*, *Penicillium chrysogenum* and *P. citrinum* were the most common fungal species on both beef burger and sausage samples. All beef burger and sausage samples were contaminated with different fungal species. Most isolated fungi were able to produce lipase enzyme.

In the present research, three *Aspergillus* species and *Monilia* sp. were observed from fuel oil contaminated soil. Colla *et al.* (2015) described that lipases from different sources may present different properties. Two *Aspergillus* species were isolated from the diesel-contaminated soil in the city of Passo Fundo, RS, Brazil and selected as good lipase producers. In addition, Mukhtar *et al.* (2015) also stated that seven different lipolytic fungal strains were found from soil samples for lipase production. Among those fungal strains, *Aspergillus niger* revealed the best results.

Fattah and Hammad (2002) reported that filamentous fungi from soil were screened and isolated for extracellular lipase production. Among ten isolated fungi, *Aspergillus niger* and *Aspergillus terreus* revealed as the highest lipase producers. Sumathy *et al.* (2012) also reported that *Aspergillus*, *Penicillium*, *Mucor*, *Geotrichum*, *Fusarium* and *Rhizopus* genera were widely used as sources of lipases.

In this study, three different genera of lipolytic fungi were observed from six different sources. They were *Aspergillus, Penicillium and Monilia* genera. Lipolytic fungi such as eight *Aspergillus* species, one *Penicillium* sp. and one *Monilia* species from pork sausage, cheese, scraped coconut shell, scraped coconut peel, tea leaves in bean oil and fuel oil contaminated soil were observed. Total ten lipolytic fungi were screened and isolated from six different sources. Among them, about 4 to 8 lipolytic fungi with the most outstanding clear zone will be selected for the future study.

Acknowledgements

I would like to express my sincere gratitude to Professor Dr. Aye Pe, Professor and Head of Botany Department, University of Yangon, for his permission to carry out this research and invaluable advice. I would like to express my special gratitude to my supervisor Dr. Bay Dar, Professor, Department of Botany, University of Yangon, for her brilliant guidance, patience, motivation and continuous great support. I extend my deepest gratitude to my co-supervisor Dr. Nu Yin Aye, Lecture, Department of Botany, University of Yangon, for giving me the opportunity to do this research, invaluable guidance and good support.

References

Atlas, R. M., (1993). **Handbook of Microbiological Media.** CRC Press, London.

Alexander, S. K. and D. Strete, (2001). **Microbiology: A Photographic Atlas for The Laboratory.** U.S.A.

Alexandra, K., (2017). **Analysis of extracellular lipase enzymes from zygomycetes fungi: enzyme production, characterization of synthetic and hydrolytic reactions.** Ph.D Thesis. Department of Microbiology. Szeged.

Barnett, H. L., (1960). **Illustrated Genera of Imperfected Fungi.** Second Edition. Printed in the United States of America.

Colla, L. M., A. M. M. Ficanha, M. J. Rizzardi, T. E. Bertolin, C. O. Reinehr and J. A. V. Costa, (2015). **Production and characterization of lipase by two new isolates of Aspergillus through solid-state and submerged fermentation.** BioMed Research International, ID 725959.

Dube, H. C., (1983). **An Introduction to Fungi. Second Revised Edition.** Printed at Vishal Printers, Delhi.

Fattah, G. M. A. and I. Hammad, (2002). **Production of lipase by certain soil fungi: Optimization of cultural conditions and genetic characterization of lipolytic strains of *Aspergillus* using protein patterns and random amplified polymorphic DNA (RAPD).** Online Journal of Biological Sciences 2 (10): p. 639-644.

Griebeler, N., A. E. Polloni., D. Remonatto, F. Arbter., R. Vardanega, J. L. Cechet, M. D. Luccio, D. d. Oliveira, H. Treichel, R. L. Cansian, E. Rigo and J. L. Ninow, (2011). **Isolation and screening of lipase-producing fungi with hydrolytic activity.** Food Bioprocess Technol 4: p. 57-586.

Kumar, A. P., K. J. Kumar and G. Narasimha, (2012). **Isolation of lipase producing fungi from groundnut oil mill effluent soil site at Nandyal.** Int J Pharm Bio Sci, 3(4): (B) p. 275-280.

Kotogan, A., B. Nemeth, C. Vagvolgyi, T. Papp and M. Tako, (2014). **Screening for extracellular lipase enzymes with transesterification capacity in Mucoromycotina strains.** ISSN (FTB-3455), p. 1330-9862.

Mukhtar, H., M. Hanif, A. U. Rehman, A. Nawaz and I. U. Haq, (2015). **Studies on the Lipase production by Aspergillus niger through solid state fermentation.** Pak. J. Bot., 47(SI): p. 351-354.

Pandey. H., A. Kestwal, D. Chauhan, S. Kumari, V. Dhalwal, G. J. Singh, P. Singh, Parul Mann, A. Sharma, G. Saxena, A. Kapoor and B. Giri, (2016). **Isolation and screening of potential fungi and standardization of a process for the production of exracellular lipase.** DU Haurnal of undergraduatae Research and Innocation (Delhi, 2016): p. 116-122.

Sumathy, R., M. Vijayalakshmi and M. Deecaraman, (2012). **Studies on lipase production from fungal strains by different inducers at varied concentrations- A comparative study.** International journal of environmental sciences, vol. 3, ISSN, p. 0976- 4402.

Wadia, T. and S. K. Jain, (2017). **Isolation, screening and identification of lipase producing fungi from oil contaminated soil of Shani Mandir Ujjain.** Int. J. Curr. Microbiol. App. Sci (2017) 6(7): p. 1872-1878.

Zohri, A. A., A. M. Moharram and R. R. S. Refaie (2014). **Mycobiota contaminating beef burger and sausage with reference to their toxins and enzymes.** Journal of Basic & Applied Mycology (Egypt) 5 (2014): p. 61-73.

Websites

www.HiMedia.com

CHARACTERIZATION OF ANTIFUNGAL COMPOUNDS FROM ISOLATED ENDOPHYTIC FUNGUS *ASPERGILLUS DURICAULIS*

May Zin Myo[1], Zar Zar Yin [2], Thandar Aung [3]

Abstract

In the present research, the antifungal compounds isolated from endophytic fungus, *Aspergillus duricaulis* was carried out by paper chromatography using the solvent 20% NH_4Cl, ethyl acetate saturated with water, n-butanol saturated with water, n-butanol-acetic acid- water (3:1:1) for the extraction of antifungal metabolites against *Candida albicans*. And then, the fungal culture filtrate was studied by the ratio of two solvents, ethyl acetate and n-butanol to fermented broth (1:1, 2:1, 3:1v/v). The equal ratio (1:1 v/v) ethyl acetate extract showed higher inhibitory effect (26.75 mm) than n-butanol extract (20.49 mm). Crude ethyl acetate extract (5.0 g) was obtained from 17 liter of fermented broth and subjected to purification over silica gel column chromatography with various solvent systems. By silica gel column chromatographic separation, compound A (aromatic primary amide, 23 mg colourless crystal) and compound B (aliphatic ester, 24 mg yellow semisolid) in hexane: ethyl acetate solvent system were isolated. These isolated compounds were characterized by R_f value, physicochemical properties, modern spectroscopic methods such as UV and FT IR. In the investigation of minimum inhibitory concentrations (MICs), it was observed that MICs value of antifungal compound A and B were 0.625 µg/mL and 1.25 µg/mL on *Candida albicans* respectively.

Keywords: paper chromatography, silica gel column chromatography, minimum inhibitory concentrations

Introduction

Endophytic fungi have the capability to produce bioactive compounds such as alkaloids, terpenoids, steroids, quinones, lignans, phenols and lactones (Lee *et al*., 2008). The recovery and purification of the product is one of the most critical aspects of industrial fermentation process. The type of extraction method, duration of extraction, temperature, and the polarity of solvent used influence the quality and the concentration of bioactive components isolated from the raw material (Annegowda *et al*., 2013). Chromatography is a useful technique for the separation of compounds from a complex mixture, such as a fungal extract.

Based on the physical and chemical properties of compounds and their affinities for certain solid phase materials (e.g., silica), a mixture can be separated into its individual compounds, or at least into mixtures containing fewer compounds with similar characteristics by selecting the appropriate elution solvent or solvent system (Harris, 2003). The most common methods of detection for early stages are: ultraviolet-visible spectroscopy (UV/Vis) that provides information on chromophores present in a compound and FT IR provides information on functional group present in a compound (Henke and Kelleher, 2016).

The minimum inhibitory concentrations (MICs) is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation (Andrews, 2001). It is well known crude extract isolated from these endophytic fungal metabolites contains complex chemical diversity which is difficult to identify and characterize. Therefore, effort has been made to characterize a bioactive molecule synthesized by isolated endophytic fungus *A. duricaulis* in this study. The aim and objectives of this study were to isolate some organic compounds from the ethyl acetate extract of *A. duricaulis*, to characterize the

[1] Demonstrator, Department of Botany, Pathein University
[2] Associate Professor, Department of Botany, Pathein University
[3] Associate Professor, Department of Chemistry, Yangon University

isolated compounds by physicochemical tests and spectroscopic techniques such as UV, FT IR and to determine the Minimum Inhibitory Concentrations (MICs) of fungal metabolites against *C.albicans.*

Materials and Methods

Paper chromatography (Tomita, 1988)

The filter paper and four solvents; 20% ammonium chloride (NH_4Cl), n- butanol saturated with water, n- butanol- acetic acid- water (3:1:1) and ethyl acetate saturated with water, were used for preliminary characterization of compound. The obtained fermented broth sample (100 µL) was applied on the paper and allowed to dry. The papers were chromatographed in each solvent. Then, bioautography was done to check the antifungal activity of each. Each paper was placed on assay agar plate. After one hour the paper was taken out, and then the plates were incubated for 24-36 hours. In this case, the inhibitory zone was measured yielding the R_f value for the corresponding bioactive compound.

Extraction of antifungal metabolites (Natarajan *et al*., 2010)

The fungus was cultivated on PGA by inoculating selected endophyte culture in 500 mL conical flask containing 250 mL of the medium. The flask was incubated at 25˚C for 5 days. After incubation period, fermentation broth of the fungus was filtered with filter paper. The filtrate was extracted with equal ratio of ethyl acetate. Then the mixture was shaken in a separating funnel. The organic layer was separated and collected.

Thin layer chromatographic analysis (Verma *et al*., 2014)

Thin layer chromatography (TLC) was performed on ethyl acetate crude extract from the culture broth of the endophyte. For this, the crude fraction was spotted (50 µL) on the TLC plate [GF_{254} silica gel precoated aluminium plate (Merck)] and chromatography was performed by employing solvent system chloroform: methanol (9:1, 8:2, 7:3 v/v), dichloromethane: methanol (9:1, 5:1, 2:1, 1:2 v/v) and hexane: ethyl acetate (9:1, 5:1, 2:1, 1:2 v/v). Spots were visualized by spraying with sulphuric acid.

Isolation of organic metabolites by silica gel column chromatography

(Simon and Gray, 1998)

According to thin layer chromatographic analysis, the ethyl acetate extract residue of isolated fungus *A. duricaulis* metabolite was developed to isolate the active compound by silica gel column chromatography with hexane: ethyl acetate as eluting solvent. Silica gel (60- 120 mesh) (ca.50g) was dissolved in Hexane: EtOAc 80:1 v/v and the column was packed by the wet method. EtOAc crude extract (3.0 g) was then passed through silica gel column and eluted with Hexane: EtOAc 80:1, 40:1, 20:1, 9:1, 5:1, 2:1, 1:1, 1:2 v/v. Fractions of each equal to 2 mL, were collected individually, the compounds presents were checked with TLC.

Characterization of isolated antifungal compounds

In an attempt to characterize the isolated antifungal compounds, the following tests were performed:

Determination of solubility of isolated compounds

Each of isolated compounds (0.5 mg) was subjected to 0.5 mL of polar and non-polar solvents such as water (H_2O), methanol (MeOH), ethyl acetate (EtOAc), chloroform ($CHCl_3$), pet-ether (PE) and Hexane (C_6H_{14}) in order to know their solubility.

Determination of some chemical properties of isolated compounds

Some coloured reagents such as 10% potassium permanganate ($KMnO_4$), iodine (I_2) vapour, Anisaldehyde, 5% sulphuric acid (H_2SO_4), 5% ferric chloride ($FeCl_3$), Lieberman Burchard, and 2,4 Dinitrophenylhydrazine (DNP) were used to study their behaviour on TLC.

Study under ultraviolet (UV- visible) spectroscopy

For the identification of isolated compounds, ultra violet absorption spectra were also recorded and examined. A Shimadzu UV- 1800 UV- visible spectrophotometer at Chemistry Department, Pathein University were used.

Study under Fourier transform (FT IR) spectroscopy

The FT IR spectra of isolated compounds were sampled with 1% KBr pellet and recorded by using Shimadzu FT IR- 8400 Fourier Transform Infrared spectrophotometer at Chemistry Department, Pathein University.

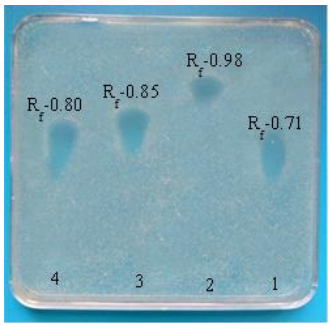
Minimum Inhibitory Concentration (MICs) of isolated compounds

Minimum Inhibitory Concentration (MICs) was carried out by two- fold serial dilution method (Domain, 1999 and Phay, 1997). The concentrations were 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.625 µg/mL and 0.3.12 µg/mL respectively. The test organism was *Candida albicans*. After incubation for 24 hours, the MICs were determined by selecting the lowest concentration of metabolite.

Results

Paper chromatography

In this study, four kinds of solvent 20% NH_4Cl, ethyl acetate saturated with water, n-butanol saturated with water, n-butanol- acetic acid- water (3:1:1) were used. According to R_f value (0.98), ethyl acetate was more extractable the antifungal metabolites than other solvent, followed by n- butanol solvent (0.85), ethyl acetate- acetic acid- water 3:1:1 (0.80) and the lowest R_f value at NH_4Cl (0.71) in Figure 1.



1. 20%NH_4Cl
2. Ethyl acetate saturated with water
3. n- butanol saturated with water
4. n-butanol-acetic acid- water (3:1:1)

Figure 1 Paper chromatography bioautographic assay

Comparison of antifungal activity of metabolite in *A.duricaulis* extracted with different volume of EtOAc and n-BuOH

Using ethyl acetate extract (1:1v/v) resulted in inhibition zone was 26.75 mm, followed by 24.75 mm and 22.69 mm in ethyl acetate extract (2:1v/v) and (3:1v/v) respectively as well as inhibitory zone 20.49 mm was found in n- butanol extract (1:1v/v), 19.53 mm in n-butanol extract (2:1v/v) and 18.84 mm in n-butanol extract (3:1v/v). Therefore ethyl acetate extract (1:1v/v) of MZF-2 showed the higher inhibition zone than n- butanol extract (1:1v/v). There was no antifungal activity at all of lower layer. These results were shown in Table 1.

Table 1 Comparison of antifungal activity of isolated fungus *A. duricaulis* extracted with different ratio of EtOAc and n-BuOH against *C.albicans*

Different ratio of solvent: fermented broth (v/v)	Inhibition diameter zone (mm)	
	EtOAc extract	n-BuOH extract
1:1	26.75	20.49
2:1	24.75	19.53
3:1	22.69	18.84

Thin layer chromatographic analysis

Thin layer chromatography (TLC) was performed on ethyl acetate crude extracted by employing solvent system: chloroform ($CHCl_3$): methanol (MeOH) (9:1, 8:2, 7:3 v/v), hexane (C_6H_{14}): ethyl acetate (EtOAc) (9:1, 5:1, 2:1, 1:2 v/v) and dichloromethane (CH_2Cl_2): methanol (MeOH) (9:1, 5:1, 2:1, 1:2 v/v). The extract showed well- separated spots on TLC by using $CHCl_3$: MeOH and C_6H_{14}: EtOAc solvent systems. No spots were observed by using CH_2Cl_2: MeOH solvent system. These results were presented in Figure 2. Therefore the solvent system C_6H_{14}: EtOAc was chosen to isolate pure compounds by silica gel column chromatography.

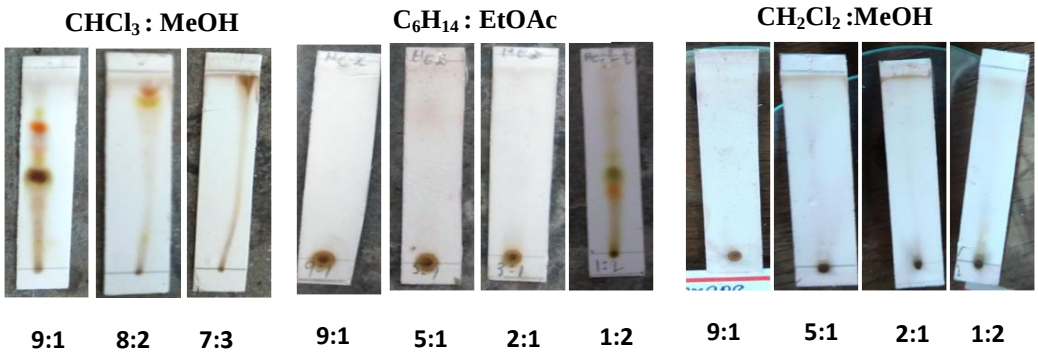
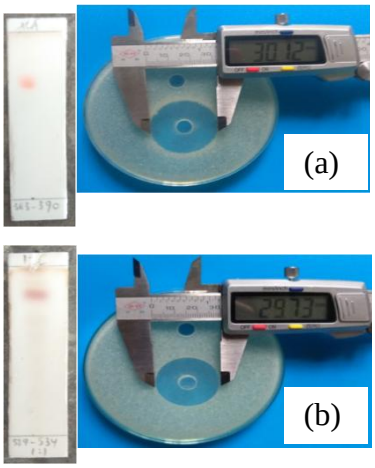


Figure 2 Thin layer chromatographic analysis with various solvent system

Isolation of some organic metabolites from ethyl acetate extract of the fermented broth of *A. duricaulis*

Gradient elution was performed successively with increasing polarity (Hexane: EtOAc, 80:1, 40:1, 20:1, 9:1, 5:1, 2:1, 1:1 and 1:2 v/v). According to the procedure in Figure 4, compound A (colourless crystal 23 mg) and compound B (semisolid in yellow 24 mg) were obtained from the respective fractions F-II and F-III. The remaining fractions FI, FIV and FV were observed as mixtures and no antifungal activity was recorded. This isolated compound A and B have significant activity on *C. albicans* with inhibitory zone 30.12 mm and 29.73mm respectively. Thin layer chromatogram of compounds (A and B) and their antifungal activity were presented in Figure 3.



Compound A

Solvent system : Hexane:EtOAc (1:1v/v)

R_f value : 0.6

Spraying agent : 5% H_2SO_4, heat

Compound B

Solvent system : Hexane: EtOAc (1:1v/v)

R_f value : 0.84

Spraying agent : 5% H_2SO_4, heat

Figure 3 Thin layer chromatogram of isolated compound and their antifungal activity against *Candida albicans* (a) compound A (b) compound B

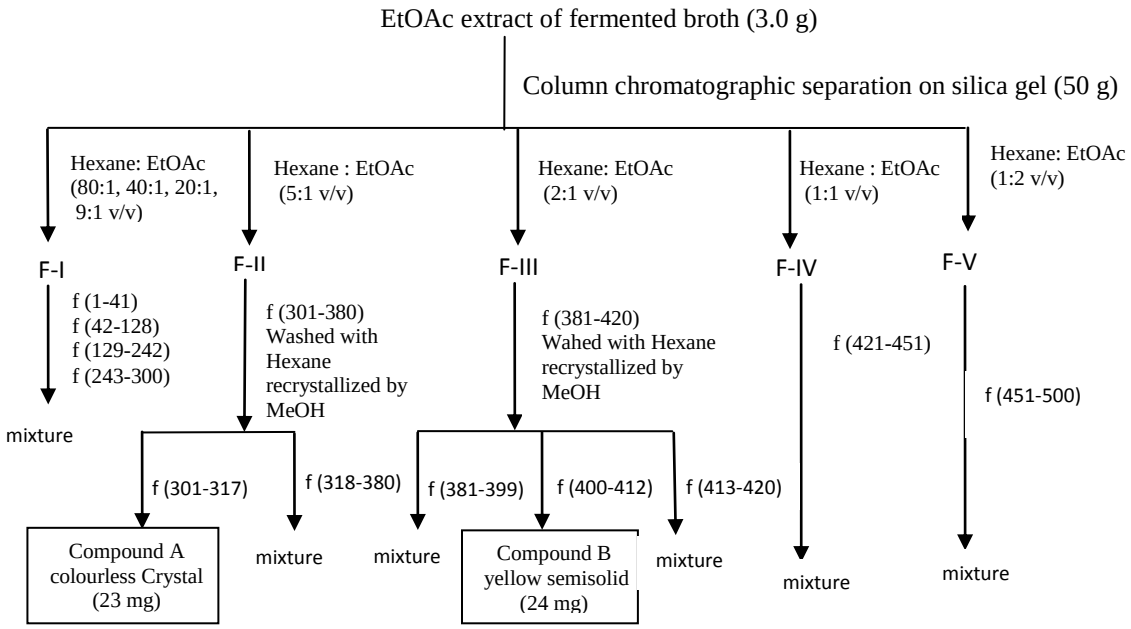


Figure 4 Isolation of organic metabolites from ethyl acetate crude extract,culture broth of isolated fungus *A.duricaulis* by column chromatography

Characterization of isolated antifungal compounds

The isolated compounds were characterized by physicochemical tests, solubility tests, modern spectroscopic techniques such as UV and FT IR. These resultant data were given as follow:

Table 2 Some chemical properties of isolated compounds

No.	Spraying agent	Observation (compounds)	
		A	**B**
1	10% $KMnO_4$	discharged	ND
2	I_2	Yellow	yellow
3	Anisaldehyde	Purple	ND
4	$FeCl_3$	ND	ND
5	Liebermann Burchard	Cherry red	pink
6	2,4 DNP	Yellow ppt	ND
7	5%H_2SO_4	pink	violet

ND- not detected

Compound A

It was soluble in EtOAc, EtOH, MeOH and $CHCl_3$ but insoluble in PE, Hexane and H_2O. R_f value of compound A was found to be 0.6 in Hexane: EtOAc (1:1 v/v) solvent system. According to the result obtained from chemical tests (Table 2), compound A gave yellow spot on TLC chromatogram with iodine vapour, purple spot with anisaldehyde followed by heating, cherry red colouration with Liebermann Burchard reagent, yellow colour with 2, 4 DNP and the colour of compound A was pink spot on TLC plate while spraying with 5% H_2SO_4 followed by heating.

The UV absorption spectrum shows peak at 296 nm. This band may be attributed to π-π^* transition (Figure 5 and Table 3). The functional groups present in compound A was studied by FT IR spectroscopy. The FT IR spectrum was shown in Figure 6 and the interpreted spectral data were illustrated in Table 4. The FT IR spectrum at compound A showed the bands at 3465 cm^{-1} and 3265 cm^{-1} due to N-H stretching of amide. Absorption band at 2960 cm^{-1} and 2853 cm^{-1} were due to -C-H stretching of alkyl group. C=O stretching of 1° amide was observed at 1685 cm^{-1} and 1603 cm^{-1}. Stretching band at 1532 cm^{-1} and 1436 cm^{-1} for C=C stretching of aromatic, and the band at 1407 cm^{-1} for C-C stretching of aromatic were observed. =C-H bending of aromatic group and C-H bending of aromatic compound were found at 1290 cm^{-1}, 1230 cm^{-1}, 1098 cm^{-1},

and 976 cm^{-1}, 909 cm^{-1} respectively. From the physicochemical properties, R_f value, UV and FT IR spectral data, isolated compound A may be aromatic primary amide.

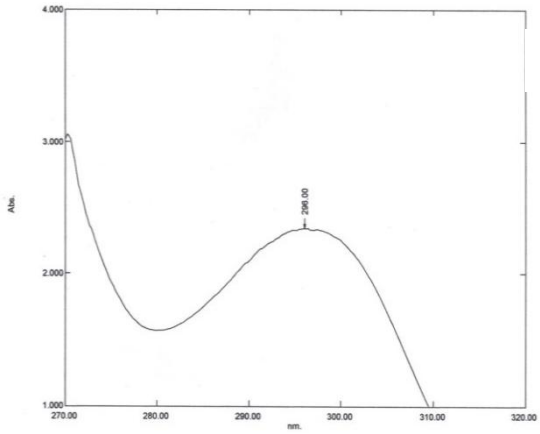


Table 3 UV spectral data of isolated compound A

Solvent used	Observed λ_{max} (nm)	Remark
MeOH	296	π-π* transition

Figure 5 UV spectrum of isolated compound A

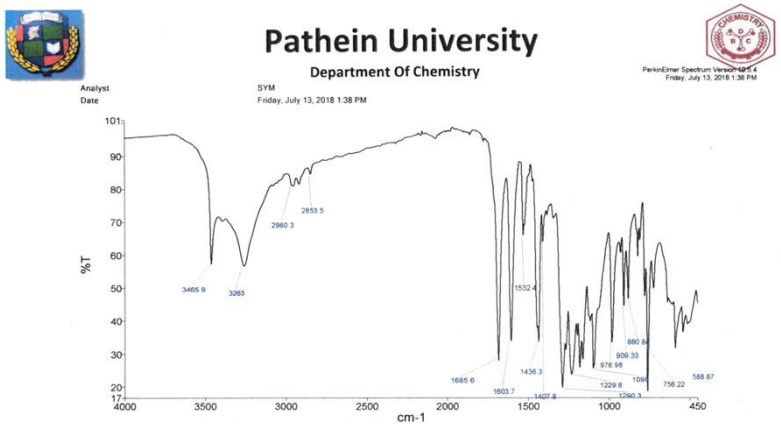


Figure 6 FT IR spectrum of isolated compound A

Table 4 FT IR spectral data of isolated compound A

Wave number (cm^{-1})	Literature* Wave number (cm^{-1})	Band assignment
3465, 3265	3500~3350	N-H stretching of amide
2960, 2853	2950~2840	-C-H stretching of alkyl group
1685, 1603	1700~15000	C=O stretching of 1° amide
1532, 1436	1600~1400	C=C stretching of aromatic
1407	~1430	C-C stretching of aromatic
1290, 1230, 1098	1250~1000	=C-H bending of aromatic group
976, 909	910~665	C-H bending of aromatic compound

*(Joseph *et al.*, 1987)

Compound B

It was soluble in EtOAc, EtOH, MeOH and $CHCl_3$ but insoluble in PE, Hexane and H_2O. Its R_f value was recorded 0.84 in Hexane: EtOAc (1:1 v/v) solvent system. Some chemical properties of isolated compound B was represented in Table 2. It showed a yellow spot on TLC plate with iodine vapour, pink colouration with Liebermann Burchard reagent and violet in colour when spraying with 5% H_2SO_4 followed by heating.

No absorption was observed in readily accessible UV region for isolated compound B. The IR results of the compound B shows the

presence of different bonds corresponding to the following functional group present in the molecular structure: stretching band at 2917 cm^{-1} and 2850 cm^{-1} for -C-H of alkyl group, stretching band at 1753 cm^{-1} for C=O of ester, 1476 cm^{-1} and 1461 cm^{-1} for C-H bending of an ester, 1372 cm^{-1} for -C-H bending of alkyl group. And the band at 1172 cm-1 was due to C-O-C stretching. These functional group of isolated compound and literature cited were shown in Table 5. All of physicochemical tested result, R_f value and spectroscopic data indicated that compound B may be aliphatic ester.

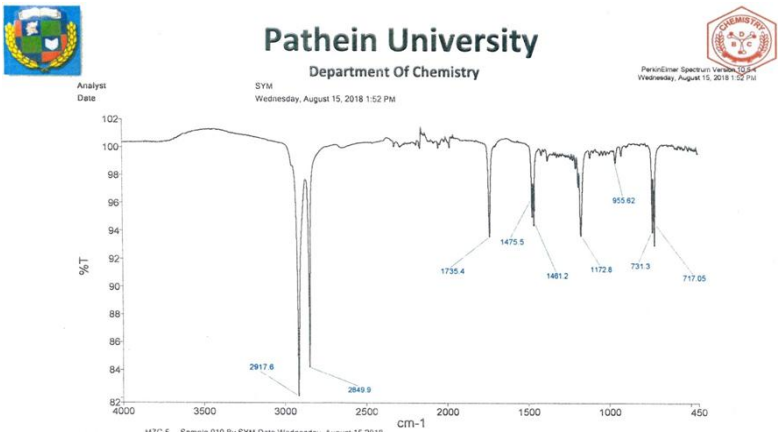


Figure 7 FT IR spectrum of isolated compound B

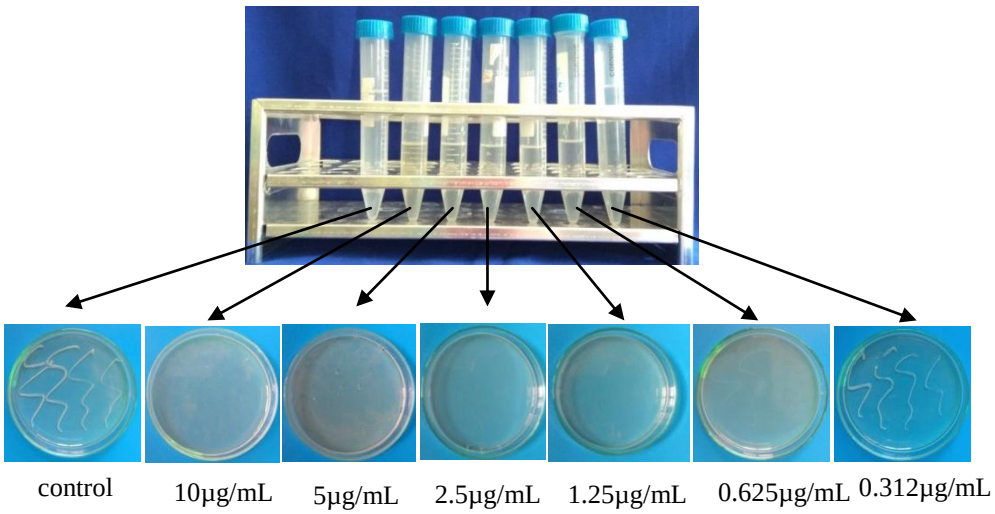
Table 5 FT IR spectral data of isolated compound B

Wave number	Literature* Wave number	Band assignment
2917, 2850	2950~2840	-C-H stretching of alkyl group
1753	1750~1720	C=O stretching of ester
1476, 1461	1480~1440	C-H bending of an ester
1372	1390~1365	-C-H bending of alkyl group
1172	1250~1050	C-O-C stretching

*(Joseph *et al.*, 1987)

Minimum Inhibitory Concentrations (MICs) of isolated compound

MICs compounds were determined by two fold serial dilution method ranging from 10 µg/ mL to 0.312 µg/ mL. MICs were read in µg/ mL after overnight incubation. It was observed that MICs value of compound A was 0.625 µg/ mL when used against *Candida albicans* and for compound B MICs value was 1.25 µg/ mL (Figure 8 to 11).



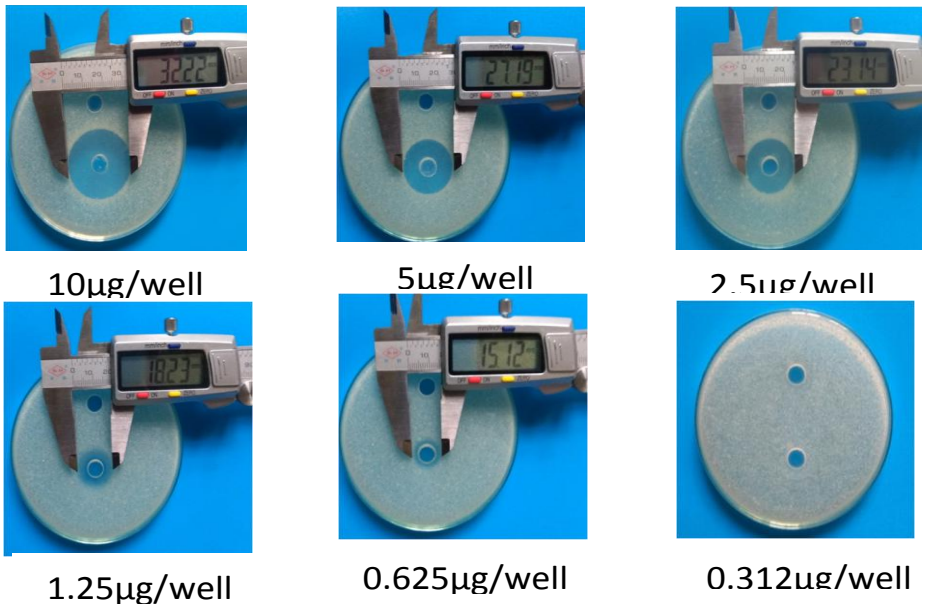


Figure 9 Minimum inhibitory concentrations of secondary metabolites from compound A on *Candida albicans* (agar well diffusion method)

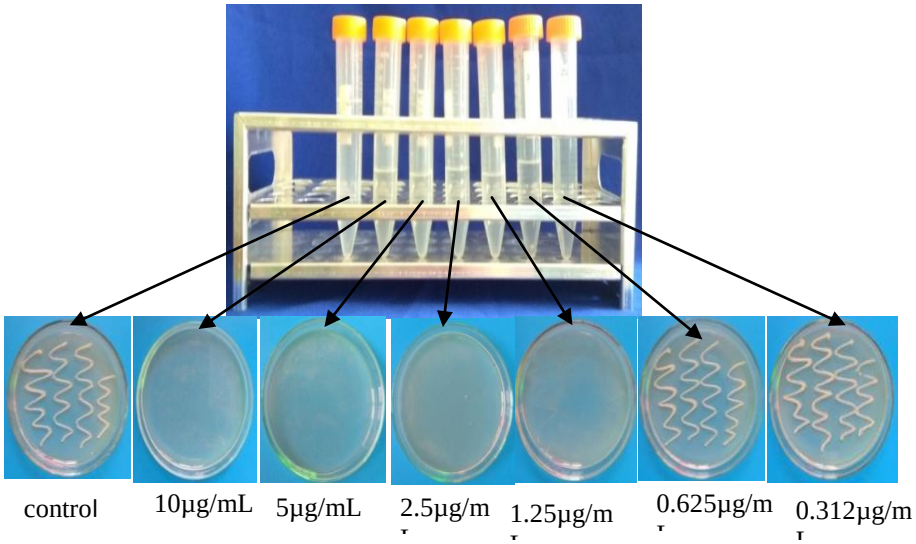


Figure 10 Minimum inhibitory concentrations of secondary metabolites from compound B on *Candida albicans* (streak method)

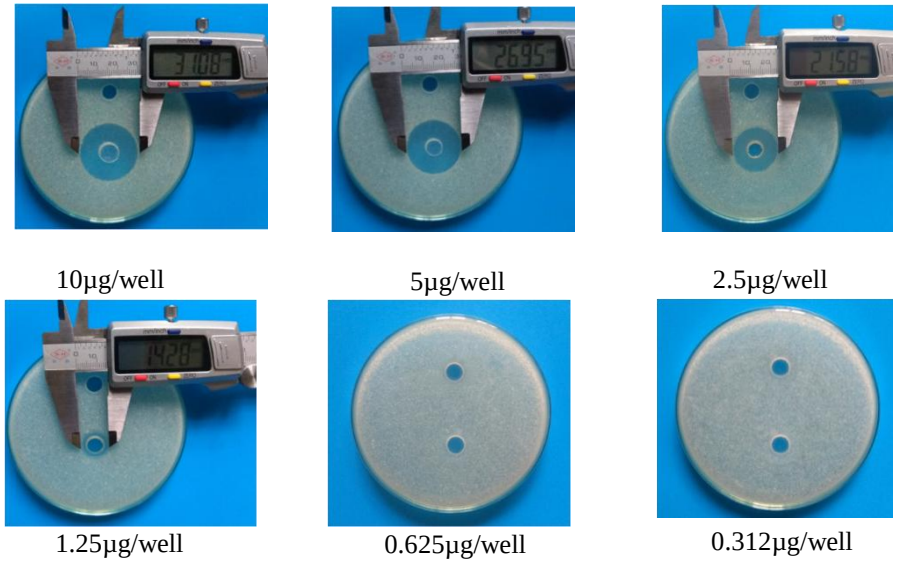


Figure 11 Minimum inhibitory concentrations of secondary metabolites from compound B on *Candida albicans* (agar well diffusion method)

Discussion and Conclusion

Fungal endophytes have been recognized as prolific producer of many chemical compounds having antibacterial, antifungal and other biological potential (Hoffman *et al*., 2008). In the investigation of paper chromatography, four kinds of different solvents were applied to observe the optimum extraction ability of secondary metabolites. According to R_f value, ethyl acetate was the excellent solvent for extracting secondary metabolites from *A. duricaulis*. There was in agreement with Garcia *et al*., 2012 who reported that ethyl acetate solvent system was

most efficient method to extract endophytic fungi principle compounds. On studying antifungal activity of *A.duricaulis* extracted with different ratio (1:1, 2:1, 3:1) of EtOAc and n-BuOH, equal ratio of ethyl acetate extract showed the highest activity with inhibition zone (26.75 mm). This result was in agreement with the description of Jain and Pundir 2011 the maximum antimicrobial metabolite was obtained by using the ratio of ethyl acetate to fermentation broth (1:1). Ayanbimpe *et al*., 2005 reported that the amount and nature of compounds produced depended on the strain of fungi and other conditions for extraction.

In this study, the optimized solvent system used was hexane: ethyl acetate at ratio 80:1, 40;1, 20:1, 9:1, 5:1, 2:1, 1:1 and 1:2. Similarly, Abdulwahid *et al*., 2013 used hexane: ethyl acetate solvent mixture in ratio of 8:2 and characterizied antibacterial compound from *Aspergillus niger*. The fractions (F) which gave the same R_f value on TLC were combined and tested their antifungal activity against *C.albicans.* The fraction (F-II) $f_{301-317}$; isolated compound A with R_f value 0.6] showed antifungal activity 30.12 mm. Inhibitory zones 29.73 mm was observed in fraction (F-III) $f_{400-412}$; isolated compound B with R_f value 0.84. The purified active compounds obtained were subjected to various examinations such as some chemical reagent tests, ultraviolet (UV) and FT IR(Fourier transform infrared). In order to these datas, the isolated compounds A and B may be aromatic primary amide and aliphatic ester respectively**.** In another study done by Yin *et al*., 2015, amine, aromatic, ketones, carboxylic acid ester, coumarin derivatives, dicarboxylic acid, heterocyclic compounds, hydrazide derivatives and imines identified were reported to exhibit antibacterial, antifungal and other biological activities. In a study of Minimum Inhibitory Concentrations (MICs) of isolated compounds, the antifungal metabolites affected on the growth of *C.albicans* at least MIC of 0.625 µg/mL for compound A and 1.25 µg/mL for compound B. A lower MIC is an indication of a better antimicrobial activity. In a study carried out by Suzuki *et al*., 1997, NF00659 A1, A2, A3, Bl and B2 novel metabolite produced by *Aspergillus* sp. showed antimicrobial activity at concentration of 1µg/ml against Gram-positive and Gram-negative bacteria and fungi.

The present study indicates that endophytic fungus *A.duricaulis* isolated from *Momordica charantia* L. had proved its capabilities of being a potential candidate in the search for an antifungal compound against antibiotic resistant human pathogenic fungi *C.albicans*. Further purification and structure elucidation of active compound and investigation its molecular mechanisms can be a promising approach for further antimicrobial drug development programs.

Acknowledgements

I gratefully acknowledge the following people: Professor Dr Kay Thi Mya, Head of Botany Department, University of Pathein and Professor Dr Wah Wah Lwin, Department of Botany, University of Pathein for their suggestion and kind understanding during this study. Dr Zar Zar Yin, Associate Professor, Department of Botany, Pathein University for her supervision, encouraging me to be eager doing this research. Dr Thandar Aung, Associate Professor, Department of Chemistry, University of Yangon, for supervising this study and for her fruitful discussion to carry out this work.

References

Abdulwahid, B.A., F.I. Al- Shaibani, S. AL- Shakarchi and S.R. Ameen. (2013). **Extraction and characterization of antibacterial compound from *Aspergillus niger***. Journal of Al-Nahrain University. 16 (4):167-174.

Andrews, J.M. (2001). **Determination of minimum inhibitory concentrations**. Journal of Antimicrobial Chemotherapy. 48:5-16.

Annegowda, H., P. Tan and M. Mordi. (2013). **TLC-bioautography-guided isolation, HPTLC and GC-MS-assisted analysis of bioactives of piper betle leaf extract obtained from various extraction techniques: *in vitro* evaluation of phenolic content, antioxidant and antimicrobial activities**. Food Analytical Methods. 6(3):715–726. doi: 10.1007/s12161-012-9470-y.

Ayanbimpe, G.M., E.O. Afolabi, R. Madaki and E.G. Solomon, (2005). **Preliminary extraction of secondary metabolites of arthropod-borne fungi**. AMERICAN Journal of Sci.6(1): 1215-1220.

Domain, A. L. (1999). **Biological properties of secondary metabolites**. 234-246.

Garcia, A., S.A. Rhoden, J. Bernardi- Wenzel, R.C. Orlandelli and J.L. Azevedo. (2012). **Antimicrobial activity of crude extract of endophytic fungi isolated from medicinal plant *Sapindus saponaria* L**. J ApplPharm Sci. 2: 35-40.

Harris, D. C. (2003). **Quantitative chemical analysis (6th ed.)**. New York: W. H. Freeman and Company.

Henke, M.T. and N.L. Kelleher. (2016). **Modern mass spectrometry for synthetic biology and structure-based discovery of natural products**. Natural Product Reports.33: 942-950 .

Hoffman, A.M, S.G. Mayer, G.A. Strobel, W.M. Hess and G.W. Sovocool. (2008). **Purification, identification and activity of phomodione, a furandione from and endophytic *Phoma* species.** Phytochemistry. 69; 1049-1056.

Jain, P. and R.K. Pundir. (2011). **Effect of fermentation medium, pH and temperature variations on antibacterial soil fungal metabolite production**. Journal of Agricultural Technology. 7 (2) : 247-269.

Joseph, B.L, F. S. Herbert , A.L. David and R.G. Cooks. (1987). **Introduction to Organic Spectroscopy**. New York, Macrnillan Publishing company.183-268.

Lee , H.S.H.H., J.H. Song, C.G. Ahn, G.P. Shin, and C. Lee. (2008). **Statistical optimization of growth medium for the production of the entomopathogenic and phytotoxic cyclic depsipeptide beauvericin from *Fusarium oxysporum* KFCC 11363P**. J. Microbiol. Biotechnol. 18: 138-144.

Natarajan, K., S. S. Rabiya and R. Sathish. (2010). **Screening for antibacterial compound in broth extracts of *Aspergillus niger* MTCC 2208**. Drug Invention Today. 2(8): 385-386.

Phay, N. (1997). Doctoral Thesis; **Studies on highly selective antibiotics**. Faculty of Agriculture, Hokkaido University, Japan.

Simon, G. and A.I Gray. (1998). **Isolation by planar Chromatography**. 209-246.

Suzuki, K., A. Kuwahara, H. Yushida, S. Fujita, T. Nishikiori and T. Nakagava. (1997). **NF00659AJ, A2, A3, BI and B2, novel antitumor antibiotics produced by *Aspergillus* sp. NF00659**. J. Antibiot. 50: 344-347.

Tomita, F.,(1988). **Laboratory Method, Hokkadio University Japan.**

Verma, A., B.N. Johri and A. Prakash. (2014). **Antagonistic evaluation of bioactive metabolite from endophytic fungus, *Aspergillus flavipes*** KF671231. J Mycol. 5, Article ID 371218.

Yin, O.C.J., I. Darah and C.L. Chong. (2015). **Bioactive Compounds from *Aspergillus terreus* MP15, an Endophytic Fungus Isolated from *Swietenia Macrophylla* Leaf.** Malays. j. med. biol. res. 2(3): 262-272.

MORPHOLOGICAL CHARACTERS OF *BARLERIA CRISTATA* L. AND *BARLERIA PRIONITIS* L. AND THEIR ANTIMICROBIAL ACTIVITIES

Sanda Myint[1], Zin Moe Moe[2], Myint Myint Khaing[3]

Abstract

The medicinal plants *Barleria cristata* L. and *Barleria prionitis* L. belong to the family Acanthaceae. *Barleria cristata* L. is known as blue bell *barleria* in English, leik-tha-ywe-pya in Myanmar. *Barleria prionitis* L. is known as porcupine flower in English, leik-sa-ywe in Myanmar. These plants are collected from Pyay Township. Comparative morphological characters, *Barleria cristata* L. is perennial mush branched shrubs; leaves simple, glabrous in two surfaces. *Barleria prionitis* L. is perennial erect spinescent herbs or undershrubs; leaves simple, exstipulate, spines 2-4 in the leaf axils, sparsely pubescent on both surfaces. In the result of antimicrobial test, various extracts of the leaves of *Barleria cristata* L. and *Barleria prionitis* L. are tested against six pathogenic microorganisms by using paper disc diffusion assay method. Antimicrobial activities of different solvent extracts (petroleum-ether, chloroform, ethyl-acetate, acetone, ethanol, methanol and water) of *Barleria cristata* L. and *Barleria prionitis* L. were tested on six pathogenic microorganisms such as *Aspergillus flavous*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae* at Department of Botany, University of Yangon.

Keywords: morphological characters, various leaves extracts, different microorganisms.

Introduction

Family Acanthaceae consists of about 250 genera and 2500 species widespread in tropical regions. *Barleria* is a large genus, pantropical genus of herbs and shrubs comprising of more than 300 species world-wide. *Barleria* is the most widespread, occurring almost throughout the entire geographic range of the genus. It distinguished by its conspicuously scarious calyx and axillary inflorescence based on scorpioid cymes, flowers weakly zygomorphic and 2-seeded (Hakimi *et al*., 2018).

Barleria cristata L. is an erect or diffuse herb, up to 1 m tall, stem appressed hairy, densely hairy at the nodes; branches and bracteoles spiny, leaves elliptic-oblong to lanceolate, acute or acuminate, hairy on both sides, flowers bluish-purple, pink or white, pubescent outside, born in 1-4 flowered axillary and terminal spikes, fruits capsules, ellipsoid or ablong, acute at both ends, 4-seeded and seeds orbicular, compressed, silky-hairy.*Barleria cristata* L. has various medicinal and therapeutic uses. *Barleria cristata* is an ornamental perennial shrub.Different parts of *Barleria cristata* L. have been used in the treatment of various diseases like anemia, toothache and cough, antimicrobial, anti- inflammatory and hepatoprotective activity. Root and leaves are used in the treatment of swelling and inflammation (Bency *et al*., 2018). *Barleria prionitis* L. is mush-branched, perennial, usually prickly shrub, stem rounded branches, leaves opposite, elliptic, acuminate, lineolate, bristletipped, entire, glabrous above, young leaves often pubescent beneath, flowers orange-yellow or cream-coloured, sessile, borne in axillary foliaceous bristle-tipped bracts, fruits capsules ovoid with a tapering beak, 2-seeded and seeds compressed, ovate, clothed with silky appressed hairs (Naidu, 2012). The whole plant and especially the roots are used as tonic and diuretic (Hakimi *et al*., 2018). Leaves, stem and root of *Barleria prionitis* possess antibacterial and anti-inflammatory activities (Aneja *et al*., 2010). *Barleria prionitis* L.

[1] Dr, Associate Professor, Depart of Botany, Pyay University
[2] Dr, Associate Professor, Depart of Botany, Pyay University
[3] Lecturer, Depart of Botany, Pyay University

Leaves and young inflorescences are diuretic. Leaf juice used in stomach disorders, urinary affections, fever and catarrh; leaf juice applied to lacerated soles of feet in rainy season and also for pimples. Leaf extract is effective in reducing blood sugar in diabetic animals (Bhogaonkar and Lande, 2012). Another shrub from the same family *Barleria prionitis,* has been mush widely researched with documented medicinal properties of the whole plant, leaves, and roots against e.g., diabetes and respiratory diseases (Pathy *et al*., 2015). The aim and objectives of this research paper were to identify the comparative morphological characters and to examine the antimicrobial activities leaves extract of *Barleria cristata* L. and *Barleria prionitis* L.

Materials and Methods

The specimens of *Barleria cristata* L. and *Barleria prionitis* L. used in this research were collected from Pyay Township Area, Bago Region. *Barleria cristata* L. flowering period is November to February. *Barleria prionitis* L. flowering period is November to March. After collecting, the plants were mounted between newspaper sheets, were dried, they were mounted into herbarium sheets. The plant identification was carried out in the Department of Botany, Pyay University to get correct family, genus and species with the help of references literature such as Hooker (1885), Kirtikar and Basu (1975), Burkill (1935), Lawrence (1964), Dassaneyake (1995), Balkwill and Balkwill (1997) and Kress (2003).

Antimicrobial activities of different solvent extracts (petroleum-ether, chloroform, ethyl-acetate, acetone, ethanol, methanol and water) of *Barleria cristata* L. and *Barleria prionitis* L. were tested on six pathogenic microorganisms such as *Aspergillus flavous*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae* at Department of Botany, University of Yangon.

Screning of antimicobial activity of crude extracts had been done by paper disc diffusion method Cruickshank (1975). Paper disc having six millimeter diameter were utilized for antimicrobial test. Among medium was prepared according to the method described by Atlas (1993). Among medium was boiled and 20-25 ml of the medium was poured into each conical flask, plugged with cotton wool and autoclaved at 121°C for 15 minutes. Then the conical flask was cooled down to 40-45°C and each of 0.1-0.2 ml of test organisms were also added into the flask and then, poured into sterilized petridishes. After solidification, paper disc impregnated with sample were applied on the agar plates and incubated at 37°C for 24 hours. Then the diameter of inhibitory zone was measured with the help of a transparent ruler.

Results

Scientific Name	:	*Barleria cristata* L. Sp. Pl. 1753.
Synnonum	:	*Barleria ciliate* Roxb.
English Name	:	Blue bell barleria
Myanmar Name	:	Leik-tha-ywe-pya
Family	:	Acanthaceae
Flowering and Fruiting period	:	November to February

Morphological characters of *Barleria cristata* L.

Perennial mush branched shrub, stem terete, glabrous. Leaves opposite and decussate, simple, petiolate, exstipulate; laminar elliptic-oblong (5.0-9.5 x 1.5-4.5 cm), the tip cuspidate, the

margin entire, the base attenuate, deep green in two surfaces, glabrous; petiole 0.5-1.0 cm long, canaliculated above and rounded beneath, glabrous. Inflorescence axillary short and dense cymes, many flowered. Flower 1.5 cm across at anthesis, bracts absent, bracteolate, pedicellate, bisexual, zygomorphic, 4 merous, hypogynous. Calyx 4 sepals, foliaceous,fused, slightly connate at the base, the outer calyx lobed oblong-lanceolate, margin spiny, inner calyx lobed linear lanceolate, margin scarious, persistent. Corolla 5 lobed, fused, infundibuliform, bilabiate, the tube 1.8-2.0 cm long, violet or puplish-blue. Stamens 4+1st, free, epipetalous, didynamous, the filament 0.5-2.0 cm long, the anther dithecous, oblongoid, longitudinal dehiscent. Ovary superior, oblong, 2 carpels, fused, 2 locules, two ovules in each locule, axile placentation, the style long and slender, the stigma bifid. Fruit loculicidal capsule, ovoid. Four seeded.

Scientific Name	:	*Barleria prionitis* L., Sp. Pl. 2:636. 1753.
Synnonum	:	*Barleria spicata*
English Name	:	Porcupine flower
Myanmar Name	:	Leik-sa-ywe; Leik-tha-ywe
Family	:	Acanthaceae
Flowering and Fruiting period	:	November - March

Morphplogical characters of *Barleria prionitis* L.

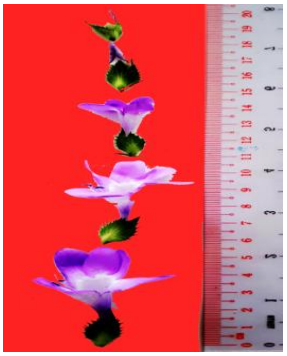
Perennial erect spinescent herbs or undershrubs, up to 40-60 cm hight, mush branched, branches subterete, pubescent; spines 2 – 4 in the leaf axils, 1.2 – 2.0 cm long, white. Leaves opposite and decussate, simple, petiolate, exstipulate; lamina elliptic oblong, cuspidateat the tip, entireat the margin, acuminate at the base, sparsely pubescent on both surfaces. Inflorescence axillary cyme, 1 – 3 flowered per flower axil, spike, spinescent. Flower bright yellow, about 2 cm in across at anthesis, bracts foliaceous, oblong-lanceolate, spinescent, persistent, sessile, zygomorphic, bisexual, 4 merous, hypogynous. Sepals 4, in opposite pair, the outer pair much larger, 1.0 x0.3 cm, broadly lanceolate, the inner pair small, 0.8 x 0.1 cm, linear lanceolate, spinescent at the tip, pubescent, persistent. Corolla 5 lobed, infundibuliform, yellow, tube about 1 cm long, slightly curved, widened upwards, lobes broadly ovate, about 1.0 x 0.8 cm, unequal, glabrous. Stamens 4, didynamous, 2 fertile, 2 staminodes, petalostemonous, the filaments of the fertile stamens longer and exserted, the filament filiform, the other stamens minute and inserted, pilose, the anthers dithecous, dorsifixed, introrse, longitudinal dehiscent. Ovary ovoid, carpels 2, syncarpous, 2-loculed, 2 ovules in each locule, axile placentation, the style long and slender, the stigma bifid, ovary superior. Fruit a loculicidal capsule, 2-seeded, accrescent calyx. Seed ovoid, compressed.

Morphological characters of *Barleria cristata* L.

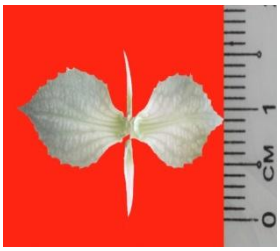
Habit

Leaves

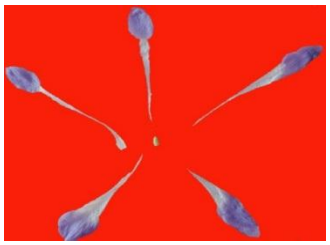
Inflorescence



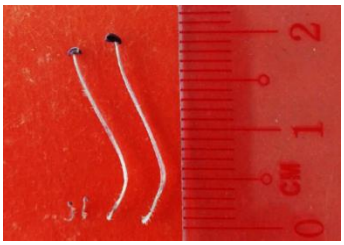
Flowers



Calyx



Corolla



Androecium

Figure 1 Morphological characters of *Barleria cristata* L.

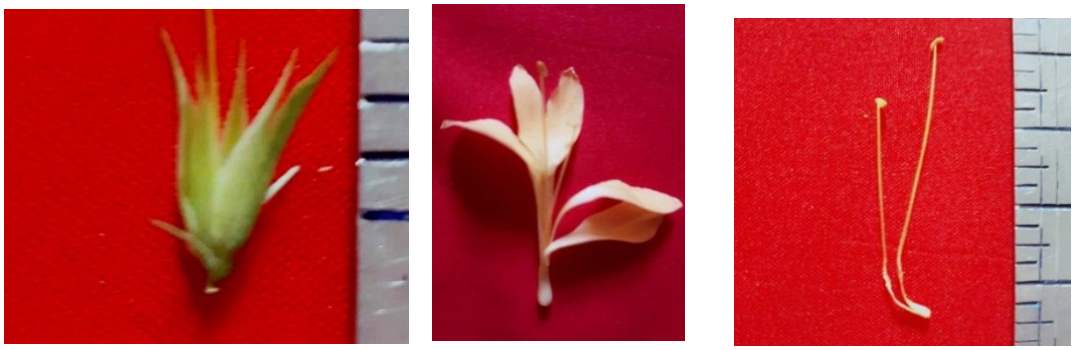
Morphplogical characters of *Barleria prionitis* L.

Habit

Leaves

Inflorescence

Flowers



Calyx **Corolla** **Androecium**

Figure 2 Morphplogical characters of *Barleria prionitis* L.

Table 1 Comparison the characteristics of *Barleria cristata* L. and *Barleria prionitis* L.

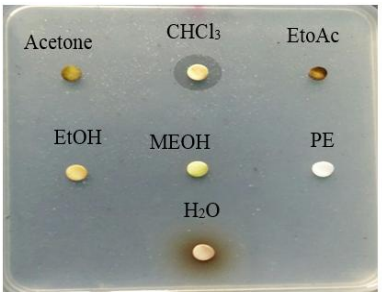
Scientific name	***Barleria cristata* L.**	***Barleria prionitis* L.**
Myanmar Name	Leik-tha-ywe-pya	Leik-sa-ywe; Leik-tha-ywe
English Name	Blue bell barleria	Porcupine flower
Habit	Perennial mush branched shrubs	Perennial erect spinescent herbs or undershrubs
Leaves	opposite and decussate, simple, exstipulate,glabrous in two surfaces	opposite and decussate, simple, exstipulate, spines 2 – 4 in the leaf axils, sparsely pubescent on both surfaces
Inflorescence	Axillarycymes, many flowered per axil.	Axillarycymes, 1-3 flowered per axil, spinescent
Flowers	Ebracteate, bracteolate, persistent,purplish blue or violet	Bracteate, bracteolate, persistent, bright yellow
Calyx	Sepals 4, the outer pair much larger, foliaceous, reticulate, spinescent at the margin; the inner pair small, pubescent.	Sepals 4, the outer pair much larger, foliaceous, spinescent at the tip, pilose; the inner pair small, pubescent
Corolla	Petals 5, fused, bilabiate,puplish-blue	Petals 5, fused, bilabiate, bright yellow
Androecium	Stamens 4+1st, didynamous, 4 fertile, one staminode	Stamens 4, didynamous, 2 fertile, 2 staminodes, pilose at the base
Gynoecium	Ovary superior, oblong, 2 carpels, fused, two ovules in each locule, axile placentation	Ovary superior, ovoid, 2 carpels, fused, two ovules in each locule, axile placentation

Table 2 Antimicrobial activities of diffusion solvent Leaves extracts of *Barleria cristata* L. and *Barleria prionitis* L.

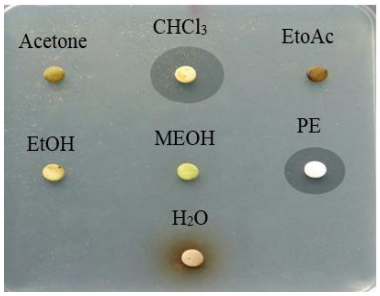
Extracts	Test Organisms											
	Aspergillus flavous		*Bacillus subtilis*		*Candida albicans*		*Escherichia coli*		*Pseudomonas fluorescens*		*Xanthomonas oryzae*	
	Barleria cristata	*Barleria prionitis*	*Barleria cristata*	*Barleria prionitis*	*Barleria cristata*	*Barleria prionitis*	*Barleria cristata*	*Barleria prionitis*	*Barleria cristata*	*Barleria prionitis*	*Barleria cristata*	*Barleria prionitis*
Pet-ether	12	12	-	12	-	-	14	-	-	14	12	12
$CHCl_3$	12	16	16	14	14	-	18	12	16	14	12	12
Me OH	-	-	-	-	-	-	-	-	-	-	-	-
Acetone	-	-	-	-	-	-	-	-	-	-	-	-
EtoAc	-	-	-	-	-	-	-	-	-	-	-	-
EtOH	-	-	-	-	-	-	-	-	-	-	-	-
H_2O	-	-	16	12	-	-	-	12	-	-	-	-

Paper disc size – 6 mm
10 mm - below (poor activity)
10 mm -14 mm (weakly activity)
14 mm - above (highly activity)

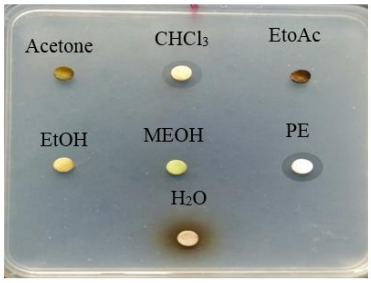
Antimicrobial activities of diffusion solvent extract of the leaves of *Barleria cristata* L.



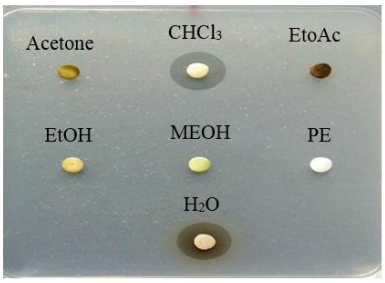
Aspergillus flavas



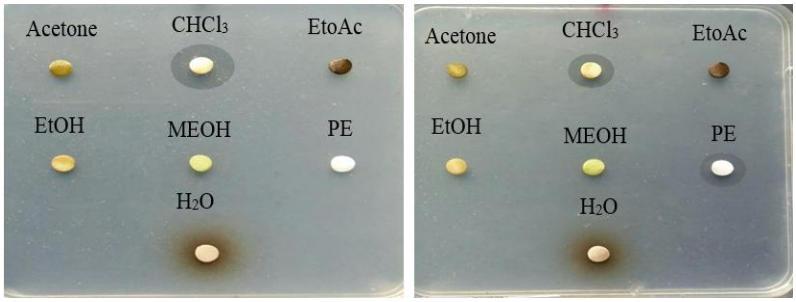
Bacillus subtilis



Candida albicans



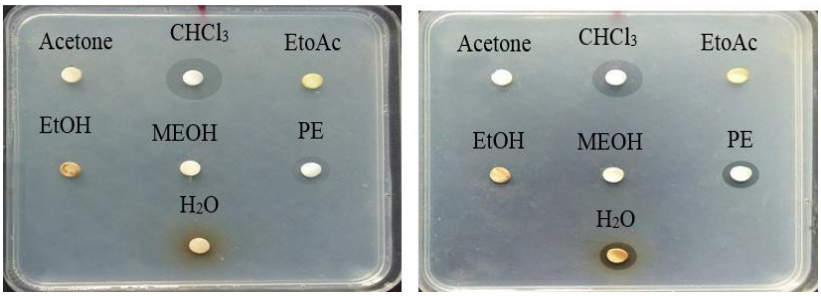
Escherichia coli



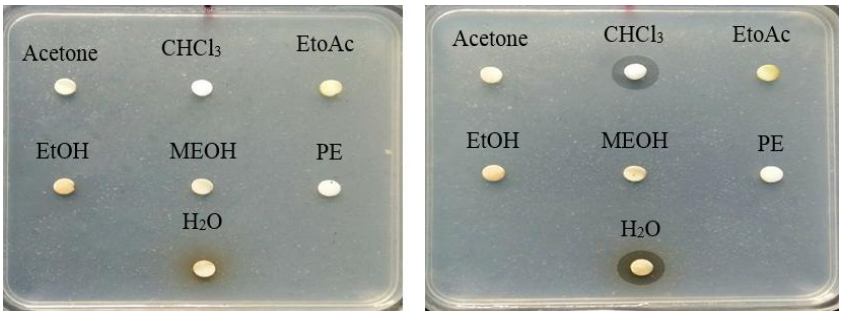
Pseudomonas fluorescens ***Xanthomonas oryzae***

Figure 3 Antimicrobial activities of diffusion solvent extract of the leaves of *Barleria cristata* L.

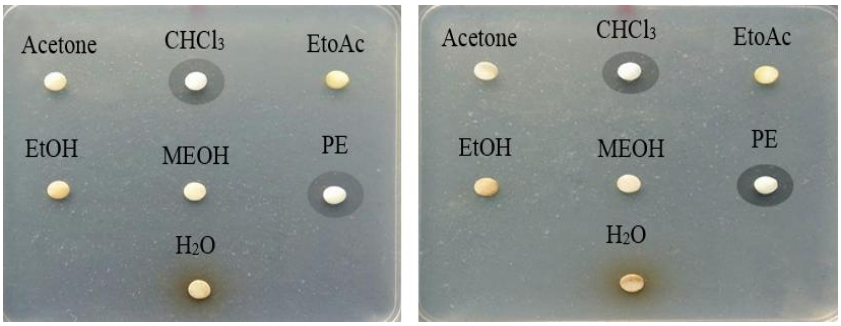
Antimicrobial activities of diffusion solvent extract of the leaves of *Barleria prionitis* L.



Aspergillus flavas ***Bacillus subtilis***



Candida albicans ***Escherichia coli***



Pseudomonas fluorescens ***Xanthomonas oryzae***

Figure 4 Antimicrobial activities of diffusion solvent extract of the leaves of *Barleria prionitis* L.

Discussion and Conclusion

The medicinal plants *Barleria cristata* L. and *Barleria prionitis* L. belong to the family Acanthaceae were collected from Pyay Township, Bago Region. In the presence investigation, comparative morphological characters and antimicrobial activities of *Barleria cristata* L. and *Barleria prionitis* L. are carried out. In morphological characters of *Barleria cristata* L. is perennial mush branched shrub, stem terete, glabrous. Leaves simple, petiolate, exstipulate; laminar elliptic-oblong, the tip cuspidate, the base attenurate, glabrous; petiole long, canaliculated above and rounded beneath, glabrous. Inflorescence axillary short and dense cymes. Flower violet or purplish-blue, bract absent, bracteolate, bisexual, zygomorphic, 4 merous, hypogynous. Calyx 4 sepals, foliaceous, fused, persistent. Corolla 5 lobed, fused, infundibuliform, bilabiate, violet or purplish-blue. Stamens $4+1^{st}$, free, petalostemonous, didynous, the filament long, the anther dithecous, dorsifixed, oblongoid. Ovary superior, oblong, 2 carpels, 2 locules, axile placentation, the style long and slender, the stigma bifid. Fruit loculicidal capsule, ovoid. Four seeded.

Barleria prionitis L. is perennial erect spinescent herbs or undershrubs, branches subterete, pubescent; spines 2-4 in the leaf axils. Leaves simple, petiolate, exstipulate; lamina elliptic oblong. Inflorescence axillary cyme, spike, spinescent. Flower bright yellow, bracts foliaceous, spinescent, zygomorphic, bisexual, 4 merous, hypogynous. Sepals 4, spinescent at the tip, pubescent, persistent. Corolla 5 lobed, infundibuliform, yellow. Stamens 4, didynamous, 2 fertile, 2 staminodes, petalostemonous, the filament filiform, pilose, the anther dithecous, dorsifixed, introrse, longitudinal dehiscent. Ovary ovoid, carpels 2, syncarpous, 2 locules, 2 ovules in each locule, axile placentation, style long and slender, stigma bifid, ovary superior. Fruit a loculicidal capsule, 2 seeded, accrescent calyx. Seed ovoid, compressed.

These characters are in agreement with those of describe by Hooker (1885); Kirtikar&Basu (1975); Burkill (1935); Lawrence (1964); Dassaneyake (1995); Balkwill M.J & Balk will, K., (1997); and Kress (2003).

In this research, antimicrobial activities of leaves extracts of *Barleria cristata* L. and *Barleria prionitis* L. are tested on six pathogenic microorganisms by using paper disc diffusion method. The result of the present study with pet-ether, chloroform, methanol, acetone, ethyl acetate, ethnol and aqueous extracts of *Barleria cristata* L. and *Barleria prionitis* L. showed the significant activities against six microorganisms.

Pet- ether, chloroform and aqueous extract of the leaves of *Barleria cristata* L. and *Barleria prionitis* L. more effective than different extracts of antimicrobial activity against six microorganisms. Methanol, acetone, ethyl acetate and ethanol extracts of leaves did not show antimicrobial activity against six microorganisms. Among them pet-ether and chloroform leaves extracts of *Barleria cristata* L. showed sensitive against *Aspergillus flavous, Xanthomonas oryzae* and more sensitive against *Bacillus subtilis, Candida albicans, Escherichia coli* and *Pseudomonas aeruginosa.* Aqueous extracts showed more sensitive against *Bacillus subtilis.* Chloroform, pet-ether and aqueous extracts showed *Aspergillus flavous, Bacillus subtilis* and *Escherichia coli* and more sensitive against *Aspergillus flavous, Bacillus subtilis* and *Pseudomonas fluorescences*.

Therefore, the present research focuses the usefulness medicinal plant *Barleria cristata* L. and *Barleria prionitis* L. on antimicrobial activity.

So, the medicinal plant of *Barleria cristata* L. and *Barleria prionitis* L. can be utilized for Myanmar traditional medicine systematically.

Acknowledgements

I would like to express to my gratitute to Professor Dr. Mie Mie Myint Shein, Head of Department of Botany, Pyay University, for providing all departmental facilities and valuable suggestions. Personally, my special thanks are due to Professor Dr. Nyo Nyo Thaung, Department of Botany, Pyay University for her valuable leading toward the successful completion of this research.

References

Aneja K. R., R. Joshi and C. Sharma. (2010). **Potency of *Barleria prionitis* L. bark extracts against oral diseases causing strains of bacteria and fungi of clinical origin.** New York Science Journal; 3(11), India.

Backer, C.A. and R.C. Bakehuizen Van Der Brink, Jr. (1965). **Flora of Java**, Vol II.Noordhoff-Groningen. The Netherland.

Balkwill, M.J. and Balkwill, K. (1997). **Delimitation and infrageneric classification of *Barleria* (Acanthaceae).** Kew Bull. 52 (3): 535-573.

Bency A., J. Lohidas and M**.** Murugan. (2018). **Preliminary Phytochemical Investigation and Antibacterial Activity of *Barleria Cristata* Linn.** International Journal of Scientific Research and Reviews. 7(3), 1063-1069. ISSN: 2279–0543.

Bhogaonkar P.Y. and S.K. Lande. (2012). **Anatomical Characterization of *Barleria prionitis* Linn. : A Well-known Medicinal herb.** Biological Forum — An International Journal, 4(1): 1-5. ISSN: 0975-1130.

Cruikshank, R.J.P. (1975). **Medicinal Microbiology**. Living Stone Ltd., London.

Dassanayake.(1995). **A Revised Handbook to Flora of Ceylon.** Vol IX

Hakimi, A. S. A., Q.Z. Faridah, A.S. Abdulwahab and A. Latiff. (2018). Pollen and seed morphology of *Barleria* L. (Barlerieae: Ruellioideae: Acanthaceae) of Yemen. *South African Journal of Botany* 116, 185–191.

Hooker, J.D.(1885). Vol-IV, **The Flora of British India**, L. Reece Co.Ltd.The Cost House, Brook, Ashford, Kent. England.

Hundley, H.G. and Chit Ko Ko. (1987). **List of Trees, Shrubs, Herbs and Principle Climbers etc**. Govt. Printing and Stationary.

Kartikar and K.R., Basu. (1975). **Indian Medicinal Plants**. Vol I, p.p 813-815, International Distribution, Indian.

Kress, J.W. and Yin Yin Kyi, Daw. (2003). **A Check List of Trees, Shrubs, Herbs and Climbers of Myanmar.** Department of Systematic Biology Botany, National Museum of Natural History Washington, DC.

Lawerence, G.H.M. (1964). **Taxonomy of Vascular Plant**. The Macmillian Company, New York.

Naidu, V.S.G.R. (2012), **Hand Book on Weed Identification.** Directorate of Weed Science Research, Jabalpur, India Pp 354.

Pathy M., T. Sharma and S. Bhatnagar. (2015). ***Barleria Cristata*: A Comparative Analysis of Phytochemical, Cytotoxic and Antioxidant Activities of and Bark Extracts.** European Journal of Pharmaceutical and Medicinal Research, 2[5], 586-593. ISSN: 3294-3211.

Shankar, M.S. and S.R. Yadav. (2010). **Revision of the Genus *Barleria* (Acanthaceae) in India.** Vol. 20(2), Department of Botany, Shivaji University, Kolhapur-416004, Maharashtra, India.

Sharma P., G.N. Sharma, B. Shrivastava and H. R. Jadhav, .(2014). Evaluation of Antioxidant Potential of *Barleria prionitis* Leaf and Stem. *American Journal of Phytomedicine and Clinical Therapeutics*: JPCT [2] [11]1177-1186. ISSN: 2321 – 2748.

Sumaya B., E. Honey, B. Anasuya1 H. Gangarayudu, M. J. Reddy and C. Girish. (2017). **Investigation of Antibacterial Activity of Different Extracts of *Barleria cristata* Leaves.** International Journal of Health Sciences & Research, Vol.7; Issue: 9; ISSN: 2249-9571.

ISOLATION AND ANTIMICROBIAL ACTIVITY OF ENDOPHYTIC CTINOMYCETES FROM DIFFERENT PARTS OF FIVE SELECTED PLANTS AND CHARACTERIZATION OF SELECTED *STREPTOMYCES* (TG-16)

Theingi Aung[1], Zar Zar Yin[2] and Kay Thi Mya[3]

Abstract

A total of eighteen endophytic actinomycetes were isolated from leaf, stem, root of five selected plants such as *Millingtonia hortensis* L., *Aloe vera* L., *Barleria strigosa* Willd., *Desmodium triquetrum* (L.)DC. and *Polygonum chinensis* L. from Pathein University Campus from June to August, 2016. This study was carried out at Biotechnological Development and Resources Centre, Pathein University. The isolated strains were designated as TG 1-18. All of the isolated strains were studied their morphological, microscopical characters and Gram staining. The antimicrobial activity of eighteen isolated strains were studied by agar well diffusion method with ten kinds of test organisms. Among them, ten strains showed the antimicrobial activity on *Agrobacterium tumefaciens, Aspergillus paraciticus, Salmonella typhi, Escherichia coli and Saccharomyces cerevisae.* TG-16 showed the highest antimicrobial activity (27.14mm) against *E. coli.* Therefore, TG-16 was selected and characterized by biochemical reactions. The selected strain TG-16 was gram positive with sporophore as hook, open-loop rectinaculum apertum type, spiral spore chain and spore smooth and oval shape. The colony morphology of TG-16 was raised, entire and circular. In the sugar fermentation test of selected strain TG-16, the acid was produced on all studied carbon sources except galatose, lactose, arabinose and positive results to methyl red, nitrate reduction, citrate utilization, catalase, arginine and casein hydrolysis, growth on streak line of potato slice and sodium chloride level at 2.5%(w/v), melanin was not produced but yellow pigment produced in casein hydrolysis medium. According to the results, TG-16 was characterized as the genus *Streptomyces.*

Keywords: Streptomyces, Antimicrobial activity, Biochemical characterization

Introduction

Endophytic bacteria are microorganisms that colonize the internal tissues of the plant without causing any external sign of infection or negative effect on their host (Schulz and Boyle, 2006). Fungi, bacteria or actinomycetes have been found in endophytic association with plants. Endophytic microorganisms can be derived from any part of the plant like bark, leaves, flowers, fruits, roots, seeds etc. Endophytes are an under-investigated group of microorganisms that represent a plentiful and renewable source of bioactive and chemically new compounds with potential for exploitation in a wide variety of medical, agricultural, and industrial realms. Actinomycetes are prokaryotic filamentous, branching bacteria with a fungal type of morphology. The physiological and biochemical characteristics in actinobacterial systematics and identification are still meaningful.

Genus Streptomyces belongs to the Streptomycetaceae family. The filaments and spores are very small usually 1µm or less in diameter. The spores are formed by the fragmentation of the filaments and are borne in straight, wavy, or helical chains. The colonies are slow-growing and often have a soil-like odour because of production of a volatile metabolite, geosmin. They produce a wide variety of pigments responsible for the colour of the vegetative and aerlial mycelia. Streptomyces species are nonmotile, catalase positive, reduce nitrates to nitrites and

[1] Lecturer, Department of Botany, Pathein University
[2] Associate Professor, Department of Botany, Pathein University
[3] Professor and Head, Department of Botany, Pathein University

degrade adenine, esculin, casein, gelatin, hypoxanthine, starch, and L-tyrosine (Hasani *et al*., 2014)

The Actinomycetes also synthesizes and excrete darkpigments, melanin or melanoid, which are considered to be a useful criterion for taxonomical studies. Melanins are frequently used in medicine, pharmacology, and cosmetics preparations (Dastager S.G *et al*.,2006). The aim and objectives of this study were to isolate endophytic actinomycetes from five selected plants and to identify the selected strain TG-16 based on their colony morphology, Gram straining, microscopical characters and biochemical reactions.

Materials and Methods

Study area and collection of plant samples

Millingtonia hortensis L., *Aloe vera* L., *Barleria strigosa* Willd., *Desmodium triquetrum* (L).DC. and *Polygonum chinensis* L. were used to isolate endophytic actinomycetes. The plants samples (leaf, stem, root) were collected from Pathein University Campus, Ayeyarwady Region from June to August, 2016. The identification of these plants were referred by (Flora of Hong Kong, 2009).

Isolation of endophytic microorganisms (Coombs and Franco 2003).

The leaves, stems and roots of selected plant samples were used to analyze. The plant samples were washed by running tap water for several times to remove soil particles and then they were cut into small pieces (0.5 ×0.5cm for leaf and 0.2cm for stem and root) and after that sterilized by sequential immersion in alcohol 70% (1 min), sodium hypochlorite 1.5% (5 min) to eliminate unwanted endophytic fungi or bacteria and finally rinsed with sterile distilled water for three times to remove surface sterilization agents. The surface sterilized plant samples were dried in the folds of sterile paper. After proper drying the surface sterilized plant materials were cultured on four different media, there were Glycerol asparagine agar medium (M-1), Yeast extract malt extract agar medium (M-2), Emerson agar medium (M-3), Starch casein agar medium (M-4) and supplemented with chloramphenicol and nystatin for 7-10 days at room temperature.

Preliminary study of antimicrobial activity of isolated actinomycetes

Eighteen endophytic actinomycetes isolates were inoculated into precultured media respectively (medium-I, Medium-II, Medium-III and Medium-IV) for 4 days at room temperature. After incubation period, the inoculated strain was transferred into the seed medium (M-2) for 3 days at room temperature. After 3 days, then seed medium(25%) was transferred into fermentation medium(Glycerol 0.2%, Peptone 0.5%, Yeast extract 0.3, Malt extract 0.3%, CaCO3 2.5% at pH 7.0) worked out for 3-10 days and evaluated by agar well diffusion method.

Screening of antimicrobial activity by agar well method (Collins 1965)

The assay medium (glucose 1%, Peptone 0.3%, agar 1.8% at pH 7.0) was utilized for test organisms. The molten sterile medium was cooled to 40-45ºC, inoculated with test organism and thoroughly mixed and then, poured into sterile petri plates and allowed to settle. And ten wells were made using sterile cork borer (8 mm in diameter) in the plate and then fermentation broths (20µL) was added to wells . The diameter of inhibition zones around each well were measured and recorded after 24 hrs incubation.

Test organisms

Agrobacterium tumefaciens NITE 09678, *Aspergillus paraciticus* IFO5123, *Bacillus subtilis* IFO 90571, *Candida albicans* NITE 09542, *E.coli* AHU 5436, *Micrococcus luteus* NITE 83297, *Pseudomonas fluorescens* IFO 94307, *Saccharomyces cerevisiae* NITE 83297, *Salmonella typhi* AHU 7943, *Staphylococcus aureus* AHU 8465 were obtained from NITE(National Institute of Technology and Evaluation, Kisarazu, Japan).

The identification of isolated bacterial strains were carried out using their colony morphology, Gram staining methods (Dubey and Maheshwari, 2002), and biochemical tests which include sugar fermentation of carbohydrate test (Cowan, 1975), Catalase test (Salle, 1948), starch hydrolysis test (soluble starch, rice powder, wheat powder, tapioca powder, sticky rice powder) (Pelezar and Chan, 1972), urea hydrolysis test (Christenson, 1946), potato plug test (Atlas 1993), nitrate reduction test (Harrigan and Mc Cance ,1966), mannitol salt broth test (Marshall 1992), methyl red test, (Bisen and Verma, 1998), citrate test (Atlas, 1993), hydrogen sulfide Test (Cowan, 1975), Voges proskaucer test (Cruickshank, 1963), Hanging slide test, Oxidase test, argenine hydrolysis, gelatin hydrolysis test (Dubey and Maheswari , 2002),casein hydrolysis test (Aneja, 1996),esterace hydrolysis test (Prescott, 2002),melanin production test (Shirling and Gottlieb 1966), salt tolerance test (Atlas, 1993), respectively.

Results

From this study, 18 isolates of endophytic actinomycetes were found. Among them 11 strains were obtained from stem of *Milllingtonia hortensis* L., two strains from stem of *Aloe vera* L., one strain from root of *Barleria strigosa* Willd. each one strains from leaf, stem and root of the *Desmodium triquetrum* (L). DC., and one strain from root of *Polygonium chinensis* L. The aerial mass color of TG-1, 3-6 were white, TG-10-13, 16, 17 were whitish blue while TG-2, 7,8, 9, 14, 15 were greenish white, creamy white, black, pale brown gray, pale blue and pale yellow respectively. The reverse color of TG-1-7, 9, 10 and 16 were pale yellow, TG-11-15 and 17 were pale brown, TG-8 and 18 were black.

The colony size of TG-1-7, 9-11, 13 and 17 were medium while TG-8, TG-14-16 and TG-18 were small and TG-12 was large. The elevation and forms of TG-1, 3, 7, 12, 17-18 were flat, TG-2, 4-6, 8, 14 and15 were convex, TG-10-11,13,16 were raised while TG-9 was umbonate. The elevation and forms of TG-1, 3, 7, 12, 17-18 were flat, TG-2, 4-6, 8, 14and 15 were convex, TG-10-11, 13, 16 were raised while TG-9 was umbonate. The spore chains of all strains generally produce flexibilis, retinaculum and spiral types. The spore chains of TG-1, 2, 9, 11,12, 15, and 17 were flexible type, TG-3,16 showed retinaculum apartum type, TG-4, 5 and 6 were spiral type and TG-8 showed biverticilliate (no spiral) type and isolated strains of TG- 7,10, 13, 14, 18 were short spore chains as shown in Figure (1 and 2).

Eleven isolated strain (TG-2, 3, 4, 5, 7, 9, 12, 13, 15 and 16) had antimicrobial activity and remaining eight isolates could not produce antimicrobial metabolites. Among them, antibacterial activity of isolated actinomycetes TG-16 exhibited the maximum inhibitory zone (27.14 mm) against *Escherichia coli* in 6 days fermentation period. These results were shown in Figure (3). The results of colony morphology, cultural characteristics, microscopical characters and some biochemical tests for the selected strains TG-16 were shown in Table 4-6 and Figure 4-12.

Table 1 Number of endophytic actinomycetes from different parts of five selected plants

Name of plants	Leaf	Stem	Root
Milliongtonia hortensis L.	-	-	TG1, TG-2, TG-3, TG-4, TG-5, TG-6, TG7, TG-8, TG-9, TG-10,TG-11
Aloe vera L.	-	TG-12, TG-13	-
Barleria strigosa Willd.	-	-	TG-14
Desmodium Triquetrum (L). DC.	TG-17	TG-16	TG-15
Polygonum chinensis L.		-	TG-18

Table 2 Colony morphology of the isolated strains

Isolated strain	Size of colony	Margin	Color		Elevation and form
			Surface	Reverse	
TG-1	Medium	Circular	White	Pale	Flat
TG-2	Medium	Circular	Greenish	Pale	Convex
TG-3	Medium	Circular	White	Pale	Flat
TG-4	Medium	Circular	White	Pale	Convex
TG-5	Medium	Circular	White	Pale	Convex
TG-6	Medium	Circular	White	Pale	Convex
TG-7	Medium	Circular	Creamy white	Pale	Flat
TG-8	Small	Circular	Black	Black	Convex
TG-9	Medium	Circular	Pale brown	Pale	Umbonate
TG-10	Medium	Circular	Whitish blue	Pale	Raised
TG-11	Medium	Circular	Whitish blue	Pale	Raised
TG-12	Large	Irregular	Whitish blue	Pale	Flat
TG-13	Medium	Circular	Whitish blue	Pale	Raised
TG-14	Small	Circular	Gray	Pale	Convex
TG-15	Small	Circular	Pale blue	Pale	Convex
TG-16	Small	Circular	Whitish blue	Pale	Raised
TG-17	Medium	Circular	Whitish blue	Pale	Flat
TG-18	Small	Irregular	Pay yellow	Black	Flat

Table 3 Zone of inhibition (in mm) of isolated strain TG-16 on five kinds of test organisms in (4-8) days fermentation peroid

Fermentation peroid	Test organisms									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
4 day	10.73	19.03	-	-	-	11.96	-	23.57	-	10.73
5 day	17.08	18.28	-	-	-	17.68	-	24.96	-	17.64
6 day	15.42	17.13	-	-	-	17.97	-	**27.14**	-	14.34
7 day	14.08	15.73	-	-	-	17.20	-	24.13	-	14.20
8 day	14.11	15.10	-	-	-	17.11	-	23.02	-	14.18

Well size=8mm

1. *Agrobacterium tumefaciens*
2. *Aspergillus paraciticus*
3. *Bacillus subtilis*
4. *Candida albicans*
5. *Micrococcus luteus*
6. *Salmonella typhi*
7. *Pseudomonas fluorescens*
8. *Escherichia coli*
9. *Staphphylococcus aureus*
10. *Saccharomyces cerevisiae*

Table 4 Cultural characteristics of selected strain TG-16 by using ISP- three medium

Medium	Cultural characteristic	
	Surface color	**Reverse color**
Yeast extract malt extract agar (ISP2)	Pale yellow	Yellow
Glycerol asparagine agar (ISP-5)	Pale yellow	Light brown
Peptone yeast ion agar (ISP-6)	Pale yellow	Light bnrown

Table 5 Biochemical test for sugar fermentation of selected strain TG-16

Sugar sources	Responces	Results	
		Acid production	**Gas production**
Sucrose	Yellow color change in medium	+	-
Xylose	Yellow color change in medium	+	-
Fructose	Yellow color change in medium	+	-
Glucose	Yellow color change in medium	+	-
Arabinose	No change in color	-	-
Lactose	No change in color	-	-
Galatose	Yellow color change in medium	+	-
Maltose	No change in color	-	-

Table 6 Biochemical tests for selected strain TG-1

No.	Biochemical Tests	Responses	Result
1	Urea hydrolysis test	No change in pink color	-
2	Mannitol salt broth test	Change red color in test broth	+
3	Nitrate reduction test	Change cherry red color in test broth	+
4	Methyl red test	Medium remain red	+
5	Voges proskaucer test	No change in color of medium	-
6	Citrate utilization	Medium change from green to blue	+
7	H_2S production	No change in color of medium	-
8	Catalase test	Release free oxygen gas bubble	+
9	Oxidase test	No change in color	-
10	Hanging slide test	No motility	-
11	Arginine hydrolysis	Change back to purple from yellow	+
12	Casein hydrolysis	Clear zone around the colony	+
13	Gelatin hydrolysis	No clear zone around the streak line	-
14	Potato plug	Growth on streak line of potato slice	+
15	Melanin production test	No pigmentation in medium	-
16	Starch Hydrolysis		
	(i) Soluble starch	Clear zone around the streak line	++
	(ii) Tapioca powder	Clear zone around the streak line	+
	(iii)Sticky rice powder	Clear zone around the streak line	++
	(iv)Wheat powder	Clear zone around the streak line	++
	(v) Rice	Clear zone around the streak line	+
17	Salt tolerance test		
	(i) 1.5%	Moderate growth	++
	(ii) 2.5%	Highest growth	+++
	(iii)3.5%	Moderate growth	++
	(iv)4.5%	Poor growth	+
	(v) 5.5%	Poor growth	+
	(vi)6.5%	Poor growth	+

positive = +, negative = –, + (poor growth), ++ (moderate growth), +++ (Hightest growth)

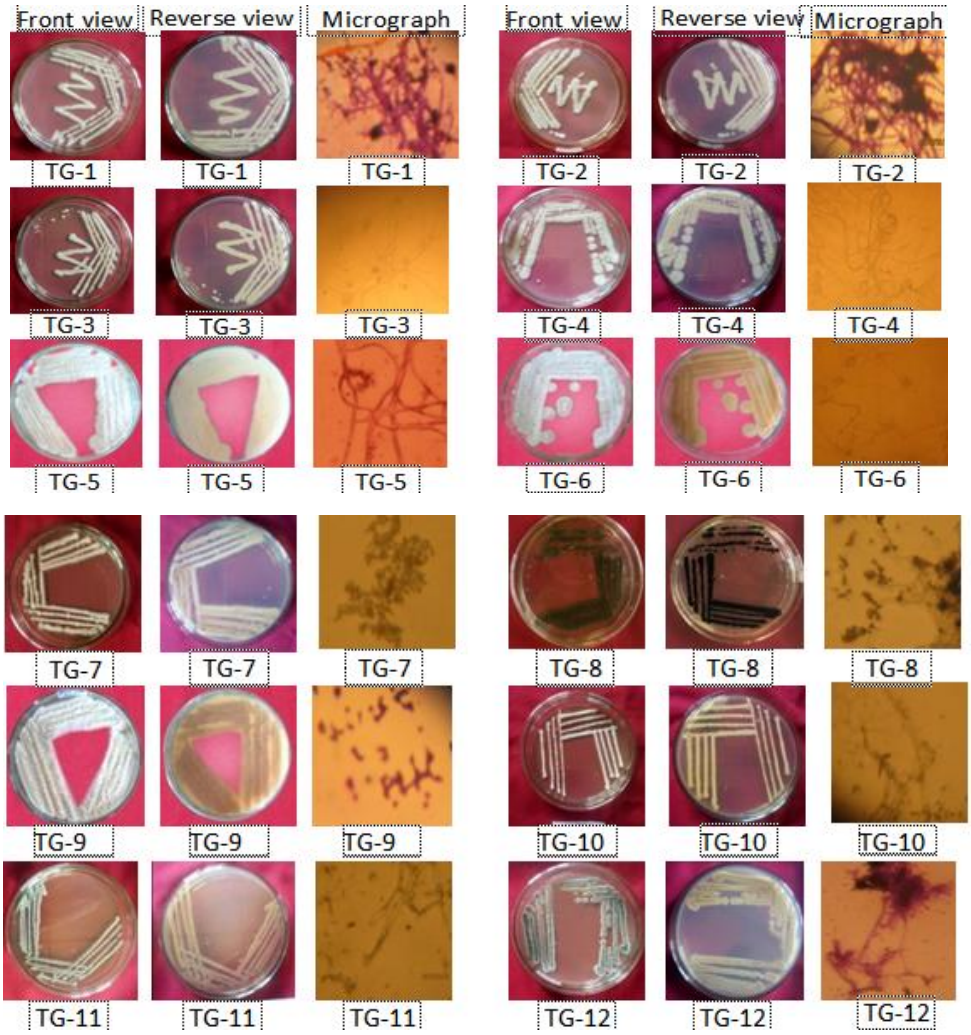


Figure 1 Cultural characteristics (surface α reverse view) and micrograph of isolated strains TG-1 to 12

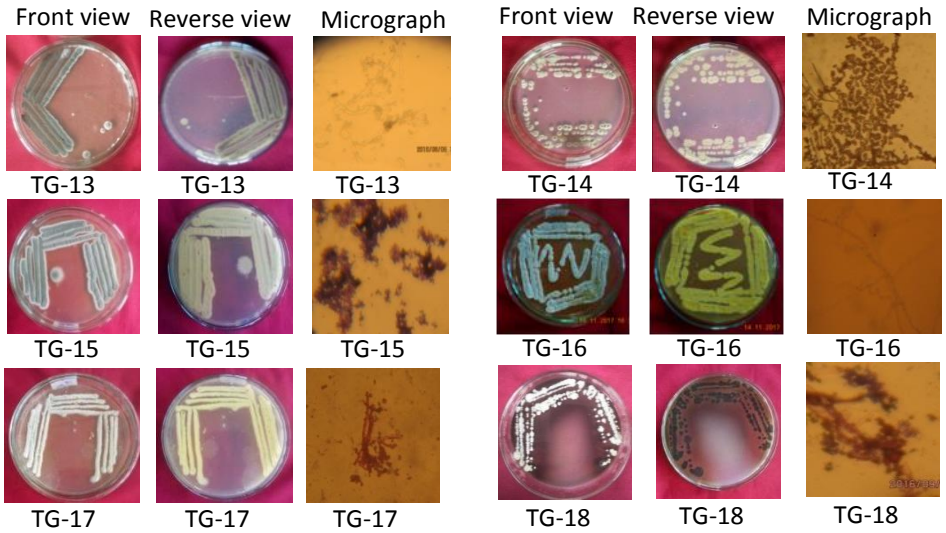


Figure 2 Cultural characteristics (surface α reverse view) and micrograph of isolated strains TG-13 to 18

Figure 3 Antimicrobial activity (in mm) of selected strain TG-16 against *Escherichia coli* at (4-8) days fermentation period

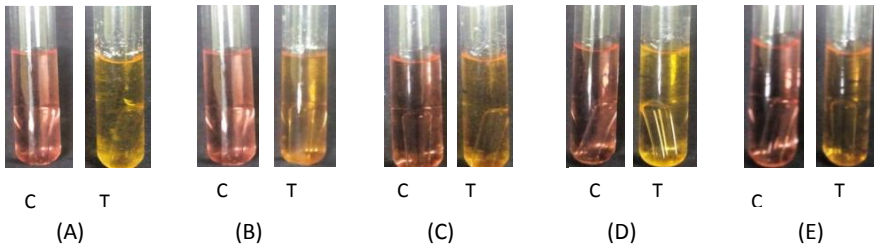


Figure 4 Biochemical test for sugar fermentation of selected strain TG-16
(A) Sucrose (B) Xylose (C) Fructose (D) Glucose (E) Maltose(All positive)

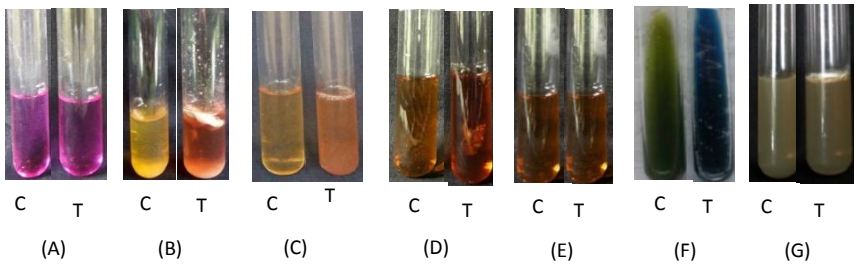


Figure 5 Biochemical test for selected strain TG-16 (A) Urea test (negative) (B)Manitol test (positive) (C) Nitrate reduction test (positive) (D) MR test (positive) (E) VP test (negative) (F) Citrate reduction test (positive) (G) Hytrogen sulphide test (negative)

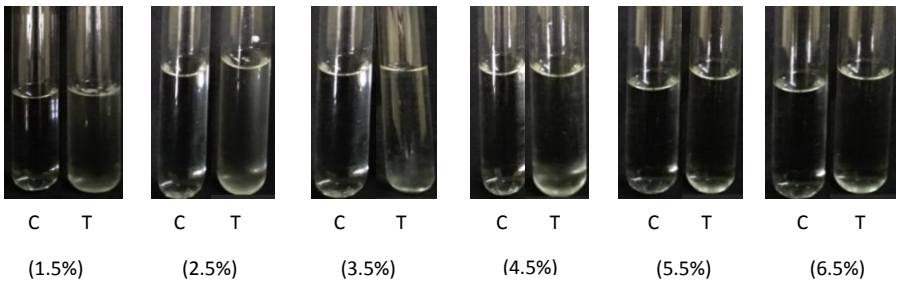


Figure 6 Sodium chloride tolerance of selected strain TG-16 (1.5%-moderate growth), (2.5%-highest growth), (3.5%-moderate growth), (4.5%-poor growth), (5.5%-poor growth) and (6.5%-poor growth)

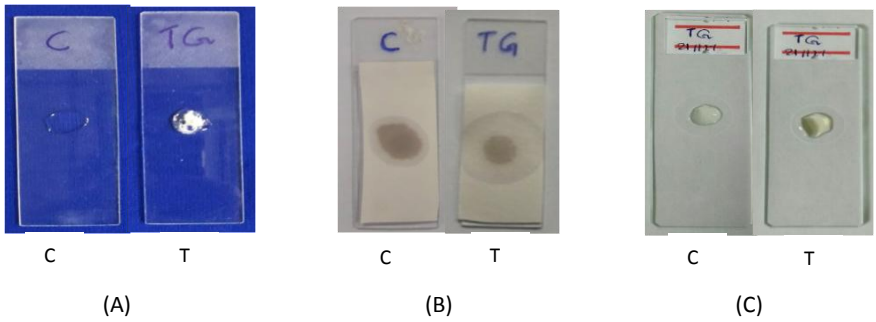


Figure 7 Biochemical test for selected strain TG-16. (A) Catalase test(positive), (B) Oxidase test(negative), (C) Molity test (negative)

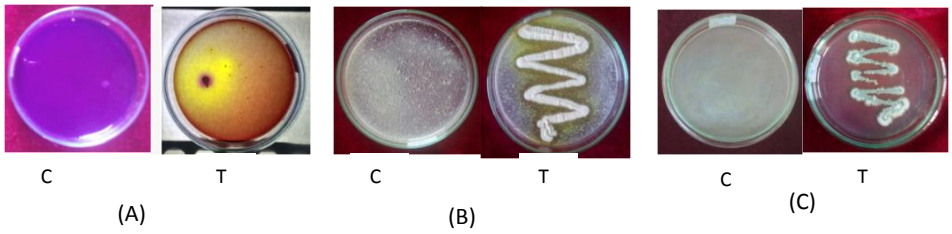


Figure 8 Biochemical test for selected strain TG-16 (A) Arginine hydrolysis (positive) (B) Casein hydrolysis (positive) and (C) Gelatin hydrolysis (negative)

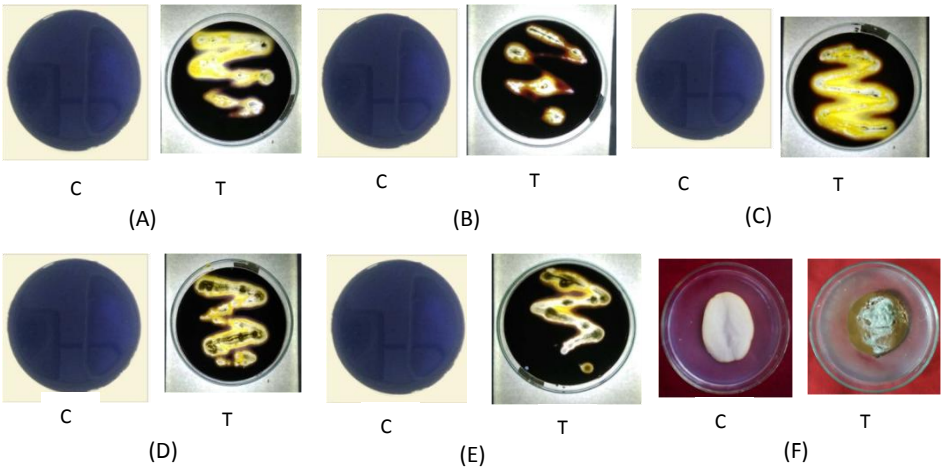


Figure 9 Biochemical test of starch hydrolysis for selected strain TG-16
(A) Soluble Starch (positive) (B) Tapioca powder (positive) (C) Sticky rice (positive) (D) Wheat flour(positive) (E)Rice (positive) and (F) potato plug test (positive)

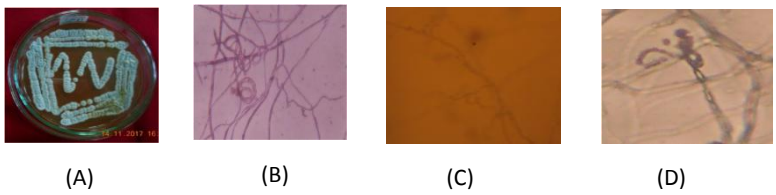
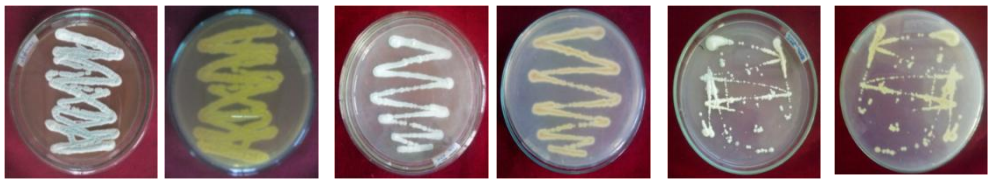
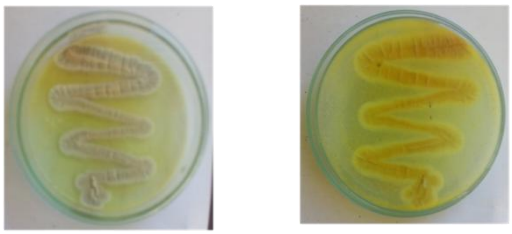


Figure 10 Cultural characteristics and Micrograph of selected strain TG-16
(A) Culture (Streaks Method) (B, C) aerial hyphae with sporophore Retinaculiaparti type (D) sporophore bearing spiralspore chain



Surface (ISP-2) Reverse Surface (ISP-5) Reverse Surface (ISP-6) Reverse

Figure 11 Cultural characteristics of selected strain TG-16 on medium (ISP-2, ISP-5, ISP-6)



Surface view of TG-16 Reverse view of TG-16

Figure 12 Yellow pigment production in casein hydrolysis test medium of TG-16

Discussion and Conclusion

The endophytic actinomycete that resides in the tissue of living plants and does not visibly harm the plants are known as endophytic actinomycetes (Stone *et al.*,2000). In this study, different parts (leave, stem,root) of five selected plant samples were collected from Pathein University Campus, Ayeyarwady Region. Eighteen endophytic actinomycetes were obtained from these plant samples, there were eleven strains from root of *M. hortensis* L., two strains from stem of *A. vera* L., one strain from root of *B, strigosa Willd.*, each one strain from leaf, stem and root of *D. triquetrum* (L).DC. , and one strain from root of *P. chinensis* L.

Out of eighteen isolates, more diverse endophytic actinomycetes were isolated from roots rather than from stem and leaf. Verma *et al*., 2009, recommended that the majority of endophytic actinomycetes have been isolated from root rather than other organs. In this study, eleven strains were obtained from root of *Millingtonia hortinsis* L. Shimizu, 2011 also reported multiple strains of endophytic actinomycetes could be isolated from a single plant.

In order to identify the isolated strains, the colony morphology, gram strains, microscopical characters were carried out. The aerial mass color of all strains were white, gray, whitish gray, whitish blue, dark greenish brown and yellow. The spore chains of all strains were generally produced flexibilis, retinaculum apertum and spiral types. Chatsuda and Kaewalin, 2015 was reported that the type of spore chain of isolated actinomycetes from *Centella asiatica* (L.)Urban. were rectus-flexibils, retinaculum-apertum and spira.

Eleven isolated strain (TG-2, 3, 4, 5, 7, 9, 12, 13, 15 and 16) had antimicrobial activity. Among them, antibacterial activity of isolated actinomycetes TG-16 exhibited the maximum inhibitory zone against *Escherichia coli.* In the study of morphological, microscopical characters and Gram staining reaction, the selected strain TG-16 was gram positive, with sporophore as

hook, open loop retinaculum apertum type. Moreover TG-16 was spiral spore chain and spore smooth and oval shape, elevation, margin and form of these strains were raised, entire and circular.

Three medium; ISP-2, ISP-5 and ISP-6 were used for the cultural characteristics of selected strain TG-16. In this study, aerial mycelium showed pale blue in all medium but reverse color observed yellow in ISP-2, light brown in ISP-5 and ISP-6. The aerial mass color of TG-16 varied from white and pale blue to different nuances of grey (from pale grey to green-grey), therefore it could be a signed to the grey series. These characters was agreement in the result of Stefka, *et al*., 2007. In the study of some biochemical tests, positive in starch hydrolysis and casein hydrolysis. Similar result was described by Venkateswara *et al*. 2015.

The other biochemical characters such as methyl red test, citrate utilization, catalase and arginine hydrolysis and nitrate reduction were positive and dextrose, fructose, lactose and sucrose were positive in acid production test and was also produced yellow pigments on starch casein hydrolysis medium. According to the above results, these characters were in the same with those of Reddy *et al*., 2011. These characters were similar to the investigation on the genus *Streptomyces* by the Actinomycetes (volume I and II) of Selman and Waksman, 1961 and Buchanam, 1964. Based on the obtained results of TG-16 was classified as belonging to the genus *Streptomyces* sp.

Detection and identification of *Streptomyces* are valuable, provides medically important bioactive compounds. The present study concluded that the selected strain belongs to *Streptomyces* sp., which also have diffusible pigment production ability. It can be used for food industries and Pharmaceutical industries as a natural colorant and might be useful in cosmetic industries.

Acknowledgements

I would like to acknowledge to the following persons who have supported for this research work: Dr Si Si Hla Bu, Rector, University of Pathein for providing me an permission to do this work, Dr Nilar Myin and Dr Than Htun Pro-rectors, University of Pathein for their suggestion and advices, Dr Kay Thi Mya, Professor and Head of Botany Department, University of Pathein and Professor Dr Wah Wah Lwin, Department of Botany, University of Pathein for their invaluable suggestions and comments offered in writing this research, Dr. Zar Zar Yin Associate Professor from Department of Botany, University of Pathein for her valuable instructions, critical reviews and suggestions for the successful completion of this research paper.

References

Aneja, K. R.(1996). **Experiments in Microbiology**, Plant pathology, Tissue Culture and mushroom cultivation. Wishwa Prakashan New Age International (P) Limited. New Delhi

Atlas, R.M. (1993). **Microbial media**. Boca Raton Ann Arbor, London Tokyo.ological fundamental

Bisen,P.S and K.Verman,1998. **Handbook of microbiology**.CBS Publishers and Distributors, New Dehli, India.

Buchanan, R.E, N.E. Gibbons, (1974). **Bergey's Mannual of Deternative Bateriology. 8th Edition; Batimor,** The Wiliams and Wilkins Company.Christensen W.B.,. **J. Bacteriol,** 52:461

Collins, CH. (1965). Microbial methods. Butterworth & Co., Publishers Ltd., Lodon.

Cruickshan,R.J.,P.Guguid and R.H.R. Swain, (1963). **Medical microbiology.** 11th ed. The English Book Society and S. Living stone Ltd., London

Chatsuda P. and K. Kaewalin, (2015). **Isolation and Screening for Endophytic Actinomycetes from *Centella asiatica* (L.) Urban**

Coombs JT. and C.M.M. Franco, (2003). **Islation and identification of action bacteria from surface sterilized wheat roots. Appl Environ Microbiol** 69(9): 5603-5608.

Dubey, R.C. and D.K. Maheswari, (2002). Practical Microbiology. S. Chand & Co., New Delhi. Dastager S.G., L.Wen-Jun, A.Dayanand, T.Shu-Kun, T. Xin-Peng,

Y.Xiao, X. Li-Hua,and J. Cheng-Lin (2006). **Seperation, identification and analysis of pigment (melanin) production in Streptomyces.** African Journal of Biotechnology Vol. 5 (8), pp. 1131-1134, 16 .

Hasani A.,A.Kariminik,K.Issazadeh, (2014). **Streptomycetes:Characteristics and TheirAntimicrobial Activities.** International journal of Advanced Biological and Biomedical ResearchVolume 2, Issue 1,: 63-75

Harrigan, W.F. & M.E. Mc Cance. (1996). **Laboratory methods in microbiology academic press inc.,** London.

Pelezar, M. J. & E. C. S. Chan. **(**1972). **Exercises in microbiology.3rd ed.** McGraw. Hill Book Co., New York. Pennsylvania State University Pesticide Education and Assessment Program.

Prescott, H. (2002). **Laboratory exercises in Microbiology**. McGraw-Hill Companies.

Reddy N.G, D.P.N. Ramakrishna, S.V. Raja Gopal, (2011)**. A morphological, physiological and Biochemical Studies of Marine Streptomyces rochei (MTCC 10109) Showing Antagonistic Activity Against Selective Human Pathogenic Mcroorganisms**. Asian journal of Biological Sciences.

Salle, A. J. (1948). **Fundamental princioles of bacteriology. Mc.** Graw Hill Book Co., Inc., New York.

Scchulz, B. and C. Boyle, (2006). **Whats are endophytes?** Microbial Root Endophytes, 9:1-13.

Selmam A. and Waksman, (1961). **Classification, Identification of Genera and Species.** The Actinomycetes, Vol. I and II. The Williams & Wilkins Company.

Shimizu, M.,(2011)**. Endophytic actinomycetes: biocontrol agents and growth promoter.** Pp. 201-210

Shirling E.B and Gottlieb D. (1966). **Methods for characterizing Streptomyces sp.** Int J Syst Bacteriol 16:313-340

Stefka A. N., S. Vanya, Y. Lyubomira, (2007). **Taxonomic Study of Streptomyces SP.Strain 34-1**. J. of Culture Collection. Volume 5.pp. 10-5

Stone, J.K., Bacon, C.W., White, J.F., (2000). **An overview of endophytic microbes: endophytism defined.** In: Bacon CW, White JF (eds) Microbialendophytes. Marcel Dekker Inc., New York, pp 3–29.

Kumud Chandra Kandpal, D.A., Umesh Kuma, T. Rashmi, T. Siva Kumar, (2012). **Isolation and screening of endophytic of actinomycetesproducing antibacterial compound from Citrus aurantifolia Fruit.** European Journal of Experimental Biology , 2 (5):1733-1737.

Venkateswara ,VS. and P. Ellaiah (2015)**. A new variant of Streptomyces species–Streptomyces azureusvargossypii from soil of Andhra Pradesh.** Department of Pharmaceutical Sciences Andhra University, IJRPC 5(1), 126-131

Verma J.P.,and J. Yadav, (2009). **Response of wheat to PGPR and organic manures in cereal and legume based cropping sequences under nascent stage of organic farming.** Ann. Plant soil Res., 11: 122-125.

ISOLATION AND ANTIMICROBIAL ACTIVITY OF BACTERIA FROM MANGROVE SOIL AND BIOCHEMICAL CHARACTERIZATION OF SELECTED BACTERIUM (ZM-7)

Zin Mar Cho[1] , Zar Yin[2] and Kay Thi Mya[3]

Abstract

Mangrove soil samples were collected from four different station of U-To Creek at Shwe thaung yan Township (Ma-Gyi), Ayeyawady Region. These samples were cultured on Nutrient Agar (NA) and American Type Culture Collection (ATCC) medium. A total of 26 bacterial colonies were obtained and 14 strains from Nutrient Agar (NA) and 12 isolates from (ATCC) medium. Isolated strains were designated as ZM 1 to 26. These isolated strains were tested by using ten different test organisms from one day to three days old culture. Five strains showed the antimicrobial activity. Among them, ZM-7 showed the highest antimicrobial activity (24.34 mm) on *Escherichia coli* and (22.63 mm) on *Candida albicans.* Therefore, ZM-7 was selected and characterized by morphological, microscopial, Gram staining and biochemical characteristics. In the colony morphology, ZM-7 was medium in size, entire, creamy, flat and creamy glistening. In the microscopical and biochemical characteristics, ZM-7 was Gram positive and short rod, catalase positive, aerobes and acid was produced in the sugar fermentation and gas did not produce. According to the results, ZM-7 was characterized as the possible genus *Bacillus.*

Keywords: Soil Bacteria, Antimicrobial activity, Biochemical characterization

Introduction

The microorganisms of mangrove are essential in the productivity, conservation, and rehabilization of mangrove ecosystem (Holguin *et al.*, 2001). Mangrove ecosystem shows diversity of microbes such as bacteria, fungi, actinomycetes etc. Bacteria includes various types like nitrogen fixing bacteria, phosphate soilubilizing bacteria, sulphate reducing bacteria, photosynthetic anoxygenic bacteria, methano bacteria, enzyme producing bacteria (Sahoo and Dhal, 2009).

Microbial research always involves the isolation and identification of microorganisms, strain preservation testing for biological activity and fermentation practice. Microbial fermentations have also been developed for the production of a wide range of pharmaceutical products (Mansi and Charlie, 2003).

The most commonly used biochemical tests involve the observation of whether or not a growth of the bacterium in liquid nutrient medium will ferment particular sugar such as glucose, lactose or mannitol. Then acid and gas may be produced which may be detected by a change in colour of un indicator dye present in the medium. Other tests determine whether the bacterium produces particular end products (eg. indole, H_2S, nitrite) when grown in suitable culture media. Many enzyme activities (such as catalase, ureas, gelatinase) are frequently measured to aid in the identification of bacteria (Mitruka and Mary, 1977).

Therefore, the present study was carried out the isolation and identification of bacterial strains from the mangrove soil. The aim and objectives of this study were to isolate the bacterial strains of mangrove soil and to identify those bacterial strains based on their colony morphology, gram staining, microscopical characters and biochemical reactions.

[1] Assistant Lecturer, Department of Botany, Pathein University
[2] Associate Professor, Department of Botany, Pathein University
[3] Professor and head, Department of Botany, Pathein University

Materials and Methods

Study area and collection of soil samples

Soil samples were collected from four different station of U-To-Creek, Shwe-Thaung Yan Sub-Township (Ma-Gyi) Ayeyawady Region. Soil smaples were collected from 0-3 cm, 1-6 cm and under 6 cm deep from each of these stations using a sterile spatual. Then, it was brought to the Biotechnological Development and Resource Centre (BDRC) and soil was analyzed by Department of Agriculture (Land Use).

Isolation of Soil Bacteria

Isolation of the bacteria from collected soil samples was done by serial dilution method. Salle 1948; Collins 1965 and Pelezer and Chan 1972, as soon as possible after soil collection in fields.

Preliminary Study on Antimicrobial Activity of Isolated Bacteria (NITE 2005)

The isolated soil bacteria were inoculated into seed medium and incubated for 3 days at 27ºC. Seed culture were transferred to the fermentation medium. After three days, the pre-culture (1%) was transferred into the fermentation medium (glucose 1.0 g, peptone 0.5 g, yeast extract 0.5 g, $MgSO_4$ $7H_2O$ 0.01 g, K_2HPO_4 0.01 g, $CaCO_3$ 0.01 g, DW 100 mL at pH 7.0 and carried out for 3-7 days and evaluated by agar well diffusion method.

Screening of Antimicrobial Activity by Agar Well Method

(Collins, 1965)

1 day old culture test broth (0.2 mL) was added to 25 mL warm assay medium (glucose 1.0 g, peptone 0.3 g, KNO_3 0.1 g, DW 100 mL, agar 1.8 g) and thoroughly mixed and poured into plate. Cork borer was used to make the wells (8 mm in diameter). And then, the fermented broth (20 µL) was carefully added into the well and incubated at room temperature for 24 -48 hours. The diameter of the zones of inhibition around each well measured and recorded after 24-48 hours incubation.

Test Organisms

Bacillus subtilis IFO 90571, *Candida albicans* NITE 09542, *Staphylococcus aureus* AHU 8465, *Escherichia coli* AHU 5436, *Pseudomonas fluorescens* IFO 94307, *Agrobacterium tumefaciens* NITE, 09678, *Aspergillus paraciticus* IFO-5123, *Micrococcus luteus* NITE-83297, *Saccharomyces cerevisiae* NITE 83297 and *Salmonella typhi* AHU-7943 were obtained from NITE 2005 (National Institute of Technology and Evaluation, Kisarazu, Japan) and PRD-Pharmaceutical Research Departmen (Ministry of Industry).

The identification of isolated bacterial strains were carried out by using their colony morphology, gram staining methods (Dubey and Maheshwari, 2002), and biochemical tests which include the motility test (Tittsler and Sandholzer, 1936), methyl red test (Aneja, 1996), sugar fermentation test (sucrose, lactose, maltose) (Atlas, 1993), nitrate reduction test (Dickey and Kelman, 1988), starch hydrolysis test (Aneja, 1996), catalase test (Dickey and Kelman, 1988), oxidase test (Dickey and Kelman, 1988), oxygen requirement (aerobic/anaerobic) (Prescott, 2002), citrate utilization test (Atlas, 1993), Voges-Proskauer VP test (Cruickshank, 1963), Urea test (Woodland, 2004), respectively.

Results

The total of 26 bacterial strains (ZM-1 to 26) were isolated from the mangrove soils. The antimicrobial activity of these strains were tested by using ten different test organisms. Five strains showed the activity on *Aspergillus paraciticus, Candida albicans, Escherichia coli, Saccharomyces cerevisiae.* Among them, ZM-7 showed the highest antibacterial activity on *E. coli.* Therefore, ZM-7 was selected and identified by colony morphology, Gram-staining and Biochemical characteristics. In the colony morphology, ZM-7 was medium in size, entire, creamy, flat and creamy glistening. In the microscopical and biochemical characteristics, ZM-7 was Gram positive and short rod, catalase positive, aerobes and acid was produced in the sugar fermentation and gas did not produce. According to the results, ZM-7 was characterized as the possible genus *Bacillus.* These results were shown in Table 1, 2 and Figure 1, 2.

Table 1 Isolated Bacteria from Soil Samples

Soil Sample No.	Nutrient Agar (NA)	American Type Culture Collection (ATCC) medium
S - 1	ZM 1 – 3 = 3	ZM 1 – 2 = 2
S - 2	ZM 4 – 5 = 2	ZM 3 – 6 = 4
S - 3	ZM 6 – 9 = 4	ZM 7 – 9 = 3
S - 4	ZM 10 – 14 =	ZM 10 –12 = 3 /12

Table 2 Colony and Cell Morphology of Isolated Bacteria

Isolated Bacteria	Size of Colony	Margin	Color	Elevation and Form	Cell Morphology	Gram Staining
ZM - 1	Medium	Entire	Pale	Flat	Cocco-bacilli	-
ZM - 2	Medium	Entire	C ream	Flat	Rod	+
ZM - 3	Medium	Entire	White	Raised	Rod	+
ZM - 4	Large	Entire	White	Flat	Cocco-bacilli	-
ZM - 5	Medium	Filamentous	White	Flat	Short-rod	-
ZM - 6	Large	Entire	White	Flat	Rod	-
ZM - 7	Large	Undulate	White	Flat	Short-rod	+
ZM - 8	Medium	Entire	White	Raised	Cocco-bacilli	-
ZM - 9	Large	Entire	White	Flat	Short-rod	-
ZM - 10	Large	Entire	Creamy	Flat	Rod	-
ZM - 11	Medium	Entire	Creamy	Flat	Rod	-
ZM - 12	Medium	Entire	Creamy	Flat	Rod	-
ZM - 13	Large	Filamentous	White	Flat	Rod	-
ZM - 14	Large	Entire	White	Convex	cocco-bacilli	-
ZM - 15	Large	Undulate	White	Flat	Rod	+
ZM - 16	Large	Entire	White	Flat	Rod	-
ZM - 17	Small	Entire	White	Flat	Rod	-

Isolated Bacteria	Size of Colony	Margin	Color	Elevation and Form	Cell Morphology	Gram Staining
ZM - 18	Large	Entire	White	Flat	Rod	-
ZM - 19	Small	Filamentous	White	Flat	Short-rod	-
ZM - 20	Large	Rhizoid	White	Flat	Rod	-
ZM - 21	Small	Entire	C ream	Flat	Rod	-
ZM - 22	Large	Filamentous	White	Flat	Short-rod	-
ZM - 23	Large	Curled	White	Flat	Rod	-
ZM - 24	Large	Filamentous	C ream	Flat	Rod	-
ZM - 25	Large	Entire	White	Flat	Rod	+
ZM - 26	Large	Undulate	White	Raised	Rod	-

Small < 2mm diameter/ between 2mm and 5mm diameter
Large > 5mm diameter + = Gram positive - =Gram negative

Table 3 Carbohydrate Fermentation of Selected Strain ZM-7

Sugar	Responces	Acid production	Gas production
Glucose	Yellow colour changes in medium	+	-
Maltose	Yellow colour changes in medium	+	-
Xylose	Yellow colour changes in medium	+	-
Galactose	Yellow colour changes in medium	+	-
Fructose	No change in colour	-	-
Arabinose	No change in colour	-	-
Lactose	No change in colour	-	-

+ = acid and gas was produced
- = acid and gas was not produced

Table 4 Biochemical Characteristics of Selected Strain ZM-7

No	Biochemical tests	Responses	Results
1	Urea hydrolysis test	No change in colour	(-)
2	Mannitol salt broth test	No change in colour	(-)
3	Nitrate reduction test	Medium change from pale yellow to orange	(+)
4	Methyl red test	Medium remain red	(-)
5	Voges proskaucer test	Medium change from yellow to red	(+)
6	Citrate utilization test	Medium change from green to blue	(+)
7	H_2S production test	Medium change from white to black	(+)
8	Catalase test	Release free oxygen gas bubble	(+)
9	Oxidase test	A little change in colour	(+)
10	Hanging slide test	No motility	(-)
11	Arginine hydrolysis	No change back to purple	(-)
12	Gelatin hydrolysis	No clear zone around the colony	(-)
13	Potato plug	Growth in streak line	(+)
14	starch hydrolysis		
	(i) Soluble starch	Clear zone around the streak line	(+)
	(ii) Tapioca powder	Clear zone around the streak line	(+)
	(iii) Sticky rice powder	Clear zone around the streak line	(+)

No	Biochemical tests	Responses	Results
	(iv) Wheat powder	Clear zone around the streak line	(+)
	(v) Rice	Clear zone around the streak line	(+)
15	Caesin hydrolysis	Clear zone around the colony	(+)
16	Esterase activity	No colour change in medium	(-)
17	Salt tolerance test		
	(i) 2% NaCl	Highest growth	(+)
	(ii) 4% NaCl	Highest growth	(+)
	(iii) 6% NaCl	Moderate growth	(+)
	(iv) 8% NaCl	Moderate growth	(+)
	(v) 10% NaCl	Poor growth	(+)

\+ = Gram positive - = Gram negative

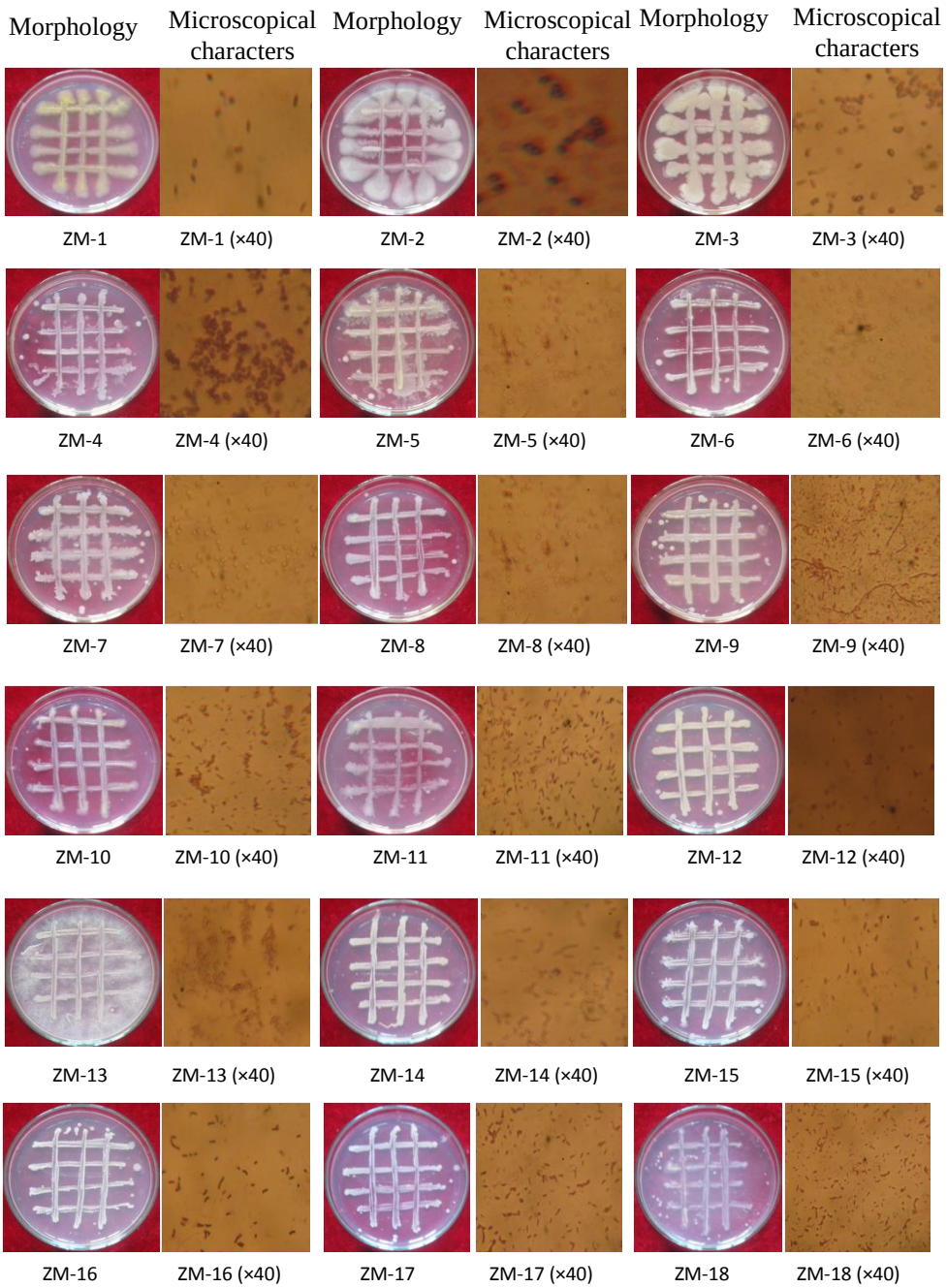


Figure 1 Cultural Character and Cell morphology of Isolated Bacteria ZM-1 to ZM-18

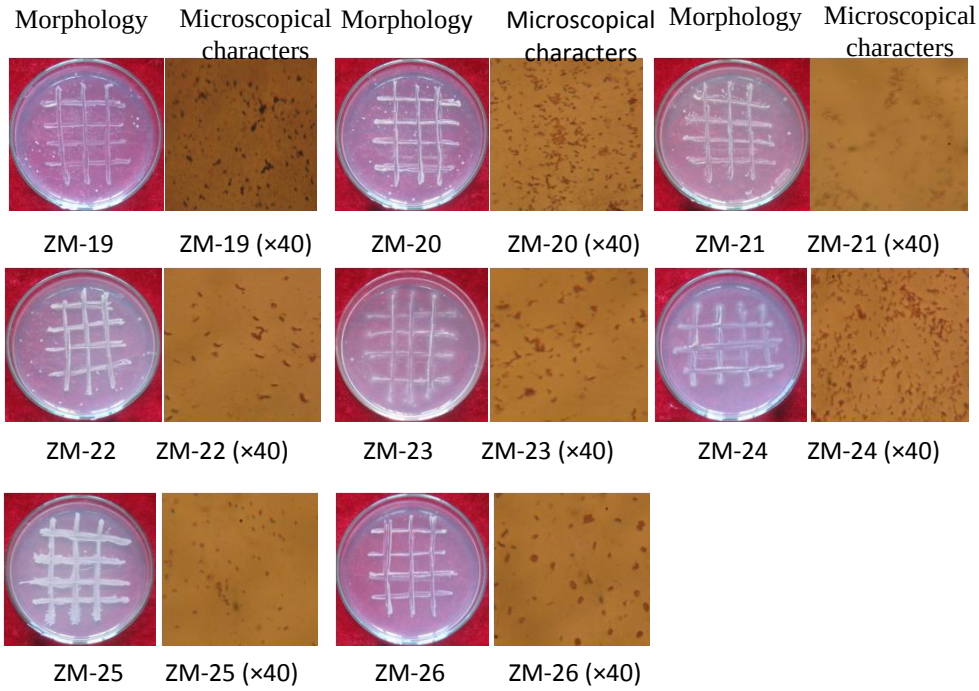


Figure 2 Cultural Character and Cell morphology of Isolated Bacteria ZM-19 to ZM-26

Antimicrobial Activity of Isolated Bacterial Strains from Mangrove Soil

Fourteen isolated bacteria (ZM-1, 2, 3, 4, 5, 6, 10, 11 and 14) could not produce antimicrobial metabolites. Five isolates (ZM-7, 8, 9, 12, 13) had antimicrobial activity and ZM-7 showed the highest antibacterial activity (24.34 mm) on *E. coli*, followed by (22.63 mm) on *Candida albicans.*

Table 6 Antimicrobial Activity of Five Selected Bacteria

No.	Isolated bacteria	Test Organisms and Antimicrobial Activity (mm)			
		Aspergillus paraciticus	***Candida albicans***	***Escherichia coli***	***Saccharomyces cerevisiae***
1	ZM-7	16.03 mm	22.63 mm	**24.34 mm**	14.45 mm
2	ZM-8	12.22 mm	-	18.30 mm	11.77 mm
3	ZM-9	-	-	-	15.00 mm
4	ZM-12	9.43 mm	-	10.75 mm	-
5	ZM-13	-	-	-	13.00 mm

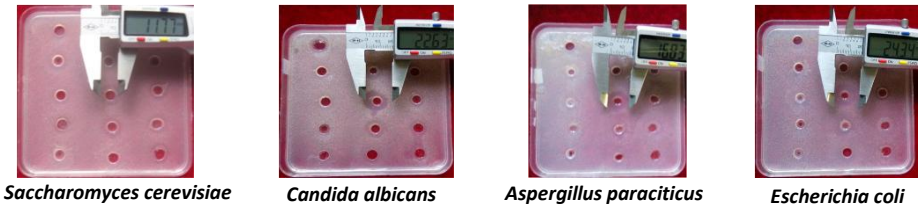
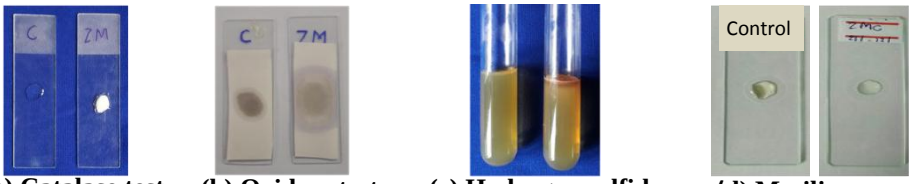


Figure 3 Antimicrobial Activity of Five Selected Bacteria



(a) Catalase test (positive) **(b) Oxidase test (positive)** **(c) Hydrogen sulfide test (positive)** **(d) Motility test (negative)**

Figure 4 Biochemical Characteristics of Selected Bacterium (ZM-7)

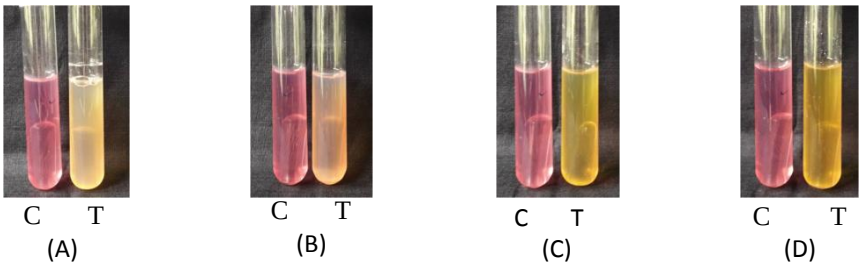


Figure 5 Carbohydrate Fermentation Test of Selected Bacterium (ZM-7) A. Glucose, B. Maltose, C. Xylose, D. Galactose (All positive)

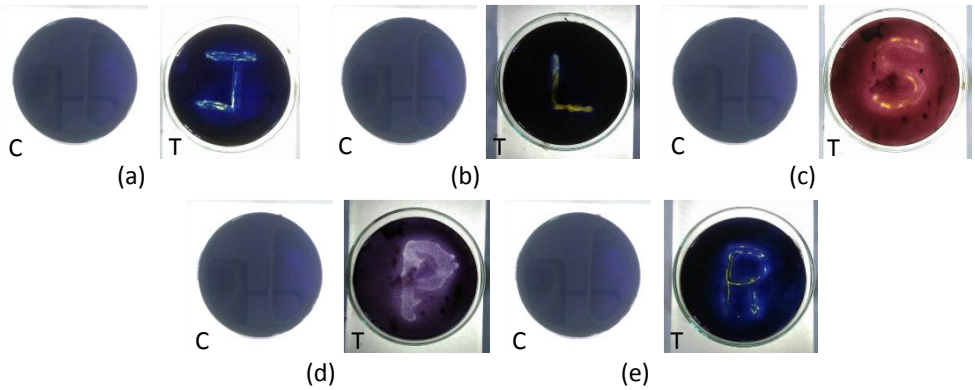


Figure 6 Starch Hydrolysis Test of Selected Bacterium ZM-7 (a) Soluble starch (positive), (b) Tapioca powder (positive), (c) Sticky rice (positive), (d) Rice (positive), (e) Wheat flour (positive)

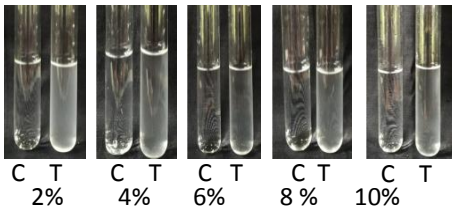


Figure7 NaCl Tolerance Test of Selected Bacterium ZM-7 (2% highest growth), (4% highest growth), (6% moderate growth), (8% moderate growth), (10% poor growth)

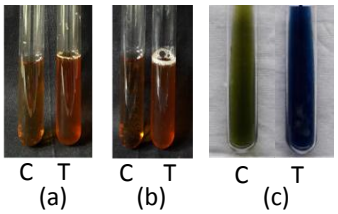


Figure 8 Biochemical Characteristics of Selected Bacterium ZM-7 (a)Methyl red (negative), (b)Voges Proskucer (positive), (c) Citrate utilization (positive)

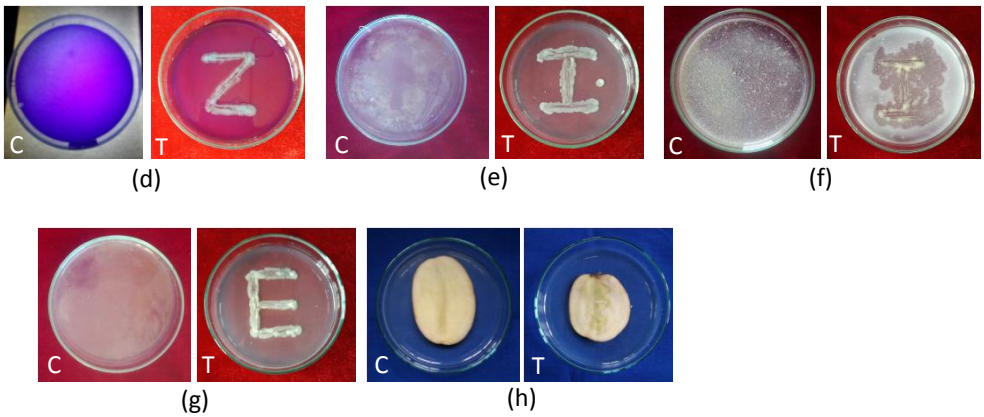


Figure 9 Biochemical Characteristics of Selected Bacterium ZM-7
(d) Arginine hydrolysis (negative), (e) Gelatin hydrolysis (negative),
(f) Caesin hydrolysis (positive), (g) Esterase (negative),
(h) Potato plug (positive)

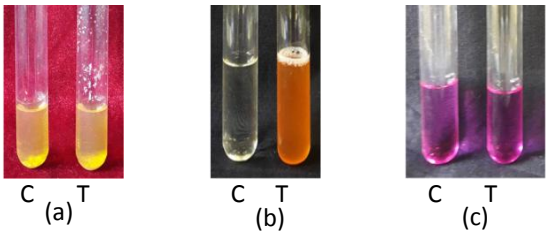


Figure 10 Biochemical Characteristics of Selected Bacterium ZM-7
(a) Mannitol test (negative), (b) Nitrate reduction (positive),
(c) Urea hydrolysis (negative)

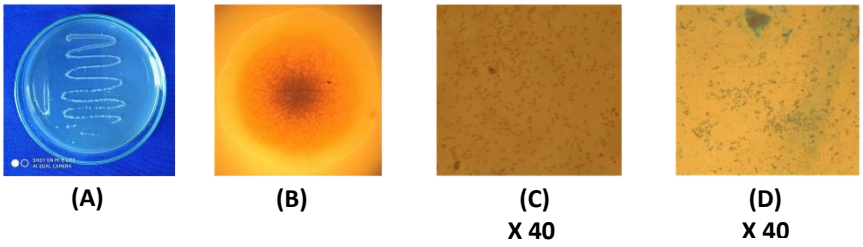


Figure 11 (A) Colony morphology (B) Single colony (C) Gram staining
(D) Spore staining of selected bacterium ZM-7

Discussion and Conclusion

In this study, 26 strains were isolated from four different samples collected from shwe-thaung yan coastal area (Ma-Gyi). Two different media were employed and it was found that 14 strains was got from NA medium and 12 strains from ATTC medium. These isolated bacteria were designated as ZM-1 to ZM-26. In the colony morphology, ZM-1 to ZM-26 were small, medium and large in size of colony and the color were cream, white, pale yellow and creamy. In the margin, ZM-1 to ZM-26 were entire, filamentous, undulate, rhizoid and curled. In the elevation and form, all strains were flat and raised and they produced pigments. Bhant, 2003

reported the bacterial colonies which were moist, small, with regular margin and were translucent from mangrove soil. Twenty isolates were found to be gram negative and 6 strains were gram positive. The results were in agreement with general rules of Haglund, 2003 that the proportion of Gram negative bacteria is much higher than the proportion of Gram positive bacteria in the ocean.

All strains were tested by using ten test organisms. Among them, strain ZM-7, showed the highest activity against *Aspergillus paraciticus, Candida albicans*, *Escherichia coli*, *Saccharomyces cerevisiae* at 2 days old culture. Therefore, ZM-7 was selected for futher study.

Mitruka and Mary, 1997 reported that in artificial classification, organisms are grouped together in a key. The first step in bacterial identification is necessary to obtain the organisms in pure culture. According to the biochemical characteristic, ZM-7 was short rod and Gram positive, spore present, catalase positive, oxidase positive, H_2S positive and motility negative, acid was produced in the sugar (glucose, maltose, xylose and galactose) except fructose, arabinose and lactose, gas not produced. In the starch hydrolysis, ZM-7 can hydrolyse soluble starch, tapioca powder, sticky rice, rice powder and wheat flour and can tolerate in NaCl 2%, 4%, 6%, 8% and 10% respectively, Methyl red negative, Voges Proskucer (VP) positive, citrate positive, esterase, arginine and gelatine hydrolysis, casein hydrolysis positive, potato plug positive, mannitol and urea hydrolysis negative, nitrate reduction positive. These characters were similar to the previous research of Buchanan 1974 and ZM-7 was classified as the genus *Bacillus* sp. Similarly observation was reported by Park *et al.*,2003 who isolated *Bacillus* spp. and identified from rotating biological contractor based on their biochemical properties Joshi *et al.*, 2007 identified *Bacillus* and studied its biochemical characteristics as well.

Thus, it would be concluded that the present findings of those isolated bacterial strains (ZM-7) can be noted as the *Bacillus* bacterial strain. Those bacterial strain would be isolated from the soil and identified as *Bacillus* spp. However, further study should be undertaken for the antimicrobial activities and biocontrol agent by using the effective bacterial strain.

Acknowledgements

Firstly, I wish to express our gratitude to Professor Dr Si Si Hla Bu, Rector, Pathein University for providing me an opportunity to do this work. I also extended my thank to Professor Dr Than Tun and Dr Nilar Myint, Pro-Rectors, Pathein University, for their valuable instruction and guidance. I would like to record my deep thank to Professor Dr Kay Thi Mya, Head of Botany Department, Pathein University and Professor Dr Wah Wah Lwin, Department of Botany, Pathein University for their suggestion and kind understanding during this study. Many thanks are due to my supervisor Dr Zar Zar Yin, Associate Professor, Department of Botany, Pathein University, for her valuable instructions, encouragement and overall supervision for the successful completion of this research paper.

References

Aneja, K. R. (1996). **Experiments in Microbiology, Plant pathology, Tissue Culture and mushroom cultivation.** Wishwa Prakashan New Age International (P) Limited. New Delhi.

Atlas R. M. Bartha R (1998). **Microbial Ecolongy.** Fundamentals and applications 4th Edition. Benjamin cummings publishing company Inc. Addison Wesley Longman Inc. pp. 300-350.

Bhat M.R, Shwewade leena, (2003). Isolation and characterization of microorganisms from mangrove soil of CBD Belapur creek, Navi Mumbai, MS India, INTERNATIONAL JOURNAL OF ENVIRONMENTAL SCIENCES Volume 3, No 6.

Buchanan, R.E.N.E. gibbons. (1974). **Bergey's Mannual of Determinative Bacteriology.** 8th Edition: Batimore, the Williams and Wilkins Company, USA.

Collins, C.H., (1965). **Microbiological Methods** (5th ed.) Butler Tanner ltd., London.

Cruickshank, R., J. P. Guguid & R. H. R. Swain. 1963. **Medical microbiology. 11th ed.** The English Language Book Society and F. and S. Living stone Ltd., London.

Dickey, R. S. & A. Kelman. (1988). **Caratovora or soft rot group.** In: Laboratory guide for identification of plant pathogenic bacteria 2nd ed. (Ed. N.W. Shaad.). Minnesota. Pp 81-84.

Dubey, R.C. and D.K. Maheswari. (2002). **Practical Microbiology.** S. Chand & Co., New Delhi.

Haglund, A.L., (2003). Depth distribution of active bacteria and bacterial activity in lake sediment. FEMS Microbiol. Ecol.,46:31-38

Joshi G. K., Kumar S. and Serma V. (2007). **Production of Moderately Haloterant, SDS stable Alkaline Protese from *Bacillus cereus* MTCC 6840 isolated from Lake Nainital, Urrtaranchal State, India.** Brazilian J. of Microibology 38:773-779

Mansi, M.E. and B. Charlie. (2003). **Fermentation microbiology and biotechnology.** British library cataloguiny in Publication Data, British. UK.

Mitruka, B.M. and J.B. Mary. (1977). **Methods of detection and identification of bacteria.** CRC Press, Inc., 18901, United State of America.

NITE, 2005. **Medium for fermentation to produce the metabolite.**

Park J., Yoon C. Kim H. Shin K. (2003). **Characterization of Proteolytic Activity of Bacteria Isolated from Rotating Biological Contactor,** J. of microbiology. The Microbiological Society of Korea. 41(2): 73-77.

Pelezar, M. J. & E. C. S. Chan. (1972). **Exercise in microbiology.** 3rd ed. Mc Graw. Hill Book Co., New York.

Prescott, H. (2002). **Laboratory exercises in Microbiology**. McGraw-Hill Companies.

Sahoo K., N.K. Dhal, 2009. **Potential microbial diversity in mangrove ecosystem,** A review Indian Journal of marine sciences, 38(2), pp 249-256

Salle, A. J. (1948). **Fundamental principles of bacteriology.** Mc. Graw Hill Book Co., Inc., New york.

Tittsler, R.P. and L.A. Sandholzer. (1936). **The use of semi-solid agar for the detection of bacterial motility.** J. Bacterial., 31:576-580.

Woodland, J. (2004). **Bacteriology.** NWFHS Laboratory Prodcedure Manual. Second Edition, pinetop, Arizona.

POLLEN MORPHOLOGY ON TEN SPECIES OF SAPINDALES FOUND IN MANDALAY REGION AND SOUTHERN SHAN STATE

Hnin Yu Maw[1], Swe Swe Linn[2] and Nwè Nwè Yi [3]

Abstract

The pollen morphology of 10 species belonging to 10 genera of Sapindales was studied. The specimens were collected from Mandalay Region and Southern Shan State from 2017 to 2018. The collected plants include 2 species of Anacardiaceae, 1 species of Sapindaceae, 5 species of Rutaceae and 2 species of Meliaceae. The examined of pollen grains were found in monad type. The morphological characters of each grain were studied. The aperture types of all the pollen grains were porate and colporate; triporate in one species, tricolporate in 5 species and tetracolporate in 4 species. The pollen shapes were found in prolate, oblate spheroidal, subprolate to prolate spheroidal. The sizes of pollen grains were small and medium. The small sizes of pollen grains was found in 6 species and medium size of pollen grains was found in 4 species.The sculpture patterns of 10 species are varied from psilate, reticulate to obscurely reticulate.

Keywords : Sapindales, Palynology, Southern Shan State.

Introduction

Palynology is the study of pollen and spores. Pollen characters which are only of limited taxonomic importance at the generic level or lower are largely ignored. Pollen characters have been grouped into seven categories, which will be treated in the following order: aperture type, pollen wall architecture, pollen-unit, polarity, symmetry, shape and grain size (Walker & Doyle 1975).

The basic palynology has contacts with cytology and genetics, morphology, physics, chemistry and other branches of science, even mathematics; to basic palynology can also be referred investigations of pollen and spore dispersal, preferably by wind and water and of the pollen and spore content of peat and sediments etc. Several types of pollen grains may even be produced by a single species (Erdtman 1952).

Pollen grains are microscopic; their detail cannot be resolved by the naked human eye unless they area at the larger end of the size range. They are measure in microns. Most pollen grain are between 20 and 80 microns. However, the smallest pollen grains are about 5 to 8 microns. In most plant pollen grains are released from the anthers of mature flowers as individuals. However, in some plant families (circa fifty) there are at least some species where the mature pollen grains are dispersed as 'tetrads' (Kesseler 2009).

According to the classification system of Byng *et al*. (2016), Sapindales is an order of flowering plants. Well-known members of Sapindales include citrus; maples, horse-chestnuts, lychees and mangos and cashews; mahogany and neem. Sapindales includes Biebersteiniaceae, Nitrariaceae, Kirkiaceae, Burseraceae, Anacardiaceae, Sapindaceae (including Xanthoceraceae), Rutaceae, Simaroubaceae and Meliaceae. However, only six out of nine family are found in Myanmar. The remaining three family, namely, Biebersteiniaceae, Nitrariaceae, Kirkiaceae are not found in Chit Ko Ko (1961) and Kress *et al.* (2003). Sapindales is about 278 species (Hundley and Chit Ko Ko 1961) and is about 285 species (Kress *et al.* 2003).

[1] Assistant Lecturer, Department of Botany, Yadanabon University
[2] Lecturer, Department of Botany, Mandalay University
[3] Professor & Head, Department of Botany, Sagaing University of Education

Pollen morphology of the family Anacardiaceae has radially symmetrical, isopolar, sub-prolate rarely prolate spheroidal or oblate-spheroidal, sexine thicker or thinner than nexine, colpal membrane granulate to spinulose (Perveen & Qaiser 2010).

Citrus species are a common and extremely important for their fruit. It is one of the genera in the family Rutaceae. *Citrus* comprises around 60 species, most of which are cultivated throughout the tropics and subtropics (Anbari *et al.* 2015).

In Meliaceae pollen descriptions of the three *Carapa* species were very similar presenting pollen grains stephanocolporate (4-6 aperture), oblate spheroidal, isopolar and circular shape (Zidko *et al.* 2016).

Sapindaceae are mostly tropical or subtropical, with a few genera extending to sub-temperate zones. Sapindaceae pollen grains are usually iso-polar or subisopolar monads, pollen grain size is usually between 20 and 30 mm, and the grains are oblate to prolate in shape (Rodriguez *et al.* 2010).

Many researchers had given an account on the classification and identification of order Sapindales in various localities in Myanmar. However, pollen morphology of the order Sapindales has not been mentioned in Myanmar. Therefore, a research on pollen morphology of order Sapindales still needed to be studied and recorded.

The aims and objectives of this research were to identify and classify the morphological variation in pollens of Sapindales, to study and record the collected species systematically from the palynological point of views and to provide the different pollen characters.

Materials and Methods

Collection of Plants

The specimens of the order Sapindales were collected from Mandalay Region and Southern Shan State from 2017 to 2018. The collected species were photographed to record their inflorescences and flowers. All the collected plants are pressed and preserved in the herbarium sheets. Locations of the collected specimens were described by using Global Position System (GPS). Identification of genera and species were carried out by referring to Backer (1965), Dassanayake (1980), Hooker (1881). Myanmar names were referred to Hundley and Chit Ko Ko (1961) and Kress and Daw Yin Yin Kyi (2003) in Myanmar.

Collection of Pollen Samples

All the fresh pollens were collected from the anthers of blooming flowers**.** The collected flowers of each species were stored in the glass vials with glacial acetic acid and the specimens were labeled.

Acetolysis of Pollen Grain

The pollen samples were acetolysed by the standard method of Erdtman (1960). The samples in glass vial were put into a test tube, then crushed with a glass rod. The acetolysis solution was mixed using a measuring cylinder; 9 parts of glacial acetic acid were added, and then 1 part of concentrated sulphuric acid was added. The acid was dropped gently down the side of the tube. 1 cc of acetolysis mixture was poured into the test tube containing the pollen

samples and stirred with a glass rod. The test tube containing the pollen sample was transferred to a water bath at 75°C for 30 minutes. The test tube was diluted with distilled water and the test tubes were put in an electric centrifuge tube for 30 minutes at 3000 rpm. This was repeated twice, decanting the water each time. After centrifuging and decanting, a few drops of dilute glycerin solution was added to the residue, then transferred and stored in air tight glass vial.

Slide Preparation

A drop of sample was taken from sample bottle with a glass rod and placed on a slide, then covered with a cover-slip. The glass slice was examined under light microscope and photomicrograph. Pollen grains were measured and recorded on their polar length (P); equatorial diameter (E); length and wide of colpi; diameter and length of pores and exine thickness. These measurements were based on 10-15 grains per sample. The pollen terminology used in identificatioin is according to Erdtman (1952), Moore & Webb (1978), Hoen (1999) and Hesse (2009).

Results

In this research, pollen morphology of 10 species belonging to 10 genera in four family of Sapindales were studied. The list of collected species were arranged according to classification system of Byng *et al.* (2016) and listed in alphabetically as shown in Table 1.

Table 1 List of the collected specimens

Order	Family	No.	Scientific Name	Myanmar Name
Sapindales	Anacardiaceae	1	*Buchanania latifolia* Roxb.	Thit si bo, Lunbo
		2	*Melanorrhoea usitata* Wall.	Thit si
	Sapindaceae	3	*Cardiospermum canescens* Wall.	Kala myetsi
	Rutaceae	4	*Aegle marmelos* (L.) Correa, Trans, L.	Okshit
		5	*Atalantia monophylla* (Roxb.) DC.	Shauk yaing,Taw shauk
		6	*Clausena excavata* Burm. f.	Pyin daw thein
		7	*Glycosmis pentaphylla* (Retz.) DC.	Taw shauk ,Obok
		8	*Micromelum pubescens* Blume	Tanyin bo, Taw pyindaw thein
	Meliaceae	9	*Azadirachta indica* A. Juss.	Tama, Tama kha
		10	*Melia azedarach* L.	Thinbaw pan tama

1. *Buchanania latifolia* Roxb., Fl. Ind. 2.1832. (Figure 1 A)

***Buchanania lanzan* Sprengel.**

Myanmar name : Thit si bo, Lunbo

English name : Almond

Flowering period : December to March

Outstanding characters

Perennial trees, stems and branches terete, brown, pubescent. Leaves simple, alternate, exstipulate. Inflorescences axillary and terminal paniculate raceme. Flowers bisexual, actinomorphic, pentamerous. Petals 5, free, oblong. Fruits drupaceous. Seeds one-seeded, brown, endospermic.

Specimens examined: Mandalay Region, Kyaukse Township, Yeywa village; 21° 40' 26" N and 96° 26' 30" E; 8 December, 2018; Hnin Yu Maw, collection no. 18.

Pollen morphology (Figure 1 B, C)

Tricolporate, prolate spheroidal, small, 19.2 – 22.8 × 18.0 – 20.4 µm in length and breadth; amb rounded triangular; colpi longicolpate, 16.8 – 19.2 × 4.8 – 6.0 µm in length and breadth; pori lalongate, 6.0 – 7.2 × 7.2 – 8.4 µm in length and breadth; exine 0.6 – 1.2 µm thick, sexine thicker than nexine; sculpturing obscurely reticulate.

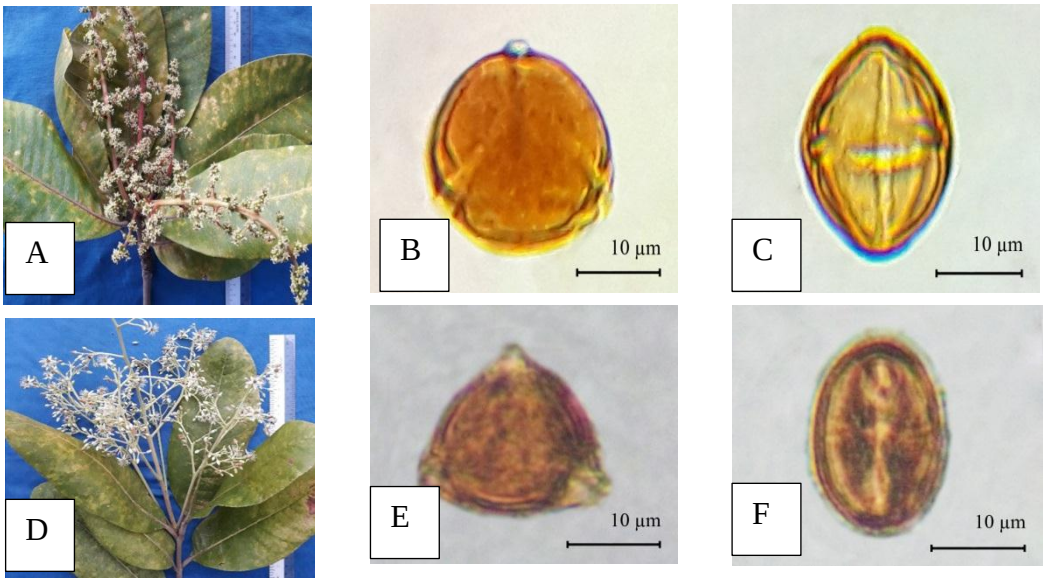


Figure 1 A. Inflorescences of *Buchanania latifolia* Roxb.
B. Polar view of *B. latifolia* Roxb.
C. Equatorial view of *B. latifolia* Roxb.
D. Inflorescences of *Melanorrhoea usitata* Wall.
E. Polar view of *M. usitata* Wall.
F. Equatorial view of *M. usitata* Wall.

3. Family – Sapindaceae

***Cardiospermum canescens* Wall., P1. As. Rar.1:14.t.14.1829 (Figure 2A)**

***C. corundum* L.f. canescens (Wall.) Radlk.in Pflan.zenr. Ht. 98. 448.1937.**

Myanmar name : Kala myetsi

English name : Ballon vine

Flowering period : May to December

Outstanding characters

Annual, tendrillar climber; stems and branches terete. Leaves bipinnately compound, imparipinnate, alternate; stipules subulate. Inflorescences axillary, umbelliform racemes, many-flowered. Flowers bisexual, zygomorphic, hypogynous, white. Sepals 4, two series, ovate in inner ,suborbicular in outer two. Petals 4, 2 series, oblong in inner, suborbicular in outer, white. Fruit capsular, ovoid globose, 3-angled, not winged, valves membranous. Seeds orbicular, black.

Specimens examined: Mandalay Region, Madaya Township, 22º 12' 46" N and 96º 65' 42" E; 2 July, 2017; Hnin Yu Maw, collection no 1.

Pollen morphology (Figure 2 B, C)

Triporate, prolate, medium, 37.2 – 42.0 × 24.0 – 28.8 µm in length and breadth; amb triangular; pori lalongate, 3.6 – 8.4 × 6.0 – 7.2 µm in length and breadth; exine about 2.4 µm thick, sexine thicker than nexine; sculpturing reticulate, the lumina heterobrochate, 1.2– 2.4 µm width; the muri simplibaculate, about 0.6 µm wide.

4. Familly- Rutaceae

***Aegle marmelos* (L.) Correa, Trans, L. Soc. Landon 5: 223. 1800. (Figure 2 D)**

***Crataeva marmelos* L., Sp. Pl. 444. 1753.**

***Bilacus marmelos* (L.) Kuntze, Rev. Gen. 1:98. 1898.**

***Belou marmelos* (L.) Lyons, Plant Names 69. 1907.**

Myanmar name : Okshit

English name : Back fruit, Golden apple

Flowering period : March to July

Outstanding characters

Perennial deciduous spinous trees. Leaves palmately 3 to 5-foliolate compound, alternate; leaflets ovate to elliptic. Inflorescences axillary, few-flowered, fascicled raceme. Flowers bisexual, actinomorphic, pentamerous, hypogynous, greenish-white. Calyx campanulate, 5-toothed, puberulent. Petals 4-5, oblong-obovate greenish white, glabrous. Fruits subgloboid, 6-10 seeded with a hard woody shell. Seeds oblongoid, wooly-pubescent, endospermic.

Specimens examined: Mandalay Region, Patheingyi Township, Yae Tagon Taung; 21° 57' 53" N and 96° 12' 53" E; 31 March, 2018; Hnin Yu Maw, collection no. 10.

Pollen morphology (Figure 2 E, F)

Tetracolporate, prolate spheroidal, small, 20.4 – 24.0 × 19.2 – 21.6 µm in length and breadth; amb quadrangular; colpi ¾ way up to the pole, 14.4 – 18.0 × 2.4 – 4.8 µm in length and breadth; pori lalongate, 2.4 – 4.8 × 3.6 – 6.0 µm in length and breadth; exine about 2.4 µm thick, sexine thicker than nexine; sculpturing reticulate, the lumina heterobrochate, 1.2 – 2.4 µm width; the muri simplibaculate, about 0.6 µm wide.

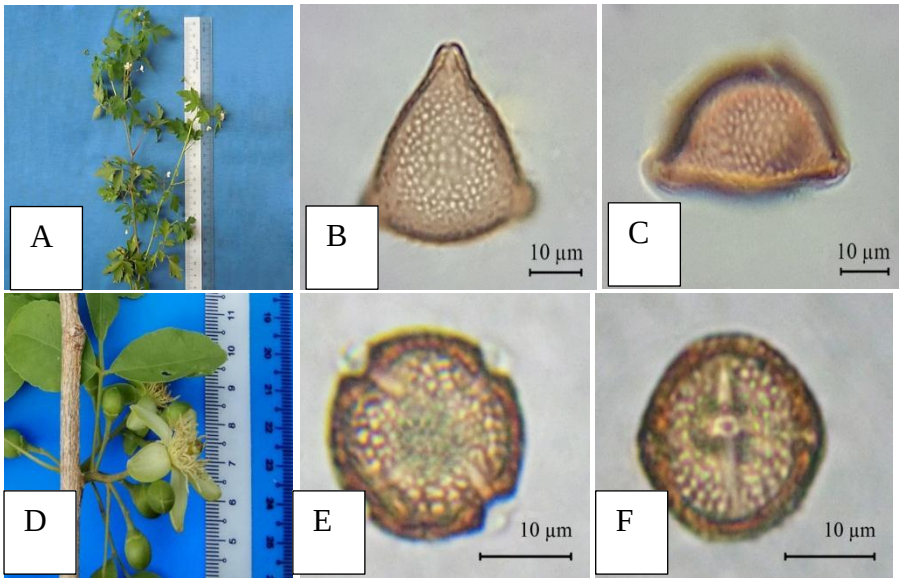


Figure 2 A. Inflorescences of *Cardiospermum canescens* Wall.
B. Polar view of *C. canescens* Wall.
C. Equatorial view of *C. canescens* Wall.
D. Inflorescences of *Aegle marmelos* (L.) Correa, Trans, L.
E. Polar view of *A. marmelos* (L.) Correa, Trans, L.
F. Equatorial view of *A. marmelos* (L.) Correa, Trans, L.

5. *Atalantia monophylla* (Roxb.) DC., Prod. 1: 535. 1824. (Figure 3 A)

***Limonia monophylla* Roxb. (non L.), Pl. Corom. 1: 59. 1795.**

***Trichilia spinosa* Willd., Sp. Pl. 2: 554. 1799.**

Myanmar name : Shauk yaing, Taw shauk
English name : Juncle lemon, Wild lime
Flowering period : September to January

Outstanding Characters

Perennial armed shrub to small tree. Leaves simple, alternate. Inflorescences axillary fasciculate raceme, many-flowered. Flowers bisexual, actinomorphic, pentamerous, hypogynous, white, fragrant. Calyx campanulate, 2-lobed or irregularly 3-lobed, pale green, pubescent. Petals 5, free, obovate-oblong. Fruits hesperidium, globoid, yellowish-green, densely glandular dotted. Seeds oblongoid, endospermic.

Specimens examined: Mandalay Region, Patheingyi Township, Yae Tagon Taung; 21° 57' 53" N and 96° 12' 53" E; 27 October, 2018; Hnin Yu Maw, collection no. 16.

Pollen morphology (Figure 3 B, C)

Tetracolporate, oblate spheroidal, small, 16.8 – 20.4 × 19.2 – 21.6 µm in length and breadth; amb quadrangular; colpi ¾ way up to the pole, 14.4 – 18.0 × 3.6 – 6.0 µm in length and breadth; pori lalongate, 2.4 – 6.0 × 4.8 – 7.2 µm in length and breadth; exine 1.2 – 2.4 µm thick, sexine thicker than nexine; sculpturing reticulate, the lumina heterobrochate, 2.4 – 3.6 µm width; the muri simplibaculate, about 2.4 µm wide.

6. *Clausena excavata* Burm.f., Fl. Ind. 89,t. 29. 1768. (Figure 3D)

Myanmar name : Pyin daw thein

English name : Curry leaf tree

Flowering period : August to February

Outstanding characters

Perennial, small tree; stems and branches terete, glabrous. Leaves unipinnately compound, imparipinnate, alternate; leaflets 7-13, blades oblong or lanceolate. Inflorescences terminal or axillary, paniculate cymes. Flowers bisexual, actinomorphic, pentamerous, hypogynous, white. Calyx cup-shaped, 5-lobed. Petals 5, oblong, pubescent. Fruits baccate, globoid, whitish-green. Seeds oblongoid.

Specimens examined: Mandalay Hill, 22º 00' 50" N and 96º 06' 38" E; 27 Aug, 2017; Hnin Yu Maw, collection no 5.

Pollen morphology (Figure 3 E, F)

Tricolporate, subprolate, medium, 32.4 – 39.6 × 28.8 – 33.6 µm in length and breadth; amb rounded triangular; colpi longicolpate, 31.2 – 36.0 × 6.0 – 10.8 µm in length and breadth; pori lalongate, 3.6 – 7.2 × 7.2 – 9.6 µm in length and breadth; exine about 1.2 µm thick, sexine thicker than nexine; sculpturing reticulate, the lumina 0.6 – 1.2 µm width; the muri simplibaculate, about 0.6 µm wide.

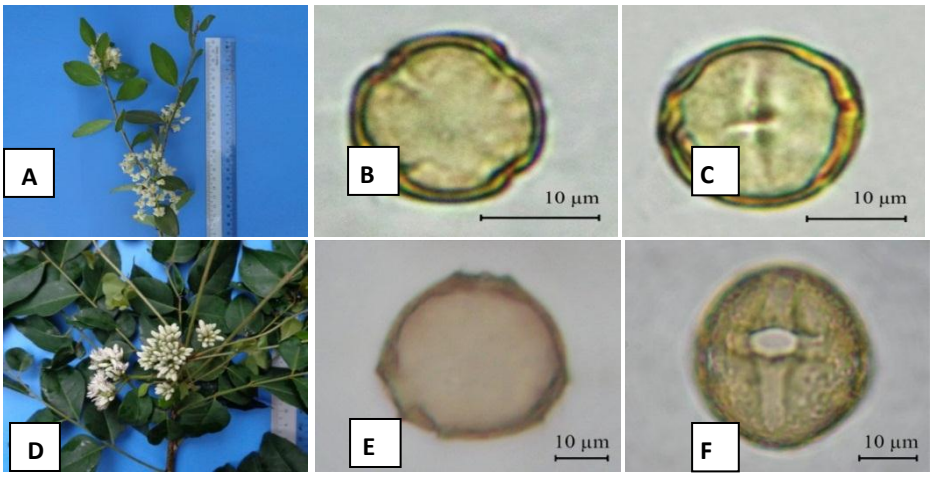


Figure 3 A. Inflorescences of *Atalantia monophylla* (Roxb.) DC.
B. Polar view of *A. monophylla* (Roxb.) DC.
C. Equatorial view of *A. monophylla* (Roxb.) DC.
D. Inflorescences of *Clausena excavata* Burm.f.
E. Polar view pollen of *C. excavata* Burm.f.
F. Equatorial view pollen of *C. excavata* Burm.f.

7. *Glycosmis pentaphylla* (Retz.) DC. Prodr. 1:538.1824. (Figure 4A)

***Glycosmis arborea* (Roxb.) DC. Prodr. 1:538.1824.**

Myanmar name : Taw Shauk, Obok

English name : Unknown

Flowering period : September to November

Outstanding characters

Perennial, unarmed shrubs. Leaves unipinnately compound, imparipinnate, alternate; leaflets 3 – 5, oblong. Inflorescences terminal or axillary, fascicled paniculate cymes, many-flowered. Flowers bisexual, actinomorphic, pentamerous, hypogynous, white. Calyx campanulate, green, glabrous. Petals 5, obovate-elliptic. Fruits baccate, globoid, white to pink, with fleshy juice. Seeds round, suboblong, green.

Specimens examined: Mandalay Region, Patheingyi Township, Ye Ta Khun Taung, 21º 57' 59"N and 96º 12' 51" E; 24 Sep, 2017; Hnin Yu Maw,collection no 8.

Pollen morphology (Figure 4 B, C)

Tricolporate, prolate spheroidal, small grains,19.2 – 24.0 × 16.8 – 21.6 µm in length and breadth; amb rounded triangular; colpi ½ way up to the pole, 8.4 – 15.0 × 4.8 – 10.8 µm in length and breadth; pori circular, 2.4 – 3.6 µm in diameter; exine 1.2 – 2.4 µm thick, sexine thicker than nexine; sculpturing obscurely reticulate.

8. *Micromelum pubescens* Blume, Bijdr. 138. 1825. (Figure 4D)

Myanmar name : Tanyin bo,Taw pyindaw thein

English name : Unknown

Flowering period : August to December

Outstanding characters

Perennial, unarmed shrubs. Leaves unipinnate compound, imparipinnate, alternate; leaflets 10-15, ovate. Inflorescences terminal paniculate corymbs, many-flowered. Flowers bisexual, actinomorphic, pentamerous, hypogynous, yellowish-green. Calyx copular, 5-lobed; tube. Petals 5, ovate-oblong. Fruit baccate, ovoid, glabrous. Seeds ellipsoid.

Specimens examined: Southern Shan State, Kalaw Township, 20º 37' 19" N and 96º 33' 23" E; 7 Aug, 2017; Hnin Yu Maw, collection no 3.

Pollen morphology (Figure 4 E, F)

Tricolporate, subprolate, small, 22.5 – 25.0 × 17.5 – 20.0 µm in length and breadth; amb rounded triangular; colpi ¾ way up to the pole, 15.0 – 19.0 × 4.0 – 7.5µm in length and breadth; pori lalongate, 2.5 – 5.0 × 3.5 – 7.5 µm in length and breadth; exine 2.0 – 2.5 µm thick, sexine thicker than nexine; sculpturing reticulate, the lumina heterobrochate, 0.25 – 0.5 µm width; the muri simplibaculate, about 0.5 µm wide.

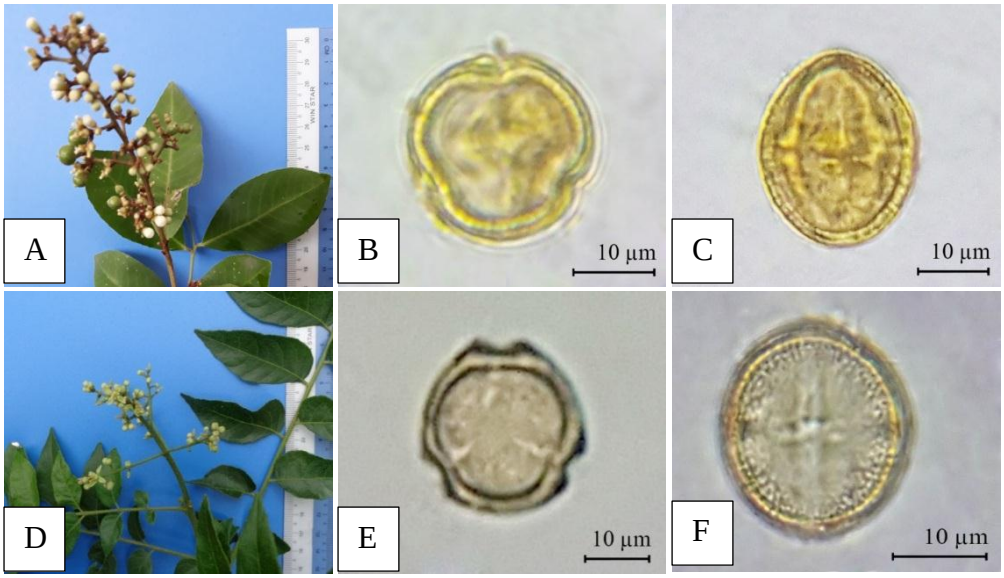


Figure 4 A. Inflorescences of *Glycosmis pentaphylla* (Retz.) DC.
B. Polar view pollen of *G. pentaphylla* (Retz.) DC.
C. Equatorial view pollen of *G. pentaphylla* (Retz.) DC.
D. Inflorescences of *Micromelum pubescens* Blume.
E. Polar view pollen of *M. pubescens* Blume.
F. Equatorial view pollen of *M. pubescens* Blume.

9. Family – Meliaceae

***Azadirachta indica* A. Juss., Mem.Mus.Hist.Nat. 19:221.1830. (Figure 5 A)**

Myanmar name : Tama, Tama Kha

English name : Neem tree, Margosa tree

Flowering period : April to September

Outstanding characters

Perennial, trees; stems and branches terete, glabrous.. Leaves unipinnately compound, imparipinnate, alternate; leaflets 10-15, alternate or opposite. Inflorescences axillary, paniculate cymes, many-flowered. Flowers bisexual, actinomorphic, pentamerous, hypogynous, white. Calyx campanulate, 5-lobed, pubescent. Petals 5, free, spathulate. Fruit drupaceous, oblongoid, 1-2 cm long. Seeds oblongoid, acute.

Specimens examined: Mandalay Region, Patheingyi Township, Yan King Taung, 21º 59' 06" N and 96º 10' 08" E; 2 Sep, 2017, Hnin Yu Maw, collection no 9.

Pollen morphology (Figure 5 B, C)

Tetracolporate, prolate spheroidal, medium, 33.6 – 39.6 × 30.0– 38.4 µm in length and breadth; amb quadrangular; colpi ¾ way up to the pole, 24.0 – 33.6 × 4.8 – 6.0 µm in length and breadth; pori circular, 6.0 – 9.6 µm in diameter; exine 2.4 µm thick, sexine thicker than nexine; sculpturing psilate.

10. *Melia azedarach* L., Sp. Pl. 1:384.1753. (Figure 5 D)

Myanmar name : Thinbaw pan tama

English name : Bead tree

Flowering period : April to July

Outstanding characters

Perennial, trees; stems and branches terete. Leaves bipinnately compound, imparipinnate; leaflets 7-12, opposite, elliptic or ovate. Inflorescences axillary, many-flowered. Flowers bisexual, actinomorphic, pentamerous hypogynous, purplish blue. Calyx tubular, 5-lobed, pubescent. Fruits drupe, 1.5-4.0 cm long, glabrous. Seeds oblong, brown.

Specimens examined: Mandalay Region, Meiktila Township, Kandawlay Village, 20º 52' 22" N and 95º 51' 47" E; 16 July, 2017, Hnin Yu Maw, collection no 2.

Pollen morphology (Figure 5 E, F)

Tetracolporate, prolate spheroidal, medium, 30.0 – 33.6 × 28.8 – 21.66 µm in length and breadth; amb quadrangular; colpi ½ way up to the pole, 16.3– 18.0 × 4.8 – 7.2 µm in length and breadth; pori circular, 6.0 – 9.6 µm in diameter; exine 0.6 – 2.4 µm thick, sexine thicker than nexine; sculpturing psilate.

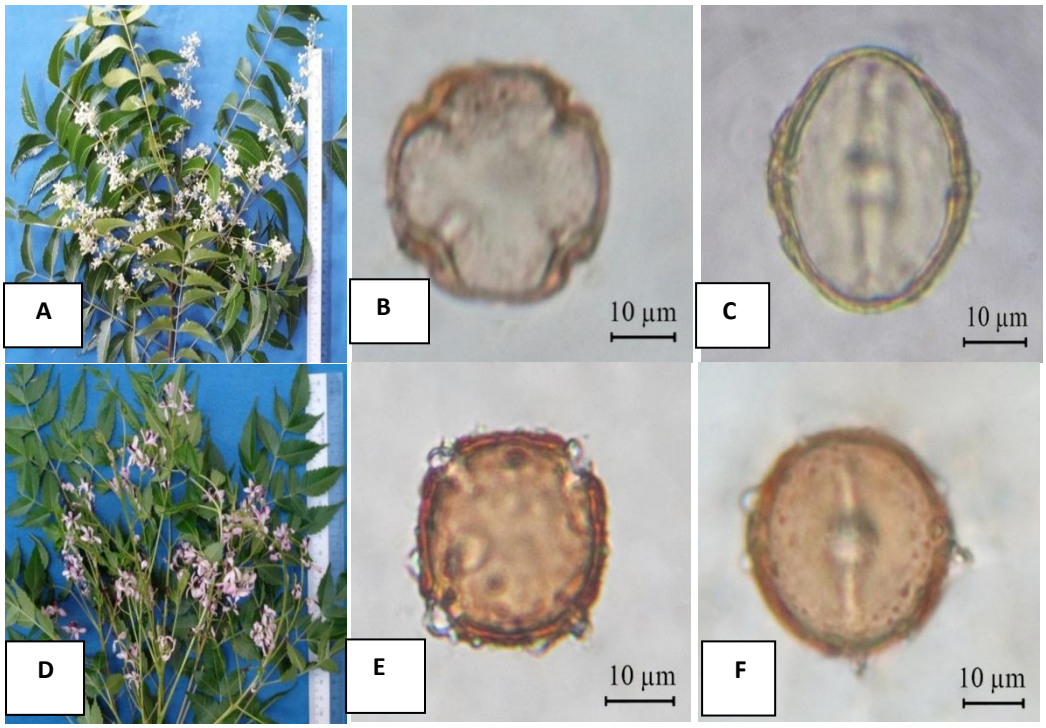


Figure 5 A. Inflorescences of *Azadirachta indica* A. Juss.
B. Polar view pollen of *A. indica* A. Juss.
C. Equatorial view pollen of *A. indica* A. Juss.
D. Inflorescences of *Melia azedarach* L.
E. Polar view pollen of *M. azedarach* L.
F. Equatorial view pollen of *M. azedarach* L.

Table 2 Pollen Morphology of 10 Species of Sapindales

No.	Scientific Name	Types of Aperture	Shape of E.V	Size of E.V(µm)	Size of pollen grains	Colpi length	Pori shape	Exine		Sculptures	Amb	Lumina (µm)	Muri (µm)
								Thickness (µm)	S>N				
1	*Buchanania latifolia* Roxb.	CP	PS	19.2-22.8 × 18.0-20.4	Small	Lon	Lalo	0.6-1.2	S>N	Or	Rounded triangular	-	-
2	*Melanorrhoea usitata* Wall.	CP	SP	16.0-25.2 × 12.0-21.6	Small	¾	Lalo	0.6-2.4	S>N	Or	Triangular	-	-
3	*Cardiospermum canescens* Wall.	P	Pro	37.2 – 42.0 × 24.0 – 28.8	Medium	-	Lalo	2.4	S>N	Re	Triangular	1.2-2.4	0.6
4	*Aegle marmelos* (L.) Correa, Trans, L.	CP	PS	20.4-24.0 × 19.2-21.6	Small	¾	Lalo	2.4	S>N	Re	Quadrangular	1.2-2.4	0.6
5	*Atalantia monophylla* (Roxb.) DC.	CP	OS	16.8-20.4 × 19.2-21.6	Small	¾	Lalo	1.2-2.4	S>N	Re	Quadrangular	2.4-3.6	2.4
6	*Clausena excavata* Burm. f.	CP	SP	32.4 – 39.6 × 28.8 – 33.6	Medium	Lon	Lalo	1.2	S>N	Re	Rounded triangular	0.6-1.2	0.6
7	*Glycosmis pentaphylla* (Retz.) DC.	CP	PS	19.2 – 24.0 × 16.8 – 21.6	Small	½	Cir	1.2-2.4	S>N	Or	Rounded triangular	-	-
8	*Micromelum pubescens* Blume	CP	SP	22.5 – 25.0 × 17.5 – 20.0	Small	¾	Lalo	2.0-2.5	S>N	Re	Rounded triangular	0.25-0.5	0.5
9	*Azadirachta indica* A. Juss.	CP	PS	33.6 – 39.6 × 30.0 – 38.4	Medium	¾	Cir	2.4	S>N	Psi	Quadrangular	-	-
10	*Melia azedarach* L.	CP	PS	30.0 – 33.6 × 28.8 – 21.66	Medium	½	Cir	0.6-2.4	S>N	Psi	Quadrangular	-	-

CP = Colporate Lon = Longicolpate P = Porate Lolo= Lolongate Psi= Psilate
Lalo= Lalongate PS = Prolate spheroidal Cir = Circular Or = Obscurely reticulate
SP = Subprolate S>N = Sexine>Nexine Pro = Prolate Re = Reticulate

Discussion and Conclusion

The pollen morphology of 10 species and 10 genera belonging to the order Sapindales found in Mandalay Region and Southern Shan State was examined. The order Sapindales consists of nine families, namely Anacardiaceae, Biebersteiniaceae, Burseraceae, Kirkiaceae, Meliaceae, Nitrariaceae, Rutaceae, Sapindaceae and Simaroubaceae. Among them, six families such as Anacardiaceae, Sapindaceae, Rutaceae, Simaroubaceae, Burseraceae and Meliaceae are found in Myanmar. In this paper, four families, Anacardiaceae, Sapindaceae, Rutaceae and Meliaceae were presented.

In the present study, 5 species are trees, 2 species are small tree, 2 species are unarmed shrubs and 1 species is armed tendrillar climber. The family Anacardiaceae including 2 genera and 2 species *Buchanania latifolia* Roxb. and *Melanorrhoea usitata* Wall. were recorded. One species of Sapindaceae was found in *Cardiospermum canescens* Wall.; 5 genera and 5 species of *Aegle marmelos* (L.) Correa, Trans, L. *Atalantia monophylla* (Roxb.) DC. *Glycosmis pentaphylla* (Retz.) DC. *Clausena excavata* Burm. f. and *Micromelum pubescens* Blume belong to family Rutaceae. In the study area, 2 genera and 2 species of family Meliaceae in *Azadirachta indica* A. Juss. and *Melia azedarach* L. were found.

According to the aperture types, three types were found in all studied species. These types of pollen aperture are tricolporate, tetracolporate and triporate. Tricolporate pollen grains present in *Buchanania latifolia* Roxb., *Melanorrhoea usitata* Wall., *Clausena excavata* Burm. f. *Glycosmis pentaphylla* (Retz.) DC and *Micromelum pubescens* Blume; tetracolporate pollen grains were found in 4 species *Aegle marmelos* (L.) Correa, Trans, L., *Atalantia monophylla* (Roxb.) DC., *Azadirachta indica* A. Juss and *Melia azedarach* L.; triporate pollen grains were found in *Cardiospermum canescens* Wall.

The shape of pollen grains are prolate spheroidal, oblate spheroidal, subprolate, prolate, suboblate and oblate. Prolate spheroidal were found in *Buchanania latifolia* Roxb., *Aegle marmelos* (L.) Correa, Trans, L., *Glycosmis pentaphylla* (Retz.) DC., *Azadirachta indica* A. Juss. and *Melia azedarach* L. Oblate spheroidal were found in *Atalantia monophylla* (Roxb.) DC., prolate pollen grain were occurred in *Cardiospermum canescens* Wall. and remaining three species are subprolate were described.

In the study area, 6 species of pollen grains are small and 4 species are medium grains. The size of small pollen grains was found in *Buchanania latifolia* Roxb., *Melanorrhoea usitata* Wall., *Aegle marmelos* (L.) Correa, Trans, L., *Atalantia monophylla* (Roxb.) DC., *Glycosmis pentaphylla* (Retz.) DC. and *Micromelum pubescens* Blume. The smallest pollen is *Melanorrhoea usitata* Wall. 16.0-25.2 × 12.0-21.6 µm in length and breadth and the largest pollen is *Cardiospermum canescens* Wall. 37.2 – 42.0 × 24.0 – 28.8 µm in length and breadth. The size of pollen grains are varied from small, medium to large or very large (Erdtman 1952). These pollen characters are agreed with the present finding.

The sculptures of pollen grains are psilate, reticulate and obscurely reticulate. species are obscurely reticlate, 5 species are reticulate and remaining 2 species are psilate.

According to the results, different types of pollen characters were investigated and recorded. This investigation will contribute not onlyto the pollen features but also varieties of the pollen morphological data in the study of the order Sapindales. The important pollen

morphological characteristics that are useful for classification and identification of flowering plants are pollen shape and type of exine sculpturing. These morphological features of pollens will support the identification and classificatioin of order Sapindales. Therefore, these pollen characters are very important and beneficial for the future researchers.

Acknowledgements

I would like to thank Dr Nu Nu Yee, Professor and Head, Department of Botany, University of Mandalay, for her permission and encouragement in doing paper. I am greatly indebted to my supervisor, Dr Nwè Nwè Yi, Professor and Head, Department of Biology, Sagaing University of Education, for her support, encouragement and precious supervision in the taxonomical point of view. I am very grateful to Dr Khin Thet Kyaw, Professor and Head, Department of Botany, Yadanabon University, for her patient directions and suggestion for this research paper.

References

Anbari, A. K. A., S. Barusrux, P. Pornpongrungrueng & P. Theerakulpisut. (2015). Pollen grain morphology of *Citrus* (Rutaceae) in Ira. International Conference on Plant, Marine and Environmental Sciences, Malaysia.

Backer, C. A. (1965). Flora of Java. Vol.2 Nordhoff. Groningen. Netherland.

Byng, J. W., M. J. M. Chase, M. J. M. Christenhusz, M. F. Fay, W. S. Judd, D. J. Mabberley, A. N. Sennikov, D. E. Soltis, P. S. Soltis & P. F. Stevens. (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants, APG IV. Botanical Journal of the Linnean Society, London. 181, 1-20.

Dassanayake, M. D. (1980-1991). A revised handbook of Flora of Ceylon Vol 5 to 13. Department of Agriculture, University of Peeradeniya. Sri Lanka.

Erdtman, G. (1952). Pollen morphology and plant taxonomy. Angiosperms. An introduction to palynology I. Almquist & Wiksell, Stocknolm. The Chronica Botanica Co. Waltham. Mass. U.S.A.

Erdtman, G. (1960). The acetolysis method. A revised description. Seven. Bot.Tidskr. 54:561-564.

Hesse, M., H. Halbritte., R. Zetter., M. Weber., R. Buchner, A. Frosh- Radiva & S. Ulrich. (2009). Pollen terminology. University of Vienna Austria.

Hoen, P. (1999). Glossary of pollen and spore terminology. Laboratory of palaeobotany and palynology. Second and revised edition.

Hooker, J. D. (1881). Flora of British India. Vol. 1-3 L. Reeve & Co. Henrietta Street, London.

Hundley, H. G. & Chit Ko Ko. (1961). List of trees, shrubs, herbs and principle climbers of Myanmar.Third revised and enlarged edition. Supdt., Govt. Printing and Stady.

Kesseler, R. & M. Harley. (2009). Pollen, The hidden sexuality of flowers, 3rd Edition.

Kress, J., A. R. Defilipps, E. Farr & Yin Yin Kyi. (2003). A checklist of the trees shrubs, herbs and climbers of Myanmar. Department of Systematic Biology, Botany National Museum of Natural History Washington, DC. U.S.A.

Moore, P. D. & J. A. Webb. (1978). An illustrated guide to pollen analysis. Great Britain. Richard Clay (The Chaueer Press) Ltd, Bungay, Suffolk.

Perveen, A. & M. Qaiser. (2010). Pollen flora of pakistan-lxvi: Anacardiaceae. Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Rodriguez, P.A, P. V. Welzen, F. Adema & R. W. J. M. Van Der Ham. (2010). Sapindaceae. Naturalis Biodiversity Center.

Walker, J. W. & J. A. Doyle. (1975). The bases of angiosperm phylogeny: palynology. Published by Missouri Botanical Garden Press. 62: 684-723.

Zidko, A., L. Antonio, Rodrigues, C. B. F. Mendonca, M. L. Absy, M. G. Ferreira, P. D. T. B. Sampaio & V. Esteves. (2016). Pollen morphology of *Carapa* species (Meliaceae) from the Brazilian Amazon. Universidade Federal do Rio de Janeiro, Museu Nacional, Botânica, Vol- 46(3) 2016: 333 – 336.

ISOLATION AND OPTIMIZATION PARAMETERS OF FERMENTATION CONDITIONS OF SELECTED SOIL BACTERIUM (SY-17) FROM MYINT THA TOWNSHIP, MANDALAY REGION

Shwe Yee Win[1], Zar Zar Yin[2]

Abstract

Soil samples were collected from six different areas of Yoar Thit village, Kin Sein Zay and Pauk Myang village of Myint Tha Township, Mandalay Region. These sample were cultured on Flo Medium, Nutrient. Agar medium, CAS medium, Glucose Peptone Agar medium and Centenum medium. The experiments were carried out at the Microbiology Laboratory of Botany Department in Mandalar Degree College. A total of 36 bacterial colonies were obtained from these soil samples. These isolated bacteria were designated as SY-1 to 36. Antimicrobial activity of all strains were carried out by agar well diffusion assay with five test organisms. Among them, SY -12, 13, 17, 22, 27, 29 and 32 strains showed different levels of antimicrobial activities. Especially, SY-17 showed the highest antifungal activity (39.06 mm) on *Candida albicans.* Therefore, SY-17 was selected and the fermentation condition of this bacterium was carried out by the study of fermentation period 3 days (27.77 mm), proper age 72 hrs (26.02 mm), size 20 % (16.86 mm), different carbon sources maltose (38.05 mm) and nitrogen sources sodium nitrate (48.19 mm), effect of fermentation medium FM 5 (29.98 mm), pH 7 (21.95 mm), temperature 25°C (39.65 mm), and shaking (34.01 mm) and static culture (26.02 mm) on antimicrobial activity against *Candida albicans*. It result of this work can provide the knowledge of nature of soil bacteria and how to select active strains exhibited against some test organisms.

Keywords: Soil bacteria, antimicrobial activity, fermentation

Introduction

Microorganisms are frequency present in soil, manure and decaying plant tissues which are able to degrade wastes that are correlated with the substrate organic matter (Alexander, 1977).

In general the majority of microbial population if found in the upper six to twelve inches of soil and the number decreases with depth.

Then number and kinds of organisms found in soil depend upon the nature of soil, depth, season of the year, state of the cultivation, reaction, organic matter, temperature moisture, aeration, etc. Soil is a primary source of microorganisms (Omalu *et al.,* 2011).

Microbes in the soil are the key to carbon and nitrogen recycling. A teaspoon of production soil generally contains between 100 million and 1 billion individual bacteria. That is as much as two cows per-acre. A ton of micrscopic bacteria may be action per core and there may be over one million species of bacteria present.

Natural products from microorganisms have been the most successful source that has found many application in the fields of medicines, pharmacy and Agriculture. Microorganisms were found to produces secondary metabolites with a diverse chemical structure and antimicrobial activities (Stachelhaus *et al.,* 1995).

Microorganisms play important roles on nutritional chains that are important for biological balance in the life on our planet being essential for the closing of nutrient and geochemical cycles such as the carbon, nitrogen, sulfer and phosphorus cycle. (Madsen, 2008).

[1] Assistant Lecturer, Department of Botany, Kyaukse University
[2] Dr, Associate Professor, Department of Botany, Pathein University

Materials and Methods

Collection of Soil Samples

Six different soil samples were collected from three different places of Yoar Thit village, Kin Sein Zay village and Pauk Myaung village in Myint Tha Township, Mandalay Region in the month of August 2017.

Isolation of Bacteria from the Soil Samples (Altas, 1993)

The soil Bacteria were enumerated by physical and chemical treatment dilution method or media such as FLO Medium, Nutrient Agar Medium, Chrome azural S **(**CAS) Medium, Glucose Peptone Agar Medium (GPA medium), Centenum Medium.

Media used for isolation of Bacteria

FLO Medium (Altas, 1993)

Casein	10.0 g
Peptone	10.0 g
K_2HPO_4	1.5 g
$MgSO_4.\ 7H_2O$	1.5 g
Agar	15.0 g
Distilled Water	1000 mL
pH	5.0

Nutrient Agar Medium (Altas, 1993)

Peptone	5.0 g
NaCl	5.0 g
Yeast extract	2.0 g
Agar	15.0 g
Beef extract	1.0 g
Distilled Water	1000 mL
pH	5.0

CAS Medium (Chrome azural S) (Altas, 1993)

Casein	10.0 g
$MgSO_4.\ 7H_2O$	1.0 g
K_2HPO_4	0.25 g
Agar	18.0 g
Distilled Water	1000 mL
pH	6.8

Glucose Peptone Agar Medium (GPA medium) (Altas, 1993)

Peptone	20.0 g
Glucose	10.0 g
NaCl	5.0 g
Agar	18.0 g
Distilled Water	1000 mL
pH	7.2

Centenum Medium (Altas, 1993)

Yeast extract	10.0 g
Sodium pyruvate	2.2 g
K_2HPO_4	1.0 g
$MgSO_4.\ 7H_2O$	0.5 g
Vitamin B12	0.02 g
Agar	20 g
Distilled Water	1000 mL
pH	7.0-7.2

Nutrient Broth Medium

(Dubey and Mahesh Wari 2007)

Peptone	5.0 g
Beef extract	3.0 g
Sodium chloride	5.0 g
Yeast extract	10.0 g
Distilled Water	1000 mL
pH	6.8-7.02

Medium used for Antimicrobial Activity

Seed and Fermentation Medium Glucose Yeast Peptone (GYP) Medium (Atlas, 1993)	
Glucose	10 g
Yeast extract	3 g
Peptone	2 g
Distilled water	1000 mL
pH	6.5

Assay Medium Glucose Yeast Peptone (GYP) Medium (Atlas, 1993)	
Glucose	10 g
Yeast extract	3 g
Peptone	2 g
Agar	16 g
Distilled water	1000 mL
pH	6.5

Serial Dilution Method of Soil Samples (Collins, 1964)

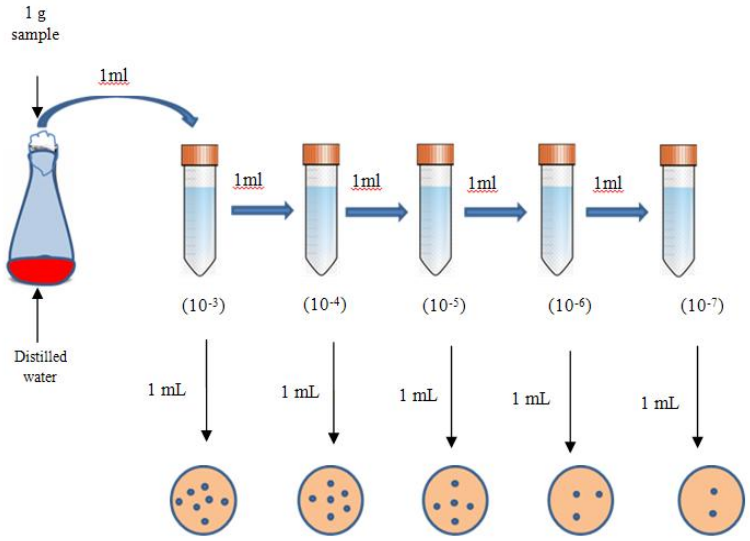


Figure 1 Serial dilution method for soil samples (Collin, 1964)

Scanning for Antimicrobial Activity of Isolated Bacteria

The isolated soil bacteria were inoculated into seed medium and incubated for 1 day at 27 ºC. Seed culture were transferred to the fermentation medium. After one day, the seed culture (1%) was transferred into the fermentation medium and carried out by static culture. Then, the fermented broth was used to check the antimicrobial activity by agar well method (Collins, 1964). Agar well having (8 mm in diameter) were utilized for antimicrobial activity.

Scanning of Antimicrobial by Agar well method (Collins, 1965)

This method was used for the antimicrobial activity by five test organisms. The assay medium (glucose – 1.0 g, yeast extract – 0.3 g, peptone – 0.2 g, agar – 1.6 g) was utilized for these bacteria. Isolated strains were subjected with antimicrobial activity by agar well method. Cork borer was used to make the wells (8 mm in diameter) in the autoclave basal antimicrobial test medium.

Well impregnated with 1-5 days old culture fermented broth (0.1 mL) were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were

measured. Clear zone surrounding the test wells indicated the presence of antimicrobial activities which inhibit the growth of the test organisms selectively.

Fermentation medium with various carbon sources (NITE, 2005)

The initial basal medium contained yeast extract (0.5 g), K_2HPO_4 (0.001 g), $MgSO_4$ (0.001 g), $CaCO_3$ (0.1 g), DW (100 mL), pH 7. After that glucose (1 g, 1.5, 2, 2.5 and 3g) was added to the initial basal medium. The medium were sterilized by autoclaving at 121ºC for 30 min, and were cooled before inoculation. And then, SY-17 was inoculated. Fermentation was carried out for 5 days and antimicrobial activity was tested by agar well diffusion method.

Fermentation medium with various nitrogen sources (NITE, 2005)

The initial basal medium used contained glucose (1g), soluble starch (0.5g), K_2HPO_4 (0.001g), $MgSO_4$ (0.001g), $CaCO_3$ (0.1g), DW (100 mL), pH 7. Peptone (1, 1.5, 2, 2.5 and 3g) was added to the initial basal medium. The medium were sterilized by autoclaving at 121°C for 30 min, and were cooled before inoculation. And then, SY-17 was inoculated. Fermentation was carried out for 5 days and antimicrobial activity was tested by using agar well diffusion method.

Fermentation Medium (FM Medium)

Fermentation was undertaken with suitable conditions of 20 % size and 72 hrs ages of inoculum with different media. Fermentation was carried out for 7 days and antifungal activity test was carried out every 24 hrs. Composition of fermentation media use on the present study. All the ingredient well dissolved in 1 liter distilled water and adjusted to pH 7.0.

Fermentation Medium -1			**Fermentation Medium-2**	
Maltose	10 g		Maltose	10 g
Sodium nitrate	3 g		Ammonium phosphate	3 g
Agar	16 g		Agar	16 g
Distileld water	1000 mL		Distilled water	1000 mL
pH	7		pH	7

Fermentation Medium -3			**Fermentation Medium-4**	
Maltose	10 g		Maltose	10 g
Potassium nitrate	3 g		Urea	3 g
Agar	16 g		Agar	16 g
Distileld water	1000 mL		Distilled water	1000 mL
pH	7		pH	7

Fermentation Medium -5			**Fermentation Medium-6**	
Sucrose	10 g		Sucrose	10 g
Sodium nitrate	3 g		Ammonium phosphate	3 g
Agar	16 g		Agar	16 g
Distileld water	1000 mL		Distilled water	1000 mL
pH	7		pH	7

Fermentation Medium -7			**Fermentation Medium-8**	
Sucrose	10 g		Sucorse	10 g
Potassium nitrate	3 g		Urea	3 g
Agar	16 g		Agar	16 g
Distileld water	1000 mL		Distilled water	1000 mL
pH	7		pH	7

Fermentation Medium -9			**Fermentation Medium-10**	
Soluble starch	10 g		Soluble starch	10 g
Sodium nitrate	3 g		Ammonium phosphate	3 g
Agar	16 g		Agar	16 g
Distileld water	1000 mL		Distilled water	1000 mL
pH	7		pH	7

The effect of pH on fermentation conditions (Hernadez*, et al*., 2005)

The pH sensitivity of the culture supernatant recovered during stationary growth phase of the isolates, pH values were adjusted ranging from 4-10 by using 0.1 M NaOH or 0.1 M HCl. The medium constituents were sterilized by autoclaving at 121°C for 30 min, and were cooled before inoculation. And then, the strain was cultured. Fermentation was carried out for 5 days and antimicrobial activity was tested by agar well diffusion method.

The effect of temperature on fermentation (Hernadez*, et al*., 2005)

The medium constituents were sterilized by autoclaving at 121°C for 30 min, and were cooled before inoculation. And then, the strain was cultured. The selected bacteria were incubated at 20°C, 25°C, 30°C, 35°C , 40°C and respectively. Fermentation was carried out for 5 days and antimicrobial activity was tested by using agar well diffusion method.

The static and shaker of fermentation conditions

Using all optimized medium components, the shake-flasks was done using (1.5g) of sucrose as a carbon sources and (1 g) of sodium nitrate as nitrogen source. The flasks were

placed in (TS-2000 A VDRL) shaker. After 5 days, the fermented broth was tested on agar well diffusion method. Precisely, the static fermented broth was also tested on the above method.

Results

Isolation of bacteria from soil samples

In the investigation, 36 bacterial strains were isolated from the six different soil sample of Myint Tha Township. Isolated bacteria SY-1-27 were obtained from Yoar Thit village, SY 28-31 from Kin Sein Zay village and SY-32-36 from Pauk Myang village. The results were shown in Table 1. The isolated bacteria were designated as SY 1-36. (The colony morphology of soil bacteria were small, medium and large in size and color were white yellow and cream. The margin of colonies were entire, undulate, lobate, rhizoid, curled and the elevation were raised and flat.) The colony morphology of soil bacteria SY-17 was circular, large, entire, cream flat, cream colour. The strain were rod shape and gram positive. The results were show in Table (2).

Table 1 Chemical Analysis of Six Different Soil Samples Collected from Myint Tha Township

Soil sample	Collected place	location	Soil Type	pH	Moisture	Total N %	Available p (ppm)	Exchangeable k (me/100g)	Available K_2OMg/ 100
S I	Yoar Thit Village	N22º25'54" E 96º 19'54"	Silty loans	8.83	3.95	0.28	3.52	0.29	13.61
S II	Yoar Thit village	N22º25'54" E 96º 99'56"	Loans	8.62	4.4	0.22	3.41	0.15	7.04
S III	Kin Sein Zay Village	N22º25' 36" E 96º 07'03"	Silty clay	8.78	2.95	0.18	2.22	0.14	6.57
S IV	Kin Sein Zay Village	N22º25' 48" E 96º 06'48"	Silty clay loans	8.20	2.99	0.32	14.23	1.16	54.43
S V	Pauk Myang village	N22º26' 02" E 96º 06'00"	Silty clay	8.49	4.23	0.22	12.08	0.37	17.36
S VI	PaukMyaung village	N22º26'12" E96º05'40"	Silty clay	9.81	4.83	0.18	13.16	1.29	60.53

Table 2 Isolation of Soil Bacterium on five different media

Soil Sample	FLO Medium	Nutrient Agar medium	CAS medium	Glucose peptone Agar medium	Centenum medium
S -1	SY-1-9 = 9	SY-16-21 = 6			
S-2	SY-10-15= 6	SY-22-27 = 6			
S-3			SY -28 = 1		
S-4				29-31 = 3	
S-5				32-33 = 2	
S-6					34-36 = 3
Total	15	12	1	5	3

Figure. Chracteristic of Isolated Bacteria SY-17

Isolated bacteria and their Antimicrobial Activity

In this study, seven selected bacterial strains were tested with five test organisms by agar well diffusion method. There are *Agrobacterium tumefaciens, Bacillas Pumilus, Bacillus subtilis, Candida albicans, Staphylococcus aureus.* Among them, SY-17 showed the best activities on *Candida albicans* (Table 3)

Table 3 Antimicrobial activity of isolated bacteria SY-17 against five test organism

No.	Isolated bacteria	Test organism and Antimicrobial activity (mm)				
		Agrobaterium tumefaciens	***Bacillus pumilus***	***Bacillus subtilis***	***Candida albicans***	***Stuphylococcus aureus***
1	SY-12	28.24	22.24	26.01	+	23.09
2	SY-13	29.97	+	+	18.54	27.55
3	SY-17	+	30.90	31.51	**39.06**	25.81
4	SY-22	+	+	16.01	22.70	20.78
5	SY-27	+	+	25.45	+	+
6	SY-29	21.28	+	23.90	+	25.45
7	SY-32	+	17.48	+	-	23.37

Effect of fermentation period on the antifungal activity of SY-17 on *Candida albicans*

SY-17 reached the highest activities 27.77 mm in 3 day period of inoculum on *Candida albicans* (Table 4, Figure 3)

Table 4 Effect of fermentation period on the antifungal activity of SY-17 on *Candida albicans*

No	Fermentation period days	Activity (clear zone , mm)
1	1	14.72 mm
2	2	16.67 mm
3	3	**27.77 mm**
4	4	22.66 mm
5	5	16.71 mm

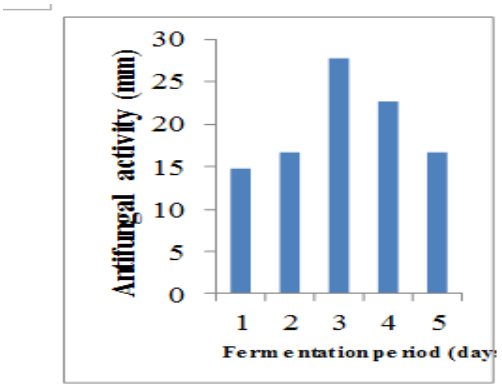


Figure 3 Effect of fermentation period on the antifungal activity of SY-17 on the *Candida albicans*

Effects of ages of inoculum on the antifungal activity of SY-17 on the *Candida albicans*

SY-17 showed the highest activities (26.02 mm) in 72 hrs age of inoculum on *Candida albicans* (Table 5 and Figure 4).

Table 5 Effects of ages of inoculum on the antifungal activity of SY-17 on the *Candida albicans*

No	Age of	Antifungal
1	24 hrs	14.99 mm
2	48 hrs	17.08 mm
3	72 hrs	**26.02 mm**
4	96 hrs	13.62 mm
5	120 hrs	13.23 mm
6	144 hrs	11.61 mm

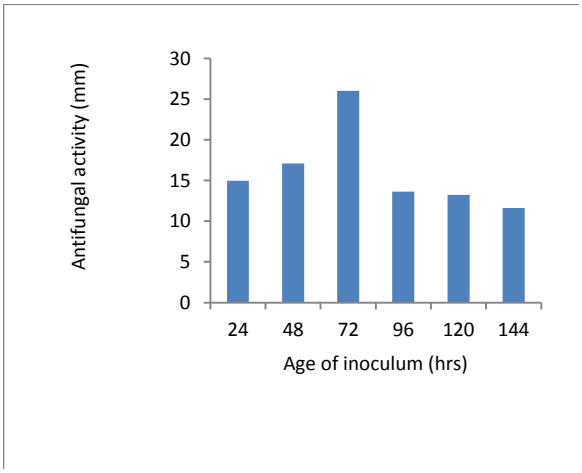


Figure 4 Effects of ages of inoculum on the antifungal activity of SY-17 on the *Candida albicans*

Effects of sizes of inoculums on the antifungal activity of SY-17 on the *Candida albicans*

In the proper size of inoculums, 20% was the most suitable activity 16.86 mm followed by 25% and 30% respectively (Table 6 and Figure 5).

Table 6 Effects of sizes of inoculums on the antifungal activity of SY-17 on the *Candida albicans*

No	Sizes of	Antifungal
1	5%	14.66
2	10%	14.70
3	15%	16.01
4	20%	**16.86**
5	25%	16.50
6	30%	16.31

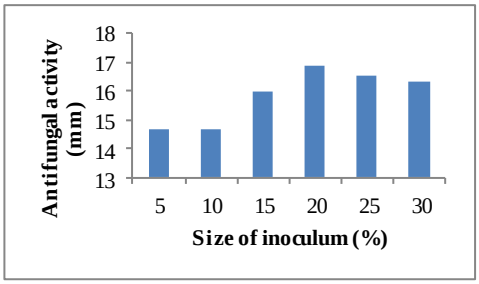


Figure 5 Effects of sizes of inoculums on the antifungal activity of SY-17 on the *Candida albicans*

Effects of carbon and nitrogen sources on the antifungal activity of SY-17 on *Candida albicans*

The maximum antifungal activity of SY-17 was influenced by addition of maltose and sodium nitrate reaching the moderate antifungal activity (38.05 mm and 48.19 mm). These results were shown in Table 7 and 8.

Table 7 Effects of carbon and nitrogen sources on the antifungal activity of SY-17 on *Candida albicans*

Sr. No	Carbon sources	Antifungal Activity(mm)	Sr. No	Carbon sources	Antifungal Activity (mm)
1	Carrot	13.14	8	Mannitol	15.79
2	Lactose	13.62	9	Xylose	15.70
3	Potato	14.77	10	Soluble starch	31.39
4	Oat	16.21	11	Rice	18.76
5	Molassess	15.93	12	Glycerol	16.41
6	Maltose	**38.05**	13	Glucose	17.11
7	Sucrose	35.18	14	Corn	16.35

Table 8 Effects of Nitrogen Sources on the antifungal activity of SY-17 on *Candida albicans*

Sr. No	Nitrogen sources	Antifungal Activity	Sr. No	Nitrogen sources	Antifungal Activity
1	Soybean	11.02	8	Peptone	15.26
2	Meat extract	14.30	9	Fish cake	14.66
3	Asparagine	14.03	10	Yeast	14.72
4	Urea	31.98	11	Ammonium sulphate	19.36
5	Ammonium phosphate	43.32	12	Gelatin	16.52
6	Sodium nitrate	**48.19**	13	Potassium nitrate	40.81
7	Casein	17.47	14	Ammonium chloride	24.16

Effects of various fermentation media on the antifungal activity of SY-17 on *Candida albicans*

In this study, FM 5 showed the maximum antifungal activity (29.98 mm) against *Candida albicans* (Tables 9).

Table 9 Effects of various fermentation media on the antifungal activity of SY-17 on *Candida albicans*

No	Fermentation media	Antifungal activity (mm)
1	FM-1	21.35
2	FM-2	15.80
3	FM-3	16.19
4	FM-4	15.18
5	FM-5	**29.98**
6	FM-6	15.58
7	FM-7	17.22
8	FM-8	15.02
9	FM-9	20.77
10	FM-10	14.82

Effects of different pH on the antifungal activity of SY-17 on *Candida albicans*

In this study, the highest antifungal activity was obtained at pH 7 (21.95 mm) against *Candida albicans* (Table 10 and Figure 6).

Table 10 Effects of different pH on the antifungal activity of SY-17 on *Candida albicans*

No	pH	Antifungal activity (mm)
1	4	-
2	5	14.13
3	6	17.31
4	7	**21.95**
5	8	21.07
6	9	18.21
7	10	14.82

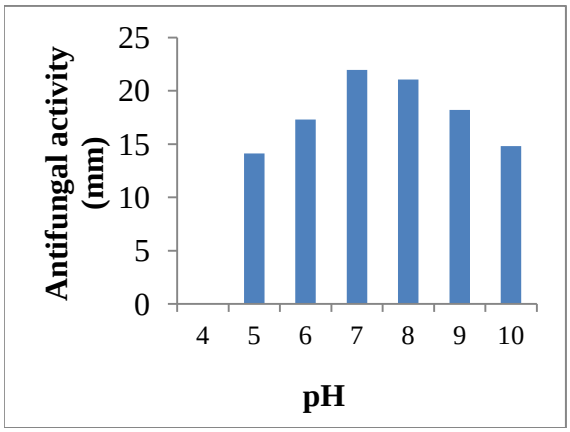


Figure 6 Effects of pH on the antifungal activity SY-17 on *Candida albicans*

Effects of different temperature on the antifungal activity of SY-17 on *Candida albicans*

In this investigation, temperature 25˚C showed the highest antifungal activity (39.69 mm) against *Candida albicans* (Table 11 and Figure 7).

Table 11 Effects of different temperature on the antifungal activity of SY-17 on *Candida albicans*

No	Temperature	Antifungal
1	20ºC	24.07
2	25ºC	**39.69**
3	30ºC	36.65
4	35ºC	35.65
5	40ºC	21.47

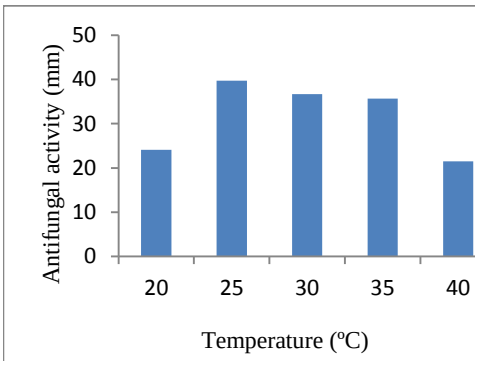


Figure 7 Effects of different temperature on the antifungal activity of SY-17 on *Candida albicans*

Effects of Static and shaking culture on the production of antifungal activity of SY-17 against *Candida albicans*

In this investigation, the antifungal activity between shaking culture and static culture were observed. The shaking culture showed the inhibitory zone 34.01 mm and the static culture showed 26.02 mm for 3 days fermentation.

Table 12 Effects of Static and shaking culture on the production of antifungal activity of SY-17 against *Candida albicans*

No	Fermentation	Antifungal activity
1	Static	**26.02**
2	Shaking	**34.01**

Static Shaking

Figure 8 Effects of Static and shaking culture on the production of antifungal activity of SY-17 against *Candida albicans*

Discussion and Conclusion

Soil contains varieties of microorganism including bacteria that can be established in any natural environment. Bacteria are the most important and abundant microorganism which is present in surrounding environment. These are very small, unicellular, primitive and non chlorophyll containing microorganisms. Dilution is one of the most important method to isolate the soil bacterium (Benson, 2001).

Soil samples were collected from six different places of Myint Tha Township, Mandalay Region. 36 bacteria were isolated from six different soil. The isolated bacteria were designated as SY-1 to SY-36.

In the present study, the antifungal activity of SY-17 was investigated by various fermentation conditions. In the fermentation period, SY-17 was found to be optimum fermented incubation period in 3 days.

Das 2006 stated that Antibacterial metabolites production by the strain was studied 1 to 4 days of fermentation. The highest amount was obtained on 3rd day of fermentation and then production was declined gradually. In the present work the isolated SY-17 was allowed to incubate for 72 hrs for the maximum production of antibacterial metabolites. And then, 20% inoculum size and 72 hrs age were the most suitable condition. The effect of various carbon and nitrogen sources were observed for the growth and maximum metabolite production. The addition of xylose, sucrose, molasses and glucose as carbon sources provided better growth and

the maximum inhibition zone resulted in maltose (38.05 mm) followed by sucrose (35.18 mm) and soluble starch (31.39 mm).

Among the nitrogen sources, the best growth of SY-17 was found on the ammonium sulphate and maximum antifungal activity was obtained in the sodium nitrate (48.19 mm) follow by ammonium phosphate (43.32 mm) and potassium nitrate (40.81 mm).

Yang *et al.,* 2006 reported that the carbon and nitrogen sources were the important constituents to be considered highly influenced on the antibiotic production by bacteria.

Ten kinds of fermentation media (FM) were utilized and the highest activity was found in FM-5 (29.98 mm). Microorganisms growing in the soil are influenced by factors such as moisture, temperature pH, carbon sources and nitrogen sources biotic factor and inhabiting factors (Davidson, *et al.,* 1998).

In the study of different temperature and pH utilization for the fermentation, the optimum temperature was found at 25°C (39.69 mm) and the highest activity was obtained at pH7.(21.95mm).

Lilly *et. al.,* 1951 reported that the maximum temperature of bacteria is 40°C and the optimum pH is 8. In the comparison between shaking culture and static culture, the antifungal activity of shaking culture (34.01 mm) was more than that of the static culture (26.02 mm). It was concluded that the present study was to observe the fermentation period of SY-17 against *Candida albicans* and to optimize the parameters of fermentation conditions of selected bacterium SY-17.

The present study concluded that the optimum conditions required for the production of bioactive metabolites by selected soil bacterium SY-17 were determined and metabolites showed better antifungal activity against human pathogen, *Candida albicans*.

Acknowledgements

Firstly, I wish to express our gratitude to Professor Dr Kay Thi Mya, Head of Botany Department, University of Pathein for providing me an opportunity to do this work. I also extended my thank to Professor Dr Wah Wah Lwin, University of Pathein for their valuable instruction and guidance. I would like to record my deep thank to Professor Dr Aye Aye Than , Head of Botany Department, University of Kyaukse and Professor Dr Tin Tin Thein, Department of Botany, University of Kyaukse for their suggestion and kind understanding during this study. Many thanks are due to my supervisor Dr Zar Zar Yin, Associate Professor, Department of Botany, University of Pathein, for her valuable instructions, encouragement and overall supervision for the successful completion of this research paper.

References

Alexander M. (1977). **Introduction to soil microbiology 2nd Edition.** John Wiley & Sons, New York. Pp. 423-437.

Atlas, R.M. (1993). **Microbiological media**. Boca Raton Ann Arbor, London Tokyo. Australia. Bacteriology. 8th Edition. Williams Wilkinson Co. Ltd. Baltimore. P. 244.

Benson, (2001). **Pure culture techniques in microbiological Applications Lab Manual in general microbiology,** the MC Graw-Itill Companies, 4, 82-88.

Collins, C.H., (1965), **Micorbiological Methods, 5th ed**. Butler and Tanner Ltd., London: pp: 49-80.

Das M. P. and Kumar S. (2013). **A comparative study on microbial growth and substrate utilization kinetics**. ISSN: 0975-766X

Davidson, E. A., Belk, e., and Boone, R. D., (1998). **Soil water content and temperature as independent and confounded factors controlling soil respiration in a temperate mixed hardwood forest,** Glob. Change Biol,, 4, 217-227

Hernandez., D., Cardell E. and Zarate, V., (2005), **Antimicrobial Activity of Lactic Acid Bacteria Isolated from Tenerife Cheese**: Initial Characterization of Plantaricin TF 711, J. App. Microbiol. vol. 99, pp.77-84.

Lilly VG, Barnett HL, (1952). **Physiology of fungi.** Mc Graw Hill Book Co. Inc., 464.

Madsen, E.L. (2008). **Environmental Microbiology:** From Genomes to Biogeochemisty (1st Ed). Malden, MA: Wiley-Blackwell Publishing 496 pp.

NITE, (2005). **Medium for fermentation to produce the metabolites.**

Omalu, I.C.J. Ayanwale, V.A., Ajalaruru, A.B., Mohammed, A..Z., Bala, J. and Chukwuemeka V. (2011). **Isolation of fungi and bacteria from housefly (Musca domestica L.) Larvae. The Internet Journal of Microbiology**.

Pelezar, M.J. and E.C.S. Chan, (1972). **Exercises in Microbiology.** 3rd ed. Mc Graw. Hill Book Co., New York.

Salle, (1948). **Fundamental Principles of Bacteriology** Mc. Graw Hill Book Co., Inc., New York.

Stachenhaus T, Schneidu A, Marahid MA, (1995). **Rational design of peptide antibiotics by targeted replacemen of bacterial and fungal domains,** Scl 269 (5220); 69-72.

Yang XF, Quiu DW,, Jiao NN, Liu Z, Yuan JJ, (2006). **Cultural medium and fermentation conditioins of *Xenorhabdus* sp.** strain D43, Chinese Journal of Vector Biology and Control, 22, 58-62.

INVESTIGATION OF NUTRITIONAL VALUES, ACUTE TOXICITY AND HYPOGLYCEMIC ACTIVITIES ON TWO CULTIVARS OF *LYCOPERSICON ESCULENTUM* MILL.

Than Than Yee[1]

Abstract

Lycopersicon esculentum Mill. belongs to the family Solanaceae. It is known as the kha yan gyin in Myanmar. Two cultivars of *Lycopersicon esculentum* Mill., cv. local and cv. 909 (Taiwan) were collected from Moe Kaung village, Sintgaing Township, Mandalay Region. Morphological and histological studies on two cultivars of *Lycopersicon esculentum* Mill. were carried out, to get their correct identification. Determination of nutritional values of two cultivars such as water, carbohydrate, crude fiber, proteins and fat were also investigated. The acute toxicity study of ethanol extracts of two cultivars on albino mice were performed. The lethal effect was not observed even use of maximum does of 2500 mg/kg. The hypoglycemic effect of ethanol extracts from two cultivars on adrenaline induced hypoglycemic albino mice were tested. These two cultivars show more reduction of hypoglycemic activity than standard drug, glibenclamide. The property of reduction of blood glucose concentration of ethanol extract cv. local and cv. 909(Taiwan) and glibenclamide were compared. From the comparison, ethanol extract of cv. local can reduce hyperglycemia better than that of cv. 909(Taiwan).

Keywords: *Lycopersicon esculentum* Mill., cultivars, nutritional values, acute toxicity, hypoglycemic activities

Introduction

Lycopersicon esculentum Mill. belongs to the family Solanaceae. The tomato is native to the Andes region of South America. The tomato plant is indigenous to the western regions of tropical South America. It is an important vegetable crop grown mainly for its popular vegetable crop; is now cultivated throughout the world for its edible fruits (Ross 2001).

Some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies. More than 400 plant species having hypoglycemic activity have been available in literature. Diabetes mellitus is a serious health problem being the third greatest cause of death all over the world, and if not treated. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs (Estari *et al.* 2013).

Diabetes is one of the most prevalence chronic diseases in the world. The number of diabetic people is expected to rise from present estimate of 150 million in 2025. For a long time, diabetes has been treated with several medicinal plants or their extract based on the folklore medicine. Nowadays herbal medicine are highly recommended for the treatment of diabetes inspite of other therapeutic option, which can produce serious side effect and in addition they are not safe during pregnancy. Therefore the search for the more effective and safer hypoglycemic agents has continued to be an important area of active research. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigation on hypoglycemic agent from medicinal plants has become more important (Malpani *et al.* 2010).

Various studies suggest that approximately 150 million people suffer from diabetes and this number may be double by the year 2025. One fifth of the diabetics will be from India. Much

[1] Dr, Lecturer, Department of Botany, Kyaukse University

of this increase will occur in developing countries and could be due to population growth, ageing, unhealthy diets, obesity and sedentary lifestyle (Faizal *et al.* 2009).

Interestingly, over the years, dietary consumption of vegetables and fruits(tomato) rich in carotenoids has been recommended for diabetic patients to be a protective factor against hyperglycemia but dietary consumption of high tomatine unripe tomatoes has not been recommended for diabetes mellitus (Akinnuga *et al.* 2010).

In Myanmar, there is no one who had investigated the tomato from the point of view of pharmacognosy. It is necessary to be carry out pharmacognostic study on *Lycopersicon esculentum* Mill. The aims and objectives of this research are to investigate the effect of the ethanol extract of *Lycopersicon esculentum* Mill. on hypoglycemic activity and to study the acute toxicity.

Materials and Methods

Collection, Identification and Preparation of *Lycopersicon esculentum* Mill. cv. local and cv. 909 (Taiwan)

The specimens of *Lycopersicon esculentum* Mill. cv. local and cv. 909 (Taiwan) were collected from Moe Kaung village, Sintgaing Township, Kyaukse District, Mandalay Region. The collected plants were taxonomically identified with the help of references literature such as Hooker (1885) and Dassanayake (1987). The fresh specimens were pressed, dried and preserved for morphological studies.

Nutritional Values

The nutritional values such as proteins, carbohydrate and fat of the dried fruit samples of two cultivars were determined at Department of Chemistry, University of Mandalay. Kjeldahl method was used for the determination of protein content. The carbohydrate content was determined by phenol- sulfuric acid colorimetric method. The fat content was determined by using Soxhlet apparatus.

Preparation of Ethanol Extracts from the Fruits of *Lycopersicon esculentum* Mill. cv. local and cv. 909 (Taiwan)

The fruits of *L. esculentum* Mill. cv. local and cv. 909 (Taiwan) were cut into small pieces and dried in the shade. Then they were ground and 50 g of air-dried powder were percolated with 95% ethanol (500 mL) for two months. The solution was filtered and concentrated. Ethanol crude extracts (2.2 g) and (2.5 g) was obtained.

The Study of Acute Toxicity and Hypoglycemic Activity of Ethanol Extracts from the Fruits of Two Cultivars on Albino Mice

The acute toxicity and hypoglycemic activity were performed at Department of Biotechnology, Mandalay Technology University.

The lethal activity and the determination of LD_{50} (Lethal dose) of the ethanol extracts from the fruits of *L. esculentum* Mill. cv. local and cv. 909 (Taiwan) were done according to the method of Birdi *et al*. (2006). Thirty albino mice of both sexes, weighing 20-25 g were used. Mice were fasted for the period of 12 hours. Mice were grouped into six and each group contains five mice. Five doses of the 95% ethanol extracts from the fruits of *L. esculentum* Mill. cv. local were given orally. The given doses of the extract were 0.5 g/kg, 1.0 g/kg, 1.5 g/kg, 2.0 g/kg and

2.5 g/kg body weight. The observation was done after one week. Similar procedures were done for ethanol extract of cv. 909(Taiwan).

The hypoglycemic activity of the ethanol extracts from the fruits of *L. esculentum* Mill. cv. local and cv. 909(Taiwan) were done according to the method of Malpani *et al*. (2010). Twenty albino mice of both sexes, weighing 20-25 g were used. Among them, sixteen mice were prepared as adrenaline-induced hyperglycemic albino mice. They were divided into five groups. Each group contains four mice. Control group was given distilled water. The mice in one group were orally given standard drug, glibenclamide, 0.5 x 10^{-3} g/kg by using syringes and needles. The mice in two groups were orally given ethanol extracts from the fruits of *L. esculentum* Mill. cv. local and cv. 909(Taiwan) (4 g/kg). The mice were subcutaneously injected with 0.2 x 10^{-3} g/kg body weight of adrenaline. The rests of mice were kept for normal condition. Then, glucose levels were measured at 45 minutes interval until 225 minutes.

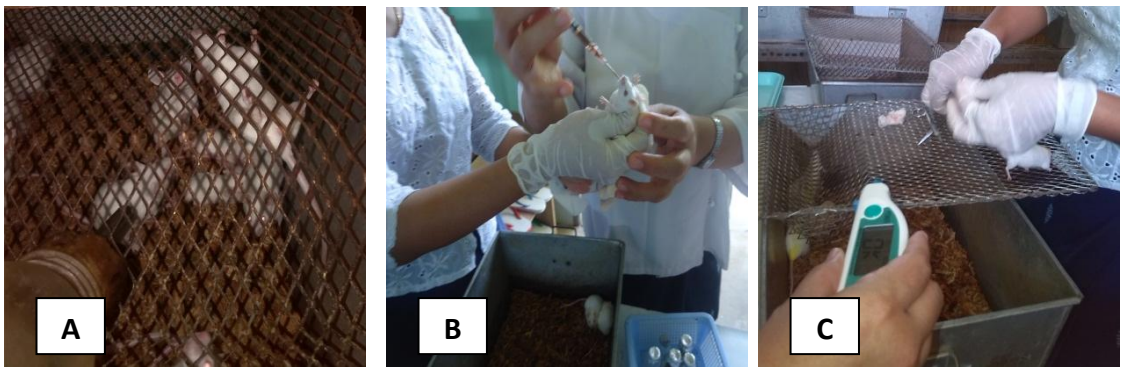


Figure 1 Test for hypoglycemia

(A) Mice test for hypoglycemic activity

(B) Administration of extract to mice

(C) Blood glucose level determination with glucometer

Results

Taxonomical Studies of *Lycopersicon esculentum* Mill. cv. local

***Lycopersicon esculentum* Mill. cv. local Gard. Dict. Ed. 8, 2.1768**

Family - Solanaceae

Local name - Kha-yan-gyin

English name - Tomato

Flowering period - October to February

Annual herbs; up to 1 m high; stems and branches terete, hispid. Leaves imparipinnate compound, basally lyrate, 12-20 cm long; lobes ovate to lanceolate, hispid on both surfaces; petioles 5-7 cm long, hispid. Inflorescences axillary, helicoids cymes with a few flowers; peduncles 2.5-3.0 cm long, hispid. Flowers bisexual, actinomorphic, hypogynous, bright yellow; 1.2-2.4 cm in diameter; pedicels 1.0-1.5 cm long, hispid. Calyx deeply 7-8 lobed, accrescent, hispid; tube 2.0-3.5 mm long, lobes lanceolate, 4.0-7.0 mm long. Corolla rotate, 7-8 lobed; tube 3.0-5.0 mm long; lobes triangular, 7-12 mm long. Stamens 7-8, free, adnate to the base of corolla

tube; filaments filiform, 0.5-1.0 mm long; anthers dithecous, basifixed, oblong; 5-8 mm long; yellow, dehiscent by 2 apical pores. Ovary superior, ovoid, 2-4 mm long, bilocular with many ovules on the axile placentae; style filiform, 6-8 mm long, pubescent at base, yellowish green; stigma simple. Berry globoid or ovoid, 4.5-7 cm in diameter, many seeded, scarlet when ripe. Seeds numerous, discoid, obscurely reniform, 4-7 mm in diameter, yellowish white.

Specimen examined : Moe Kaung Village, Sintgaing Township, Kyaukse District, Than Than Yee, October 17, 2015

Figure 2 Morphological Characters of *Lycopersicon esculentum* Mill. cv. local
(A) Habit (B) Inflorescence (C) L.S of flower

Morphological Studies of *Lycopersicon esculentum* Mill. cv. 909 (Taiwan)

Annual erect herbs; up to 1.2 m high; stems and branches terete, pubescent. Leaves imparipinnate compound, basally lyrate, 12-20 cm long; lobes ovate to oblong, pubescent on both surfaces; petioles 5-7 cm long, pubescent. Inflorescences axillary, helicoids cymes with a few flowers; peduncles 2.8-3.2 cm long. Flowers bisexual, actinomorphic, hypogynous, yellow; pedicels 1.2-1.8 cm long, pubescent. Calyx deeply 5 lobed, accrescent, pubescent, tube 2.5-3.8 mm long, lobes lanceolate, 5.0-7.5 mm long. Corolla rotate, 5 lobed, tube 5-7 mm long; lobes triangular, 8-12 mm long. Stamens 5, free, adnate to the base of corolla tube; filaments filiform, 1.0-1.5 mm long, pale yellow; anthers dithecous, basifixed, oblong, 5-9 mm long, yellow, dehiscent by two apical pores, Ovary superior, oblongoid, 3-5 mm long, bilocular with many ovules on the axile placentae; style filiform, 7-9 mm long, pubescent at base, pale yellow; stigma simple. Berry oblongoid or ovoid, 5-7cm long, many seeded, scarlet when ripe. Seeds numerous, discoid, obscurely reniform, 5-6 mm in diameter, yellowish white.

Specimen examined : Moe Kaung Village, Sintgaing Township, Kyaukse District, Than Than Yee, October 9, 2016.

Figure 3 Morphological Characters of *Lycopersicon esculentum* Mill. cv. 909 (Taiwan)
(A) Habit (B) Inflorescence (C) L.S of flower

Determination of Nutritional Values

The content of some nutrients such as protein, carbohydrate and fat were determined. According to the results, carbohydrate was found as major constituents in two cultivars**.** The results were shown and compared in Table 1.

Table 1 Nutritional Values of cv. local and cv. 909 (Taiwan) Sample

No.	Types of Nutrient	Amount (%)	
		cv. local	cv. 909 (Taiwan)
1	Water	91.17	89.06
2	Carbohydrate	56.40	61.80
3	Crude fiber	19.9	23.1
4	Protein	16.17	18.25
5	Fat	2.25	1.50

Determination of Acute Toxicity of Ethanol Extracts from the Fruits of *Lycopersicon esculentum* Mill. cv. local and cv. 909 (Taiwan)

The mice were treated with different doses of ethanol extract from the fruits of *L. esculentum* Mill. cv local. The different mice were also treated with different doses of ethanol extract from the fruits of *L. esculentum* Mill. cv. 909 (Taiwan). After one week acute toxicity on mice were studied. The results were shown in Table 2 and 3.

It was found that all mice were alive when even 2.5 g of ethanol extract from the fruits of *L. esculentum* Mill. cv local was given to the mice. Therefore, the LD_{50} of the extract supposed to be much more than 2.5 g/kg body weight.

Table 2 Effect of Ethanol Extract of *Lycopersicon esculentum* Mill. cv. local on Acute Toxicity

Groups	Number of Mice	Dosage (oral) mg/kg Body wt.	Ratio of Dead and Tested	% of Death
1	5	Distilled water	0/5	0%
2	5	Ethanol extract 0.5 g/kg body wt.	0/5	0%
3	5	Ethanol extract 1.0 g/kg body wt.	0/5	0%
4	5	Ethanol extract 1.5 g/kg body wt.	0/5	0%
5	5	Ethanol extract 2.0 g/kg body wt.	0/5	0%
6	5	Ethanol extract 2.5 g/kg body wt	0/5	0%

Diet = Stock diet & distilled water
Observation period= one week

Table 3 Effect of Ethanol Extract of *Lycopersicon esculentum* Mill. cv. 909 (Taiwan) on Acute Toxicity

Groups	Number	Dosage (oral) mg/kg Body wt.	Ratio of Dead and Tested	% of Death
1	5	Distilled water	0/5	0%
2	5	Ethanol extract 0.5 g/kg body wt.	0/5	0%
3	5	Ethanol extract 1.0 g/kg body wt.	0/5	0%
4	5	Ethanol extract 1.5 g/kg body wt.	0/5	0%
5	5	Ethanol extract 2.0 g/kg body wt.	0/5	0%
6	5	Ethanol extract 2.5 g/kg body wt	0/5	0%

Diet = Stock diet & distilled water
Observation period= one week

It was found that all mice were alive when even 2.5 g of ethanol extract from the fruits of *L. esculentum* Mill. cv. 909 (Taiwan) was given to the mice. Therefore, the LD_{50} of the extract supposed to be much more than 2.5 g/kg body weight.

Determination of Hypoglycemic Activity of Ethanol Extracts from the Fruits of *Lycopersicon esculentum* Mill. cv. local and cv. 909(Taiwan)

Blood Glucose Concentration of Normal Mice

The mean blood glucose concentration of the four albino mice at 0 min, 45 min, 90 min, 135 min, 180 min and 225 min after subcutaneous injection of adrenaline tartrate were 82.75±25.12 mg/dL, 110±12.25 mg/dL, 107.5±15.02 mg/dL, 100.25±12.52 mg/dL, 98.75 ±9.91 mg/dL and 87.25±14.41 mg/dL (Table 4).

Table 4 Blood Glucose Concentration of Normal Mice

Mouse code	Blood glucose concentration (mg/dL)					
	0 min	45 min	90 min	135 min	180 min	225 min
1	47	123	124	89	105	70
2	84	104	101	109	109	101
3	103	117	115	113	98	102
4	97	96	90	90	83	76
Sum	331	440	430	401	395	349
Mean	82.75	110	107.5	100.25	98.75	87.25
SD	25.12	12.25	15.02	12.52	9.91	14.41
SEM	12.56	6.125	7.51	6.26	4.955	7.205

The maximum blood glucose concentration was found at 45 minutes and blood glucose level was gradually decreased from 90 minutes to 225 minutes.

Effect of Distilled Water on the Adrenaline-induced Hyperglycemic Albino Mice

The mean blood glucose concentration of the four albino mice at 0 min, 45 min, 90 min, 135 min, 180 min and 225 min after subcutaneous injection of adrenaline tartrate were 80.75±24.25 mg/dL, 189.5±53.81 mg/dL, 157.75±30.97 mg/dL, 81.0±31.18 mg/dL, 65.25±23.76 mg/dL and 76±9.97 mg/dL (Table 5).

Table 5 Effect of Distilled Water on the Blood Glucose Concentration of Individual Adrenaline-induced Hyperglycemic Mice

Mouse code	Blood glucose concentration (mg/dL)					
	0 min	45 min	90 min	135 min	180 min	225 min
1	50	213	156	60	41	76
2	85	179	124	98	88	89
3	79	120	152	50	42	61
4	109	246	199	116	90	78
Sum	323	758	631	324	261	304
Mean	80.75	189.5	157.75	81.0	65.25	76
SD	24.25	53.81	30.97	31.18	23.76	9.97
SEM	12.125	26.905	15.485	15.59	11.88	4.985

The blood glucose concentrations were found to increase at 45 minutes after injection of adrenaline tartrate and gradually decrease from 90 minutes to 180 minutes. Increase in blood glucose concentration was found at 225 minutes.

Effect of Standard Drug, Glibenclamide 0.5 mg/kg (0.5×10^{-3} g/kg) on the Adrenaline Induced Hyperglycemic Albino Mice

The mean blood glucose concentration of the four albino mice treated with standard drug, glibenclamide 0.5×10^{-3} g/kg at 0 min, 45 min, 90 min, 135 min, 180 min and 225 min after subcutaneous injection of adrenaline tartrate 0.2×10^{-3} g/kg were found to be 81.5±25.67 mg/dL, 100.75±50.11 mg/dL, 61±29.98 mg/dL, 54.5±18.12 mg/dL, 76.25±13.48 mg/dL and 85.25 ±18.50 mg/dL (Table 6).

The decrease in blood glucose concentration was found after 45 minutes and increase after 135 minutes.

Table 6 Effect of Standard Glibenclamide on Blood Glucose Concentration of Individual Adrenaline Induced Hyperglycemic Mice

Mouse code	Blood glucose concentration (mg/dL)					
	0 min	**45 min**	**90 min**	**135 min**	**180 min**	**225 min**
1	90	82	55	51	83	113
2	111	149	83	55	86	82
3	50	39	21	34	53	61
4	75	133	85	78	83	85
Sum	326	403	244	218	305	341
Mean	81.5	100.75	61	54.5	76.25	85.25
SD	25.67	50.11	29.98	18.12	13.48	18.50
SEM	12.835	25.055	14.99	9.06	6.74	9.25

Effect of Ethanol Extract from the Fruits of *Lycopersicon esculentum* Mill. cv. local 4g/kg on Adrenaline Induced Hyperglycemic Mice

The mean blood glucose concentration of the four albino rats treated with ethanol extract from the fruits of *L. esculentum* Mill. cv. local (4g/kg) at 0 min, 45 min, 90 min, 135 min, 180 min and 225 min after subcutaneous injection of adrenaline tartrate 0.2 x 10^{-3} g/kg were 80.5±36.19 mg/dL, 161±99.54 mg/dL, 94.75±62.63 mg/dL, 50.75±31.36 mg/dL, 35.25± 16.78 mg/dL and 37±8.75 mg/dL (Table 7).

Table 7 Effect of Ethanol Extract from the Fruits of *Lycopersicon esculentum* Mill. cv. local on Blood Glucose Concentration of Individual Adrenaline-induced Hyperglycemic Mice

Mouse code	Blood glucose concentration (mg/dL)					
	0 min	**45 min**	**90 min**	**135 min**	**180 min**	**225 min**
1	130	253	174	97	62	41
2	54	195	107	42	37	44
3	53	20	25	28	20	22
4	85	176	73	36	22	41
Sum	322	644	379	203	141	148
Mean	80.5	161	94.75	50.75	35.25	37
SD	36.19	99.54	62.63	31.36	16.78	8.75
SEM	18.095	49.77	31.315	15.68	8.39	4.375

The decrease in blood glucose concentration was found after 45 minutes. Maximum decrease was found at 180 minutes.

Effect of Ethanol Extract from the Fruits of *Lycopersicon esculentum* Mill. cv. 909 (Taiwan) 4g/kg on Adrenaline- induced Hyperglycemic Mice

The mean blood glucose concentration of the four albino mice treated with ethanol extract from the fruits of *L.esculentum* Mill. cv.909(Taiwan) 4g/kg at 0 min, 45 min, 90 min, 135 min, 180 min and 225 min after subcutaneous injection of adrenaline tartrate 0.2 x 10^{-3} g/kg were 81.5±29.59 mg/dL, 220.25±178.65 mg/dL, 156.25±105.81 mg/dL, 72.25±49.01 mg/dL, 68±32.23 mg/dL and 64.5±27.13 mg/dL (Table 8).

The highest blood glucose concentration was found at 45 minutes and gradually decreased at 225 minutes.

Table 8 Effect of Ethanol Extract from the Fruits of *Lycopersicon esculentum* Mill. cv. 909 (Taiwan) on Blood Glucose Concentration of IndividualAdrenaline- induced Hyperglycemic Mice

Mouse code	Blood glucose concentration (mg/dL)					
	0 min	**45 min**	**90 min**	**135 min**	**180 min**	**225 min**
1	70	158	144	60	72	86
2	120	475	302	143	119	93
3	86	189	130	56	37	25
4	50	59	49	30	44	54
Sum	326	881	625	289	272	258
Mean	81.5	220.25	156.25	72.25	68	64.5
SD	29.59	178.65	105.81	49.01	32.23	27.13
SEM	14.795	89.325	52.905	24.505	16.115	13.565

Comparison Between Control, Treatments and Normal Groups

Mean blood glucose concentration of three mice groups such as glibenclamide, extract cv. local and cv. 909(Taiwan) were compared with that of control and presented in Table 9-11.

Table 9 Mean Blood Glucose Concentration of Control and Standard Drug, Glibenclamide on Adrenaline-induced Hyperglycemic Mice

Group of mice (n=4)	Blood Glucose Level (mg/dL)					
	0 min	**45 min**	**90 min**	**135 min**	**180 min**	**225 min**
Control	80.75	189.5	157.75	81.0	65.25	76
Glibenclamide	81.5	100.75	61.00*	54.50	76.25	85.25

Results were expressed mean . *P <0.05, *= significant (P<0.05)

Table 10 Mean Blood Glucose Concentration of Control and Ethanol Extract from the Fruits of *Lycopersicon esculentum* Mill. cv. local on Adrenaline-induced Hyperglycemic Mice

Group of mice (n=4)	Blood Glucose Level (mg/dL)					
	0 min	45 min	90 min	135 min	180 min	225 min
Control	80.75	189.5	157.75	81.0	65.25	76
Extract cv local	80.5	161.00	94.75*	50.75	35.25	37.00*

Results were expressed mean . *P <0.05, *= significant (P<0.05)

Table 11 Mean Blood Glucose Concentration of Control and Ethanol Extract from the Fruits of *Lycopersicon esculentum* Mill. cv. 909 (Taiwan) on Adrenaline-induced Hyperglycemic Mice

Group of mice (n=4)	Blood Glucose Level (mg/dL)					
	0 min	45 min	90 min	135 min	180 min	225 min
Control	80.75	189.5	157.75	81.0	65.25	76
Extract cv. 909 (Taiwan)	81.5	220.25	156.25	72.25	68.00	64.50

Results were expressed mean . *P <0.05, *= significant (P<0.05)

At 45 min after subcutaneous injection of adrenaline tartrate, glibenclamide gave the lowest blood glucose level. Significant decrease in blood glucose level of glibenclamide was found at 90 min ($p<0.05$) when compared with that of the control. Significant decrease in blood glucose level of extract cv. local were found at 90 min ($p<0.05$) and 225 min ($p<0.05$) when compared with that of the control. The blood glucose levels of extract cv. 909(Taiwan) were similar to that of control.

Percent Reduction of Hyperglycemia

Glibenclamide 0.5 x 10^{-3} g/kg gave percent reduction of hyperglycemia 39.45% for 90 minutes, 45.91% for 135 minutes, 24.32% for 180 minutes and 15.38% for 225 minutes.

Ethanol extract from the fruits of *L. esculentum* Mill. cv. local (4g/kg) shows percent reduction of hyperglycemia 41.15% for 90 minutes, 68.48% for 135 minutes, 78.11% for 180 minutes and 77.02% for 225 minutes.

Ethanol extract from the fruits of *L. esculentum* Mill. cv.909 (Taiwan) (4g/kg) shows percent reduction of hyperglycemia 29.06% for 90 minutes, 67.2% for 135 minutes, 69.13% for 180 minutes and 70.72% for 225 minutes.

Comparison of Percent Reduction of Hyperglycemia Between Ethanol Extracts from the Fruits of *Lycopersicon esculentum* Mill. cv. local and cv. 909 (Taiwan) and Standard Drug, Glibenclamide

Percent reduction of hyperglycemia between ethanol extract from the fruits of *L. esculentum* Mill. cv. local and cv. 909 (Taiwan) and standard drug, glibenclamide were compared each 45 minutes starting from 90 minutes. The results were shown in Table 12 and 13.

The percent reduction of ethanol extract from the fruits of *L. esculentum* Mill. cv. local increase from 135 minutes (68.48%) to 180 minutes (78.11%). The maximum percent reduction was found at 180 minutes (78.11%). After maximum condition, percent reduction decreased at 225 minutes (77.02%).

The percent reduction of ethanol extract from the fruits of *L. esculentum* Mill. cv. 909 (Taiwan) increase from 90 minutes (29.06%) to 225 minutes (70.72%).

The maximum percent reduction of glibenclamide was found at 135 minutes (45.91%) and gradually decreased up to 225 minutes (15.38%).

From the above observation, ethanol extracts of cv. local and cv. 909 (Taiwan) are more tendency to the reduction of hyperglycemia than glibenclamide. Besides, ethanol extract of cv. local can be reduce hyperglycemia better than that of cv. 909 (Taiwan).

Table 12 Comparison Between Blood Glucose Concentration (± SD) of Control Treatments and Normal Groups

Group of mice (n=4)	Blood Glucose Level (mg/dL)					
	0 min	**45 min**	**90 min**	**135 min**	**180 min**	**225 min**
Control	80.75±24.25	189.5±53.81	157.75±30.97	81.0±31.18	65.25±23.76	76±9.97
Glibenclamide	81.5±25.67	100.75±50.11	61.00±29.98*	54.50±18.12	76.25±13.48	85.25±18.50
Extract cv local	80.5±36.19	161.00±99.54	94.75±62.63*	50.75±31.36	35.25±16.78	37.00±8.75*
Extract cv 909(Taiwan)	81.5±29.59	220.25±178.65	156.25±105.81	72.25±49.01	68.00±32.23	64.50±27.13
Normal	82.75±25.12	110±12.25	107.5±15.02	100.25±12.52	98.75±9.91	87.25±14.41

Results were expressed as Mean ± SD *P < 0.05

Student's 't' test is significant at P < 0.05 , *= significant (P< 0.05)difference compared to control.

Table 13 Percent Reduction of Ethanol Extract from the Fruits of *L. esculentum* Mill. cv local and cv. 909(Taiwan) and Glibenclamide on Adrenaline- induced Hyperglycemic Mice

Group of mice (n=4)	Percent reduction of hyperglycemia (%)			
	90 min	135 min	180 min	225 min
Extract cv local (4g/kg)	41.15	68.48	78.11	77.02
Extract cv 909(Taiwan) (4g/kg)	29.06	67.20	69.13	70.72
Glibenclamide (0.5 mg/kg)	39.45	45.91	24.32	15.38

Discussion and Conclusion

Lycopersicon esculentum Mill. are widely cultivated through the tropical region of Myanmar. It is one of the species in Solanaceae family. In the present work, the morphological, nutritional values and hypoglycemic activities of *Lycopersicon esculentum* Mill. cv. local and cv. 909 (Taiwan) were presented.

In morphological studies, cv. local is annual herbs and the stems are branches terete, hispid. Leaves are imparipinnate compound, basally lyrate, hispid on both surfaces. These characters are in agreement with those mentioned by Dassanayake (1987).

The habits of *Lycopersicon esculentum* Mill. cv. 909 (Taiwan) is annual erect herbs and stems are branches, pubescent. Leaves imparipinnate compound, basally lyrate and pubescent on both surfaces. These characters are agreed with those given by Dassanayake (1987).

For cv. local, ovary is ovoid, bilocular and many ovules in each locule on the axile placentae. The fruits are berry, globoid or ovoid scarlet when ripe. Which are in agreement with those given by Hooker (1885).

For cv. 909 (Taiwan), ovary is oblong and bilocular with many ovules on the axile placentae. Fruits is berry, oblongoid or ovoid, many seeded, scarlet when ripe. These characters are agreed with those mentioned by Hooker (1885).

According to the results, the water content in cv. 909 (Taiwan) was found to be 89.06% and that in cv. local was 91.17%. The cv. 909 (Taiwan) contains 1.5% fat and 2.25% was found in cv. local. The water and fat content in cv. 909 (Taiwan) sample was smaller than that of cv. local. These amounts were agreed with those mentioned by Gupta (2011). The carbohydrate content of the cv. 909 (Taiwan) was 61.8% and that of cv. local was 56.4%. The amount of crude fiber of cv. 909 (Taiwan) was 23.1% and cv. local contains 19.9%. The amount of protein in cv. 909 (Taiwan) was 18.25% and that of cv. local was 16.17%. These data are agreement with those given by Nispetiye (2009).

In pharmacological studies, the acute toxicity and hypoglycemia effect of the fruits of *L. esculentum* Mill. cv. local and cv. 909(Taiwan) were investigated. From the acute toxicity determination with mice model, there were no lethal effect was observed with maximum permissible dose of 2.5 g/kg body weight. Therefore, the medium lethal dose, LD_{50} of the extracts supposed to be much more than 2.5 g/kg body weight per mice orally. The ethanol

extracts of *L. esculentum* Mill. cv. local and cv. 909(Taiwan) showed harmless effect on the albino mice. It is clear that ethanol extracts of tomato were free from acute toxic effect.

According to Wilde (2012), tomato contains a substance called lycopene. Tomatoes also contain vitamin C and vitamin E. The tomato can be used for diabetes management. Controlling blood sugar level is an important part of diabetes management.

Adrenaline was used to induce hyperglycemia in albino mice and blood glucose concentrations were measured at different times (each 45 minutes) according to the method of Malpani *et al*. (2010).

It was evident that for the mice treated with glibenclamide, significant decrease in blood glucose level was found at 90 min ($p<0.05$) when compared with that of the control. For the mice treated with ethanol extract from the fruits of *L. esculentum* Mill. cv. local, significant decrease in blood glucose level were found at 90 min ($p<0.05$) and 225 min ($p<0.05$) when compared with that of the control.

From the comparison of percent reduction of hypoglycemia, the percent reduction of glibenclamide was gradually decreased from 135 minutes to 225 minutes. For the ethanol extracts from the fruits of *L. esculentum* Mill. cv. local, the percent reduction was increased from 45 min to 180 minutes but decreased at 225 minutes. The percent reduction of ethanol extract from the fruits of *L. esculentum* Mill. cv. 909(Taiwan) increase from 90 minutes to 225minutes.

The ethanol extract of cv. local can be maximum percentage at 180 minutes (78.11%). After that, the blood glucose concentration gradually decrease (percent reduction decrease). The ethanol extract of cv. local has more tendency to the reduction of hyperglycemia than glibenclamide.

From the comparison of two cultivars, ethanol extract of cv. local shows higher hyperglycemic activity than that of cv. 909(Taiwan).

. Diabetes is one of the most prevalence chronic diseases in the world. In this research work, the extracts of *L. esculentum* Mill. cv. local and cv. 909(Taiwan) respond hypoglycemic effect in diabetic mellitus at short period of dietary intake and there is no toxic effect. Therefore, the consumers may benefit by eating tomatoes.

Acknowledgements

My sincere thanks are due to Dr Aye Aye Than, Professor and Head, Kyuakse University for her kind suggestion, constant encouragements and for providing the facilities during my research work.

References

Akinnuga, A.M. , B. Olubayode and O. Adeniyi. (2010). Hypoglycaemic Effects of Dietary Intake of Ripe and Unripe *Lycopersicon esculentum* (Tomatoes) on Streptozotocin-Induced Diabetes Mellitus in Rats. Benue State University, Benue State, Nigeria.

Birdi, T.J., B. Sukumaran and P. Daswani. (2006). Approach toward the preclinical testing and standardization of medicinal plants. Foundation for medicinal Research. India.

Dassanayake, M.D and F.R. Fosbers. (1987). Flora of Ceylon. Vol (VI). Amerind Publishing Co. Ltd. . New Delhi.

Estari, M. and E. Sucharitha. (2013). Evaluation of antidiabetic activity of medicinal plant extracts used by tribal communities in rural areas of Warangal district, Andhra Pradesh, India.

Faizal, P., A. Sukoco and E. Widodo. (2009). A study on the Hypoglycemic and Hypolipidemic Effects of an Ayurvedic Drug Rajanyamalakadi in Diabetic Patients. M.G University, Wuhan city, China.

Gupta, A., A. Kawatra and S. Sehgal. (2011). Physical-chemical properties and nutritional evolution of newly developed tomato genotypes. African Journal of Food Science and Technology. Vol. 2 (7), 167-172.

Hooker, J.D. (1885). The Flora of British India. Vol (IV). L. Reeve & Co., ltd. London.

Malpani, S. N, K.P. Manjunath, H. Sholapur, R. V. Savadi and S.S. Darade. (2010). Antidiabetic Activity of *Cassia fistula*. Bark in Alloxan Induced Diabetic Rats. Department of Pharmacognosy, Karnataka, India.

Nispetiye. (2009). Nutritional values for dried tomatoes.

Ross, I.A. (2001). Medicinal Plants of the World. Vol 2, Humana Press, Totowa, New Jersey.

Wilde, M. (2012). Using a combination of diet, exercise, herbs and micronutrient supplements it is possible to reduce blood sugar levels.

ISOLATION OF SOIL BACTERIA FROM NGATHAICHAUNG TOWNSHIP AND THEIR ANTIMICROBIAL ACTIVITIES

Khin Min Min Kyaw[1], Zar Zar Yin[2]

Abstract

Soil samples were collected from three different areas of Ngathaichaung Township, Ayeyawady Region. These samples were cultured on Glucose Peptone Agar medium, Flo Agar medium and Dextrose Casein Peptone Agar medium. A total of 15 bacterial colonies were isolated from these soil samples. 5 isolates were obtained from Glucose Peptone Agar medium, 7 isolates from Flo Agar medium and 3 isolates from Dextrose Casein Peptone Agar medium. Isolated bacteria were designated as KM. In the colony morphology, the size of isolated bacteria were medium, large and very large. The margin of colonies were entire, lobate and undulate. Cell morphology of isolated strains were studied by Gram staining, colony morphology and shape of cell strains were short rod, other strains were long rod chain and rod chain. Three strains were Gram positive and other strains were Gram negative. Moreover, antimicrobial activities of all isolated strains were carried out by agar well diffusion assay with seven test organisms. Among them, 6 isolated strains showed the antimicrobial activity. KM-13 showed the higher antibacterial activity (27.77 mm and 27.63 mm) against *Escherichia coli* and *Bacillus pumilus.* Especially this strain exhibited the highest antifungal activities (31.17 mm) on *Malassezia furfur* followed by (25.46 mm) on *Candida albicans.* And then, KM-11 also showed the antibacterial activity (21.40 mm) on *Bacillus subtilis.*

Keywords: Soil bacteria, culture medium, antimicrobial activity

Introduction

Soil is a primary source of microorganisms. Soil bacteria and fungi have played a significant and an important role in antibiotic discovery.

The numbers and species of microbes in soil is depend on environmental conditions like nutrient avability, soil texture, presence of moisture in soil and type of vegetation cover and their number varies according to the type of environmental condition (Atlas and Bartha, 1998).

Natural products having novel structures have been observed to possess useful biological activities, soil is a natural reservoir for microorganisms and their antimicrobial products (Dancer, 2004).

Natural products from microorganisms have been the most successful source that has found many applications in the fields of medicine, pharmacy and agriculture. Most of the antibiotics in current use for the treatment of various infectious diseases are microbial products (Tawiah *et al.,* 2012). Studies on soil bacteria and fungi have shown that these microorganisms are potentially rich source of unique bioactive substances (Fenical, 1993).

Numerous antibiotics have been isolated from a variety of microorganisms, however studies are still being conducted to identify novel antibiotics effective against pathogenic fungi and bacteria (Cavalcanti, *et al.,* 2006).

Antimicrobial agents play the most important role in the treatment of bacterial infections and wide spread efforts have been carried out by many scientists in order to screen for novel antibiotic producing microbes (Oskay, *et al.,* 2004).

The aim and objectives of this research were to collect the three different soil samples from Ngathaichaung Township, Ayeyawady Region, to isolate the diversity of bacteria from soil samples on three different culture media, to investigate the colony characters of isolated bacteria,

[1] Dr, Lecturer, Department of Botany, Kyaukse University

to observe the microscopic characters of isolated soil bacteria and to study the preliminary study of antimicrobial activity of isolated bacteria on seven test organisms.

MATERIALS AND METHODS

Collection of Soil Samples

Three different soil samples were collected from three different places of Ngathaichaung Township, Ayeyawady Region in July, 2018. These soil samples were carried out at the laboratory of Biotechnology and Development Centre of Pathein University.

Isolation of the collected soil samples was done in laboratory as soon as possible after soil collection in fields. Serial dilutions of plating and streaking techniques were used to isolate the microorganisms from soil according to Salle (1948); Collins (1965) and Pelezer and Chan (1972).

Isolation of Bacteria from Soil Samples by Serial Dilution Method

Serial dilutions of plating and streaking techniques described by Salle (1948), Collins (1965), and Pelezar and Chan (1972) were used for the isolation of bacteria species from soil.

The sterilized medium in conical flask was cooled down to about 45ºC and separately poured into each of the petridish containing the respective soil dilution. The inoculated plates were shaken clock-wise and anticlockwise direction for about 5 minutes so as to make uniform distribution of the bacterial inoculums. When the agar was solidfied, the inoculated plates were inverted and incubated at room temperature for 3-5 days.. Various types of colonies developed on the inoculated plates. They were separately streaked over another set of petridishes containing the same sterile medium. Each of the discrete colonies visible in the second set of inoculated plates was separately transferred to sterile respective medium.

Isolation of Pure Culture from Plate to Slants

For pure culture from plate to test tube, about 100 mL of culture media were separately distributed into test tubes. These tubes were plugged with cotton wool and sterilized by autoclaving then at 15 pounds pressure per square inch for 15 minutes at 121ºC. The sterilized media were cooled down. Each of the separate colonies on petri-dish was taken out to streak on the slant medium to obtain pure cultures (Atlas, 1993).

Gram Staining Method (Collins, 1965)

A smear of bacterial cells was prepared on a clean glass slide and the smear was then allowed to air-dry followed by a mild heat fixation. Crystal violet solution was added onto bacterial smear and incubated for one minute. The smear was washed with water Mordant Gram's iodine Solution was then added on bacterial smear and incubated for one minute. The smear was decolonized by washing with 95% ethyl alcohol and rinsed with water. Finally, safranin was used as counter for one minute and washed with water. Cell were then air dried and studied under microscope.

Preliminary Study on Antimicrobial Activities of Isolated Bacteria

The isolated soil bacteria were inoculated into seed medium and incubated for 1 day at room temperature. Seed culture were transferred to the fermentation medium. After one day, the

seed cultured (1%) was transferred into the fermentation medium and carried out by static culture. Then, the fermented broth was used to check the antimicrobial activity by agar well method (Collins, 1965). Agar well having (8 mm in diameter) were utilized for antimicrobial activity.

Test Organisms

Escherichia coli AHU 5436, *Bacillus subtilis* IFO 90571, *Bacillus pumilus* IFO 90571, *Candida albicans* NITE 09542, *Pseudomonas fluorescens* IFO 94307, *Staphylococcus aureus* AHU 8465, *Malassezia furfur* UY were obtained from NITE (National Institute of Technology and Evaluation, Kisarazu, Japan), PRD (Pharmaceutical Research Development, Ministry of Industry) and UY (University of Yangon).

Agar well method (Collins, 1965)

This method was used for the antimicrobial activity by seven test organisms. The assay medium (peptone 0.5 g, NaCl 0.5 g, yeast extract 0.2 g, beef extract 0.1 g and agar 1.5 g) was utilized for these bacteria. Isolated strains were subjected with antimicrobial activity by agar well method. Cork borer was used to make the wells (8 mm in diameter) in the autoclave basal antimicrobial test medium.

Well impregnated with 1-5 days fermented broth (0.1 mL) were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured. Clear zone surrounding the wells indicated the presence of antimicrobial activities which inhibit the growth of the test organisms selectively.

Results

The total of 15 bacterial strains such as KM-1, KM-2, KM-3, KM-4, KM-5, KM-6, KM-7, KM-8, KM-9, KM-10, KM-11, KM-12, KM-13, KM-14 and KM-15 were isolated from the three different area of Ngathaichaung Township. The results showed that the colonies morphology of those isolated strains (KM-1-15) were medium and large in sizes; undulate, lobate, entire in margins; cream, white, pale brown and purple in color; raised and flat in elevation and form; mucous, dry and greasy in pigments on agar, respectively. Those bacterial strains were short-rod, rod-chain and long rod chain in their cell morphologies and their antimicrobial activities were also tested.

Table 1 Colony morphology of the isolated strains

Isolated strains	Size of colony	Margin	Color	Elevation and form	Pigment on agar
KM-1	Large	Undulate	Mucous	Flat	Mucous
KM-2	Medium	Lobate	Mucous	Raised	Mucous
KM-3	Large	Entire	Dry	Raised	Dry
KM-4	Large	Undulate	Dry	Flat	Dry
KM-5	Large	Undulate	Dry	Flat	Dry

Isolated strains	Size of colony	Margin	Color	Elevation and form	Pigment on agar
KM-6	Large	Entire	Purple	Flat	Mucous
KM-7	Large	Undulate	White	Flat	Dry
KM-8	Medium	Entire	White	Raised	Dry
KM-9	Large	Undulate	Cream	Flat	Dry
KM-10	Large	Undulate	Cream	Flat	Mucous
KM-11	Large	Undulate	Cream	Flat	Greasy
KM-12	Large	Undulate	Pale brown	Flat	Greasy
KM-13	Large	Undulate	Cream	Flat	Mucous
KM-14	Large	Entire	Cream	Flat	Mucous
KM-15	Medium	Entire	Cream	Flat	Mucous

Table 2 Cell morphology of the isolaed strains

Isolated strains	Gram staining	Cell morphology
KM-1	-	Long rod chain
KM-2	-	Short rod
KM-3	-	Rod chain
KM-4	-	Long rod chain
KM-5	-	Rod chain
KM-6	-	Rod
KM-7	-	Rod chain
KM-8	-	Short rod
KM-9	-	Rod
KM-10	-	Short and chain
KM-11	-	Short rod
KM-12	-	Short rod
KM-13	+	Rod
KM-14	+	Short rod
KM-15	+	Short rod

(-) = Gram negative (+) = Gram positive

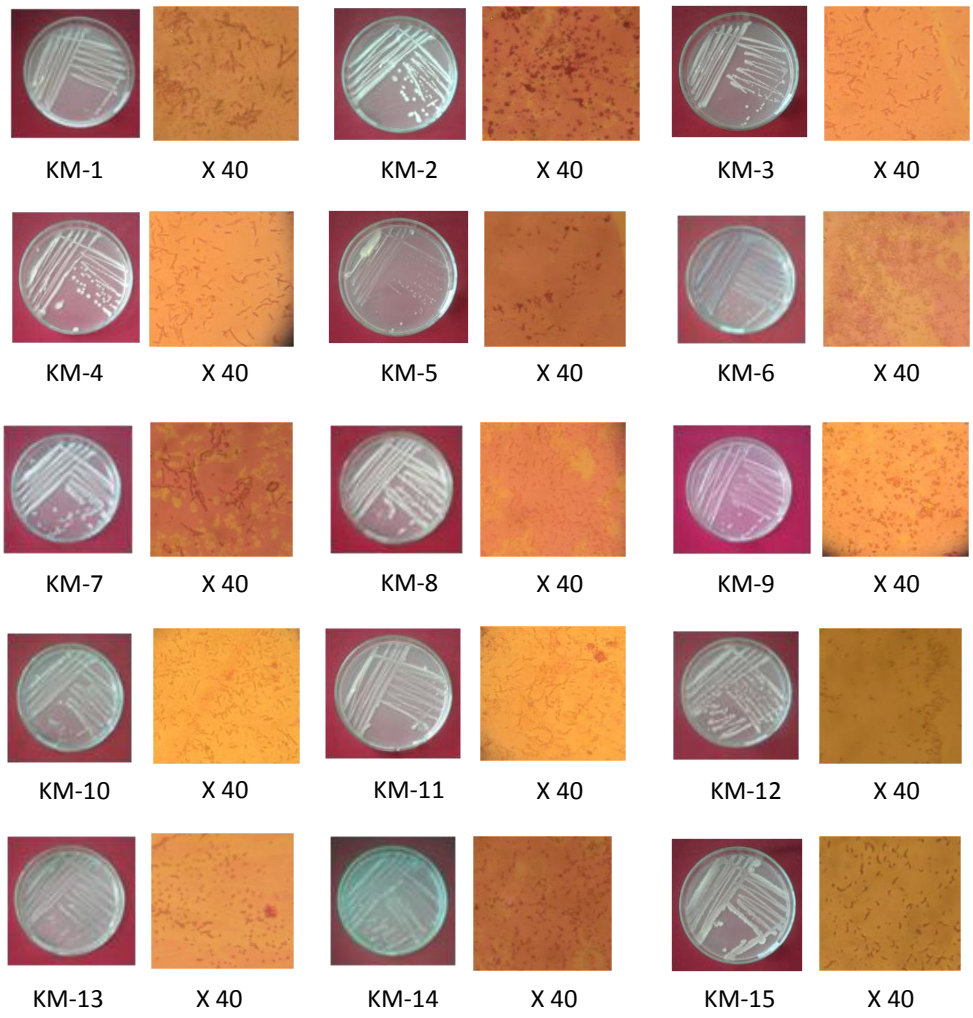


Figure 1 Cultural characteristic and cell morphology of isolated bacteria

Antimicrobial Activities of Isolated Bacterial Strains

Ten isolated bacterial strains were tested from 15 isolated bacteria. Among them, 6 isolated bacterial strains showed the different level of antimicrobial activities on 5 test organisms, except *Pseudomonas fluorescens* and *Staphylococcus aureus.*

Table 3 Antimicrobial Activities of 6 Isolated Bacteria Against Five Test Organisms

No	Isolated bacteria	*Escherichia coli*	*Bacillus subtilis*	*Bacillus pumilus*	*Candida albicans*	*Malassezia furfur*
1	KM-6	13.28 mm	+	18.01 mm	19.61 mm	19.73 mm
2	KM-9	22.72 mm	19.98 mm	+	21.14 mm	23.77 mm
3	KM-10	27.21 mm	19.95 mm	26.62 mm	+	19.38 mm
4	KM-11	17.78 mm	**21.40 mm**	16.74 mm	+	27.76 mm
5	KM-13	**27.77 mm**	+	**27.63 mm**	**25.46 mm**	**31.17 mm**
6	KM-15	14.73 mm	+	26.15 mm	16.07 mm	21.69 mm

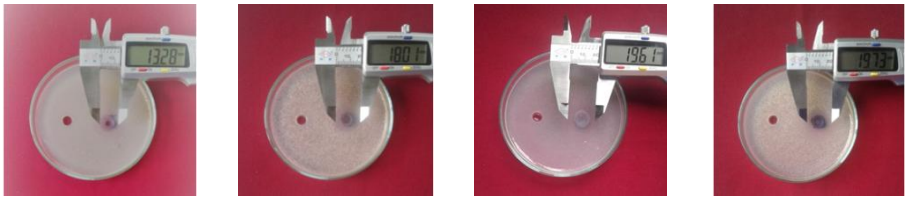


Figure 2 Antimicrobial activities of KM-6 against (A) *Escherichia coli*, (B) *Bacillus pumilus*, (C) *Candida albicans* and (D) *Malassezia furfur*

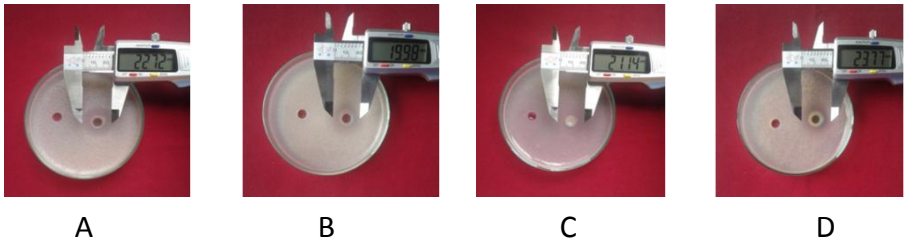


Figure 3 Antimicrobial activities of KM-9 against (A) *Escherichia coli*, (B) *Bacillus subtilis*, (C) *Candida albicans* and (D) *Malassezia furfur*

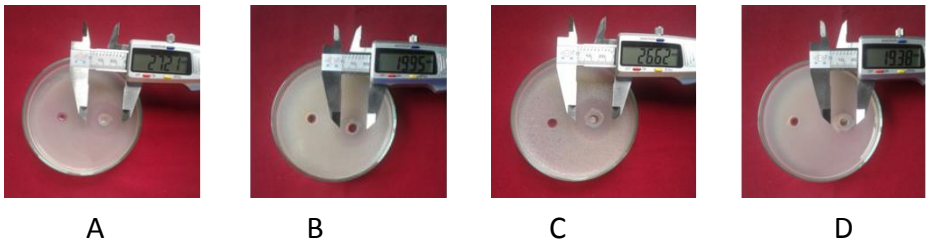


Figure 4 Antimicrobial activities of KM-10 against (A) *Escherichia coli*, (B) *Bacillus subtilis*, (C) *Bacillus pumilus*, and (D) *Malassezia furfur*

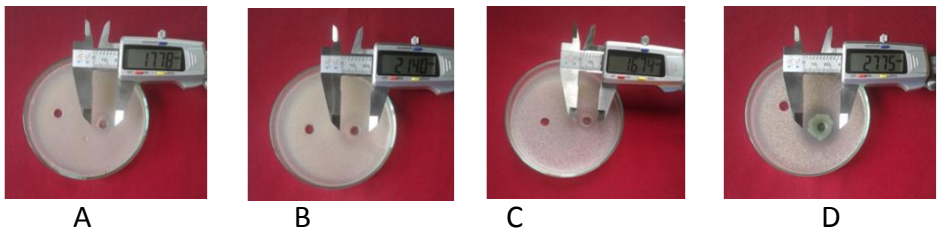
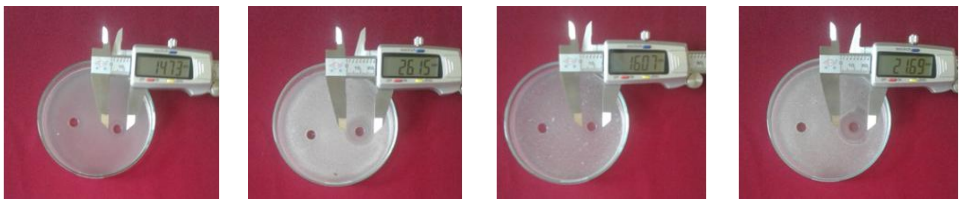


Figure 5 Antimicrobial activities of KM-11 against (A) *Escherichia coli*, (B) *Bacillus subtilis*, (C) *Bacillus pumilus*, and (D) *Malassezia furfur*

A B C D

Figure 6 Antimicrobial activities of KM-13 against (A) *Escherichia coli*, (B) *Bacillus pumilus*, (C) *Candida albicans* and (D) *Malassezia furfur*



A B C D

Figure 7 Antimicrobial activities of KM-15 against (A) *Escherichia coli*, (B) *Bacillus pumilus*, (C) *Candida albicans* and (D) *Malassezia furfur*

Discussion and Conclusion

Bacteria grow in many different environments and specific niches in the soil. The number and type of bacteria present in a particular soil would be greatly influenced by geographical location such as soil temperature, soil type, soil pH, organic matter contents, cultivation, aeration and moisture content (Davies, 1999).

Microorganisms which have capacity to produce more antibiotics can survive for longer time than the other producing antibiotics in fewer amounts. Antibiotics produced by microorganisms have been very useful for the cure of certain human diseases caused by bacteria, fungi and protozoa (Walsh, 2003).

The World Health Organization stated that there is a serious lack of new antibiotics to fight the increasing risk of antimicrobial resistance, which represents a global health emergency (Kmietowicz, 2017). Antibiotics and other bioactive compounds have been isolated from microorganisms in different environments (Charousova *et al.*, 2017).

In the present study of the isolation of bacteria, 15 strains were isolated from three different soil samples collected from Ngathaichaung Township, Ayeyarwady Region. Three different media were employed in the isolation of bacteria and 15 isolates were obtained. These bacterial strains were designated as KM-1 to KM-15.

In the colony morphology, all isolated bacteria were medium and large and the color were cream, white, purple and pale brown. The margin of colonies were entire, lobate and undulate.

Cell morphology of isolated strains were studied by Gram staining, colony characters and shape of cell. Among them, all strains were short rods, long rods, short rods chain and long rods chain. Three strains were Gram positive and other strains were Gram negative.

In the preliminary antimicrobial activity, 6 isolated strains showed different levels of antimicrobial activity on five test organisms except *Pseudomonas fluorescens* and *Staphylococcus aureus.* KM-13 showed the higher antibacterial activity (27.77 mm and 27.63 mm) against *Escherichia coli* and *Bacillus pumilus* than other strains. Especially, this strain exhibited the highest antifungal activity (31.17 mm) on *Malassezia furfur* followed by (25.46 mm) on *Candida albicans.* And then, KM-11 also showed the antibacterial activity (21.40 mm) on *Bacillus subtilis.*

Antibiotics and other bioactive compounds have been isolated from microorganisms in different environment (Charousova *et al.*, 2017; Devi *et al.*, 2017).

It was concluded that the present research was to isolate the diversity of bacteria from soil samples on three different culture media and to study the preliminary study of antimicrobial activity of isolated bacteria on seven test organisms.

Acknowledgements

Firstly, I wish to express our gratitude to Professor Dr Si Si Hla Bu, Rector, Pathein University for providing me an opportunity to do this work. I also extended my thank to Professor Dr Than Tun and Dr Nilar Myint, Pro-Rectors, Pathein University, for their valuable instruction and guidance. I would like to express my thank to Daw Than Than Naing, Principle, Pathein Education College for her encouragement and suggestions. I would like to record my deep thank to Professor Dr Kay Thi Mya, Head of Botany Department, Pathein University and Professor Dr Wah Wah Lwin, Department of Botany, Pathein University for their suggestion and kind understanding during this study. Many thanks are due to my supervisor Dr Zar Zar Yin, Associate Professor, Department of Botany, Pathein University, for her valuable instructions, encouragement and overall supervision for the successful completion of this research paper.

References

Atlas RM and Barha R. (1998). **Fundamentals and applications.** In: Microbial ecology. 4th edition, pp. 174-217. Benjamin/ Cummings Science Publishing, New York.

Cavalcanti MA, Oliveira LG, Fernandes MJ and Lima DM. (2006). **Filamentous fungi isolated from soil in districts of the Xingo region,** *Braz. Acta Bot. Bras.* 20, 831-837

Charousova I., Steinemetz H., Medo J. Javore-Kova S. & Wink J., (2017). **Soil mycobacteria as a potential source of polyketide-peptide substances.** Folia Microbiologica, 62(4):305-315

Collins, C.H. (1965). **Microbiological Methods.** Butter Worth and Co., Publishers Ltd, Landon.

Dancer, (2004). **How antibiotics can make us sick: the less obvious adverse effects of antimicrobial chemotherapy.** The lancet infectious diseases. 4:611-619.

Davies, C. and B. Williams, (1999). **Genus *Bacillus* in Bergeys manual of systematic bacteriology sneath,** ptt.Ed William and Wikins company Bultimore.

Devi S.I., Lotjem H., Devi E.J., Potshangbam M., Ngashangva N., Bora J., Sahoo D. & Sharma C., (2017). **Biomining the forest ecosystem of North East India for**

Fenical, W. (1993). **Chemical studies of marine bacteria: developing a new resource.** *Chem Rev*, 93:1673-1683.

Kmietowicz Z., (2017). **Few novel antibiotics in the pipeline,** WHO warns. British Medical Journal, 358:j4339. https://doi.org/10.1136/bmj.j4339

Oskay. M, A.U. Tamer, C. Azeri, (2004). **African Journal of Biotechnology,** 3(9); 441-446.

Pelezar, M.J, and E.C.S Chan, (1972). **Exercise in Microbiology.** 3rd ed. MMCG raw. Hill Book Co., New York

Salle, (1948). **Fundamental principles of Bacteriology** Mc. Graw Hill Book Co., Inc., New York.

Tawiah, A.A., Gbedema, S.Y., Adu F., Boamah, V.E. and Annan, K. (2012). **Antibiotic producing microorganisms from River Wiwi, Lake Bosomtwe and the Gulf of Guinea at Doakor Sea Beach, Ghana** BMC Microbiology, 12: 234-241.

Walsh, C. (2003). **Antibiotics; actions, origins, resistance.** American Society for Microbiology (ASM).

MORPHOLOGICAL IDENTIFICATION AND PHYTOCHEMICAL INVESTIGATION OF *CASSIA OCCIDENTALIS* L. LEAVES AND ITS ANTIMICROBIAL ACTIVITY

Ni Ni Htun[1]

Abstract

Cassia occidentalis L., belonging to family Fabaceae, is a medicinal plant used as a traditional medicine for the treatment of various diseases. The present study was designed to evaluate the preliminary phytochemical constituents and antimicrobial activity of leaves of *Cassia occidentalis* L. The specimen were collected from Banmaw Township, Kachin State. The morphological characters of this plant have been studied in detail and identified by the available literatures. The dried leaf powder of *Cassia occidentalis* L. was subjected to phytochemical analysis in order to find out the presence of phytochemical constituents. It contained many chemical groups included alkaloids, glycoside, reducing sugar, saponin, steroid, terpenoids, carbohydrate, tannin, phenolic compound, flavonoid, starch, protein and amino acid. For antimicrobial activity, the leaf powder of *Cassia occidentalis* L. was extracted with seven different solvents. The extracts were used to carryout antimicrobial screening in vitro on six different types of microorganisms by agar well diffusion method. It was found that the ethyl acetate extract showed most significant antimicrobial activity on *Pseudomonas aeruginosa*. The phytochemical investigations and antimicrobial properties of leaves of *Cassia occidentalis* L. prove its importance as a valuable medicinal plant.

Keywords : Phytochemical, Antimicrobial, *Cassia occidentalis*

Introduction

Traditional medicine, making use of herbs in different preparations, is greatly relied upon especially by rural dwellers, for the treatment of various ailments. Nowadays, there is growing trend of people moving from synthetic drugs to herbal cure. The plant under investigation is *Cassia occidentalis* L. *Occidentalis* species belongs to the genus *Cassia* and the Family Fabaceae. It is called Stink Weed, Stinking or Negro Coffee (Nuhu, 2008). *Cassia* is one of the largest genera with about 250 species and it is famous genus for its ornamental value and medicinal usage (Cronquist, 1981). *Cassia* plants are widely distributed and well known in Myanmar for their medicinal and ornamental values with brightly-coloured flowers. The potential of the leaf extract of *C. occidentalis* may be related to its antioxidant activity. The extract contains flavonoids which are powerful antioxidant polyphenolic compounds (Nuhu, 2008). The root of *Cassia occidentalis* L. is useful in ringworm and scorpion sting. The leaves are used in cough, asthma, stomachic and fevers. The seeds are also used as a substitute for coffee (Kirtikar and Basu, 1975).

The active principle of many drugs found in plants is phytochemical. The medicinal value of these phytochemicals is because of the presence of chemical substance that produces definite physiological action on the human body. Some of the valuable one include: - Alkaloids, tannins, saponins, glycosides, flavonoids, phosphorus and calcium for cell growth, replacement, body building (Harbone, 1973). *Cassia occidentalis* L. was found to be contain many groups of chemical substance.

[1] Dr, Associate Professor, Botany Department, Banmaw University

The development of resistance to current antibiotics by disease causing microbes has reinforced research for discovery of new ones. Current trends in drug development process are focused on natural sources, especially source of plant origin due to some proven correlation between the folkloric medicinal uses of some of these plants to biological activity (Kunle, 2009). *C. occidentalis* is an ayurvedic plant with huge medicinal importance. Leaves of this plant have ethno medicinal importance like paste of leaves is externally applied on healing wounds, sores, itch, cutaneous diseases, bone fracture, fever, ringworm, skin diseases and throat infection (Burkill, 1995).

It is the intention of this research work to identify the relevant phytochemical compounds of the plant part that produces the antimicrobial effect and to determine the antimicrobial activity of the plant extract to correlate to its medicinal use.

Materials and Methods

Morphological Studies

Collection, Identification and Preparation

In this study, *Cassia occidentalis* L. was collected during flowering period (December to March) from some areas of Banmaw Township. After the collection, all the vegetative and reproductive parts of the fresh specimens were studied, measured in detail and recorded. The relevant data for taxonomic description of the species were also recorded. Based on the resulting data, the plants were identified with the help of literatures (Backer; 1968, Burkill; 1935, Hooker; 1881, Dassanayake (1981) and Hu-Qi-ming (2009).). All the necessities were documented by photographs. Then, the leaves of this plant were thoroughly washed with water, dried in shade and crushed and powdered with a grinding machine. This powder was stored in the airtight container for further study.

Preliminary phytochemical investigation

For the phytochemical study, powdered leaves of *Cassia occidentalis* L. were used to find out the presence or absence of phytochemical constituents. The preliminary phytochemical tests were carried out at the Department of Botany, Banmaw University according to the methods of British Pharmacopoeia (1968), Marini Bettolo *et. al.* (1981) and Trease and Evans (2002).

Antimicrobial activity of different solvent extracts from *Cassia occidentalis* L. leaves

Antimicrobial activity of different solvent extracts from Cassia occidentalis L. leaves were tested on six microorganisms by agar well diffusion method at Central Research and Development Centre (CRDC).

Preparation of the crude extracts

About 5 g of the powdered leaves was extracted with 20 ml of each solvent (ethanol, methanol, pet-ether, chloroform, acetone, ethyl acetate and water) respectively. The powder sample with respectively solvents was placed in water bath for 6 hours. The crude extracts were then filtered. After filtration, the extracts were dried on water bath to obtained concentrated substances.

Test organisms

The test organisms used in this study were *Bacillus subtilis* (JAP – 0225215), *Bacillus pumalis* (IFO – 12102), *Candida albican* (IFO – 1060), *Escherichia coli* (ATCC – 25922), *Pseudomonas aeruginosa* (IFO – 3080) and *Staphylococcus aureus* (ATCC – 12277).

Antimicrobial screening

In this method, nutrient agar was used as culture media. For initial screening, according to the method nutrient agar was prepared described by Cruickshank, 1975. Nutrient agar was boiled and poured into the test tube and plugged with cotton wool and sterilized in an autoclave at 121°C for 15 minutes. Then, the tubes were cooled down to 30 – 35°C and poured into sterilized petri-dishes and 0.1 – 0.2 ml of microbial suspension from nutrient broth were added into the dishes. The agar medium was allowed to solidify for 2 – 3 hours, then 10 mm agar well was made by the help of sterilized agar well cutter. After that, about 0.2 ml of sample dissolved in their respective solvents was introduced into the agar well and incubated at 37°C for 24 hrs. After incubation for 24 hrs, the inhibition zone which appeared around the agar well indicated the presence of antimicrobial activity. Then, the zones of inhibition diameter including 10 mm agar well were measured with the aid of a transparent ruler. At the same time, the controlled experiments were prepared with only solvent for the comparison with plant extracts.

Results

Morphological studies

Scientific Name	:	*Cassia occidentalis* L.
Commons Name	:	Coffee Senna
Myanmar Name	:	Kazaw-boke
Family	:	Fabaceae
Subfamily	:	Caesalpinoideae

Flowering and fruiting Period: December to March

Taxonomic description

Annual, herb, stem erect, 1-2 meters long, 0.5-1.5 cm. thickness at its basal region, branching at nodes spirally. Young stem is green in colour and furrowed, while the mature stem is light brown to dark in colour. Branches many, ascending and smooth. The internode is 2-4 cm. long. Leaves: alternate, paripinnately compound, 9-13-20cm. long, petiolate, petiole (rachis) pulvinate, grooved or nearly round, glabrous, 5-12cm. long, showing dark purplish colour in the grooved portion and greenish on the opposite side. Leaflets:3-5 pairs, opposite, unequal, the lower most smallest and ovate, the superior ones longer, 2.5-8 cm. broad, very short stalk, ovate, oblong to ovate, lanceolate, acute or acuminate, base usually rounded and somewhat oblique, glabrous above and pubescent beneath. The leaves possess a very foetid odour. Inflorescences: racemes few –flowered, axillary, and also forming terminal panicle; bracts caduceus. Flower: Yellow, 1 to 2 cm in diameter, complete, bisexual, regular, actinomorphic, hypogynous. Calyx: sepals 5, free, oblong, glabrous, brownish green. Corolla: petals 5, free, obovate, shortly clawed, yellow. Androecium: stamens 10, free, 6-fertile, 4-sterile, inserted; filaments filiform unequal,

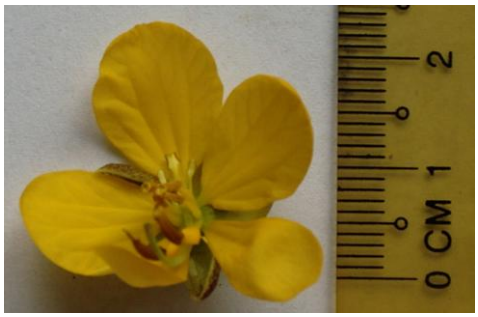
yellow, glabrous; anther dithecous, basifixed, curved, yellow, dehiscent by apical pores. Gynoecium: carpel 1, linear, pubescent, unilocular, many ovules in each locule, marginal placentation; style long filiform, curved, glabrous; stigma capitate. Fruit: flat pods 10-12 cm. long with 10-30 seeds. Areolate seeds are pointed at end and blunt at the other (as shown in Figure.1).

Habit

leaf

Pulvinate petiole

Inflorescence



Flower

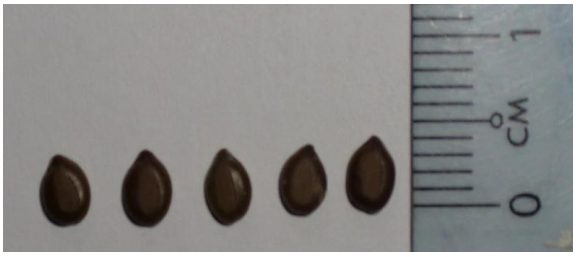
Sepal

Petal

Stamen

ovary

Fruits



Seeds

Figure 1 Morphological characters of *Cassia occidentalis* L.

Preliminary phytochemical investigation

Preliminary phytochemical test on the leaves of *Cassia occidentalis* L. was investigated and the presence or absence of phytochemical constituents in this plant were presented in Table 1 and Figure 2.

Table 1 phytochemical test on the leaves of *Cassia occidentalis* L.

No.	Constituents	Extract	Test Reagent	Observation	Result
1.	Alkaloid	3% HCL	Mayer's reagent Hager reagent Wagner's reagent.	White ppt. Yellow ppt. Reddish Brown ppt.	+
2.	Glycoside	EtOH	1 ml of water and NaOH sol:	Yellow colour	+
3..	Phenolic compound	H_2O	3% ferric chloride sol:	green colour	+
4.	Flavonoid	EtOH	HCL, Mg turning	Pink colour	+
5.	Steroid	EtOH	$CHCL_3$+conc. H_2SO_4	Green colour	+
6.	α- amino acid	H_2O	Dry and sprayed with Ninhydrin reagent	Pink spot	
7.	Terpenoid	EtOH	$CHCL_3$+ conc. H_2SO_4	Pink colour	+
8.	Starch	H_2O	I_2 solution	blue-black ppt.	+
9.	Reducing sugar	H_2O	Benedict Solution	Brick red ppt.	+
10.	Saponin	H_2O	Distilled water	Frothing	+
11.	Tannin	H_2O	5% ferric chloride sol:+ dil H_2SO_4	Yellowish-brown ppt.	+
12.	Carbohydrate	H_2O	Fehling sol: A+B	Red colour ppt.	+
13.	Protein	H_2O	Sodium hydroxide sol: + 3% copper sulphate sol:	Red or violet colour	+

+ = Present – = Absent

According to the present study, it is found that the leaves of *Cassia occidentalis* L. contained alkaloid, flavonoid, steroid, terpenoid, glycoside, carbohydrate, saponin, tannin, resin, polyphenol, protein and starch. These tests were shown in Figure 2.

Figure 2 Preliminary phytochemical test of the leaves of *Cassia occidentalis* L.

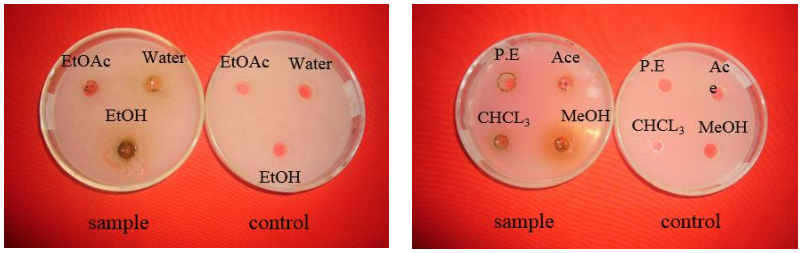
Antimicrobial activity

Screening of antimicrobial activity of leaves of *Cassia occidentalis* L. was carried out by using different solvents namely pet-ether, chloroform, methanol, acetone, ethyl acetate, ethanol and water. The diameter of inhibition zones that appeared were given in Table 2.

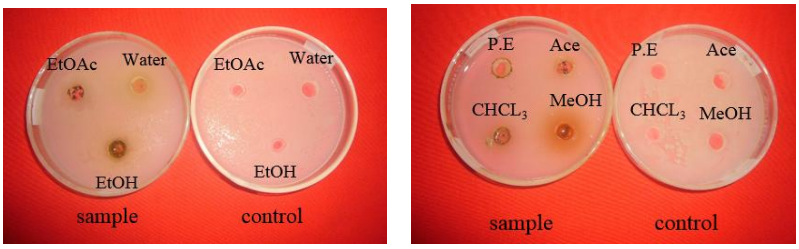
Table 2 Inhibition zone exhibited by different extracts of leaves of *Cassia occidentalis* L. against six microorganisms

No.	Extract	Microorganisms					
		B. subtilis	***S. aureus***	***P. aeruginosa***	***B. pumilus***	***C. albican***	***E. coli***
1.	Pet-ether	-	-	-	-	-	-
2.	Chloroform	-	-	-	14 mm	12 mm	-
3.	Methanol	14 mm	14 mm	13 mm	12 mm	13 mm	14 mm
4.	Acetone	14 mm	15 mm	15 mm	14 mm	14 mm	12 mm
5.	Ethyl acetate	-	-	30 mm	-	-	14 mm
6.	Ethanol	14 mm	13 mm	-	-	11 mm	13 mm
7.	water	12 mm	-	-	-	11 mm	-

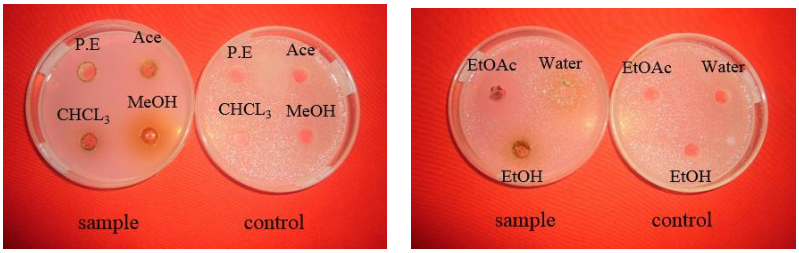
Agar well = 10 mm - = No activity



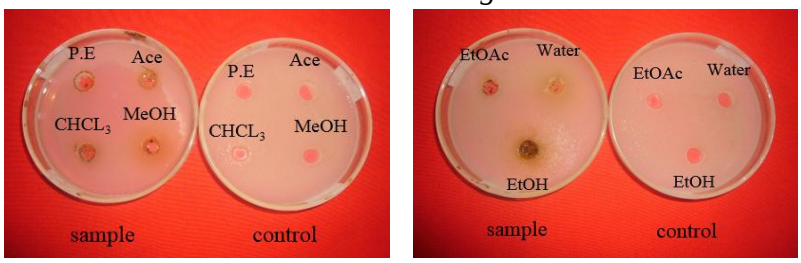
Bacillus subtilis



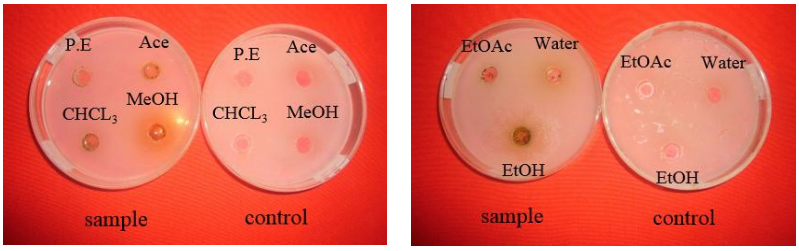
Staphylococcus aureus



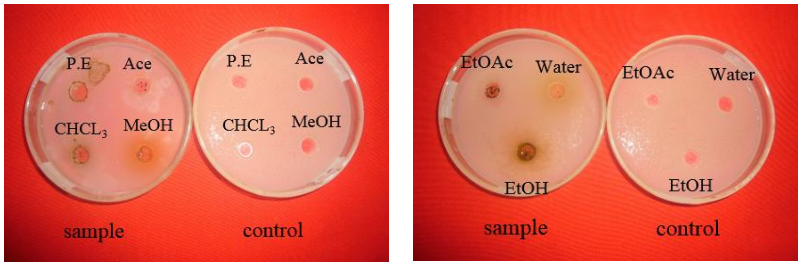
Pseudomonas aeruginosa



Bacillus pumalis



Candida albicans



Escherichia coli

Figure 3 Antimicrobial test of different solvent extracts from leaves of *Cassia occidentalis* L.

In this experiment, pet-ether extract was found to be insensitive to all tested organisms. All other extracts did not show significantly the antimicrobial activity on tested organisms except that ethyl acetate extract showed the highest activity especially more sensitive against *P. aeruginosa* (inhibition zone 30 mm). Controlled experiment containing only solvent did not show inhibitory activities on any of the organisms as shown in Figure 3.

Discussion and Conclusion

Research on *Cassia occidentalis* L. was made from two aspects such as phytochemical study and antimicrobial study about diseases caused by microorganisms. The specimens were collected from Banmaw Township, Kachin State. It is an erect herb and commonly found in road sides. It belongs to the family Fabaceae. The common name is Kazaw Boke. This plant has been widely used as traditional medicine. Entire parts of the plant have medicinal values. The roots, leaves and seeds are the parts of the plant used.

Crude drugs are usually obtained from wild sources and are mostly collected by illiterate and unskilled people unaware of their botanical information, authentication and standardization parameters. This usually affects the safety of the final product. For safe and efficacious herbal medicine production, appropriate control of starting material is extremely crucial (Kumar, 2014). *Cassia occidentalis* is a plant with potentially limitless uses and is of importance to properly establish a partial monograph for its correct identification. The morphological characters of *Cassia occidentalis* mentioned in result were in accordance with those described by Backer (1968), Burkill (1935), Hooker (1881), Dassanayake (1981) and Hu-Qi-ming (2009).

The preliminary phytochemical test revealed that Alkaloid, flavonoid, steroid, terpenoid, glycoside, carbohydrate, saponin, tannin, resin, polyphenol, protein and starch are present in leaves of *Cassia occidentalis*. The result of this study indicated that the leaves of this plant contain some major bioactive compounds needed for organisms. So, this plant proved very active.

In the antimicrobial activity, the leaves of *Cassia occidentalis* were extracted with different solvents. The extracts were used to carry out antimicrobial screening on *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albican* and *E. coli*. The result showed that pet-ether extracts are not effective against all of the test organisms and methanol and acetone extract are effective against all of the test organisms. The highest activity (zone of inhibition in diameter is about 30 mm) was demonstrated by the ethyl acetate extract against *Pseudomonas aeruginosa*. Curickshank (1975) stated that *Pseudomonas aeruginosa* causes urinary tract infection, respiratory system infection, bone and joint infection, chronic lung, eye infection, burn infection. Therefore, it is recommended that the different components detected in leaves of this plant should be isolated and tested against the susceptible microorganism (*Pseudomonas aeruginosa*) in order to arrive at the most potent structure. Further in-depth research has to be carried out to use the phytochemicals in pharmaceutical industry as a substitute for medicine.

Acknowledgements

I would like to express to my gratitute to Professor Dr. Pyone Yi, Head of Botany Department, Banmaw University, for providing all departmental facilities and valuable suggestions. Personally, my special thanks are due to Professor Dr. Myat Myat Ku, Head of Botany Department, Banmaw University for her valuable leading toward the successful completion of this research.

References

Backer, C.A. and R.C.B. Van Den Brink, (1965). **Flora of Java**.Vol: II, The Netherlands N.V.P. Noondhoff-Groningen.

Burkill, I.H.M.A., F.L.S. (1935). **A Dictonary of the Economic Products of the Malay Peninsula**. Government of the straits settlements and Federated Malay States by the Crown Agents for the Colonies Millbank, London.

Burkill, H. M. (1995). **The useful plants of West Tropical Africa**. Royal botanic garden kewl (u,k)

British Pharmacopeia, (1968). Published Under the Direction of the General Medical Council. Medicinal Act: 1956. Lodon. Willian Clowes and Sons Limited.

Cronquist, A. (1981). **An integrated system of classification of flowering plants**. Columbian university press: New York.

Cruickshank, R. (1975). **Medicinal Microbiology**. Churchill Living Stone Ltd., London.

Dassanayake, M.D. and W.D. Claylon, (1981). **Flora of Cyelon**, Vol.III, A .A. Balkema / Rotterdam/Brookfield.

Hooker, J.D. (1881). **Flora British India**. Vol.I. Reeve & Co.,Ltd. London.

Hu-Qi-ming. (2009). **Flora of Hong Kong**, Vol.III, Hong Kong Herbarium, Agriculture, Fisheries Conserval on Department.

Hundley and Chit KoKo. (1987). **List of Trees, Shrubs, Herbs and Principal Climbers, etc**, Government Printing Press, Yangon.

Harborne, J.B. (1973). **Phytochemical Methods A Guide to Modern Techniques of Plant Analysis**. London New York: Chempman and Hall.

Kirtikar, K.R., Basu B.D. (1965). **In : Ocimum sanctum in Indian Medicinal Plants**, LB Basu, Affahabad.

Kress and Yin Yin Kyi, Daw. (2003). **A Checklist of the Trees, Shrubs, Herbs and Climbers of Myanmar**, Department of Systematic Biology - Botany, National Museum of Natural History Washington, DC.

Kumar D, Kumar A, Prakash O. (2014). Pharmacognostic Investigation of Clerodendrum phlomidis Linn. f. Root. *The Journal of Tropical Life Science*. 4(2)-96-100.

Kunle O. F and Egharevba, H.O. (2009). **Preliminary studies on vernonia ambigua : Prhytochemistry and Antimicrobial screening of the whole plant.** Ethnobotanical Leaflets, 13: 1216-21.

Lawrence, G.H.M. (1951). **Taxonomy of Vascular Plants**, Macmillan C., New York.

Marini Bettolo, G. B., M. Nicolet tic and M. Patmia. (1981). Plant Screening by Chemical Chromatographic Procedure Under Field Conditions. *Journal of Chromatogram*.

Nuhu AA and R Aliyu. (2008). Effects of Cassia occidentalis aqueous leaf extract on biochemical markers of tissue damage in rats Tropical. *Journal of Pharmaceutical Research*, 7 (4): 1137-1142

Trease, G.E. and W.C. Evans. (2002). **Pharmacognosy**. 15th Ed., Harcourt Publishers Limited. Landon.

World Health Organization. (1990). **Medicinal Plants in Vietnam**. Western Pacific Manila: WHO Regional Office.

MORPHOLOGICAL CHARACTERS AND PHYTOCHEMICAL INVESTIGATION OF *CAPSICUM FRUTESCENS* L.(LEAVES) AND ITS ANTIMICROBIAL ACTIVITY

Sandar Sann[1]

Abstract

A medicinal plant *Capsicum frutescens* L. was selected for the present study. Myanmar name Moe- myaw-ngayoke belongs to the family Solanaceae. The specimens were collected from Nampha quarter, Banmaw Township in Kachin State from January to September, 2018. The collected plants were identified by the literature references to confirm its identity. The present research was conducted to study the morphological characters, qualitative analysis and antimicrobial activity of the *Capsicum frutescens* L. In morphological study, inflorescences were axillary and terminal cymes and flowers were pentamerous and hypogynous. In qualitative analysis, alkaloids, glycosides, flavonoids, phenolic compounds, steroids, starch, protein, reducing sugar, α-mino acids and carbohydrates were found to be present in the leaves of *Capsicum frutescens* L. In antimicrobial tests, the various solvent extracts of powdered leaves were tested on six microorganisms by using agar well diffusion methods at the CRDC. In this experiment, ethyl acetate extract of leaves showed the highest activity especially more sensitive against *Bacillus subtilis, Staphylococcus aureus, Bacillus pumalis and Candida albicans.* Therefore, the leaves of *Capsicum frutescens* L. may serve as a source of natural antimicrobial agents to be used in food and medicinal purposes.

Keywords: *Capsicum frutescens*, phytochemicals, and antimicrobial properties

Introduction

The family Solanaceae is one of the most important families of flowering plants economically, floristically, ethnobotanically and scientifically (Perry, 1980).

Solanaceae is a family of about 94 genera and more than 2950 species, also called as nightshades or potato family. It contains many species of economic use, such as food: tomatoes, potatoes, pepper and eggplants (Miller, 1754). A medicinal plant *Capsicum frutescens* L. belongs to the family Solanaceae. The specimens were collected from Nampha quarter, Banmaw Township in Kachin State.

Capsicum frutescens L. is currently native to the majority of central America as well as Northern and Western South America. It spread quickly throughout the tropical and subtropical regions and still grows wild today (Mabberley, 1987). The plant *C. frutesces* is used for various problems with digestion including upset stomach, intestinal gas, stomach pain, diarrhea, and cramps. It is also used for conditions of the heart and blood vessels including poor circulation, excessive blood clotting, high cholesterol, and preventing heart disease (Rose koffi-Nevry, et. al 2012). In Myanmar, it is used for indigestion, anorexia, obesity, cough, fever, edema, ulcers, arthritis, bronchitis and work as antibiotic (Ashin-na-ga-thein, 1976).

In the present study, morphological characters, preliminary phytochemical investigation and antimicrobial studies had been undertaken. As a result, leaves of *C. frutescens* L. revealed the presence of important active constituents and antimicrobial properties. Thus the leaves of *C. frutescens* L. may serve as a source of natural antimicrobial agents to be used in food and medicinal systems.

[1] Dr, Associate Professor, Department of Botany, Banmaw University

Therefore, the aim of this study is to find the medicinal plant scientifically which has effective medicinal values and also to investigate the active constituents of the medicinal plant, to evaluate their specific values and to find out the greatest activity of leaves extracts on six pathogenic microorganisms.

Materials and Methods

Morphological study of *Capsicum frutescens* L.

Capsicum frutescens L. used in this study were collected from Nampah quarter, Banmaw Township. For the identification of their morphological characters, the vegetative and reproductive parts of the plant were selected and collected at their flowering period to fruits and seeds. The specimens were identified and confirmed with the help of literature cited in Hooker (1875-1897), Johnson (1934), Kirtikar and Basu (1935), Cooke (1958), Rendle (1967), Roxburgh (1971), and Kress et. al (2003).

The collected specimens were properly dried, crushed and pounded into powdered form. This powder was stored in the airtight container for another study.

Preliminary phytochemical test

The preliminary photochemical investigation on the powdered leaves of *Capsicum frutescens* L. was carried out to determine the presence or absence of alkaloids, glycosides, phenolic compounds, flavonoids, steroids, terpenoids, α-amino acids, starch, reducing sugar, saponins, tannins, carbohydrates and protein. The methods of Marini Bettalo G.B. et. al (1981), and Trease and Evans (2002) were applied for investigation of phytochemicals. These experiments were carried out at the Department of Botany, Banmaw University.

Antimicrobial activity of different solvent extracts from leaves of *Capsicum frutescens* L.

Extraction and examination of antimicrobial activity

The dried powder sample of leaves was extracted with pet-ether, chloroform, ethyl acetate, acetone, ethanol, methanol and water. The various solvents extracts of leaves were tested on six pathogenic microorganisms such as *Bacillus pumalis, Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*.These experiments were carried out at the Central Research and Development Centre.

The study of antimicrobial activities was performed by using agar-well diffusion method according to Cruickshank (1975). Nutrient agar was prepared and boiled, and then 20-25 ml of the medium was poured into a test-tube and plugged with cotton wool and autoclaved at 121°C for 15 minutes. Then the tubes were cooled down at (30-35°C) and the medium was poured into sterilized petridishes and 0.1-0.2 ml of test organisms were also added into the dishes. The agar was allowed to set for 2-3 hours. After this, 10 mm agar well was punched with the help of sterilized cork borer. After that, about 0.2 ml of sample was introduced into the agar well and incubated at 37°C for 24 hours. The inhibitory zone appeared around the agar well, indicating possesses of antimicrobial activity. Then, the diameter of inhibitory zone including 10 mm agar well were measured with the help of a transparent ruler.

Similarly, the controlled experiments using solvent only were prepared for the comparison with plant extracts.

Results

Morphological studies

Scientific name - *Capsicum frutescens* L.

Myanmar name - Moe-myaw-ngayoke

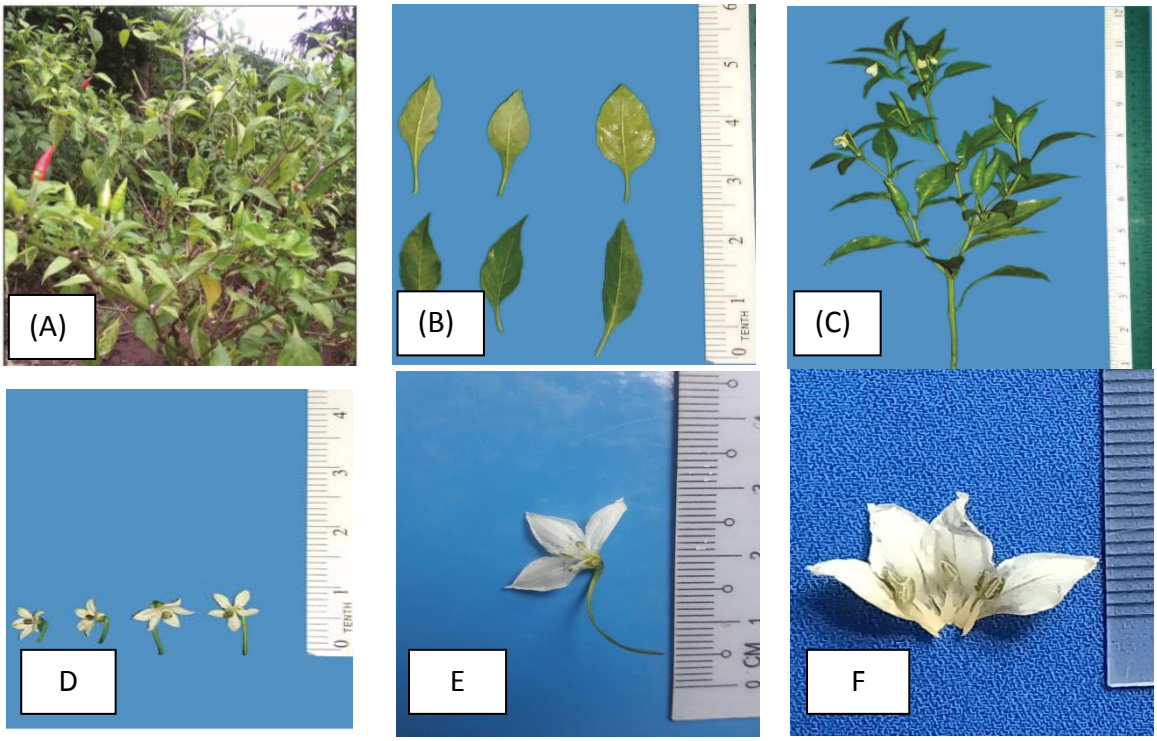
English name - Red pepper

Family - Solanaceae

Useful parts - Fruits, leaves, roots

Distinctive characters of *Capsicum frutescens* L.

Perennial, shrubby herbs, about 130 cm high; stems and branches angular with longitudinal ridges, erect, stout, glabrous, green. Leaves simple, alternate, ovate, 2-5 cm long × 1-3 cm wide; tip acute, margin entire; base oblique; glabrous, petioles 0.5 – 3 cm long, exstipulate. Inflorescences axillary and terminal cymes with up to 3 flowers; peduncle about 0.1 cm long. Flowers ebracteate, ebracteolate, complete, bisexual, regular, actinomorphic, pentamerous, cyclic, pedicel about 1 cm long; hypogynous. Sepals (5), synsepalous, cup-shaped about 1 cm long, valvate, sepaloid, persistent, pubescent, inferior. Petals (5), synpetalous, campanulate about 1 cm long, valvate, petaloid (greenish white), inferior. Stamens 5, free; filament about 6 mm long; pale-green, epipetalous; anther oblongoid about 4 mm long, dithecous, introrse, basifixed, longitudinal-slit; inferior. Ovary ovoid, oblique about 2 mm long, glabrous; bicarpellary, syncarpous, bilocular, axile placentation, many ovules in each locule; style filiform about 7 mm long; slightly curved at the apex; stigma simple, green; superior. Berries, oblongoid, 3 cm long × 0.5 cm wide, green, red at the maturity. Seeds numerous, compressed, discoid, yellow, endospermic. Flowering and fruiting almost throughout the year. The results were shown in figure (1).



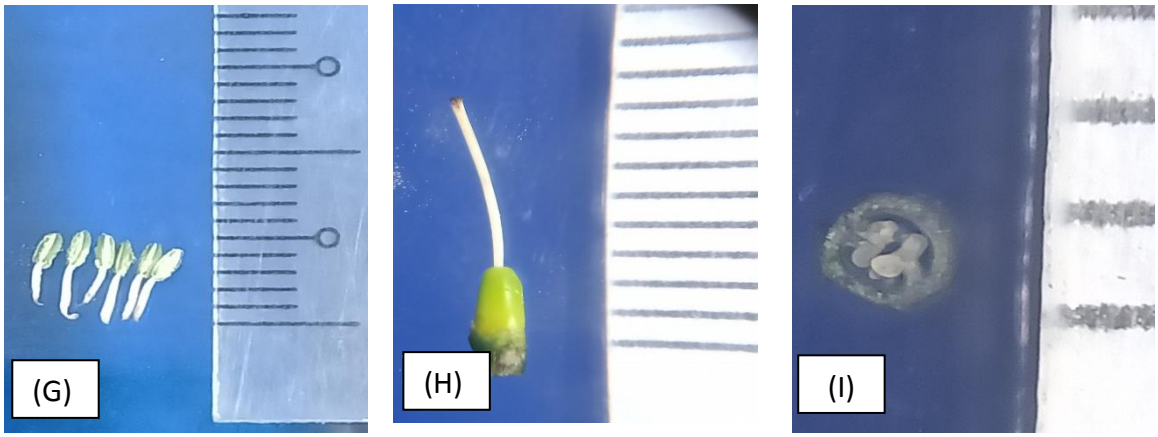


Figure 1 Morphological characters of *Capsicum Frutescens* L.

(A) Habit (B) Leaves (upper and lower surfaces)
(C) Inflorescence (D) Flowers
(E) L.S of flowers (F) Corolla with stamens
(G) Stamens (H) Gynoecium
(I) T.S Ovary

Preliminary photochemical investigation of leaves of *Capsicum frutescens* L.

The preliminary phytochemical investigation was carried out on the powdered leaves to determine the present or absent of alkaloids, glycosides, phenolic compounds, flavonoids, steroids, terpenoids, α-amino acids, starch, reducing sugar, tannins, saponins, carbohydrates and protein. The results were shown in figure (2) and table (1).

Table 1 Preliminary phytochemical test of leaves of *Capsicum frutescens* L.

No	Constituents	Extract	Test Reagents	Observation	Remark
1.	Alkaloids	3%HCL	1. Mayer's reagent 2. Hager's reagent 3. Wagner's reagent	White ppt Yellow ppt Reddish Brown ppt	+
2.	Glycosides	Ethanol EtOH	1 ml of water and sodium hydroxide	Yellow Colour	+
3.	Phenolic compounds	H_2O	3% Ferric chloride solution	Green Colour	+
4.	Flavonoids	Ethanol EtOH	Small pieces of Mg, few drops of HCl	Pink Colour	+
5.	Steroids	Ethanol EtOH	$CHCL_3$ and Conc: H_2SO_4	Green	+
6.	α-amino acids	H_2O	Dry and sprayed with Ninhydrinreagent and kept in over at 110°C	Pink Spot	+

No	Constituents	Extract	Test Reagents	Observation	Remark
7.	Terpenoids	Ethanol EtOH	$CHCL_3$ and Conc: H_2SO_4	Pink	+
8.	Starch	H_2O	Iodine Solution	Bluish black ppt	+
9.	Reducing sugar	H_2O	Benedict Solution	Brick red ppt	+
10.	Saponins	H_2O	Distilled Water	Frothing	+
11.	Tannins	H_2O	5%Ferric Chloride solution and sulphuric acid	No Yellowish brown ppt	-
12.	Carbohydrates	H_2O	1 ml of a mixture of equal parts of felling's solution A and B	Brick red ppt	+
13.	Protein	H_2O	NaOH Sol: and 3% $CuSO_4$ Sol:	Red or violet colour	+

(+) Presence (-) Absence

The tests indicated that, alkaloids, glycosides, phenolic compounds, flavonoids, steroids, terpenoids, α-amino acids, starch, reducing sugar, saponins, carbohydrates and protein were found to be present and tannin was absent in the leaves of *Capsicum frutescens* L.

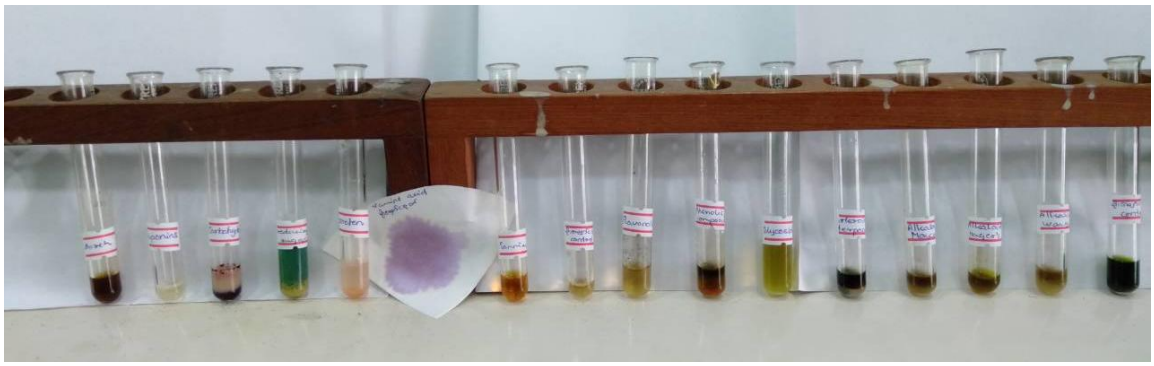


Figure 2 Preliminary phytochemical investigation of leaves of *Capsicum frutescens* L.

Antimicrobial activity of various solvent extracts of *Capsicum frutescens* L.

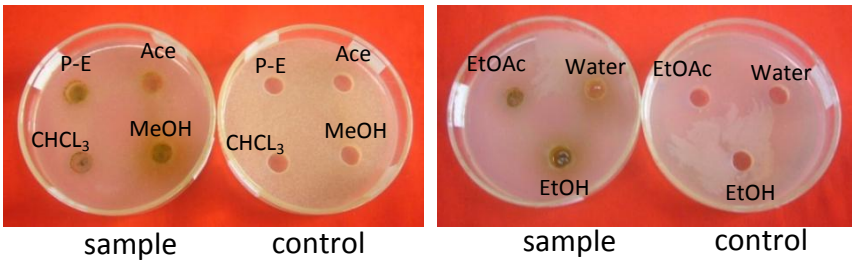
Antimicrobial activity of various solvent extracts such as pet-ether , chloroform, ethyl acetate, acetone, ethanol, methanol and aqueous extract were tested on six microorganisms. The results were shown in Table (2) and Figure (3).

Table 2Antimicrobial activity of various solvent extracts of leaves of *Capsicum frutescens* L.

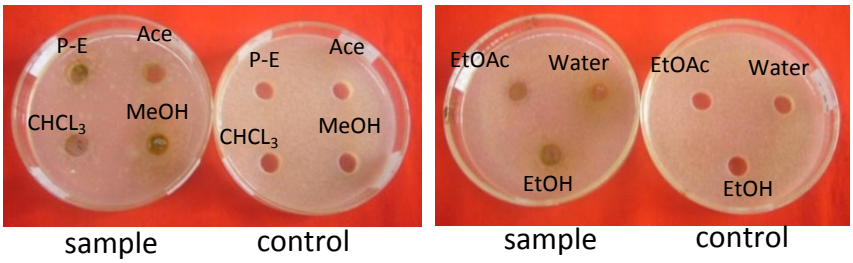
Sample	Solvents	*B.subtilis*	*S.aureus*	*P.aeruginosa*	*B.pumilis*	*C.albicans*	*E.coli*
Leaves	Pet-ether	-	-	-	-	-	-
	$CHCl_3$	-	-	-	11mm	-	11mm
	MeOH	-	-	-	12mm	19mm	12mm
	Acetone	-	-	-	12mm	19mm	-
	EtOAc	19mm	19mm	12mm	19mm	19mm	-
	EtOH	19mm	13mm	13mm	19mm	13mm	-
	Water	-	-	19mm	-	-	-

Agar well - 10 mm

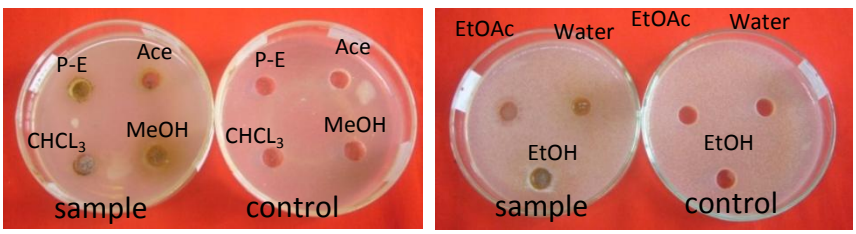
In this experiment, methanol and acetone extracts of leaves showed the highest activity especially more sensitive against *Candida albicans*. Ethyl acetate extract of leaves showed the highest activity especially more sensitive against *Bacillus subtilis, Staphylococcus aureus, Bacillus pumilis and Candida albicans*. Ethanol extract of leaves showed the highest activity on *Bacillus subtilis* and *Bacillus pumilis*. Moreover aqueous extract of leaves showed the greatest activity on *Pseudomonas aeruginosa.*



Bacillus subtilis



Staphylococcus aureus



Pseudomonas aeruginosa

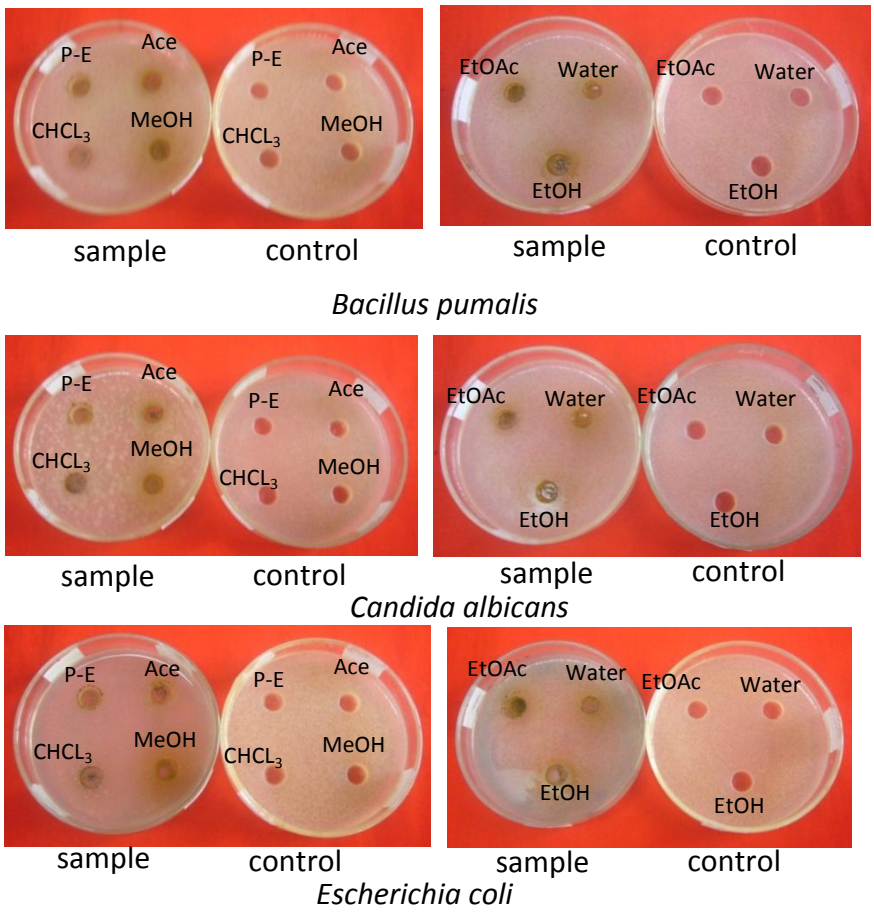


Figure 3 Treatment of various extracts from the leaves of *Capsicum frutescens* L.

Discussion and Conclusion

A medicinal plant *Capsicum frutescens* L. belongs to the family Solanaceae. The specimens were collected from Nampha quarter, Banmaw Township in Kachin State. In the present investigation, the morphological studies on both vegetative and reproductive parts of the plant, preliminary phytochemical analysis and antimicrobial activity of the leaves had been undertaken.

As a results of morphological studies, C. *frutescens* L. was perennial, shrubby herbs; stems and branches angular with longitudinal ridges, erect, glabrous. Leaves were simple, alternate, ovate, tip acute, margin entire; base oblique, petiolate, exstipulate. Inflorescences were axillary and terminal cymes with up to 3 flowers. Flowers were ebracteate, ebracteolate, bisexual, actinomorphic, pentamerous, hypogynous. Sepals were five, synsepalous, valvate, persistent, pubescent, inferior. Petals were five, synpetalous, campanulate, valvate, inferior. Stamens were five, free, epipetalous, anther oblongoid, dithecous, introrse, basifixed, inferior. Ovary was ovoid, oblique, glabrous, bicarpellary, syncarpous, bilocular, axile placentation, style filiform, stigma simple, superior. Fruits were berries, oblongoid. Seeds were numerous, compressed, discoid, endospermic. These characters are in agreement with those mentioned by Hooker (1875-1897), Johnson (1931), Kirtikar and Basu (1935), Cooke (1958), Rendle (1967), Roxburgh (1971), and kress et. al (2003).

The preliminary phytochemical investigation was carried out on the powdered leaves. These tests indicated that the leaves contained alkaloids, glycosides, phenolic compounds, flavonoids, steroids, terpernoids, α-amino acids, starch, reducing sugar, saponins, carbohydrates and protein. Tannin was absent in leaves of *Capsicum frute*scens L.According to Vinayaka (2010), phytochemical analysis revealed saponins, tannis, alkaloids, glycosides and steroids in methanol extract. These compounds are medicines for treating various diseases in human being (Website (1)).

The antimicrobial activity of various solvent extracts such as pet-ether, chloroform, ethyl acetate, acetone, ethanol, methanol and aqueous extract were tested on six microorganisms. In this experiment, methanol and acetone extracts of leaves showed the highest activity especially more sensitive against *Candida albicans*. And then, ethyl acetate extract of leaves showed the greatest activity especially more sensitive against *Bacillus subtilis, Staphylococcus aureus, Bacillus pumilis* and *Candida albicans*. Ethanol extract of leaves showed the highest activity on *Bacillus subtilis* and *Bacillus pumilis.* Finally, aqueous extract of leaves showed only the highest activity on *Pseudomonas aeruginosa* and pet-ether extract of leaves did not show the activity on all test microorganisms. According to Vinayaka (2010), *S.aureus* was found to be more susceptible to the methanolic extract followed by *P.aeruginosa.* From this finding, nevertheless, it can be inferred that leaves of C. *frutescens* L. can be effective in the formulation of medicine for the treatment of disease caused by *S. aureus, B.pumilis, P.aeruginosa*, and *C. albicans* such as wound infections, pneumonia, urinary tract infection, respiratory system infection, soft tissue infections, ear infection, eye infection, skin infections, vaginal candidiasis, sores and ring worm.

Therefore, the results of this present study on morphology can give a few information on the systematic study on a member of the family Solanaceae. Moreover, the leaves of *C.frutescens* L. included many chemical constituents. They are employed for medicinal purposes. And then the leaves of *C.frutescens* L. may serve as a source of natural antimicrobial agents to be used in food and medicinal system. Finally the main objective of the present research work was that *C. frutescens* had medicinal values not only in fruit but also in leaves.

Acknowledgements

I would like to express to my gratitude to Professor Dr. Pyone Yi, Head of the Botany Department, Banmaw University, for providing all departmental facilities and valuable suggestions. Personally, my special thanks are due to Professor Dr. Myat Myat Ku, Department of Botany, Banmaw University for her valuable guidance leading toward the successful completion of this research.

References

Ashin-na-ga-thein. (1976). *Pon-pya-say Abidan*. (vol.1). Myanmar: Mingalar Press, Yangon.

Cooke, T.C.I.E. (1958). *Flora of the presidency of Bombay*, (vol.II). Calcutta shri Gourange Press, Private Ltd.

Cruickshant, R., Duguid, J.P., Marmior, B.P. & Swaim, R.H. (1975). *Medical microbiology*. London: Churchill Livingstone Ltd.

Hooker, J.D. (1875-1897). *The Flora of British India* (vol.VII). L.Reeve and Co.Ltd. London.

Johnson, A.M. (1931). *Taxonomy of the flowering plants*. London: New york.

Kirtikar, K.F. and B.D. Basu, (1935). *Indian Medicinal plants.* (2nd edt.) India: Allalhabad.

Kress, W.J., R.A. Defilipps, E. Farr and Yin Yin Kyi. (2003). *A Check list of the Trees, Shrubs, Herbs and Climbers of Myanmar*. Department of Systematic Biology Botany, National Museum of Natural History, Washington DC: USA.

Marbberley D.J. (1987). *The plant book*. Cambridge University Press; 707.

Marini Bettalo, G.B. (1981). Plant screening by chemical and chromatographic procedure under field condition. *Journal of chromatography*, 31, 14-17.

Miller P. (1754). *The Gardener's dictionary*, Abridged 4th end. Published for the author, London.

Perry, L.M. (1980). *Medicinal plants of East and South East Asia*. MIT Press, Cambridge, Massachusetts, 620 pp.

Rendle, A.B. (1967). *The classification of flowering plants* (vol.II). London. The syndics of the Cambridge University Press.

Rose Koffi-Nevry, (2012). *Antibacterial Activity of Two Bell Pepper Extracts*.

Roxburgh, W.M.D. (1971). *Flora of India or Descriptions of Indian Plants*. New Delhi: Today and Tomorrow's Printers and Publishers, 22 B/5, Original Road.

Trease, G.E. and Evans, W.C. (2002). *Pharmacognosy*. (11th edt). London: Cassell and Collier Macmillan Publishers Ltd.

Vinayaka KS, Nandini KC, Rakshitha MN, Martis Ramva, Shruthi J, Heade Shruthi V. (2010). *Pharmacognosy Journal* 2 (12), 486 – 491.

Website (1) http://www.breastcancer.org>phytochem.

TAXONOMIC STUDY ON NINE SPECIES OF FAMILY FABACEAE IN KYAING TONG UNIVERSITY CAMPUS

Tin Tin Maw[1]

Abstract

The present study deals with the members of family Fabaceae growing in Kyaing Tong University Campus. All species are collected during January to December in 2018. Some Fabaceae from Kyaing Tong University Campus have been collected, identified and then morphological characteristic were studied. In this study, nine species belonging to eight genera of family Fabaceae were identified and systematically arranged with relevant photographs. Artificial key to the species, detail description of the individual species has also been described. In addition, their flowering period, Myanmar names and English names were also described. Comparable characteristic of the species were constructed according to their different characters.

Keywords: Taxonomy, Fabaceae, Kyaing Tong University Campus

Introduction

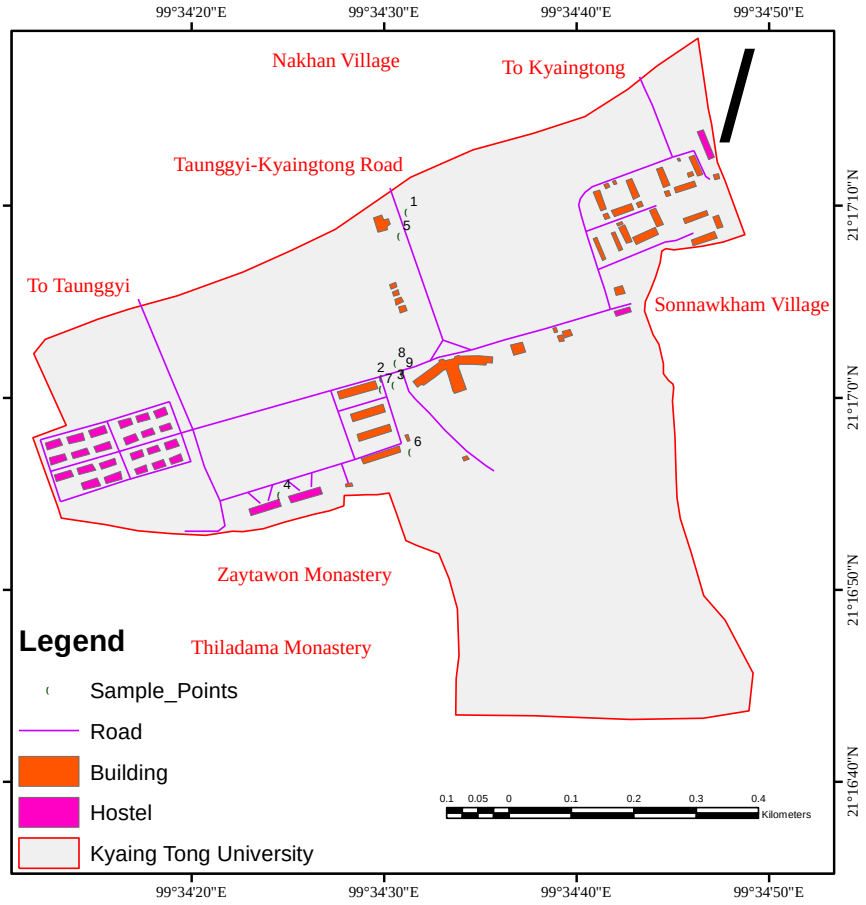
Kyaing Tong Township is situated in Golden Triangle of Eastern Shan State of Myanmar. Kyaing Tong University Campus is located in Kyaing Tong Township. Kyaing Tong University Campus is bounded by Soannawkham village in the east, Taunggyi-Kyaing Tong Road in the west, Zaytawon monastery and Thiladama monastery in the south and Nahkam village in the north. It lies between 21°16'40"-21°17'20" North Latitude and 99°34'10"-99°34'50" East Longitude. Kyaing Tong University Campus lies 800 meter above sea level. The area is about 0.72 kilometer square.

During the period from January to December 2018, an average monthly rainfall is 23.31 inches and 10 rainy days. This area almost gets minimum rain fall in January and February. The average maximum temperature is 28.78° C and average minimum temperature is 17.18° C. The coldest month of this area is February (10.6° C). The warmest month is April (32.4° C). The maximum percentage of humidity in August is 88 and the minimum percentage of humidity in February is 45. The predominant bedrocks are limestone. The major soil types are red-gray and yellow-grey sandy soils cover with mountain area and alluvial soils cover flat land and low land area. Kyaing Tong University Campus is in the mountain deciduous forest region.

The Fabaceae or Leguminosae commonly known as the legume, pea, or bean family are large and economically important family of flowering plants. It includes trees, shrubs and perennial or annual herbaceous plants, which are easily recognized by their fruit (legume) and their compound, stipulate leaves. Many legumes have characteristic flowers and fruits. The family is widely distributed and is the third-largest land plant family in terms of number species, with about 751 genera and 19,000 known species (http//en.m.wikipedia.org > wiki > Fabac.). Kress *et. al*. (2003) recorded 84 genera and 509 species in the checklist of Myanmar. In the present study 9 species belonging to 8 genera of family Fabaceae had been identified and fully described. *Indigofera linnaei* Ali. was commonly found in this area. *Crotalaria juncea* L. was rarely found in the study area.

[1] Dr, Associate Professor, Department of Botany, Kyaing Tong University

The aims and objectives of the present research are mainly to record the knowledge on the natural resources in study area, to give valuable information of Fabaceae to other researchers and to provide a partial fulfillment of the family Fabaceae in Eastern Shan State of Myanmar.



1. *Crotalaria juncea* L.
2. *Desmodium gangeticum* (L.) DC.
3. *Desmodium heterocarpon* (L.) DC.
4. *Indigofera linnaei* Ali.
5. *Millettia macrostachya* Collett & Hemsley
6. *Pueraria montana* (Lour.) Merr.
7. *Smithia conferta* Smith.
8. *Tadehagi triquetrum* (DC.) Ohashi
9. *Vigna umbellata* (Thunb.) Ohwi & Ohashi

Figure 1 Location map of Kyaing Tong University Campus

(**Source:** Department of Geography, Kyaing Tong University)

Materials and Methods

Some members of Fabaceae were collected from Kyaing Tong University Campus. The specimens were collected from January to December, 2018. The specimens were kept immediately into the plastic bags to identify and classify systematically. The collected specimens

had been observed and noted in detail. In addition to construction of artificial key to the species, all the resulting species are systematically arranged into genera according to alphabetically. The specimens were recorded by photographs. The collected specimens were identified with the references of Flora of British India, Vol. 2 (Hooker, 1879), Flora of Java (Backer, 1965), Flora of Ceylon, Vol. 7 and Vol. 10 (Dassanyake, 1991 and 1996), Flora of Hong Kong, Vol.2 (Anonymous, 2008), Flora of China (Wu. *et.al*., 2010) and Flora of West Pakistan, (Ali. and Nasir, 1973-1977)

Results

Table 1 List of the collected species of Fabaceae (Subclass : Magnoliidae)

Order	Family	Scientific names	Myanmar names
Fabales	Fabaceae	1. *Crotalaria juncea* L.	Pan-paik-san
		2. *Desmodium gangeticum* (L.) DC.	Than-byet-gyi
		3. *Desmodium heterocarpon* (L.) DC.	Unknown
		4. *Indigofera linnaei* Ali.	Meyaing
		5. *Millettia macrostachya* Collett & Hemsley	Ye-thinwin
		6. *Pueraria montana* (Lour.) Merr.	Unknown
		7. *Smithia conferta* Smith.	Unknown
		8. *Tadehagi triquetrum* (DC.) Ohashi	Lauk-the
		9.*Vigna umbellata* (Thunb.) Ohwi & Ohashi	Unknown

Taxonomic descriptions

Fabaceae (Lindl. 1836)

1. *Crotalaria juncea* L. Sp. Pl. 2: 714.1753.

Myanmar name : Pan-paik-san

English name : Sunn hemp; India hemp

Flowering period : December to May

Annual erect herbs, 2.5 m high; stems and branches terete, ribbed, appressed silky pubescent. Leaves unifoliate compound, alternate; stipules linear, 2.5 mm long, caducous; petioles 3-5 mm long; blades oblong to linear-lanceolate, 6-9.5 cm by 1.5-3 cm, attenuate at the base, entire along the margin, acuminate and mucronate at the apex, pilose on both surfaces, more densely beneath. Inflorescences terminal racemes, 10-20-flowered, 25- 30 cm long; peduncles 10-16 cm long. Flowers yellow, 2-2.2 cm in diameter, bracts linear, 3-5 mm long, persistent; bracteoles linear acuminate, 2-4 mm long, inserted at base of calyx tube, pubescent; pedicels 5-8 mm long, tomemtose. Calyx bilabiate, 5-lobed, densely rusty pilose; tube 5-7 mm long; lobes lanceolate, 1.3-1.5 cm long, curved. Corolla papilionaceous, exserted, tinged with reddish; standard suborbicular, 1.8-2 cm by 1.5-2 cm, with 2 appendages at base, clawed; wings obovate-oblong, 1.5-2 cm by 0.9 cm; keels falcate, 1.8-2.1 cm by 1 cm, twisted beak. Stamens

10, monadelphous; filaments 1-1.5 cm long; anthers dithecous, dimorphic; articulate anthers 2 mm long; ovoid anthers 1 mm long. Ovary oblong, 7-9 mm long, hairy, unilocular, with many ovules on the marginal placentae; style 4-4.5 mm long, incurved, ciliated along both sutures; stigma simple. Pods sessile, oblong-cylindrical, 2.5-3.5 cm by 1-1.5 cm, 8 to 15-seeded, inflated, rusty pubescent. Seeds obliquely-cordiform, 4-6 mm long, light brown to black, smooth to papillose. (Figure 2. A)

Distribution : A native of India but widely distributed elsewhere in the tropics (Rudd as cited in Dassanayake 1991). According to Kress *et. al.* (2003), this species was distribution in Ayeyarwady Division, Bago Division, Mon State, and Yangon Division of Myanmar.

Specimen examined : Eastern Shan State; Kyaing Tong Township, Kyaing Tong University Campus, N 21°17'9.66", E 99° 34'31.20", 803 meter, Dr. Tin Tin Maw, 20.3.2018, collected no. 5.

2. *Desmodium gangeticum* (L.) DC., Prod. 2: 327. 1825.

Hedysarum gangeticum L., Sp. Pl. 746. 1753.

Myanmar name : Kyemi-hpo, Than-byet-gyi

English name : Sal leaves desmodium

Flowering period : October to December

Erect to ascending shrubs, to 1.5 m high, stems stout, woody, slightly angular, short grey hairy, much branched, densely downy while young. Leaves unifoliate compound, alternate, stipules subulate, 0.7-1.5 cm long, sparsely white long hairy, persistent; petioles 1-2 cm long, canaliculate above, appressed grey hairy; leaflets ovate-oblong or broadly elliptic, 2.5-8 cm by 2-5 cm, obtuse at the base, entire and ciliate along the margin, acuminate at the apex, subcoriaceous, puberulous above, sparsely appressed grey hairy beneath. Inflorescences axillary or terminal racemes or panicle, many flowered, 15-30 cm long; peduncles 1.5-3.5 cm long, densely white hairy. Flowers 2-5 in a fascicle, white or purple, 4-5 mm in diameter; bracts setaceous, about 5 mm long, fugacious, white long hairy; pedicels about 2-4 mm long, densely hooked hairy. Calyx campanulate, 5-lobed, densely hooked hairy; tube about 2 mm long; lobes setaceous, longer than the tube. Corolla papilionaceous; standard obovate, about 4 by 4 mm, broadly rounded at the apex; wings about 5 mm by 3 mm; keels about 6 mm by 4 mm. Stamens 10 (9+1), diadelphous; free filaments about 3 mm long; anthers dithecous, uniform. Ovary oblong, about 2.5-4 mm long, sparsely white hooked hairy; style filiform; stigma capitate. Pods lomentum, 6 to 8 jointed, falcate, 10 cm by 3 mm, compressed, glabrous or minute hooked hairy, the upper suture straight, the lower deeply indented. (Figure 2. B)

Distribution : Old world tropics; Africa, Ceylon, India, Indo-China, southern China, Malaysia, northern Australia, naturalized in the West Indies (Rudd as cited in Dassanayake 1996). Kress *et. al.* (2003) noted that this species was widely distributed in the checklist of Myanmar.

Specimen examined : Eastern Shan State; Kyaing Tong Township, Kyaing Tong University Campus, N 21°17'1.08", E 99°34' 29.88", 810 meter, Dr. Tin Tin Maw, 15.12.2018, collected no. 9.

3. *Desmodium heterocarpon* (L.) DC., Prod. 2: 337. 1825.

Hedysarum heterocarpon L., Sp. Pl. 747. 1753.

Myanmar name : Unknown

English name : Asian tick trefoil; carpon desmodium; tick clover

Flowering period : July to October

Perennial, prostrate herbs or subshrubs, 30-150 cm high; stems and branches terete, much branched from base of stem, more or less appressed hairs. Leaves trifoliate compound, alternate; stipules subulate, 5 mm long; petioles 1.5-2 cm long; leaflets obovate, elliptic or oblong 1.5-6 cm by 1-3 cm,obtuse or rounded at the base, entire along the margin, rounded or obtuse, emarginate, mucronate at the apex, glabrous above, appressed pubescent beneath. Inflorescences terminal or axillary dense racemes, many flowered, 2.5-7 cm long, white uncinate hairs or yellowish or white straight appressed hairs; peduncles 1-2.5 cm long. Flowers purple, 5-6.5 mm in diameter, usually in pairs; bracts ovate, long acuminate, 3-6 mm long, concave, hairs; pedicels 3-7 mm long. Calyx campanulate, 4-lobed, sparsely pubescent; tube 1.5-2 mm long; lobes longer than the tube. Corolla papilionaceous, exserted, standard obovate, 5-6 mm by 5-6 mm, shortly clawed; wings obovate, 4-5 mm long, clawed, auriculate; keels oblong, 5-6 mm long, distinctly curved, obtuseat the apex. Stamens 10 (9+1), diadelphous; free filaments 3-4 mm long, anthers dithecous, uniform. Ovary narrowly oblong, 3-4 mm long, pubescent, unilocular, with few ovules on the marginal placentae; style 4-5 cm long; stigma capitate. Pods lomentum, 15-30 mm by 2.5-3 mm, 4 to 6 jointed, upper suture shallowly undulate, sparely uncinate hairy or glabrous; joints quadrate. Seeds rectangular, 1.5-2.5 mm by 1.3-1.7 mm, rim-aril prominent. (Figure 2. C)

Distribution : Widely spread in the Old World, Africa, Ceylon, India, Indichina, China, Korea, Japan, Malesia, Australia and Pacific Islands (Pedley as cited in Dassanayake 1996).

Specimen examined : Eastern Shan State; Kyaing Tong Township, Kyaing Tong University Campus, N 21°17'0.30", E 99°34'30.12", 809 meter, Dr. Tin Tin Maw, 6.10.2018, collected no. 8.

4. *Indigofera linnaei* Ali, Bot. Not. 111: 549. 1958.

Myanmar name : Meyaing

English name : Birdsville Indigo

Flowering period : September to November

Annual, procumbent herbs; stems and branches slender, densely appressed-pubescent. Leaves unipinnate compound, imparipinnate, alternate; stipules setaceous, 4-6 mm long, tomentose; petioles 2-3 mm long, pubescent; racheae 1.5-4 cm long, appressed-pubescent; leaflets 7 to 11, alternate, obovate-oblong or oblanceolate, 5-20 mm by 3-8 mm, accrescent upwards, cuneate at the base, entire along the margin, obtuse to truncate at the apex with mucronate, appressed-pubescent on both surfaces. Inflorescences axillary head-like racemes, 5-20-flowered, 6.5-8 cm long; peduncles 4-5 cm long, appressed-pubescent. Flowers reddish pink, 5-6 mm in diameter; bracts subulate, 1-2 mm long; pedicels 1-1.5 mm long. Calyx campanulate, 5-lobed, strigose outside; tube 1 mm long; lobes setaceous, 2 mm long, subequal.

Corolla papilionaceous, exserted; standard obovate, 4-5 mm by 3-5 mm, bright red, glabrous; wings obliquely-oblong, 3-5 mm by 3 mm, pink, clawed, shortly auriculate; keel 4-5 mm long, pink. Stamens 10 (9+1), diadelphous; free filaments 0.5 mm long, appressed-pubescent; anthers dithecous, uniform. Ovary oblongoid, 1-1.5 mm by 0.8 mm, glabrous, unilocular with 2-3 ovules on the marginal placentae; style 2-3 mm long, glabrous; stigma capitate. Pods oblong-cylindrical, 4-6 mm by 2 mm, 2 to 3-seeded, short-beaked, brown, appressed-pubescent. Seeds rounded or barrel-shaped, about 1.5 mm long, brown, glabrous. (Figure 2. D)

Distribution : Ceylon, India and Pakistan to Southeast Asia and Australia, usually in dry, barren places (Velva E. Rudd as cited in Dassanayake 1991). Kress *et. al*. (2003) treated this species as *Indigofera enneaphylla* L. and he state that distributed in Mandalay Division.

Specimen examined : Eastern Shan State; Kyaing Tong Township, Kyaing Tong University Campus, N 21°16'54.96", E 99°34'24.60", 802 meter, Dr. Tin Tin Maw, 2.9.2018, collected no. 7.

5. ***Millettia macrostachya*** Collett & Hemsley, J. Linn. Soc., Bot. 28: 41. 1890.

Myanmar name : Ye-thinwin

English name : Chinese wisteria

Flowering period : March to May

Deciduous tree, up to 10 m high; stems and branches terete, woody, glabrous; bark pale brown with shallow cracks. Leaves unipinnate compound, imparipinnate, alternate; stipules small, caducous; petioles 5-9 cm long, pubescent; leaflets 7-9, opposite, broadly oblong to obovate elliptic, 8-14 cm by 4-7 cm, broadly cuneate and slightly oblique at the base, entire along the margin, acute at the apex, glabrescent on both surfaces; stipels tiny point; petiolules 4-7 mm long, pubescent. Inflorescences axillary pseudoracemes, many-flowered, 33-45 cm long, longer than subtending leaf, thick, straight, slightly puberulent; peduncles 6-9 cm long; rachis node whorled with 3-7 flowers clustered on a 2-4 mm long spur. Flowers purple, 2 cm in diameter; bracts and bracteoles small, brown puberulent, caducous; pedicels 3-5 mm long, pubescent. Calyx campanulate 5-lobed, pubescent; tube 4-5 mm long, lobes lanceolate, acuminate, 3-4 mm long. Corolla papilionaceous, exserted; standard orbicular, 2 cm by 2 cm, with green calluses at base, auriculate; wings oblong, 2 cm by 7 mm, auriculate; keels falcate-oblong, 2 cm by 6 mm, auriculate. Stamens 10 (9+1), diadelphous; free filament filiform, 1.3 cm long; anthers dithecous, uniform. Ovary linear, 6-7 mm long, pubescent, unilocular, with many ovules on marginal placentae; style curved, 6-7 mm long; stigma capitate. Pods linear, 8-12 cm by 1.2-1.5 mm, leathery, apex beaked, 3 to 4-seeded; sutures thickened. Seeds ellipsoid, 5 mm by 4 mm, olive-green. (Figure 2. E)

Distribution : China, Myanmar and Thailand (https:// global species. org > ntaxa). Kress *et al.* (2003) recorded that this species was distributing in Kachin State, Kayin State, Mandalay Division and Shan State of Myanmar.

Specimen examined : Eastern Shan State; Kyaing Tong Township, Kyaing Tong University Campus, N 21°17'8.46", E 99°34'30.84", 802 meter, Dr. Tin Tin Maw, 12.3.2018, collected no. 4.

6. *Pueraria montana* (Lour.) Merr., Trans. Amer. Philos. Soc., ser. 2. 24 (2) : 10, 210. 1935.
Dolichos montanus Lour. Fl. cochinch. 440.1790.

Myanmar name : Unknown

English name : Japanese arrowroot

Flowering period : January to March

Robust climber, with tuberous roots; stems up to 8 cm long, woody at base, yellow hirsute throughout. Leaves trifoliate compound, alternate; stipules dorsifixed, ovate-oblong 1.8-2 cm by 8-10 mm, striate; petioles 6-20 cm long; leaflets broadly ovate or obliquely ovate, 7-19 cm by 5-15 cm, acute or oblique at the base, entire along the margin, acuminate at the apex, yellowish adpressed hairs. Inflorescences axillary raceme, many-flowered, 15-40 cm long, peduncles 1-2 cm long. Flowers purple or blue, 9-10 mm in diameter; bracts linear-lanceolate to linear, 1 cm long, brown yellowish hairs, caducous; bracteoles lanceolate, 5-6 mm long, brown yellowish hairs; pedicels 3-4 mm long. Calyx campanulate 5-lobed, brown yellowish hairs; tube 3-4 mm long; lobes lanceolate, acuminate, 4-6 mm long, subequal. Corolla papilionaceous, exserted; standard obovate, 8-10 mm by 9-10 mm; with yellow callosity at base, shortly clawed; wings falcate, 9-10 mm by 3 mm, with linear auricles at base; keels falcate-oblong, 7-8 mm by 4 mm, with very small and acute auricles. Stamens 10 (9+1), diadelphous; free filament filiform, 6 mm long; anthers dithecous, uniform. Ovary linear, 5 mm long, pubescent, unilocular, with many ovules on marginal placentae; style curved, 3-3.5 mm long; stigma capitate. Pods elliptic, 2.5-6 cm by 7-8 mm, flattened, constricted between the seeds, 2 to 6-seeded, brown hirsute. Seeds mostly many, ovoid-oblong or angular, 1-1.5 mm long, black-brown. (Figure 2. F)

Distribution : Asia-China, Japan, Korea, Myanmar, Thailand, Laos, Vietnan, Malaysia, Indonesia, Philippine, New Guinea, the Solomon Island (tropical. theferns. info > viewtropical > id).

Specimen examined : Eastern Shan State; Kyaing Tong Township, Kyaing Tong University Campus, N 21°16'57.60", E 99°34'30.96", 818 meter, Dr. Tin Tin Maw, 14.2.2018, collected no. 2.

7. *Smithia conferta* Smith in Rees, Cyclop. 33: n. 2. 1816.

Myanmar name : Unknown

English name : Paired flowered smithia

Flowering period : July to August

Annual, procumbent-decumbent or erect herbs; stems slender, many branched, woody 20-90 cm long, glabrous. Leaves unipinnate compound, paripinnate, alternate; stipules lanceolate, about 1 cm long, striate, scarious, persistent, appendage bilobed; lobes uequal; petioles 2-3 mm long, bristly; rachis 1-3.5 cm long, sparsely long hirsute; leaflets 3-7 pairs, opposite, subsessile, obovate-oblong, 4-12 cm by 3-4 mm, oblique at the base, entire along the margin, obtuse-rounded and mucronate at the apex, glabrous above, bristly along the margin and throughout on the midrib beneath. Inflorescences axillary, scorpioid racemes, 3-4 cm long, solitary or in pair, peduncles short. Flowers yellow, 5-8 mm in diameter; bracts ovate-elliptic, 2-3 mm long, acute, scarious, persistent; bracteoles ovate-elliptic, 1.5-2.5 mm long, acute, scarious, persistent; pedicels 2-3 mm long hispid. Calyx bilabiate, 5-lobed, a tuft of bristle only at the tip

of back; tube 1-1.5 mm long; upper lip broadly ovate, 5-7 mm by 5.5 mm, acute, lower lip ovate, 5-7 mm by 3-4 mm. Corolla papilionaceous, exserted; standard orbicular, 7-10 mm by 8-10 mm; emarginate at apex, shortly clawed; wings obovate, 9-10 mm by 3.5-4 mm, auricle at base, clawed; keels boat-shaped, 7.5-8.5 mm by 3.5-4 mm, truncate at apex, auricle at base, clawed. Stamens 10 (5+5), diadelphous; staminal colum 4-7 mm long, filament 2-2.5 mm long; anthers dithecous, uniform. Ovary linear, 2-3 mm long, glabrous, unilocular, with few ovules on marginal placentae; style 5-6 mm long; stigma pointed. Pod lomentum, more or less straight, included, 5 to 6 joined; joints suborbicular, 2 mm by 2 mm, papillose. Seeds subreniform, 0.8-1.3 mm by 0.6-1.6 mm. (Figure 2. G)

Distribution : Ceylon and southern India, in moist open areas at elevation up to about 1300 meter (Velva E. Rudd as cited in Dassanayake 1991).

Specimen examined : Eastern Shan State; Kyaing Tong Township, Kyaing Tong University Campus, N 21°17'0.06", E 99°34'29.52", 813 meter, Dr. Tin Tin Maw, 25.8.2018, collected no. 6.

8. *Tadehagi triquetrum* (DC.) Ohashi, in Ginkgouna 1: 295. 1977.

Desmodium triquetrum DC., Prod. 2: 326. 1825.

Myanmar name : Lauk-the; Shwe-gu-than-hlet

English name : Trefle gross

Flowering period : November to January

Erect shrubs, sometimes similar to small trees, about 2 m high; stems triquetrous, acutely angled, stout, woody, tomentose; branches zigzag, densely downy when young. Leaves unifoliate compound, alternate; stipules large, lanceolate, 1.5-1.8 cm by 4-5 mm, sharply acuminate at the apex; petioles winged, 2.5-4 cm by 4-5 mm, puberulous; leaflets lanceolate, 7-11 cm by 1.5 -3.5 cm, obtuse at the base entire and ciliate along the margin, acuminate at the apex, subcoriaceous, glabrous above, densely appressed white silky hairy beneath. Inflorescences axillary and terminal racemes, many-flowered, 8-30 cm long; peduncles triquetrous, 3-9 cm long, with short hooked hairs. Flowers pinkish purple, 5-8 mm in diameter; bracts lanceolate 3 mm by 1 mm, hairy, persistent; pedicels 4-6 mm long, with dense hooked hairs. Calyx campanulate, 5-lobed, with white long straight hairs and short hooked hairs; lobes setaceous, longer than the tube, the lowest one longer. Corolla papilionaceous, exserted; standard orbicular, 6-8 mm by 6 mm, cuneate at the base, glabrous; wings 6 mm by 4 mm; keels 4 mm by 2 mm, connate. Stamens 10 (9+1), diadelphous; free filaments 2-3 mm long; anthers dithecous, uniform. Ovary oblongoid, 7 mm by 1 mm, densely villous, unilocular, with few ovules on the marginal placentae; style long, curved at the tip; stigma capitate. Pods lomentum, 7 to 10 jointed, 2.5-3 cm by 3-6 mm, compressed, shallowly constricted, strongly caudate at the apex, with dense white hairs. (Figure 2. H)

Distribution : Kress *et. al.* (2003) recorded that this species was distributed in Chin State, Kachin State, Kayin State, Mandalay Division, Sagaing Division, Shan State and Yangon Division of Myanmar.

Specimen examined: Eastern Shan State; Kyaing Tong Township, Kyaing Tong University Campus, N 21°17'1.86", E 99°34'30.66", 807 meter, Dr. Tin Tin Maw, 4.1.2018, collected no. 1.

9. *Vigna umbellata* (Thunb.) Ohwi & Ohashi, Jap. Bot. 44: 31. 1969.

Dolichos umbellatus Thunb; Trans. Linn. Soc. London 2: 339. 1794.

Myanmar name : Unkown

English name : Rice bean, Oriantal bean

Flowering period : January to March

Annual, twining herbs; stems slender, 1-3 m long, pilose with yellow hairs when young, later glabrescent. Leaves trifoliate compound; alternate, stipules peltate to lanceolate-oblong, 1-1.5 cm long; petioles 5-10 cm long; leaflets ovate or ovate-lanceolae, 5-10 cm by 2.5-6 cm, broadly cuneate or obtuse at the base, entire or slightly lobed along the margin, acute at the apex, sparsely pubescent on the both surface. Inflorescences erect axillary raceme, 2-3-flowered, 6-21 cm long; peduncles 5-20 cm long. Flowers bright yellow, 1.2-2cm in diameter, bracts lanceolate 4-5 mm long, caducous; bracteoles linear, 1.5 mm long; pedicels 5-6 mm long. Calyx campnulate, 5-lobed, tube 4 mm long, lobes deltoid, 1.5-4 mm long, ciliate. Corolla papilionaceous; standard suborbicular, 1.2-1.5 cm by 1.6 cm, emarginate, wings obovate, 1.5 cm by 1.6 cm, short clawed; keels obliqely-oblong, 1.4 cm by 1.2 cm, with an incurved beak and horn-like pocket on one side. Stamens 10 (9+1), diadelphous; filaments 5-6 mm long; anthers dithecous, uniform. Ovary cylinder, 3-3.5 mm long, glabrous, unilocular with many ovules on the marginal placentae; style 1 cm long, lower style constricted at ovary insertion and upper flat; incurved, bearded on the inner side; stigma beaked. Pods linear-terete, 6-10 cm by 0.5 cm, 6 to 12-seeded, green when young, black-brown at maturity, glabrous. Seeds oblong, 5-10 mm by 2-5 mm, brown or black, motted, glabrous. (Figure 2. I)

Distribution : Throughtout India, Malaysia and the Philippine. Cultivated throughout tropical Asia and parts of Africa (Velva E. Rudd as cited in Dassanayake 1991).

Specimen examined : Eastern Shan State; Kyaing Tong Township, Kyaing Tong University Campus, N 21°17'1.32", E 99°34'30.96", 813 meter, Dr. Tin Tin Maw, 27.2.2018, collected no. 3.

Table 2 Comparable characteristics of species in Fabaceae

No.	Scientific names	Habit	Leaves	Calyx	Stamens	Anthers	Pods
1.	*Crotalaria juncea* L.	herb	unifoliate	bilabiate	monadelphous	dimorphic	not jointed
2.	*Desmodium gangeticum* (L.) DC.	shrub	unifoliate	campanulate	diadelphous (9+1)	uniform	jointed
3.	*Desmodium heterocarpon* (L.) DC.	herb	trifoliate	campanulate	diadelphous (9+1)	uniform	jointed
4.	*Indigofera linnaei* Ali.	herb	unipinnate	campanulate	diadelphous (9+1)	uniform	not jointed
5.	*Millettia macrostachya* Collett & Hemsley	tree	unipinnate	campanulate	diadelphous (9+1)	uniform	not jointed
6.	*Pueraria montana* (Lour.) Merr.	climber	trifoliate	campanulate	diadelphous (9+1)	uniform	not jointed
7.	*Smithia conferta* Smith	herb	unipinnate	bilabiate	diadelphous (5+5)	uniform	jointed
8.	*Tadehagi triquetrum* (DC.) Ohashi	shrub	unifoliate	campanulate	diadelphous (9+1)	uniform	jointed
9.	*Vigna umbellata* (Thunb.) Ohwi & Ohashi	herb	trifoliate	campanulate	diadelphous (9+1)	uniform	not jointed

An artificial key to the studied species:

1. Stamens monadelphous; anthers dimorphic; pods inflated.....1. *Crotalaria juncea*
1. Stamens diadelphous; anthers uniform; pods not inflated.....2
    2. Trees; pods leathery.....5. *Millettia macrostachya*
    2. Shrubs, herbs, climbers; pods not leathery.....3
3. Pods jointed.....4
3. Pods not jointed.....7
    4. Leaves unipinnate compound; calyx bilabiate; stamens 5+5; pods included. .....7. *Smithia conferta*
    4. Leaves unifoliate or trifoliate compound; calyx campanulate, stamens 9+1; pods exserted.....5
5. Leaves trifoliate compound; calyx 4-lobed.....3. *Desmodium heterocarpon*
5. Leaves unifoliate compound; calyx 5-lobed.....6
    6. Petioles winged; branches zigzag.....8. *Tadehagi triquetrum*
    6. Petioles wingless; branches not zigzag.....2. *Desmodium gangeticum*
7. Procumbent herbs; leaves unipinnate compound.....4. *Indigofera linnaei*
7. Robust climbers or twining herbs; leaves trifoliate compound.....8
    8. Inflorescences many-flowered; flowers purple to blue; standard with yellow callosity; pods constricted between the seeds, brown hirsute.....6. *Pueraria montana*
    8. Inflorescences 2-3-flowered; flowers bright yellow; standard without yellow callosity; pods terete, glabrous.....9. *Vigna umbellata*

Discussion and Conclusion

The present study deals with the plants growing in Kyaing Tong University Campus. Totally, 9 species belonging to 8 genera of family Fabaceae under subclass Magnoliidae had been studied in the present paper. The genera in this research paper are *Crotalaria, Desmodium, Indigofera, Millettia, Pueraria, Smithia, Tadehagi* and *Vigna* under the family Fabaceae. The genera are arranged according to alphabetically.

Among the species in the present study, the species of *Indigofera linnaei* Ali. is commonly found in this area. The species *Crotalaria juncea* L. is rarely found. Among the 9 species, *Millettia macrostachya* Collett & Hemsley is tree, *Desmodium gangeticum* (L.) DC. and *Tadehagi triquetrum* (DC.) Ohashi. are shrubs, *Pueraria montana* (Lour.) Merr. is climber, the rest species are herbs. Leaves of *Crotalaria juncea* L., *Desmodium gangeticum* (L.) DC. and *Tadehagi triquetrum* (DC.) Ohashi are unifoliolate compound, *Desmodium heterocarpon* (L.) DC., *Pueraria montana* (Lour.) Merr. and *Vigna umbellata* (Thunb.) Ohwi & Ohashi are trifoliolate compound, others are unipinnate compound. The calyx of *Crotalaria juncea* L. and *Smithia conferta* Smith are bilabiate, but the rest species are campanulate. Except *Crotalaria juncea* L. is monadelphous stamens, others are diadelphous stamens. Pods of *Desmodium gangeticum* (L.) DC., *Desmodium heterocarpon* (L.) DC., *Tadehagi triquetrum* (DC.) Ohashi and *Smithia conferta* Smith are jointed, but those of others species are not jointed.

In this study, the observed characters for all species are agreement with the references cited by identified authors. *Pueraria montana* (Lour.) Merr. and *Smithia conferta* Smith have been colleted in study area, but this two species were not found in other researchers and Myanmar by Kress et. al. (2003).

Millettia macrostachya Collett & Hemsley is used for ornamental plants. All 9 species are also medicinally important plants. *Smithia conferta* Smith included the IUCN (International Union for the Conservation of Nature) Red list of threatened species.

According to the data collected, it can be noted that 9 species from 8 genera of Fabaceae are distributing. The collected species are identified and described with comments on their scientific names, Myanmar names and coloured plates. It is hoped that this research of present investigation give valuable information of Fabaceae to other researchers in various field of study. Finally, it is also hoped that this research paper will provide a partial fulfillment of the family Fabaceae in Eastern Shan State of Myanmar.

A. *Crotalaria juncea* L.

B. *Desmodium gangeticum* (L.) DC.

C. *Desmodium heterocarpon* (L.) DC.

D. *Indigofera linnaei* Ali.

E. *Millettia macrostachya* Collett & Hemsley

F. *Pueraria montana* (Lour.) Merr.

G. *Smithia conferta* Smith

H. *Tadehagi triquetrum* (DC.) Ohashi

I. *Vigna umbellata* (Thunb.) Ohwi & Ohashi

Figure 2

Acknowledgements

I would like express my sincere to Dr. San San Mar, Rector and Dr. Mya Nyunt, Pro-rector, Kyaing Tong University, for their permission to conduct this research paper. I wish to express deepest thanks to Dr. San San Oo, Professor and Head, Dr. Moe Moe Lwin, Professor, Department of Botany, Kyaing Tong University, for their invaluable suggestion and permission to this paper.

References

Ali, S.I. and Y.J. Nasir, (1973-1977) *Flora of West Pakistan*, Department of Botany, University of Karachi, Karachi.

Anonymous, (2008) *Flora of Hong Kong*, Vol. 2, Agriculture, Fisheries and Conservation Department, Printed in Government logistics Department, Hong Kong.

Backer, C.A & R.C. Bakhuizen Van Den Brink, (1963) *Flora of Java***,** Vol. 1-2 Netherlands, Rijksherbarium, Lelyden, N.V.P. Noordhoff.

Brandis, D., (1906) *Indian Tree*, Assisted by Indian Foresters, Archibald Constable & Co. Ltd. 16 James Street Haymarket S.W. London.

Brummitt, R.K., (1992) *Vascualr Plant Families and Genera***.** Royal Botanical Garden, Kew, Printed and bound by Whistable Litho Ltd., Great Briantain.

Dassanayake, M.D., (1991, 1996) *A Revised Handbook to the Flora of Ceylon***,** Vol. 7 and 10, University of Peradeniya, New Delhi.

Hooker, J.D., (1879) *Flora of British India,* Vol. 2, L. Reeve & Co. 5 Henrietta street, Covent Garden London.

Hutchinson, J. (1967) *Key to the Families of Flowering Plants of the World,* Claredon Press Oxford.

Kress, J., *et al*., (2003) *A Checklist of the Trees, Herbs and Climbers of Myanmar,* Department of Systematic Biology-Botany. National Museum of Natural History, Washington DC, USA.

Lawrence, George H.M., (1951) *Taxonomy of Vascular Plants*, the Macmillan Company, New York.

Rodford, Elbert E., (1986) *Fundamental of Plant Systematic*, Haper & Sons Company, New York.

Subrahmanyam, N.S., (1995) *Modern Plant Taxonomy*, India, Reader in Botany Sri Venketashwara Collage, University of Delhi.

Wu, C.Y. P.H. Raven, & D.Y Hong. Eds. (2010) *Flora of China*, Flora of China (Fabaceae) 10: 1-642, Science Press & Missouri Botanical Garden Press, Beijing & St. Louis.

Website

https:// global species. org > ntaxa
tropical. theferns. info > viewtropical > id
http//en.m.wikipedia.org > wiki >Fabac.

TAXONOMY AND POLLEN MORPHOLOGY OF NINE SPECIES IN FABACEAE

Thin Thin Aye[1], Swe Swe Lin[2]

Abstract

Taxonomy and pollen morphology of 9 species belonging to 7 genera of the family Fabaceae were studied. The specimens were collected from Magway Region during 2017- 2018. The collected species are two species of *Acacia, Caesalpinia* and one species of *Aeschynomene, Butea, Clitoria, Mucuna, Vigna*. Taxonomic descriptions, artificial key to the species was presented. According to the resulting data, perennial tree are *Acacia, Butea*; small tree is *Caesalpinia sappan* L.; shrubs is *Aeschynomene americana* L.; lianas is *Caesalpinia bonduc* (L.) Roxb and rest species are herbs. The types of leaves are compound. The trifoliolate leaves also occurred in *Butea, Mucuna, Vigna* and the remaining species are uni or bipinnate. The pollen grains were found as polyads and monads. Polyads was observed in *Acacia* and the other species were monads. The shape, size and sculpture are important criteria for the study of pollen morphology. There are two types of aperture and three types of exine ornamentation. Psilate pollens are observed in *Acacia catechu* (L.f) Willd., *Clitoria ternatea* L., and the other species are reticulate. The sizes of pollens are small, medium and large.The smallest pollen was found in *Aeschynomene* and largest pollen observed in *Mucuna*. Pollen of each species was presented in polar view and equatorial view.

Keywords: Taxonomy, pollen morphology

Introduction

Fabaceae is the third largest family of flowering plants. Fabaceae includes three subfamilies: Mimosoideae, Caesalpinioideae, and Faboideae (Anonymous 2012). The Fabaceae is very large group with worldwide distribution. Legumes are one of the important plant groups, being the source of numerous pulses, soil rotation plants, oil, timber trees, gums and dyes (Simpson 2006). The Mimosaceae is 17 genera and 93 species. Caesalpiniaceae is 26 genera and 124 species. Papilionaceae is 84 genera and 510 species in the checklist of Myanmar (Kress *et al.* 2003). Fabaceae is about 650 genera and 18,000 species worldwide distribution (Langran *et al*. 2010).

The word "Palynology" was coined by Hyde and Willams as a suitable for "the science of pollen grains and spores". It comes from the Greek work palynein meaning "to spread". Palynology is the morphology of pollen, fine structure of their wall, particularly of its outermost layer, the exine. (Erdtman 1985). The exine is the outer layer of living pollen grain. It is composed of sporopollenin. Sporopollenin is very chemically stable and it is resistant to almost all kinds of environmental damage (Briggs & Brady 2000).

The examination of pollen grains, both recent and ancient, can be of value in a range of scientific studies. Taxonomy, genetic, evolutionary studies, honey studies, allergy studies, forensic science, tracing vegetation history, climate change studies (Moore *et al.* 1991). The palynological research can be either basic or applied. To the basic aspects belongs the pollen morphology in relation to taxonomy and the applied aspects belong to geopalynology, aeropalynology, iatropalynology and melitopalynology (Bhojwanii &Bhatnagar 2005). Angiosperms have two basis pollen grains, monosulcate and tricolpate. Palynologists agree that the first flowering plants probably had monosulcate pollen grains. Tricolpate types are characteristics of the advanced dicotyledons (Walker & Doyle 1975).

[1] Dr, Lecturer, Department of Botany, University of Magway
[2] Dr, Lecturer, Department of Botany, University of Mandalay

Materials and Methods

The plants were collected from Magway Region 2017- 2018. All the collected species are recorded by photographs during flowering times. Identification of collected specimens was carried out by using floristic literature of Dassanayake (1991), Kress *et al*. (2003) and Langran *et al*.(2010).Pollen samples were collected from the anther of blooming flowers. Pollen samples were acetolysed by Erdtman method (1952). The pollen samples in a glass vials were crushed with a glass rod and acetic acid was added. The mixture of glass vial was transferred into a test tube. The test tube was put in a water bath. The material was transferred to a centrifuge. After centrifuging, the polliniferous material was transferred to be stored in a bottle and labeled. The identification of pollen is referred to Erdtman (1952), Erdtman (1969), Erdtman (1971), Moore *et al.* (1991), Hoen (1999), Paldat (2005), Pascoe (2007), Hesse *et al*. (2009).

Results

Pollen morphology of nine species in Fabaceae has been studied in this paper.

A. Artificial key to the species

1. Flowers actinomorphic ----------2
1. Flowers zgyomorphic----------3
    2. Bark brown, inflorescence pedunculate spike----------1.*Acacia catechu*
    2. Bark pale yellow, inflorescence globose head----------2.*Acacia leucophloea*
3. Perennial ----------4
3. Annual----------6
    4. Flower reddish orange, stamen diadelphous----------4. *Butea monosperma*
    4. Flower yellow, stamen free----------5
5. Lianas, pods spiny----------5. *Caesalpinia bonduc*
5. Small tree, pods glabrous----------6.*Caesalpinia sappan*
    6. Stipules peltate, anther basifixed----------9. *Vigna trilobata*
    6. Stipules lanceolate, anther dorsifixed----------7
7. Trifoliolate, seed elliptic-oblong----------8. *Mucuna hirsuta*
7. Unipinnate, seed reniform----------8
    8. Twinning herbs, flower bright blue ----------7.*Clitoria ternatea*
    8. Erect shrubs, flower pinkish yellow ----------3.*Aeschynomene americana*

B. Artificial pollen key to the species

1. Grain polyads ----------2
1. Grain monads---------- ----------3
    2. Sculpture psilate ----------1.*Acacia catechu*
    2. Sculpture reticulate----------2.*Acacia leucophloea*

3. Aperture porate----------4
3. Aperture colporate----------5
    4. Operculum absent----------7. *Clitoria ternatea*
    4. Operculum present ----------9. *Vigna trilobata*
5. Angulaperturate----------6
5. Planaperturate----------8
    6. Pori lolongate----------4.*Butea monosperma*
    6. Pori lalongate----------7
7. Grain size small----------3.*Aeschynomene americana*
7. Grain size large ----------8.*Mucuna hirsuta*
    8. Amb triangular, sexine as thick as nexine ----------5.*Caesalpinia bonduc*
    8. Amb circular, sexine thicker than nexine ----------6. *Caesalpinia sappan*

1. *Acacia catechu* (L.f) Willd., Sp. Pl. 4: 1079. 1806. (Figure 1. A)

***Mimosa catechu* L. f, Sp. Pl. 439. 1782**

Myanmar name : Sha

English name : Black Catechu

Flowering period : June-September

Perennial, tree, woody; stems and branches terete, recurved prickles just below the nodes, tannin present, bark brown, pubescent. Leaves bipinnately compound, paripinnate, alternate, green, glabrous; stipules spinous, acute and curved at the apex, glabrous; petioles terete, with gland near the first pair of pinnae, glabrous, pulvinous; rachis terete, 6.0-8.0 cm long, pale green, glabrous; leaflets linear, 0.2-0.5 cm by 0.1-0.2 cm, obtuse at the base, entire along the margin, retuse at the apex, glabrous on both surfaces. Inflorescences axillary pedunculate spike, many-flowered. Flowers bisexual, actinomorphic, pentamerous, hypogynous, pale greenish-yellow, sessile; bracts minute; bracteoles absent. Calyx 5-lobed, campanulate. Corolla 5-lobed, campanulate; tube slightly pubescent, pale greenish-yellow, lobes oblong, cream color. Stamens numerous, free, exserted; filament filiform; anther dithecous, dorsifixed, longitudinal dehiscence. Ovary superior, linear-oblong, unilocular, one ovules in each locule on marginal placentae; style filiform; stigma simple. Pods oblong, compressed, brown, dehiscent.

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

Polyads, 12-celled, bilateral, flattened, medium, 45.0-47.5×43.8-50.0 µm in length and breadth; each grain triporate, small, 13.8-16.3×11.3-18.8 µm in length and breadth; amb tri-quadrangular; exine 1.3-2.5µm thick, sexine thicker than nexine; sculpturing psilate.

2 *Acacia leucophloea* (Roxb.) Willd., Sp. Pl. 4(2): 1083. 1806. (Figure 1. D)

***Mimosa leucophloea* Roxb., Pl. Corom. 2: 27, t. 150.1798.**

Myanmar name : Hta naung

English name : White-barked Acacia

Flowering period : June-September

Perennial, tree, pale yellowish bark, broadly umbelliform crown, trunk of young tree often thorny sucker; stem woody; branches cylindrical, pubescent. Leaves bipinnately compound, alternate; stipule spinous, straight or recurved, dark or brown; petiole terete, with gland above near the first pair of pinnae, tomentose; rachis 2.0-9.0 cm long, pubescent; leaflets linear, 0.3-0.8 cm by 0.1-0.3 cm, oblique at the base, entire along the margin, obtuse at the apex, sparsely pubescent on both surfaces; stipel absent. Inflorescences globose head, aggregate into terminal panicles, 18-28 cm long, tomentose. Flower bisexual, actinomorphic, pentamerous, hypogynous, pale yellow, pubescent; involucres bracts, pale brown, glabrous within, pubescent without. Calyx 5-lobed, campanulate; lobes triangular, yellowish green, pubescent. Corolla 5-lobed, campanulate; lobes very short; yellowish-green, puberulous. Stamen numerous, free, exserted, pale yellow, anther dithecous, dorsifixed, longitudinal dehiscence, yellow. Ovary superior, linear, puberulous, one ovules in each locule on the marginal placentae; style short; stigma simple. Pods linear falcate, compressed, straight or slightly curved, pale yellowish-brown.

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

Polyads, 16-celled, bilateral, medium, 43.8-47.5×46.3-56.3 µm in length and breadth; each grain triporate, small, 10.0-16.3×11.3-17.5 µm in length and breadth; amb tri-quadrangular; exine 1.3-2.5 µm thick, sexine as thick as nexine; sculpturing reticulate, the lumina heterobrochate about 0.6-1.3 µm in width, the muri simplibaculate about 0.6 µm wide.

3. *Aeschynomene americana* L. Sp. Pl, 2: 713. 1753. (Figure 2. A)

Myanmar name : Tawpe

English name : Unknown

Flowering period : October - December

Annual, erect shrubs; stems and branches cylindrical, slightly ribbed, reddish-green, velutinous. Leaves unipinnately compound, paripinnate, alternate; stipules lanceolate, green, velutinous, base auriculate, apex acute, caducous; petioles terete, 0.3-0.5 cm long, reddish-green, velutinous; racheae terete, 3-7 cm long, reddish-green, villous; stipels absent; petiolule terete, pale green, velutinous; leaflets linear-oblong, 0.6-1.0 cm by 0.1-0.2 cm, oblique at the base, entire along the margin, obtuse at the apex with mucronate, green, margin reddish, velutinous on both surfaces. Inflorescences axillary racemes; peduncle terete, 1-3 cm long, green, velutinous. Flowers bisexual, zygomorphic, pinkish-yellow, about 0.8 cm in diameter at anthesis; pedicels terete, green, velutinous; bracts paired, ovate to acute, serrate with red margin, velutinous, persistent; bracteoles linear-ovate, striate, green, velutinous; lobes very short, serrate along the margin, pale green, velutinous. Corolla papilionaceous; standard orbicular, large, 0.5-0.8 cm by 0.3-0.4 cm, pink with yellow bloth at base, glabrous; wings obliquely obovate, 0.5-0.7 cm by 0.2 -0.3 cm, pinkish yellow, glabrous; keels obtuse, slightly incurved, 0.3-0.5 cm by 0.2-0.3 cm, pinkish yellow, glabrous. Stamens 10, diadelphous, anther uniform, dithecous, dorsifixed,

yellow, longitudinal dehiscence. Ovary superior, linear, velutinous, unilocular with few ovules on the marginal placentae; style filiform, incurved, about 0.2cm long, white, glabrous; stigma simple. Pods linear, compressed, 4-8 jointed, slightly curved, lower suture incised, flattened, brown, velutinous. Seeds reniform, small, brown, glabrous.

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

Tricolporate, oblate, small, 12.5-16.3×17.5-22.5 μm in length and breadth; amb rounded, angulaperturate; colpi syncolpate; pori lalongate, 5.0-6.3×6.3-7.5 μm in length and breadth; exine 1.3-2.5 μm thick, sexine as thick as nexine; sculpturing reticulate, lumina heterobrochate, 0.6 -1.3 μm width, muri simplibaculate, about 0.3 μm wide.

4. *Butea monosperma* (Lam.)Taub in Engler&Prantl, Nat, Pflanzenfam. 3(3)366. 1894. (Figure 2. D)

***Erythrina monosperma* Lam., Enc. 2: 391.1786**

Myanmar name : Pauk

English name : Flame of the forest, Parrot tree

Flowering period : February-March

Perennial trees bark grayish-black; stems and branches terete, pubescent. Leaves trifoliolate compound, alternate; stipules linear-lanceolate, green, caducous, pubescent; petioles terete, 7-18 cm long, green, pubescent; stipels subulate, green, pubescent; petiolules terete, green, pubescent; leaflets 3, obovate or suborbicular, 10-16 cm by 8-14 cm, unequal, broadly cuneate at the base, entire along the margin, rounded or emarginate at the apex, coriaceous. Inflorescences axillary or terminal fasiculate raceme, many-flowered; peduncles terete, 8-40 cm long, green, with many tubercles, tomentose. Flowers bisexual, zygomorphic, pentamerous, hypogynous, reddish orange, about 2 cm in diameter at anthesis, showy; pedicel terete, rusty densely velutinous; bracteoles linear, rusty velutinous, caducous. Calyx 5-lobed; tube campanulate, dark green, velutinous; lobes acute, the upper one completely united, dark green, velutinous or densely silvery gray on both surfaces. Corolla papilionaceous; standard ovate-oblong, recurved, 3.5-4.5 cm by 1.6-2.5 cm, reddish-orange, densely white pubescent, clawed; wings narrowly falcate, 4.0- 4.5 cm by 1.2-1.5 cm, reddish-orange, pubescent; keel broadly falcate, beaked, 4.5-5.0 cm by 1.7-2.0 cm, connate into an ridge, densely silvery gray velutinous. Stamens 10 diadelphous; filaments filiform, glabrous; anther dithecous, dorsifixed, oblong, longitudinal dehiscence. Ovary superior, linear, villose, densely velutinous, curved, unilocular with one ovules on the marginal placentae; style filiform, pale yellow, glabrous; stigma subcapitate. Pods oblong, flat, dehiscent, brown, densely silvery gray pubescent. Seeds reniform, pale brown, compressed, glabrous.

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

Tricolporate, oblate spheroidal, medium, 33.8-47.5×35.0-48.8 μm in length and breadth; amb rounded triangular, angulaperturate; colpi longicolpate, 28.8-43.8×5.0-8.8 μm in length and breadth; pori lolongate, 8.8-12.5×7.5-11.3 μm in length and breadth; operculum about 1.3 μm thick; exine 1.3-2.5 μm thick, sexine thicker than nexine; sculpturing reticulate, lumina heterobrochate, 0.6 -1.0 μm width, muri simplibaculate, about 0.3 μm wide.

5. *Caesalpinia bonduc* (L.) Roxb., FL.Ind, ed. Carey. 2: 362. 1832. (Figure 3.A)

***Guilandina bonduc* L., Sp. Pl. 1: 381. 1753.**

***Caesalpinia crista* L., Sp. Pl., ed. 2, 1:545 . 1762.**

Myanmar name : Kalein

English name : Unknown

Flowering period : August to October

Perennial, aculeate lianas; stems and branches terete, with straight or recurved prickles, pubescent, prickle hard, falcate. Leaves bipinnately compound, paripinnate, alternate; stipules leafy, large, 3-5 lobed, ovate, persistent; petioles terete, 9.5-12.0 cm long, green, pulvinate, spiny pubescent; racheae eglandular, with incurved pairs of prickles beneath; primary rachis terete, 35-60 cm long, with hooked spine, green, 6-8 pairs of pinnae, pubescent; secondary rachis terete, 6-13 cm long, green, pubescent, with recurved prickles; leaflet numerous, oblong, 6-12 paired per pinna, opposite, 2.0-3.8 cm by 1.2-2.0 cm, oblique at the base, entire along the margin, rounded to acute at the apex, mucronate, pubescent on both surfaces. Inflorescences axillary racemes, many-flowered, densely flower at upper part and sparsely in lower part; peduncles 17-35 cm long, with densely straight spines, green, pubescent. Flowers unisexual, zygomorphic, yellow; pedicel terete, yellowish-green, pubescent; bracts subulate, yellowish-green, pubescent, caducous. Sepals 5, elliptic, 5-7 mm by 3-4 mm, ferruginous hairy on both surfaces, yellowish-green; petals 5, Caesalpinaceous, free, oblong or oblanceolate, 8-10 mm by 3 mm, yellow, clawed. Stamens 10, free, inserted; filaments filiform, pale green, wolly hairy at the base; staminodes in female flowers, anther dithecous, dorsifixed, yellow. Ovary hairy, unilocular with 1-2 ovules on the marginal placentae, with spine, shortly stipitate; style hairy; stigma ciliate; sterile pistil in male flower, flower rudimentary, hairy. Pods obovoid, spiny, 5-7 cm by 3.0 -4.5 cm, apex rounded with style rement, beaked, dehiscent, hairy spine. Seeds subglobose, 1 or 2, smooth, grey.

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

Tricolporate, suboblate, medium, 28.8-33.8× 37.5-42.5 μm in length and breadth; amb triangular, planaperturate; colpi longicolpate, 25.0-31.3×13.8-18.8 μm in length and breadth; pori lolongate, 7.5-15.0×3.8-7.5 μm in length and breadth; operculum 1.3-2.5 μm thick; exine 2.5-3.8 μm thick, sexine as thick as nexine; sculpturing reticulate, lumina heterobrochate, 1.3-2.5 μm thick; muri simplibaculate, about 0.6 μm wide.

6. *Caesalpinia sappan* L. Sp. Pl. L: 381.1753. (Figure 3. D)

Myanmar name : Tein nyet

English name : Unknown

Flowering period : July-September

Perennial; small tree; stems and branches terete, brownish green, recurved prickles, glabrous. Leaves bipinnately compound, paripinnate; alternate; stipules spiniform, pale green, caducous; petioles terete, 3-5 cm long, green, sparsely pubescent; racheae 15-25 cm long, green, pinnae 8-13 pairs, armed with recurved prickles at the base; stipel absent; petiolules terete, green, pubescent; leaflets elliptic-oblong, rhomboid, 1.0-1.5 cm by 0.5-0.8 cm, oblique at the base,

entire along the margin, retuse or rounded at the apex, glabrous above, sparsely hairy beneath, green. Inflorescences axillary or terminal raceme, many-flowered; peduncles terete, 10-30 cm long, green, glabrous, recurved prickles. Flowers bisexual, zygomorphic, pentamerous, 1.5-2.0 cm in diameter at anthesis, yellow; bracts lanceolate, green, puberulent, caducous; pedicels terete, yellowish-green, sparsely pubescent; bracteoles absent. Sepals 5, unequal, ovate, 0.5-1.0 cm long, yellowish-green, tomentose. Petals 5, Caesalpinaceous, broadly obovate, 0.8-1.2 cm by 0.5-0.9 cm, yellow, glabrous, red striated. Stamens 10, free, slightly exserted, all fertile; filaments filiform, unequal, pale yellow, glabrous top, ciliated at the base; anthers dithecous, dorsifixed, longitudinal dehiscence. Ovary superior, oblong, unilocular with few ovules on the marginal placentae, glabrous; style slender, curved, yellow, glabrous; stigma truncate. Pods obliquely oblong, compressed, beaked, 2- 4 seeded, 4-5 by 2.0-2.5 cm, glabrous. Seeds oblong, slightly compressed, pale brown, glabrous.

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

Tricolporate, suboblate, medium, 35.0-43.8 × 40-45 μm in length and breadth; amb circular, planaperturate; colpi longicolpate, 25-35 × 12.5-18.8 μm in length and breadth; pori lolongate, 6.3-10.0 × 2.5 -5.0 μm in length and breadth; operculum 1.3-2.5 μm thick; exine 3.8-50 μm thick, sexine thicker than nexine; sculpturing coarsely reticulate; lumina heterobrochate, 1.3-3.8 μm width; muri simplibaculate, 0.6-1.8 μm wide.

7. *Clitoria ternatea* L. Sp. Pl. 2: 753, 1753. (Figure 4. A)

Myanmar name : Aung me' nyo

English name : Butterfly pea, Blue pea, Asian pigeon-wings

Flowering period : June-October

Annual, twinning herbs; stems and branches terete, green, pubescent. Leaves unipinnately compound, imparipinnate, alternate; stipules linear-lanceolate, green, pubescent, persistent; petioles 2.0-3.0 cm long, cylindrical, green, pubescent; rachis 5.0-10.0 cm long, cylindric, pale green, pubescent; petiolule pale green, pubescent; leaflets 5-7, ovate-elliptic, 2.5-5.0 cm by 2.0-4.0 cm, obtuse at the base, entire along the margin, retuse at the apex, pubescent on both surfaces. Inflorescences axillary solitary cyme; peduncles cylindric, green, pubescent. Flowers bisexual, zygomorphic, pentamerous, bright-blue, 2-3 cm in diameter at anthesis; pedicel cylindric, pale green, pubescent; bracts lanceolate, with 2 minute bracts at the junction; bracteoles ovate-orbicular, pale green, persistent, pubescent. Calyx 5-lobed; tube campanulate, pale green, pubescent without, glabrous within, sub equal. Corolla papilionaceous, standard broadly obovate, 4.0-4.5 cm by 3.0-3.5 cm, bright-blue, with a pale yellow bloth, pubescent; wings oblong, 2.0 cm by 1.3 cm, claw long, bright blue at the apex, glabrous; keel boat-shaped 2.0 cm by 0.5 cm pale yellowish-green, pubescent without, glabrous within. Stamens 10, diadelphous; free, inserted; filaments filiform, pubescent, yellowish-green; anther dithecous, dorsifixed, uniform, pale yellow, longitudinal dehiscence. Ovary superior, linear, unilocular with few-ovules in the locule on the marginal placentae; style long, filiform, curved, stigma simple. Pods linear and compressed, green, tapering to a point, dehiscent, 6- to-10 seeded, pale yellow, pubescent. Seed reniform, brown, glabrous.

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

Triporate, oblate, medium, 28.8-35.0×50.0-58.8 µm in length and breadth; amb rounded triangular, angulaperturate; pori lolongate, 11.3-20.0×7.5-12.5 µm in length and breadth; exine 1.3 -2.5 µm thick, sexine as thick as nexine; sculpturing psilate.

8. *Mucuna hirsuta* Wight & Arn., Prod. 254. 1834. (Figure 4. D)

Myanmar name : Khwe la ya

English name : Velvet bean

Flowering period : October to December

Annual, twining herbs; stems cylindrical, densely brownish pubescent, green. Leaves trifoliolate compound, alternate; stipules lanceolate, green, tomentose, caducous; petioles terete, canaliculate above, 5-25 cm long, green pubescent; stipels acute, tomentose, green, caducous; petiolules terete, hirsute, pale brownish-green; leaflets 3, ovate rhomboid, 10.0-17.5 cm by 7.5 -14.0 cm, oblique or obtuse at the base, entire along the margin, acuminate at the apex, brownish pubescent on both surfaces, green. Inflorescences axillary pendulous racemes; many flowered; peduncles terete, 8-17 cm long, tomentose, green. Flowers bisexual, zygomorphic, pentamerous, 0.6-0.9 cm in diameter at anthesis, deep purple; pedicels terete, green tomentose; bracts lanceolate, caducous; bracteoles minute, fugacious, caducous. Calyx fused, campanulate, 5-lobed; tube reddish-green, tomentose; lobes acuminate, upper 2 lobes united, reddish-green, pubescent. Corolla papilionaceous; standard obovate, auriculate, 1.8 cm by 1.7 cm, deep purple; wings oblong, incurved, 2.3-2.5 cm by 1.0 cm, adherent to keel, glabrous, deep purple; keels linear-oblong, slightly longer than or equal to wings, apex beaked, 2.5 cm by 0.5 cm, glabrous, deep purple. Stamens 10, diadelphous, exserted, staminal sheath purplish-white, glabrous; anthers dimorphic, dithecous, longitudinal dehiscence. Ovary villous, oblong, about 0.5 cm long, unilocular, one ovules in each locule on the marginal placentae, brownish pubescent, green; style filiform, glabrous, white; stigma small, capitate. Pods oblong, thick, 7.5-10.5 cm by 1.2-1.5 cm, dehiscent, slightly curved at the tip, covered with densely golden hair. Seeds elliptic-oblong, glabrous, black, shining.

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

Tricolporate, subspheroidal, large, 57.5-70.0×56.3-68.8 µm in length and breadth; amb triangular, angulaperturate; colpi ¾ way up to the pole, 31.3-40.0×5.0-7.5 µm in length and breadth; pori lalongate 12.5-15.0×21.3-25.0 µm in length and breadth; exine 2.5-3.8 µm thick, sexine as thick as nexine; sculpturing reticulate; lumina heterobrochate, 3.8-7.5 µm width; muri simplibaculate, 0.6-1.3µm wide.

9. *Vigna trilobata* (L.) Verdc, Taxon 17:172. 1968. (Figure 5. A)

***Dolichos trilobatu*s L., Mant. Pl. 1:101. 1767.**

Myanmar name : Taw mat pe

English name : Unknown

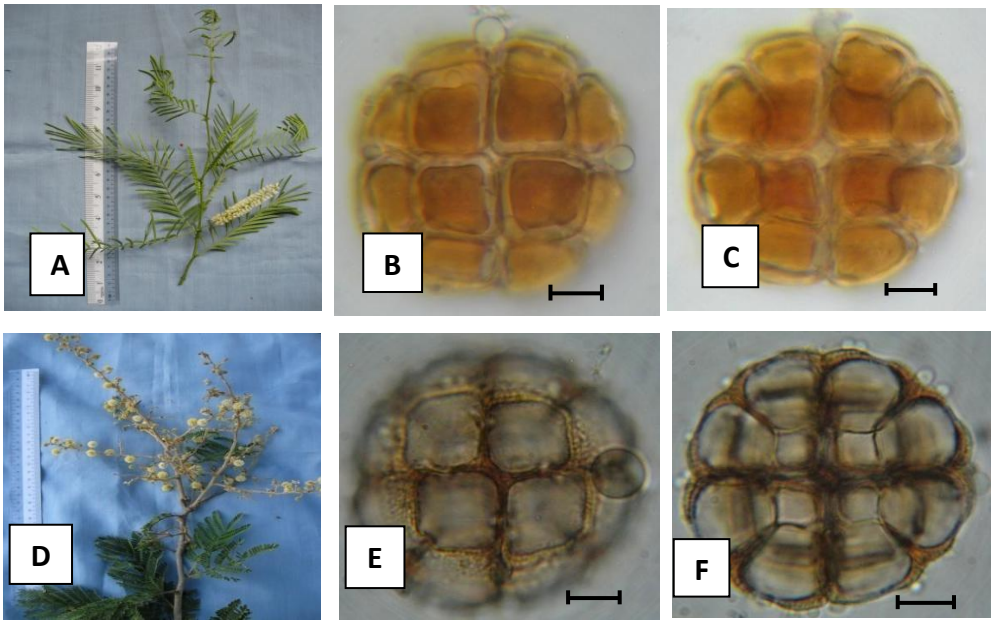
Flowering period : January-April

Annual, procumbent herbs; stems and branches slender, terete, ribbed, reddish-green, sparsely pubescent. Leaves trifoliolate compound, alternate; stipules peltate, green, pubescent,

persistent; petioles laterally compressed, 4-9 cm long, canaliculate, green, sparsely pubescent; stipels minute, ovate, green, glabrous; petiolules terete, green, pubescent; leaflets usually with shallow lobes, rhomboid or ovate, 3-lobed, 2.5-5.0 cm by 1.5-4.0 cm, green, obtuse at the base, entire along the margin, obtuse at the apex, sparsely pubescent on both surfaces. Inflorescences axillary raceme, erect, few-flowered; penducles terete, 12-28 cm long, green, sparsely pubescent. Flowers bisexual, zygomorphic, pentamerous, hypogynous yellow, about 8 mm in diameter at anthesis, showy; pedicel very short, green, pubescent; bracts ovate, green, pubescent; bracteoles obliquely lanceolate, green, glabrous. Calyx campanulate, 5-lobed; tube campanulate, green, glabrous; lobes minute, linear, pale green, glabrous. Corolla papilionaceous; standard orbicular, 4-6 mm by 5-8 mm, yellow above, brownish-green beneath; wings obliquely oblong, 4-7 mm long, yellow, clawed, glabrous; keel falcate, beaked, 6-7 mm by 2-3 mm, pale yellowish-white, glabrous, clawed, adhering to the wings. Stamens 10, diadelphous; filament filiform, white, glabrous; anther dithecous, basifixed, uniform, longitudinal dehiscence. Ovary superior, linear, green, pubescent, unilocular with one ovules in each locule on the marginal placentae; style filiform, white, glabrous; stigma globose. Pods linear, straight, 6-12 seeded, dehiscent, glabrous. Seeds brown, cylindric, truncate at both ends, glabrous.

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

Triporate, oblate spheroidal, medium, 22.5-30.0×23.8-31.3 µm in length and breadth; amb rounded triangular, angulaperturate; pori lolongate, 5.0-7.5×3.8-6.3 µm in length and breadth; operculum about1.3 µm thick; exine 2.5-3.8 µm thick, sexine thicker than nexine; sculpturing coarsely reticulate, lumina heterobrochate, 2.5-5.0 µm width, muri simplibaculate, about 1.3 µm wide.



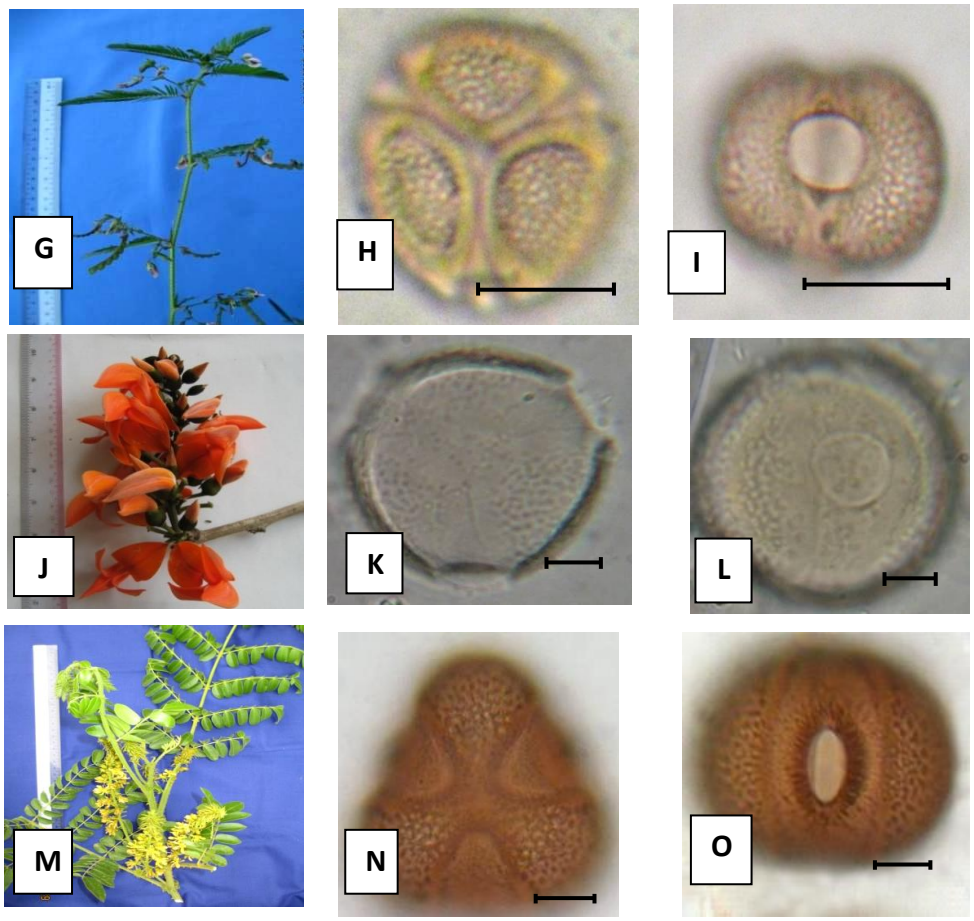
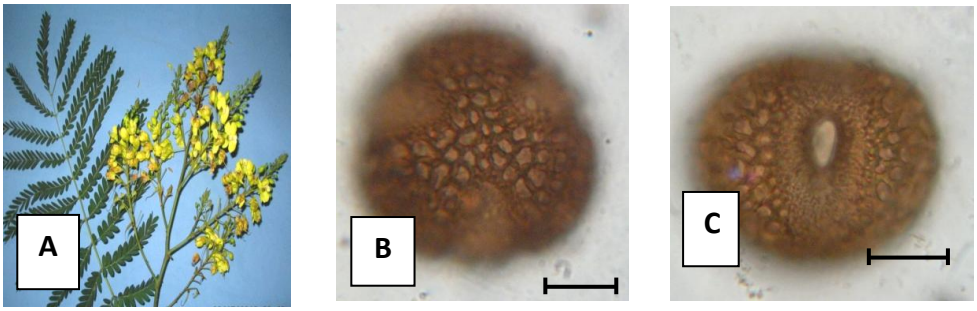


Figure 1 A. Inflorescences of *Acacia catechu* (L.f) Willd.
B &C Polar & Equatorial view pollen of *A. catechu* (L.f) Willd.
D. Inflorescences of *Acacia leucophloea* (Roxb.) Willd.
E &F Polar & Equatorial view pollen of *A. leucophloea* (Roxb.) Willd.
G. Inflorescences of *Aeschynomene americana* L.
H & I Polar & Equatorial view pollen of *A. americana* L.
J. Inflorescences of *Butea monosperma* (Lam.) Taub
K &L Polar & Equatorial view pollen of *B. monosperma* (Lam.) Taub
M Inflorescences of *Caesalpinia bonduc* (L.) Roxb.
N &O Polar & Equatorial view pollen of *C. bonduc* (L.) Roxb.
Scale bar = 10 µm



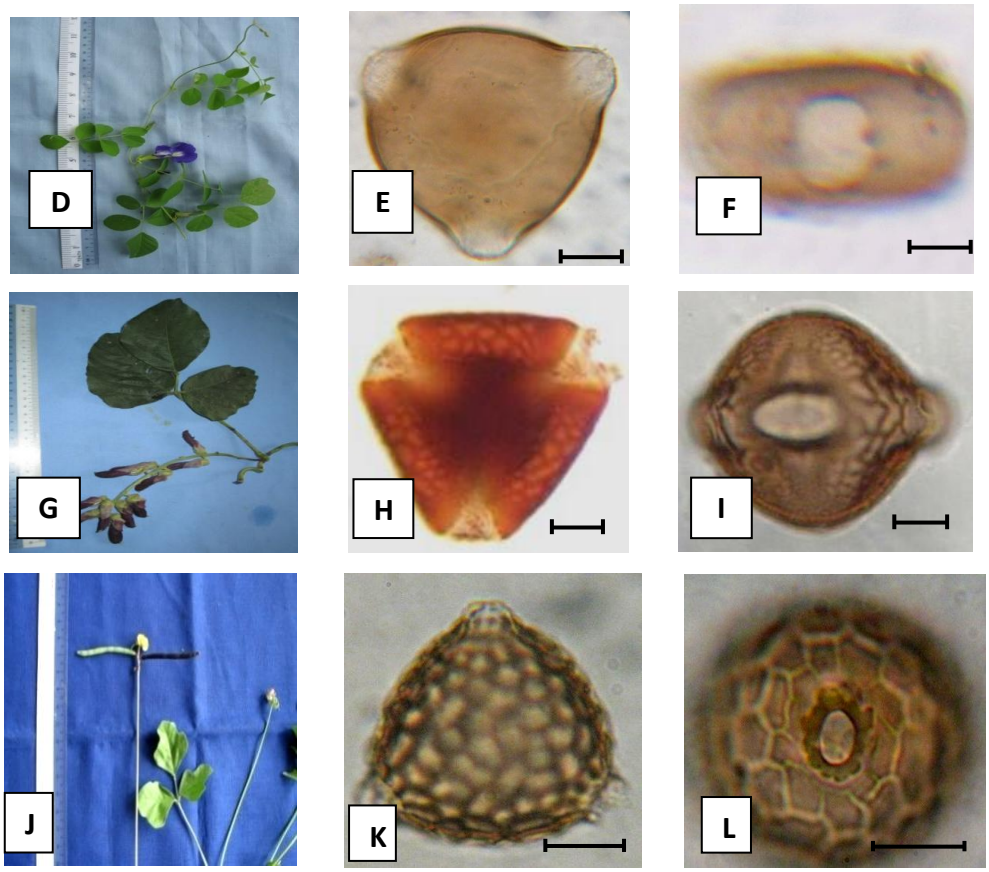


Figure 2 A. Inflorescences of *Caesalpinia sappan* L.
B &C Polar & Equatorial view pollen of *C. sappan* L.
D. Inflorescences of *Clitoria ternatea* L.
E &F Polar & Equatorial view pollen of *C. ternatea* L.
G. Inflorescences of *Mucuna hirsuta* Wight & Arn.
H & I Polar & Equatorial view pollen of *M. hirsuta* Wight & Arn
J. Inflorescences of *Vigna trilobata* (L.) Verdc
K &L Polar & Equatorial view pollen of *V. trilobata* (L.) Verdc

Scale bar = 10 µm

Discussion and Conclusion

In this research, taxonomy and pollen morphology of 9 species belonging to 7 genera of the family Fabaceae were studied. According to the resulting data, habits of this species are tree *Acacia*, *Butea*, small tree is *Caesalpinia sappan* L.; shrubs is *Aeschynomene americana* L.; lianas is *Caesalpinia bonduc* (L.) Roxb. and remaining species are herbs. The trifoliolate leaves also occurred in *Butea*, *Mucuna*, *Vigna* and the remaining species are uni or bipinnate. The inflorescences types of studied species are mostly raceme, rarely cymose *Clitoria ternatea* L.; pedunculate spike and globose head. Flowers are actinomorphic in *Acacia* and the other species are zygomorphic. Stamens are found in diadelphous and free.

Pollen morphology was classified on the basic of aperture, size, shape and sculpturing pattern.The resulting data of the pollen morphology were presented Figure 1- 5. In this research, the types of pollen are found polyads and monad. *Acacia* is polyads and the remaining species

are monad. The types of aperture in monad are mostly colporate and rarely porate. Porate pollen occurs in *Clitoria ternatea* L., *Vigna trilobata* (L.) Verdc and the rest species are colporate. Perveen and Qaiser (1998) stated that family Fabaceae is a eurypalynous. The pollen grains are mostly colporate, rarely colpate or porate. Sculpture reticulate type is most common.

In this study, the shapes of pollen grains are oblate spheroidal, subspheroidal, oblate and suboblate. Suboblate are found in *Caesalpinia*; oblate are *Aeschynomene americana* L., *Clitoria ternatea* L.; one species of subspheroidal is *Mucuna hirsuta* Wight & Arn.and the remaining species are oblate spheroidal. In polar view is triangular, rounded triangular and circular. The sculpturing patterns of the pollen are psilate, reticulate and coarsely reticulate; psilate is found in *Acacia catechu* (L.f) Willd., *Clitoria ternatea* L.; coarsely reticulate is occurred in *Caesalpinia sappan* L., *Vigna trilobata* (L.) Verdc and other species are reticulate pollen.

Walker and Doyle (1975) stated that the sculpture patterns on appearance are also varied significantly from one species to another species. Inaperturate pollen is relatively more primitive character than that of mono-, di-, tri- and polyaperturate.

Pollen grains of the genus *Caesalpinia* is tricolporate, amb circular, triangular, aperture reticulate. Graham (1998) mentioned that pollen characters of genus *Caesalpinia* are tricolporate, amb circular and aperture reticulate which characters are agreed with present result. Pollen grains of *Caesalpinia sappan* L. is tricolporate, amb circular and coarsely reticulate. Rao and Lee (1970) stated that the pollen grain of *Caesalpinia sappan* L. is tricolporate, triangular in polar view, coarsely reticulate, which characters are agreed with present result.

Walker and Doyle (1975) described that angiosperm have two basic pollen grains, monosulcate and tricolpate. Pollen of the monosulcate type is characteristics of primitive dicotyledons, majority of monocotyledons, cycads and pteridosperms. Palynologists agree that the first flowering plants probably had monosulcate pollen grains. Tricolpate types are characteristics of advanced dicotyledons. Faegri *et al*. (1964) stated that the structure and sculpturing of the exine provide characters of great diagnostic value. There are many other characters which may be of equal or even greater importance in the identification of pollen grains.

These pollen characters will be supported for identification and classification. All these interesting pollen features are undoubtedly important and beneficial for the future taxonomic studies. Moreover, this research is provided the knowledge of pollen morphology of Fabaceae to botanist and others scientists who are interested.

Acknowledgements

I would like to think Dr Nu Nu Yee, Professor and Head, Department of Botany, University of Mandalay, for her kind permission to do this research. My heartful thank to Dr Aye Aye Kyi, Professor and Head, Department of Botany, University of Magway, for her kind suggestions. I especially to thanks to Dr Swe Swe Linn, Lecturer, Department of Botany, University of Mandalay for her valuable advice the knowledge concerned with pollen. I would like to express my supervisor Dr Nwe Nwe Yi, Professor, Department of Biology, Institute of Sagaing University, for her guidance, valuable advice and my research.

References

Anonymous. (2012). Fabaceae. Wikimedia foundation Inc., a non-profit organization.

Bhojwani, S.S & S.P. Bhatnagar. (2005). The embryology of angiosperm. Vikas publishing house PVT LTD., Masjid Road, Jangpura, New Delhi.

Briggs, D. & J. Brady. (2000). Correlation between the structure and function of pollen grains of four species of angiosperm. Smith College.

Dassknayake, M.D. (1980-1991). A revised handbook of Flora of Ceylon Vol 1 and 7 Fabaceae. Department of Agriculture, University of Peradeniya. Sri Lanka.

Erdtman, G. (1952). Pollen morphology and plant taxonomy. Angiosperms. An introduction to palynology. Almquist & Wiksell, Stocknolm. The Chronica Botanica Co. Waltham. Mass. U.S.A.

Erdtman, G. (1969). Handbook of palynology, morphology, taxonomy, ecology. Hafner Publishing Co., Inc. New York.

Erdtman, G. (1971). Pollen morphology and plant taxonomy. Hafner Publishing Co., Inc. New York.

Erdtman, G. (1985). Palynology. Vol 22. Advances in Botanical Research. Academic Press.

Faegri, K., J. Iversen, & H.T. Waterbolk. (1964). Text book of pollen analysis. International booksellers & Publishers Limited. Denmark.

Graham, A. (1998). Studies on neotropical paleobotany. XI Late tertiary vegetation and environments of southeastern Guatemala. Amecican Journal of Botany, Department of Biological Science, Kent State University, Kent.

Hesse, M., H. Halbritter., R. Zetter., M. Weber., R. Buchner., A. Frosh-Radiva & S. Ulrich. (2009). Pollen Terminology. University of Vienna, Austria.

Hoen, P. (1999). Glossary of pollen and spore terminology, second and revised edition Utrecht. Netherland.

Kress, J., R. A. Defilipps., E. Farr. & Yin Yin Kyi. (2003). A checklist of the trees, shrubs, herbs and climbers of Myanmar. Department of Systematic Biology- botany. National Museum of Natural History Washington, DC. U.S.A.

Langran, X., C. Dezhao., Z. Xiangyun & H. Puhua. (2010). Flora of China. Vol 10 Fabaceae. MBG Press and Science Press, Beijing. China.

Moore, P. D., J. A. Weeb. & M.E. Collinson. (1991). Pollen analysis. Second edition. Oxford Black well Scientific Publications London.

Paldat. (2005). Illustrated handbook on pollen terminology. University of Vienna, Rennweg 14.A 1030 Wein. Austria.

Pascoe, M.S. (2007). Pollen images of selected British flora: Towards development of a National database. Department of Biology & Enviromental study, University of Sussex.

Perveen, A. & M. Qaiser.(1998). Pollen flora of Parkistan VIII. Leguminosae. (Subfamily: Papilionoideae). Department of Botany, University of Karachi. Karachi-Parkistan.

Rao, A.N & Y.K. Lee. (1970). Studies on Singapore pollen. Department of Botany, University of Singapore, Pacific science.

Simpson, M. G. (2006). Plant Systematic. Elsever academic press. 84 Theobald's Road, London. WCIX 8RR.UK.

Walker, J.W. & J.A. Doyle. (1975). The bases of angiosperm phylogeny. Ann. Missovri Bot. Gard. 62-664-723.

MORPHOLOGY, MICROSCOPICAL CHARACTERS, PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITYOF *TADEHAGI TRIQUETRUM* (L.) OHASHI

Khin Min MinThwe[1]

Abstract

Tadehagi triquetrum (L.) Ohashi is one of the medicinal plants under the family Fabaceae and it is well known as "laukthay" in Myanmar. This plant was collected from Myitkyina Township, Kachin State. Morphological and microscopical characters, phytochemical constituents and antimicrobial activities were investigated so as to certain their correct identification. The plant was perennial erect shrub, woody and reddish green angular branches with hairs on the angles. The corolla were papilionaceous and the fruits were jointed. In microscopical study, paracytic stomata were found on both surfaces. The vascular bundle was collateral type and endarch. The powdered leaves were tested for the phytochemical constituents and observed that alkaloids, flavonoids, phenolic compounds, steroids, saponins, tannins, glycosides and carbohydrates were present. The alkaloids, flavonoids, terpenoids, phenolic compounds, steroids, saponins, tannins, glycosides and carbohydrates were present in powdered root. In antimicrobial activity, the various solvent extracts of leaves and roots were tested by agar well diffusion method. The ethyl acetate extract of leaves and the methanol extract of roots showed the highest activity.

Key words: *Tadehagi triquetrum*, morphology, anatomy, antimicrobial activity

Introduction

Herbal medicine has a great tradition of maintaining human health for centuries. A majority of the world's population living in the developing countries still relies on herbal medicine to meet its health care needs (WHO, 1999). Medicinal plants are abundant in Myanmar. Eighty five percent of the population lives in rural areas. Most of people use the traditional medicinal plants for the treatment of diseases (Medicinal plants of Myanmar, 2000). *Tadehagi triquetrum* (L.) Ohashi,a species of Fabaceae, was distributed in Chin, Kachin, Kayin, Mandalay, Sagaing, Shan, Yangon areas of Myanmar (Kress *et al*., 2003). Allen, (1981) stated that it is widespread in all South Asian, East Asian, and Southeast Asian countries. The maximum height of this shrub tree is 3m. Leaves alternate,linear-oblong, ovate with a tapering tip. Flowers are small and pale purplish in color. Fruit is hairy and distinctly jointed.

The whole plant is used medicinally as an antipyretic, as a diuretic, for invigorating the spleen, and for promoting digestion. Chemical constituents from *Tadehagi triquetrum* (L.) Ohashi and their antihyperlipidemic activities was studied by Allen(1981). The traditional clinics have been using this medicinal plant practically in the past several decades. However, the systematic and comprehensives investigation is found to be lacking. Therefore, this research was conducted for safety and efficacy in treating lungs tonic and tuberculosis.

Aim of the present research work is to examine the medicinal plant scientifically which have effective medicinal values. The objectives are to identify and study the morphological and microscopical characters of *Tadehagi triquetrum* (L.) Ohashi, to perform the preliminary phytochemical test, and to evaluate the antimicrobial activity.

[1] Dr, Lecturer, Department of Botany, University of Myitkyina

Materials and Methods

The leaves and roots of *Tadehagi triquetrum* (L.) Ohashi were collected from the Myitkyina Township, Kachin State, from December 2017 to December 2018. Fresh specimens of vegetative and floral parts were used for identification. The leaves and roots were dried in shade for several days. When completely dried, these were pulverized by grinding machine to get the powder and stored in an air tight container for the microbiological studies.

For the microscopical studies, the lamina, midrib, petiole, stem and root from the fresh specimens were observed according to methods of Esau (1953) and Pandey(1993).

For phytochemical investigation, the leaves and roots were air dried at room temperature for two weeks. After the samples were completely dried, these were pulverized by grinding machine to get the powder and stored in an air tight container to prevent moisture changes and contamination.

Preliminary phytochemical examination for the leaves was carried out to detect the organic compounds qualitatively according to British Pharmacopoeia, (1965). Alkaloids test, Flavonoids test, Terpenoids test, Phenolic compounds test, Steroids test, Saponins test, Tannins test, Glycosides test, and Carbohydrates test were according to Marini-bettolo(1981), Unani Medicine (1987) and Trease and Evans (1980).

Antimicrobial activity of crude extracts of *Tadehagi triquetrum* (L.) Ohashi by Agar well diffusion method. Antimicrobial activity of crude extracts from the leaves and roots of *Tadehagi triquetrum* (L.) Ohashi have been investigated.

Antimicrobial activity of the leaves and roots of *Tadehagi triquetrum* (L.) Ohashi were conducted by using chloroform, methanol, ethyl acetate, 95% ethanol extracts. In this study, agar well diffusion method was used to screen the antimicrobial activity. These experiments were conducted in the Laboratory of Development Center for Pharmaceutical Technology (DCPT).

The powders of leaves and roots were extracted by using chloroform, methanol, ethyl acetate, 95% ethanol for about 2 weeks and then filtered. The solvents were then evaporated by using water bath to obtain a paste.The different solvents extracts were tested against six pathogenic microorganisms by using agar well diffusion method described by Cruickshank (1975). The strains of six pathogenic microorganisms were *Bacillus subtilis* (JAP-0221215), *Bacillus pumilus* (IFO-12102), *Staphylococcus aureus* (ATCC-12277), *Pseudomonas aeruginosa* (IFO-3080), *Candida albicans* (IFO-1060), and *Escherichia coli* (ATCC-25922).

Results

Morphological characters

Tadehagi triquetrum (L.) Ohashi, Ginkgoana 1:290.1973. (Figure 1)

Myanmar name: Laukthay

English name : Trefle Gross

Family : Fabaceae

Flowering period: October to December

Perennial erect or ascending shrub, about 1.5 cm high; branches angular, hairs on the angles, woody and reddish green.Leaves unifoliate compound, alternate and stipulate; stipules lanceolate; petioles distinctly winged, glabrous and green; leaflets oblong-lanceolate. Inflorescences terminal and many-flowered; peduncles triquetrous, tomentose and green. Flowers bisexual, zygomorphic; bracts lanceolate, pubescent, reddish green; bracteoles acute and minute, pale red.Calyx 5-lobed; tube campanulate, hairy and reddish green; lobes deltoid to linear. Corolla papilionaceous, exserted; standard orbicular; keel lanceolate, glabrous and pale purple. Stamens 10, diadelphous; staminal tube, pale green and glabrous; anthers uniform, dorsified, dithecous longitudinal slit and pale brown. Ovary oblongoid, densely villous and reddish green, unilocular, 6-9 ovuled on the marginal plancentae; style curved, glabrous and pale green; stigma simple. Pods flattened, linear, indehiscent, seeded softy pubescent with hairs and green. Seeds elliptic, small, glabrous and brown.

Figure1 Morphological characters of *Tadehagitriquetrum* (L.) Ohashi

A. Habit B. Inflorescence

C. Fruits D. Roots

Microscopical Characters

Lamina

In surface view, the cuticle is smooth and the epidermal cells of both surfaces are thin-walled parenchymatous, irregular wavy. The upper epidermal cells are slightly wavy and lower epidermal cells are wavier. Paracytic stomata are pressed on both surfaces and more abundant on the lower surface. The stomata are oval in outline with two reniform shaped guard cells and contain abundant chloroplasts. In transverse section, the cuticle was present on both surfaces. The epidermal cells were thin-walled and barrel shaped parenchymatous cells. The mesophyll was composed of palisade and spongy parenchymatous cells. Two layered thick palisade cells were right angle to the upper epidermis, elongated and compactly arranged with numerous

chloroplasts. The spongy mesophyll cells were placed beneath the palisade parenchyma consisting of 2-3 layers thick. They were rounded to elongated cells and contained numerous chloroplast (Figure 2 C).

Midrib

The epidermal cell of both surfaces are parenchymatous and rectangular in shaped along the length of the midrib. Unicellular uniseriate trichomes are present similar to those of the lamina.

In transverse section, concave in the lower surface and convex in the upper surface were covered with thick cuticle (Figure 2 D). The epidermal cells were thin-walled, barrel-shaped parenchymatous cells and compactly arranged. The upper epidermal cells were larger than the lower epidermal cells. The angular collenchymatous cells were present 1-2 layers in thickness below the upper epidermis. They were rounded to isodiametric in shape. The parenchymatous cells were 3-5 layers in thickness above the vascular bundle and 4-6 layers in thickness below the vascular bundle. The vascular bundle was crescent shaped in outline and collateral type. The four bundles was surrounded by a sheath of sclerenchymatous cells.

Petiole

In transverse section, the petiole was oval in outline (Figure 2 E). The cuticle layer was thick. The epidermal cells were barrel-shaped parenchymatous cells and compactly arranged. The cortex was made up of 6-7 layered parenchymatous cells. They were thin-walled and oval to rounded cells. The vascular bundles are semi-circular shaped four bundles and embedded in the parenchtmatous tissues. The arrangement was collateral type. A large pith region located in the center.

Stem

In transverse section, the young stem was circular in outline (Figure 2 F). The epidermal cells were barrel-shaped parenchymatous and one layer thick. The cortex was made up of collenchymatous cells towards the peripheral region. The collenchymatous cells were angular type and consisted of 4-6 layers. Endodermis located below the cortex. The cells were thin-walled, barrel-shaped parenchymatous cells and one layer thick. The pericycle fibre formed as a ring around the four vascular bundles, ectopholic siphonostele type. The vascular bundle were rounded.

Root

In transverse section, the roots are circular in outline (Figure 2 G). Cortex is made up of several layers of parenchymatous cells and vascular bundles are evenly distributed. At maturity, the epidermal cells are thin-walled and rectangular in shape. The cortex is composed of three layers. Phellem or cork cells are three to four layers with thin-walled and compact rectangular cells, phellogen or cork cambium consists of two to four layers and phelloderm or secondary cortex composed of five to seven layers, thin-walled parenchymatous and irregular arranged. Xylem towards the inner and phloem outside the xylem. Alternating layers of vascular bundles are collateral type. Storage parenchymatous cells are present betweeen the vascular bundles.

Preliminary phytochemical investigation

The preliminary phytochemical investigation the leaves of *Tadehagi triquetrum* (L.) Ohashi indicated the presence of alkaloids, flavonoids, phenolic compounds, steroids, saponins, tannins, glycosides, carbohydrates. The terpenoids was absent. The preliminary phytochemical investigation was shown in Table 1.

The preliminary phytochemical investigation of the roots of *Tadehagi triquetrum* (L.) Ohashi indicated the presence of alkaloids, flavonoids, terpenoids, phenolic compounds, steroids, saponins, tannins, glycosides and carbohydrates. The preliminary phytochemical investigation was shown in Table 2.

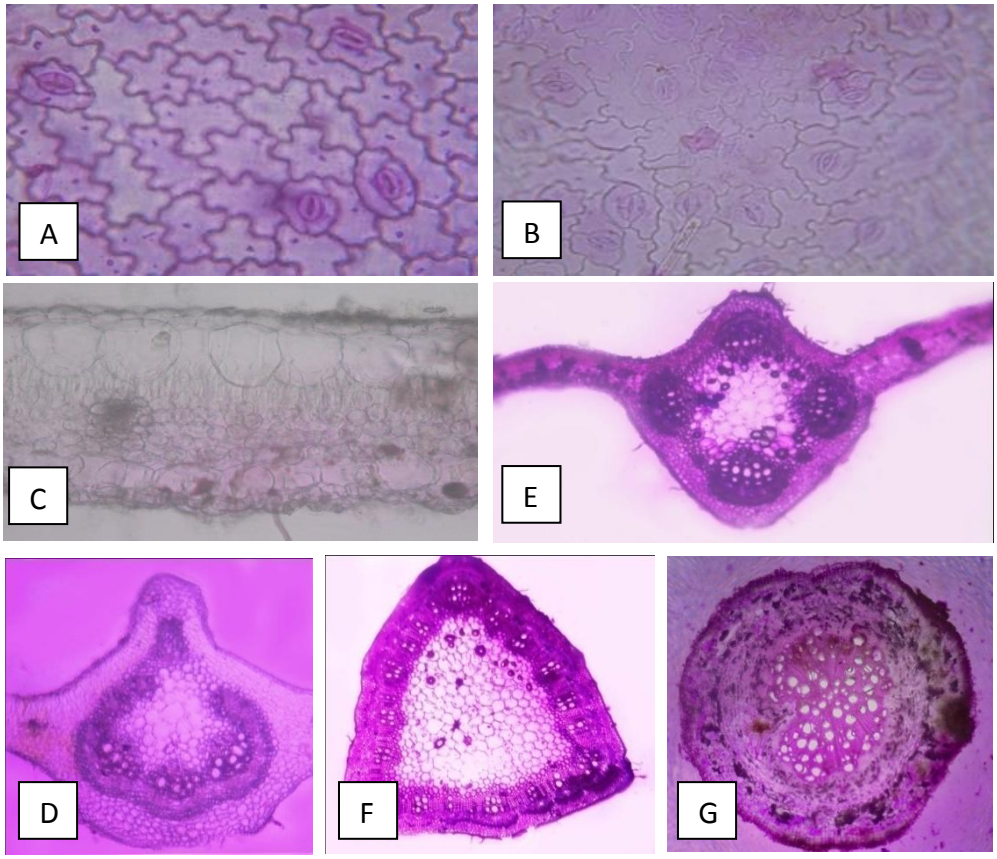


Figure 2 Microscopical characters of *Tadehagi triquetrum* (L.) Ohashi

A. Surface view of lamina showing upper epidermal cells

B. Surface view of lamina showing lower epidermal cells

C. Transverse section of lamina

D. Transverse section of midrib showing trichomes

E. Transverse section of petiole

F. Transverse section of stem

G. Transverse section of mature root

Table 1 Preliminary Phytochemical test on the leaves

No	Tests	Extracts	Test reagents	Observations	Results
1	Alkaloids	1%HCl	Dragendroff's reagent	Yellow ppt.	+
			Wagner's reagent	Orange ppt.	+
			Mayer's reagent	White ppt.	+
2	Flavonoids	EtoH	Mg turning, conc:HCL	Yellow brown	+
3	Terpenoids	EtoH	$CHCL_3$,Conc:H_2SO_4	No colour	-
4	Phenolic compounds	EtoH	5% $FeCL_3$	Reddish browncolour	+
5	Steroids	$CHCl_3$	Acetic anhydride, Conc:H_2SO_4	Green colour	+
6	Saponins	H_2O	Distilled water	Marked frothing	+
7	Tannins	H_2O	5% $FeCL_3$	Dark brown	+
8	Glycosides	H_2O	10% Lead acetate	White colour	+
9	Carbohy-drates	H_2O	10% α naphthol, Conc:H_2SO_4	Violet color ring	+

Table 2 Preliminary Phytochemical test on the roots

No	Tests	Extracts	Test reagents	Observations	Results
1	Alkaloids	1% HCL	Dragendroff's reagent	Yellow ppt.	+
			Mayer's reagent	Orange ppt.	+
			Wagner's reagent	White ppt.	+
2	Flavonoids	EtOH	Mg turning,Conc:HCL	Yellow coloration	+
3	Terpenoids	EtOH	$CHCL_3$,Conc:H_2SO_4	Reddish brown colour	+
4	Phenolic compounds	EtOH	5% $FeCL_3$	Black colour	+
5	Steroids	$CHCL_3$	Acetic anhydride, Conc:H_2SO_4	Green colour	+
6	Saponins	H_2O	Distilled water	Marked frothing	+
7	Tannins	H_2O	5% $FeCL_3$	Dark brown	+
8	Glycosides	H_2O	10% Lead acetate	White pp	+
9	Carbohy-drates	H_2O	10% α naphthol, Conc:H_2SO_4	Violet color ring	+

Antimicrobial activity of different solvent extracts from the leaves and root of *Tadehagi triquetrum* (L.) Ohashi

Antimicrobial activity of different solvent extracts from the leaves and roots of *Tadehagi triquetrum* (L.) Ohashi by using agar well diffusion method. The diameter of inhibition zones that appeared were given in Table3,4 and Figure 3,4.

According to this experiment the antimicrobial activities of *Tadehagi triquetrum*(L.) Ohashi were investigated. In the present study, the extracts of all these plants displayed different activity on the tested microorganisms. The results were showed that ethyl acetate extracts of *Tadehagi triquetrum*(L.) Ohashi leaves and methanol extracts of *Tadehagi triquetrum* (L.) Ohashi roots were highly effective extract against all tested microorganisms causing maximum inhibition zone.

Chloroform extract of leaves showed the minimum inhibition zone 11-13 mm against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli.* Methanol extract showed the minimum inhibition zone 13-14 mm against *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Methanol extract showed the medium activities 15 mm zone against *Bacillus pumalis, Candida albicans* and *Escherichia coli.* Ethyl acetate extract of *Tadehagi triquetrum* (L.) Ohashi were highly effective extract 23-40 mm against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escheriachia coli.* Ethanol extract was showed the minimum inhibition zone 13-14 mm against *Staphlococcus aureus* and *Pseudomonas aeruginosa.* Ethanol extract was showed medium activities 15-17 mm zone against *Bacillus subtilis, Bacillus pumilus, Candida albicans* and *Escherichia coli* as shown in Table 3 and Figure 3.

Choloform extract of root showed minimum activities 12-13 mm zone against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli.* Methanol extracts of *Tadehagi triquetrum* (L.) Ohashi was showed highly effective extract against maxium inhibition zone 28-45 mm against *Bacillus subtilis, Staphyloccus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli.* Ethyl acetate extract was showed medium activities 16-18 mm zone against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and Escherichia coli.* Ethanol extract showed medium activities 16-18 mm zone against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and Escherichia coli* as shown in Table 4 and Figure 4.

Table 3 Antimicrobial activity of different solvent extracts from the leaves

Solvent	**Test Organisms**					
	Bacillus subtilis	***Staphylococcus aureus***	***Pseudomonas aureginosa***	***Bacillus pumilus***	***Candida albicans***	***Escherichia coli***
Chloro-form	12 mm (+)	12 mm (+)	11 mm (+)	11 mm (+)	13 mm (+)	12 mm (+)
Methanol	14 mm (+)	14 mm (+)	13 mm (+)	15 mm (++)	15 mm (++)	15 mm (++)
Ethyl acetate	25 mm (+++)	23 mm (+++)	40 mm (+++)	33 mm (+++)	35 mm (+++)	40 mm (+++)
Ethanol	15 mm (++)	14 mm (+)	13 mm (+)	16 mm (++)	17 mm (++)	16 mm (++)

Agar well – 10 mm; 10 mm ~ 14 mm (+);

15 mm ~ 19 mm (++); 20 mm above (+++)

Table 4 Antimicrobial activity of different solvent extracts from the roots

Solvent	Test Organisms					
	Bacillus subtilis	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Bacillus pumilus***	***Candida albicans***	***Escherichia coli***
Chloro-form	12 mm (+)	12 mm (+)	12 mm (+)	12 mm (+)	12 mm (+)	13 mm (+)
Methonol	28 mm (+++)	33 mm (+++)	45 mm (+++)	35 mm (+++)	45 mm (+++)	30 mm (+++)
Ethyl acetate	17 mm (++)	17 mm (++)	17 mm (++)	18 mm (++)	16 mm (++)	17 mm (++)
Ethanol	17 mm (++)	17 mm (++)	17mm (++)	18 mm (++)	16 m (++)	17 mm (++)

Agar well – 10 mm; 10 mm ~ 14 mm (+)

15 mm ~ 19 mm (++); 20 mm above (+++)

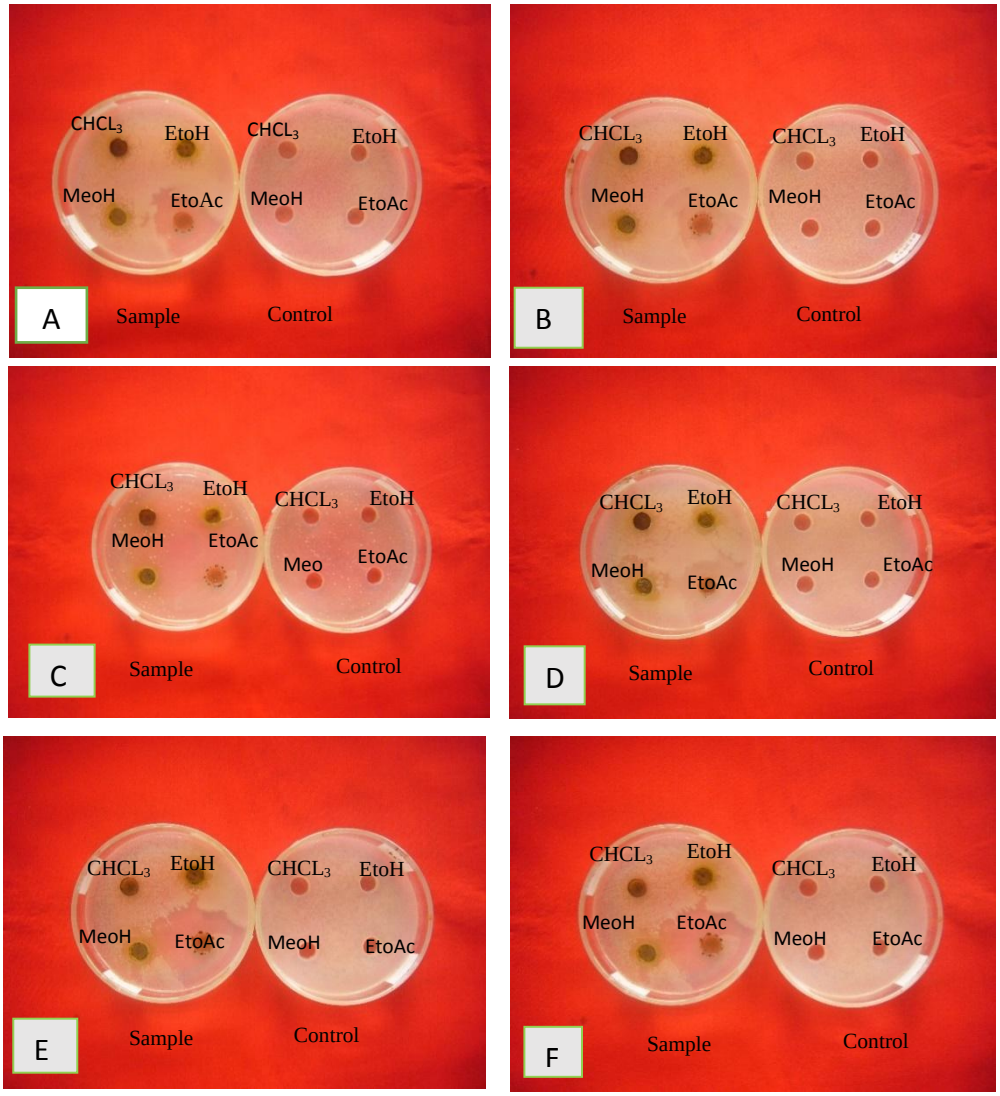


Figure 3 Antimicrobial screening of the leaves extracts from *Tadehagi triquetrum* (L.) Ohashi
A. *Bacillus subtilis*; B. *Staphylococcus aureus*
C. *Pseudomonas aeruginosa*; D. *Bacillus pumilus*
E. *Candida albicans*; F. *Escherichia coli*

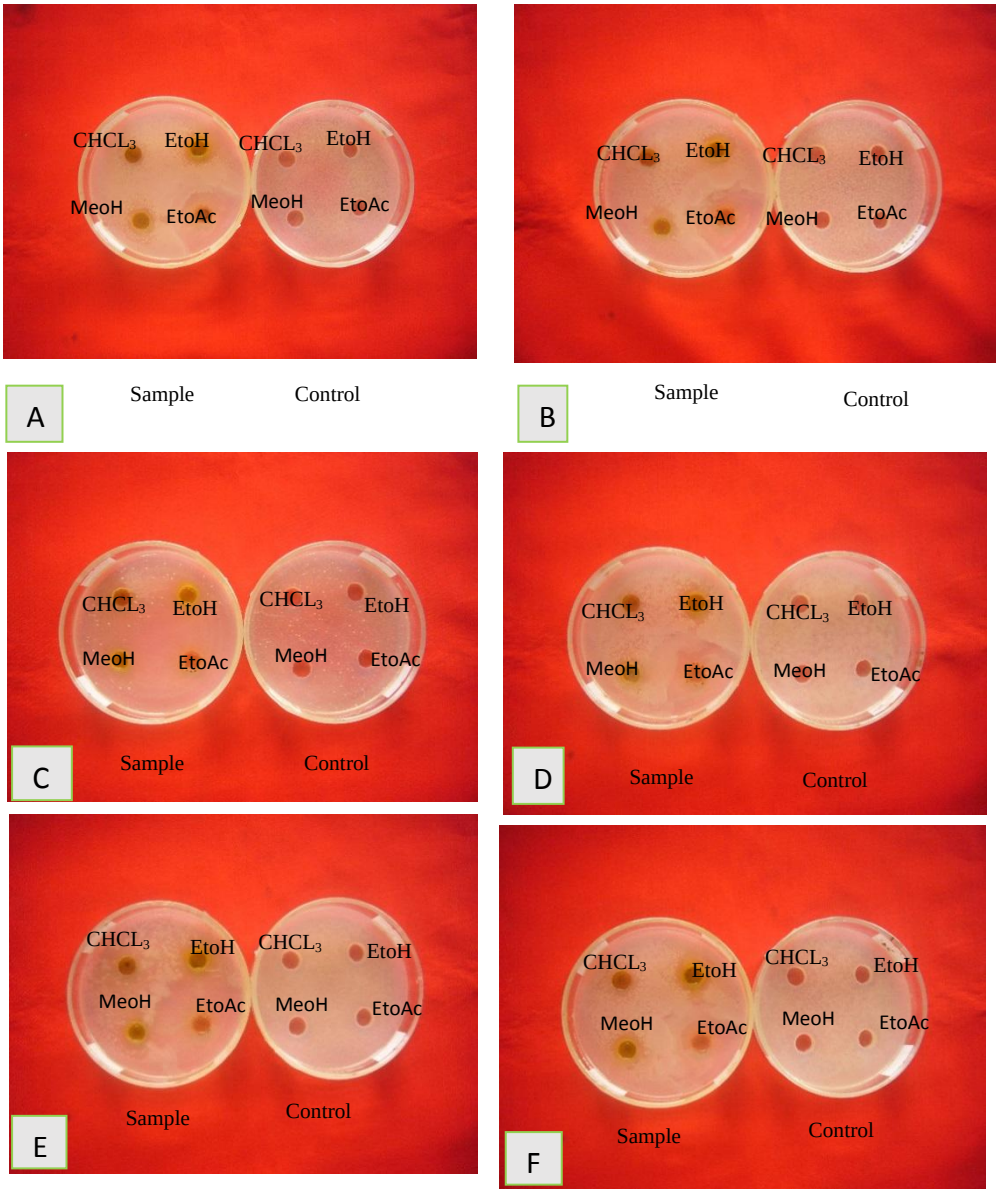


Figure 4 Antimicrobial screening of the roots extracts from *Tadehagi triquetrum* (L.) Ohashi
A. *Bacillus subtilis*; B.*Staphylococcusaureus*
C. *Pseudomonas aeruginosa*; D. *Bacillus pumilus*
E. *Candida albicans*; F. *Escherichia coli*

Discussion and Conclusion

Tadehagi triquetrum (L.) Ohashi is wildely distributed in China, Kachin, Kayin, Mandalay, Sagaing, Shan, Yangon areas of Myanmar (Kress *et al*., 2003). The morphological and microscopical study of the plant revealed that it is a herbaceous shrublet. The leaves are unifoliate with winged petioes, broadly lanceolate flowers are small with slender pedicel, stamens are diadelphouswith uniform anther; stigma are capitates, and pods are flat. These characters are in agreement with Allen (1981) and Dassanyake (1980). In microscopical study, the epidermal cells were wavy and trichomes were presentin leaflets. Paracytic type stomata were present, more in upper surface than lower surface. These characters are in agreement with Esau (1953).

Medicinal uses to expel worms treats spasms in infants, indigestion, piles, and absccesses digestion (decoction of whole plant); for hemorrhoids Leaves, as a poultice on bruises and drunk daily for chronic coughs and tuberculosis (decoction of roots); to treat kidney complaints (infusion of roots); eaten or used in baths for gastro-intestinal and urinary problems ranging from an upset stomach to hepatitis infusion or decoction of roots (Anmin *et al*., 2003).

The prelimary phytochemical test indicated that alkaloids, phenolic, saponin, glycoside, tannin, carbohydrates, flavonoids and steroid were present in the leaves and alkaloids, phenolic, saponin, glycoside, tannin, carbohydrate, flavonoid, terpenoid and steroid were present in the roots. These characters are in agreement with Kimura (1996).

Antimicrobial activities of aqueous and organic solvents extracts of *Tadehagi triquetrum* (L.) Ohashi are tested in against six different microorganisms. However, the leaves showed antimicrobial activity on Ethyl acetate extract *Pseudomonas aeruginosa* and *Escherichia coli* were the highest activity. The roots showed antimicrobial activity on Methanol extract *Pseudomonas aureginosa* and *Candida albicans* were the highest activity.This results will provide to clinician because the determination of the antimicrobial susceptibility of pathogen is important in aiding the clinician to select the most appropriate agent for the treating that disease. From the findings the extracts of this plant could be possible the therapeutic agent for the treating infectious diseases caused by six tested microorganisms.

The *Tadehagi triquetrum* (L.) Ohashi is a usual Chinese herbal medicine. This paper studied on the antimicrobial activity of *Tadehagi triquetrum* (L.) Ohashiextract and its preservative function on cut carnation. The results showed that *Tadehagi triquetrum* (L.) Ohashi extract had some effects both on bacilli and fungi (Chen, 2007).

At present, the government of Myanmar also encourage for development of scientific research on herbal and traditional medicine. Thus, further investigation on propagation of this plant and application of its medicinal uses should upgrade to confirm its activities.

Acknowledgements

First of all, I would like to mention, my deepest gratitude to Dr. Khin Win Naing, Professor and Head of Botany Department, University of Myitkyina for her kind permission to carry out the research in the department.

I would like to thank Dr. Seine Nyoe Nyoe Ko, Professor, Botany Department, University of Myitkyina for their valuable suggestions and encouragements.

I thanks also extented to Dr. Soe Myint Aye, Pro Rector, University of Myitkyina for his kind guidance and encouragements.

My deep appreciation to Daw Cherry Aung, Assistant Manager, Pharmaceutical Research Deputy, for her encouragements and invaluable help in testing the bacterial activity of my sample.

I would like to thank Dr. Tu Tu Wai, Lecturer, Chemistry Department, University of Myitkyina for her kind help and instruction of chemistry portion for this research.

References

Allen EK, Allen ON. (1981). **The leguminosae: A source Book of characteristics, Uses and Nodulation.** University of Wisconsin press, Madison pg.568.

Anminc C, Yingfu M, Yuan G, Zhemin G. (2003). **Encyclopedic Reference of Traditional Chinese Medicine**. Springer Berlin pg-564.

Bristh Pharmacopoeia (1965). **The pharmaceutical press,London and bradford.**17B 100 msbury square, London W.C.I.

Central Council for Research in Unani Medicine (1987). **Phytochemical Standard of Unani.**

Chen, J, X. (2007). **Potential of agricultural residues and hay for bioethanol production**. Appl Biochem Biotechnol 142 (3):276-90.

Cruick Shank, R., j.P. Duguid, B.P Marmion, and R.H.A. Swan (1970). **Medicinal Microbiology.**Churchill living stone Ltd; London.

Dassanyake, M.D. (1996). **Flora of ceylonVol.X.**Amerind Publishing Co.Rt. Ltd., New Delhi.

Esau, K. (1953). **Plant Anatomy.** John Wiley and Sons, Inc. New York. London.

Kress, W.J.R.A Defilipps. E. Earr& Yin YinKyi (2003). **A Checklist of the trees,shrubs, herbsandclimbers of Myanmar.** Department of Systematic Biology Botany, National, Museum of Natural History Washington DC. USA.

Kimura,T. (1996). **International Collation of Traditional and Folk Medicine**. Northeast Asia Part 1, World Scientific publishing Co. Singapore.Pg.62.

Marini Bettolo,G.B. (1981). **Plant screening by chemical and chromatographic procedure under field condition.** Journal Chromatography.

Medicinal Plants of Myanmar (2000). **Ministry of Health of China Department of Traditional Medicine.**

Meskin, Mark S.(2002). **Phytochemicals in Nutrition and Health.** CRC Press.p.123.ISBN 978-1-58716-083-7

Pandey, S.N & A.C chanda. (1993). **Plant Anatomy and Embroyology**.Vikas Publishing House (Put, Ltd)

Trease and Evans (1980). **Pharmacognosy.** 11th Edition, Balliere London.

World Health Organization (1999). **W.H.O Monographs on Selected Medicinal Plants.**Geneva,1.

TAXONOMIC STUDY ON TWELVE SPECIES OF ROSACEAE FOUNDED IN WAINGMAW TOWNSHIP, KACHIN STATE

Depar Myint[1], Soe Myint Aye[2], Sanda Htun[3]

Abstract

The research deals with taxonomic study on some member of rosaceous plants distributed in Waingmaw Township in Kachin State. The area lies between 25°22' and 25°44' North latitude and 97°12' and 97° 24' East longitude. The total area was 1883.17 sq km and the elevation ranges from 146 m to 1992 m above sea level. The member of rosaceous plants from Waingmaw Township were collected, preserved and identified from June 2017 to March 2019 in Department of Botany, University of Myitkyina. These species are *Chaenomeles speciosa* (Sweet) Nakai, *Neillia sinensis* Oliv., *Prunus cerasoides* D.Don., *Prunus mume* (Siebold) Siebold & Zucc., *Prunus persica* (L.) Batsch, *Pyrus pyrifolia* (Burm. f.) Nakai, *Rosa chinensis* Jacq, *Rosa clinophylla* Redout & Thory, *Rubus corchorifolius* L. f., *Rubus ellipticus* var. *obcordatus* Focke., *Rubus reflexus* Ker Gawl., *Rubus rosifolius* Sm. were included. Among them, *Chaenomeles speciosa* (Sweet) Nakai, *Neillia sinensis* Oliv, *Pyrus pyrifolia* (Burm. f.) Nakai, *Rosa chinensis* Jacq. and *Rubus reflexus* Ker Gawl. are not recorded in previous list of Myanmar. These species are very valuable for compilation of Flora of Myanmar.

Keywords : Taxonomic study, Rosaceae, Waingmaw Township, Kachin State

Introduction

The present research deals with the members Rosaceae growing Waingmaw Township in Kachin state. Rosaceae is a large family of perhaps 110 genera and 3100 species, widespread but best represented in the Northern Hemisphere, mainly in the temperate and arctic climate. The major genera in the world are *Rubus* (750 species), *Potentilla* (500), *Prunus* (430), *Crataegus* (240), *Cotoneaster* (230), *Sorbus* (230), *Rosa* (225), *Alchemilla* (220), *Spiraea* (100), *Pyrus* (60), *Malus* (55), *Geum* (40) and *Fragaria* (15). The members are herbs (*Alchemilla*, *Fragaria*), shrubs (*Rosa, Rubus*), trees (*Prunus*, *Malus, Pyrus*), rarely climbing (some species of *Rosa*), often with prickles and thorns (Gurcharan 2010). The family is worldwide but with maximum development in the temperate to sub-tropical region of the northern hemisphere. Rosaceae family is valued both for its genera of bush and tree fruits, edible fruit, medicinal useful and for many popular horticultural ornamentals (Heywood 2007).

The Rosaceae are distinctive in having usually stipulate leaves (often adnate to petiole) and an actinomorphic, generally pentamerous flower with hypanthium present, variable in gynoecial fusion, ovary position and fruit type. The family is very economically important as the source of many cultivated fruits including *Fragaria* (strawberry), *Malus* (apples), *Prunus* (almond apricot, cherry, peach, plum), *Pyrus* (pear), *Rubus* (blackberry, raspberry) (Simpson 2006).

Waingmaw Township is located east bank of Ayeyawaddy river. The total area of Waingmaw Township is 1883.17 square miles and established by 13 quarters, 3 towns and 170 village tracts within the township. It lies between 25°22'-25°44' North latitude and between 97°12'-97°24' East longitude. The elevation of Waingmaw is 146 m (481') to 1992 m (6534') above the sea level. If a good growth of forest vegetation anywhere in the world depends on three

[1] Assistant Lecturer, Department of Botany, University of Myitkyina
[2] Prorector, University of Myitkyina
[3] Associate Professor, Department of Botany, University of Myitkyina

main factors such as optimal temperature, good rainfall and fertile soil. These three main factors are found in Waingmaw Township.

Rosaceae is mostly common in ornamental plants and some cultivated species for edible purpose were recorded in Myanmar. The resource of Rosaceae as wild is very attractive for recording as interesting species in Myanmar. The aim and objectives of this research is to identify and classify the members of Rosaceae distributed in Waingmaw Township, to record the taxonomic characters of various kinds of rosaceous plants, to contribute the floristic information of natural rosaceous plants resources and to ascertain the nomenclature of rosaceous plant resources distributed in Waingmaw Township.

Materials and Methods

The rosaceous plants were collected in Waingmaw Township during the years 2017 to 2019. All the collected specimens were recorded by digital images while flowering period. Precise locations of the specimens collections were recorded by using Global Positioning System (GPS) Map Navigator. Field notes were recorded by habitat types and peculiar plants characters. Then, the specimens were kept immediately into the plastic bags and carried to the lab to identify and classify systematically.

Identification of the specimens was carried out by referring the floras or manuals, and checklist of the particular region. The families of the collected specimens were determined by referring to literature of Hutchinson (1967) and Geesink (1981). Identification of genera and species were carried out by referring to the available literature such as Hooker (1897), Brandis (1907), Dassanayake (1981), Lingdi, L. & G. Cuizhi, L. Chaoluan, C. Alexander, B. Bartholomew and A.R. Brach. (2003), Volume-9, and Qi-ming and De-Lin (2008).

The nomenclatural data was referred to Index Kewensis by which the names and synonyms of plants up to the rank of species being confirmed. All of nomenclatural studies were finalized by referring to the web site of International Plant Names Index (www.ipni.org) and online Botanical Database of Tropicos Plants (www.tropicos.org). Myanmar names and their distribution were followed to the Checklist of Hundley & Chit Ko Ko (1987) and Kress *et al.* (2003).

The study species under the family were systematically arranged alphabetically. The dried specimens were mounted with a label of field data on a herbarium sheet. They were kept in the Herbarium of Botany Department, University of Myitkyina for references and other academic purposes.

Results

Altogether 12 species belonging to 6 genera of Rosaceae were resulted (Table 1). The morphological and taxonomic characteristics were described. The detailed characters of photographic records were stated in Figures 1 to 12.

Table 1 List of the species of Rosaceae distributed in Waingmaw Township

No.	Scientific Names	Common Names	Local Names	Specimens Location
1.	*Chaenomeles speciosa* (Sweet) Nakai	Quince	Chinsaw Ga, Ma Kwar	Lu Myan village, N Latt 25°23'42.455" and E Long 097° 54'19.775", Elevation 790 m
2.	*Neillia sinensis* Oliv.	Chinese neillia	Unknown	Lu Htaung village, N Latt 25° 22'20.464" and E Long 097° 54'12.666", Elevation 1356 m
3.	*Prunus cerasoides* D.Don	Byin-byimg, Chai-ri, Panni, Pannu	Cherry	La Jaung village, N Latt 25°23'41.001" and E Long 097° 55'10.877", Elevation 1250 m
4.	*Prunus mume* (Siebold) Siebold & Zucc	Sow Thistle, Plum	Maman	Kampaitee village, N Latt25°25'47.428" and ELong097°56'55.054", Elevation 1503 m
5.	*Prunus persica* (L.) Batsch	Me-man, Phai-zong, Shanzi	Peach	Kampaitee village, N Latt 25° 25'10.113" and E Long 097° 54'20.775", Elevation 1222 m
6.	*Pyrus pyrifolia* (Burm.f.) Nakai	Pear	Thit taw yaing	La Jaung village, N Latt 25° 23'42.008" and E Long 097° 54'17.878", Elevation 1265 m
7.	*Rosa chinensis* Jacq.	China Rose	Hnin si yaing	La Jaung village, N Latt 25° 25'49.721" and E Long 097° 55'17.355", Elevation 1205 m
8.	*Rosa clinophylla* Redout & Thory	Kahpalap, Myitkaing	Hnin si yaing	Madain village, N Latt 25° 22'10.101" and E Long 097° 32'24.111", Elevation 180 m
9.	*Rubus corchorifolius* L. f.	Kaiching	Unknown	Kampaitee village, N Latt 25° 24'38.899" and E Long 097° 06'57.321", Elevation 1995 m
10.	*Rubus ellipticus* var. *obcordatus* Focke.	Chaya, Lingsan, Shaga, Sumwe	Nga u su pin	Kampaitee village, N Latt 25° 25'27.340" and E Long 097° 54'47.410", Elevation 1270 m
11.	*Rubus reflexus* Ker Gawl.	Raspberry	Unknown	La Jaung village, N Latt 25° 25'41.001" and E Long 097° 55'10.877", Elevation 1250 m
12.	*Rubus rosifolius* Sm.	Raspberry	Namjawshalum	La Jaung village, N Latt 25° 22'57.587" and E Long 097° 44'11.395", Elevation 671 m

1.***Chaenomeles speciosa*** (Sweet) Nakai, J. Jap. Bot. 4:331.192. (Figure 1, A-F)

Cydonia speciosa Sweet, Hort. Suburb. Lond.113.1818.

Flowering period : November to February

Perennial deciduous shrubs, up to 2-5 m high. Leaves simple, alternates petiolate; stipules conspicuous, reniform, glabrous; blades oblong-elliptic, reniform at the base, slightly emerginate at the apex. Inflorescences axillary, solitary cyme. Flowers bisexual, dark pink; pedicels subsessile. Hypanthium campanulate. Sepals 5, reddish-green, fused. Petals 5, free, orbicular, caducuous. Stamens numerous; anthers dithecous. Carpels 5, fused, within the hypanthium, style 5, pentalocular, with many ovules on the axile placenta. Fruits pomiferous.

2. ***Neillia sinensis*** Oliv., Icon. Pl. 16:, pl. 1540. 1886. (Figure 1, G-L)

Flowering period : June to August

Perennial; climbing subshrubs, up to 2-3 m high. Leaves simple, hastate, alternate; stipules ovate, pubescent; petiolate, blades ovate, pubescent on veins lower surfaces. Inflorescences terminal, racemose. Flowers bisexual, white; pedicelate. Hypanthium campanulate. Sepals 5, lanceolate-ovate, alternate petals, pubescent. Petals 5, free, caducuous, white, ovate. Stamens numerous; anthers dithecous. Carpels 1, 2 ovules, pendulous placenta. Fruits follicular.

3. ***Prunus cerasoides*** D.Don, Prod. Fl. Nepal 239. 1825. (Figure 2. A-F)

Flowering period : November to February

Perennial; deciduous, tree, up to 12-15 m high. Leaves simple, fascicles, alternate; stipulate, caducuous; petiolate, gland two dots at the base; blades lanceolate, glabrous, acuminate at the apex. Inflorescences axillary, fascicles, cyme. Flowers bisexual, pale pink; pedicelate. Hypanthium campanulate. Sepals 5, triangular. Petals 5, free, obovate, pink, caducuous. Stamens numerous, filaments pink; anthers dithecous. Carpels 1, oblong, glabrous, one ovule, on the pendulous placenta. Fruits drupaceous.

4. ***Prunus mume*** (Siebold) Siebold & Zucc. Fl. Jap. 1:29.pl.11. 1836. (Figure 2, G-L)

Armeniaca nume Siebold, Verh. Batav. Genootsch. Kunst. 12(1):69. 1830.

Flowering period : January to March

Perennial small tree, deciduous, up to 3-5 m high. Leaves simple, alternate, petiolate; stipules caducuous; petiole with glands; blades ovate-oblong, serrate and red-gland along the margin, acuminate at the apex,. Inflorescences axillary, cymose, solitary or 2 in a fascicle. Flowers bisexual, white; pedicels sessile. Hypanthium campanulate. Sepals 5, oblong-ovate. Petals 5, free, white, orbicular. Stamens numerous, free; anthers dithecous. Carpels 1, densely sliky pubescent, 2 ovules, on the pendulous placenta. Fruits drupaceous.

5. ***Prunus persica*** (L.) Batsch, Beytr. Entw. Cewachsreich. 1:30.1801.(Figure 3, A-F)

Amygdalus persica L., Sp. Pl. 1: 472. 1753.

Flowering period : February to April

Perennial small tree, deciduous, up to 4-5 m high. Leaves simple, alternate, petiolate; stipules caducous; blades ovate-elliptic. Inflorescences terminal or axillary, solitary. Flowers bisexual, dark pink; pedicels stout. Hypanthium campanulate. Sepals 5, ovate-oblong. Petals 5, free, ovate-

orbicular, crenate along the margin. Stamens numerous, free; anthers dithecous. Carpels 1, densely pubescent, one-ovulate on the pendulous placenta. Fruits drupaceous.

6. ***Pyrus pyrifolia*** (Burm.f.)Nakai, Bot.Mag.Tokyo 40(479): 564.1926. (Figure 3, G-L)

Ficus pyrifolia Burm.f., Fl. Indica. 226.1768.

Flowering period : February to March

Perennial small tree, up to 5-7 m high. Leaves simple, alternate, stipulate; petiolate; blades ovate, reddish green at young. Inflorescences terminal or axillary, raceme umbellate. Flowers bisexual, white; pedicelate. Hypanthium urceolate. Sepals 5, triangular. Petals 5, free, caducous, white, broadly ovate. Stamens numerous; anthers dithecous. Carpels 5, fused, within the hypanthium; slightly pubescent, 2 ovulate on the basal placenta. Fruits pomeferous.

7. ***Rosa chinensis*** Jacq., Observ. Bot. 3:7, t.55. 1768. (Figure 4, A-F)

Flowering period : March to June

Perennial climbing shrubs, up to 1-2 m high. Leaves compound, imparipinnate; alternate; stipules adnate; petiolate; blades ovate. Inflorescences terminal, corymb. Flowers bisexual, pink; pedicelate. Sepals 5, ovate. Hypanthium ovoid. Petals numerous, free, obovate. Stamens numerous; anthers dithecous. Carpels many, free, within the hypanthium; unilocular, one-ovulate on the basal placenta. Fruits hip.

8. ***Rosa clinophylla*** Redout & Thory, Roses 1:43. 1837. (Figure 4, G-L)

Rosa involucrata Roxb. ex Lindl., Fl. Ind. 2:53. 1832.

Flowering period : February to May

Perennial erect shrubs, up to 2-3 m high. Leaves compound, imparipinnate; alternate, petiolate; stipules adnate, fimbriate; leaflets 3- to 7-paired; blades elliptic. Inflorescences terminal, cymose. Flowers bisexual, white; pedicels pubescent; involucra bracts ovate, fimbriate along the margin, pubescent. Hypanthium obovoid, densely pubescent. Sepals 5, ovate. Petals 5, free, broadly obovate, white, emerginate at the apex. Stamens numerous; anthers dithecous. Carpels many, free, within the hypanthium, densely silky pubescent, unilocular, one-ovulate on the basal placenta. Fruits hip.

9. ***Rubus corchorifolius*** L.f., Suppl. Pl. 263.1781. (Figure 5, A-F)

Flowering period : February to March

Perennial shrubs, up to 1-2 m high. Leaves simple, alternate; stipules adnate; petiolate; blades ovate, silky pubescent. Inflorescences axillary, solitary. Flowers bisexual, white; pedicels silky pubescent. Hypanthium campanulate. Sepals 5, ovate, pubescent. Petals 5, free, obovate. Stamens numerous; anthers dithecous. Carpels many, free; unilocular, 1-ovulate on the basal placenta, pubescent. Fruits aggregate.

10. ***Rubus ellipticus*** var. ***obcordatus*** Focke, Biblioth. Bot. 17 (Heft 72(2)):199. 1911. (Jun 1911). (Figure 5, G-L)

Flowering period : January to April

Perennial erect shrubs, up to 2-3 m high. Leaves compound, trifoliolate, alternate; stipules adnate, pubescent; petioles densely brownish pubescent; blades obcordate, cordate at the apex,

densely brownish pubescent. Inflorescences terminal or axillary, paniculate cyme. Flowers bisexual, white; pedicellate. Hypanthium campanulate. Sepals 5, ovate, persistent. Petals 5, free, obovate. Stamens numerous, slightly curved; anthers dithecous, dorsifixed. Carpels many, free; unilocular, one-ovulate on the basal placenta. Fruits aggregate.

11. ***Rubus reflexus*** Ker. Gawl., Bot. Reg. 6:461. 1820 (Figure 6, A-F)

Flowering period : May to July

Perennials climbing scandent shrubs, up to 1-3 m high. Leaves simple, hastate, alternate; stipulate; petiolate; blades auriculate-oblong. Inflorescences terminal or axillary, paniculate cyme. Flowers bisexual, white; pedicellate, densely pubescent. Hypanthium obconical, densely rusty pubescent. Sepals 5, ovate, pubescent. Petals 5, free, antisepalous, obovate. Stamens numerous; anthers dithecous. Carpels many, unilocular, one-ovulate on the pendulous placenta. Fruits aggregate.

12. ***Rubus rosifolius*** Sm., Pl., Icon.Ined. 3: pl.60. 1791. (Figure 6, G-L)

Flowering period : February to April

Perennial shrubs; up to 1-3 m high. Leaves compound, imparipinnate; alternate; stipulate; petiolate; leaflets 2- to 4-paired; blades elliptic-ovate. Inflorescences terminal or axillary, cymose. Flowers bisexual, white; pedicellate, with gland hair. Hypanthium urceolate. Sepals 5, ovate. Petals 5, free, obovate. Stamens numerous, free; anthers dithecous. Carpels numerous, free, unilocular, one-ovulate on the pendulous placenta. Fruits aggregate.

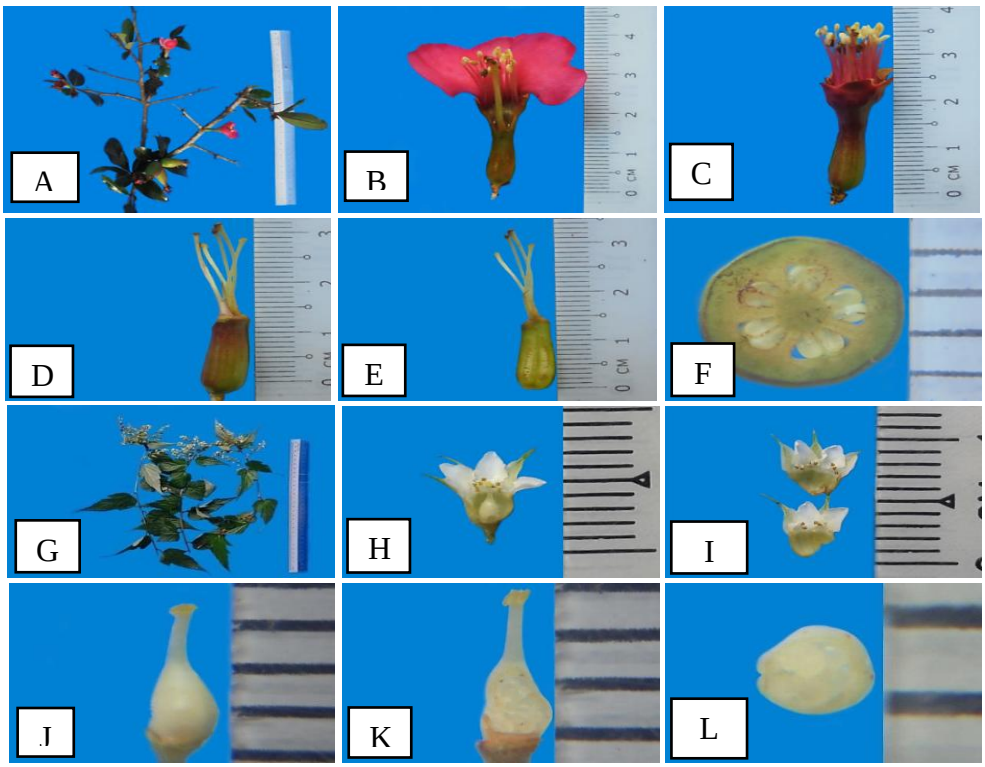


Figure 1 *Chaenomeles speciosa* (Sweet) Nakai, **A**.- Inflorescence, **B**.- L.S of flower, **C**.- Stamens **D**.- Pistal, **E**.- L.S of ovary, **F**.- T.S of ovary; ***Neillia sinensis*** **Oliv**.,**G**.- Inflorescence, **H**.- L.S of flower, **I**.- Stamens, **J**.- Pistal, **K**.- L.S of ovary, **L**.- T.S of ovary

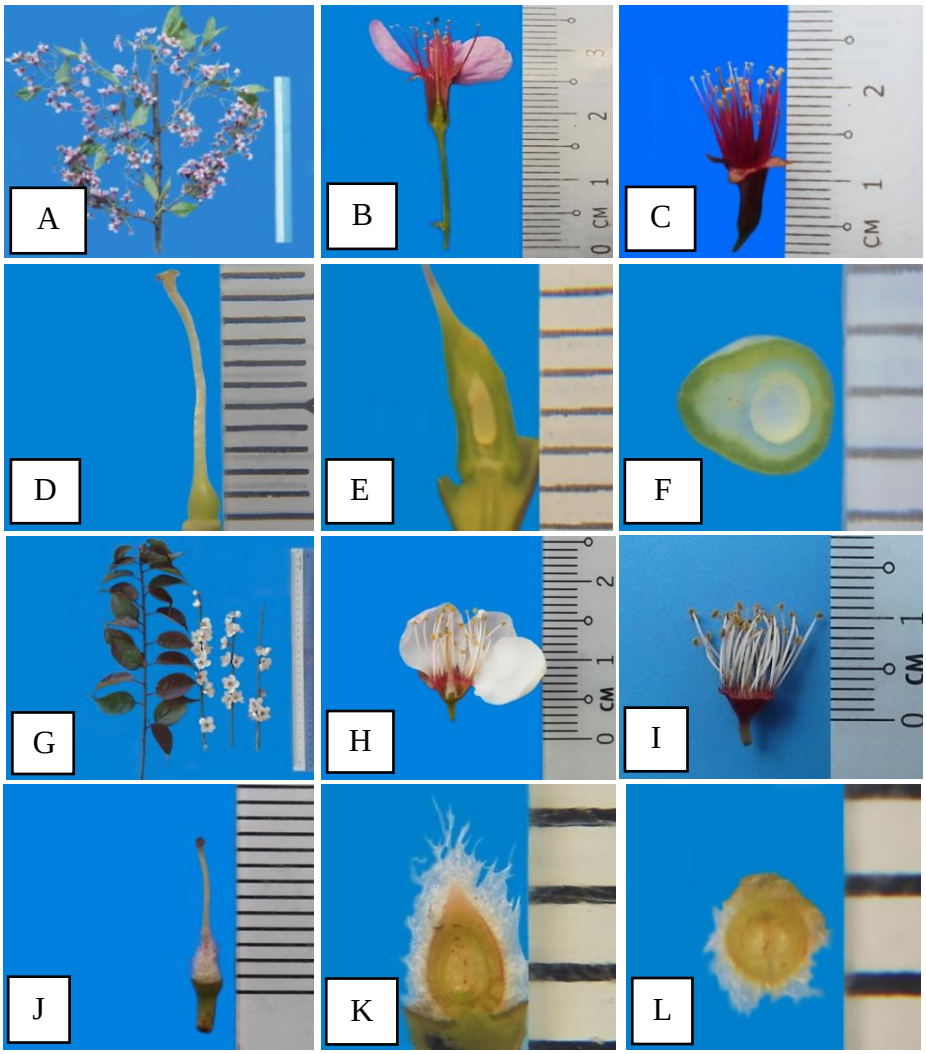
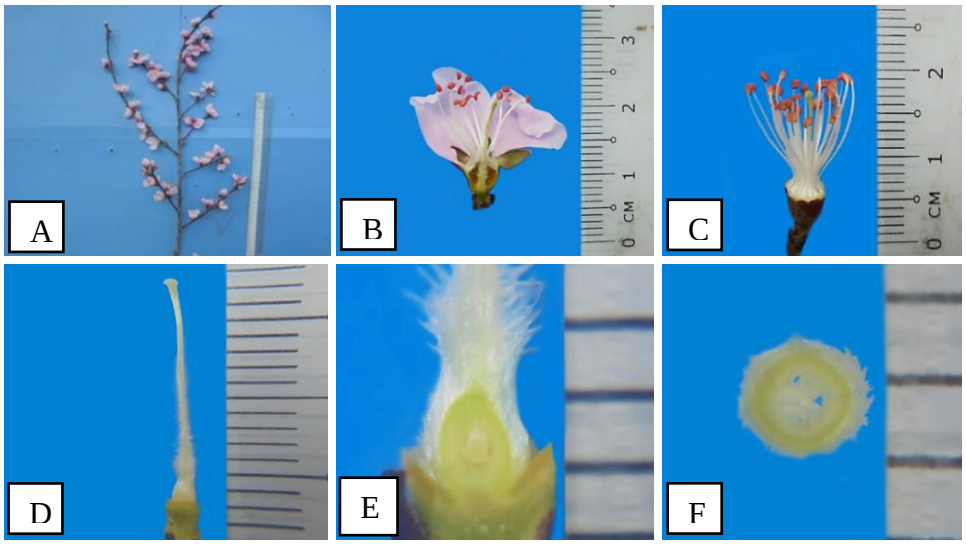


Figure 2 *Prunus cerasoides* D.Don, **A**.-Inflorescence, **B**.- L.S of flower, **C**.- Stamens, **D**.–Pistil, **E**.- L.S of ovary, **F**.- T.S of ovary; ***Prunus mume*** **(Siebold) Siebold & Zucc**., **G**.- Inflorescence, **H**.- L.S of flower, **I**.- Stamens, **J**.- Pistil, **K**.- L.S of ovary, **L**.- T.S of ovary



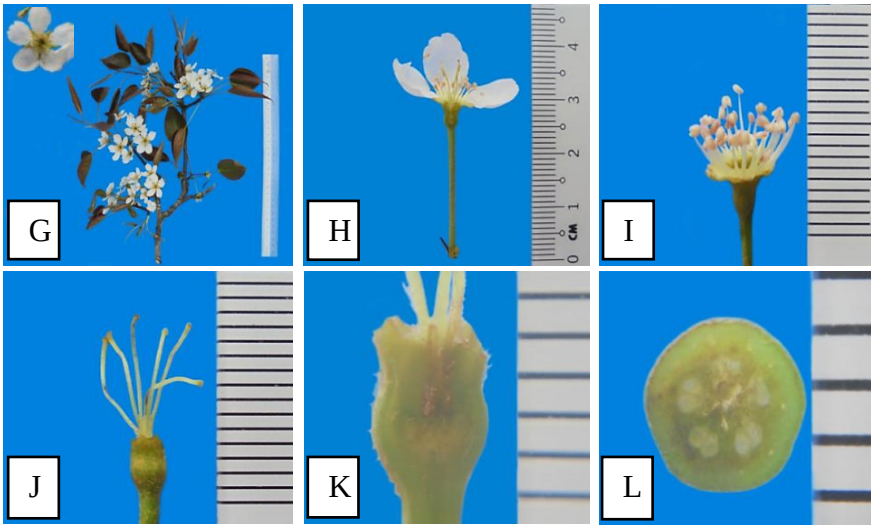


Figure 3 *Prunus persica* (L.) Batsch, **A**.- Inflorescence, **B**. -L.S of flower, **C**. Stamens **D**.- Pistil, **E**.- L.S of ovary; **F**.- T.S of ovary; ***Pyrus pyrifolia* (Burm.f.) Nak** **G**.- Inflorescence, **H**. -L.S of flower, **I**.- Stamens, **J**.- Pistil, **K**.- L.S of ovary, **L**.- T.S of ovary

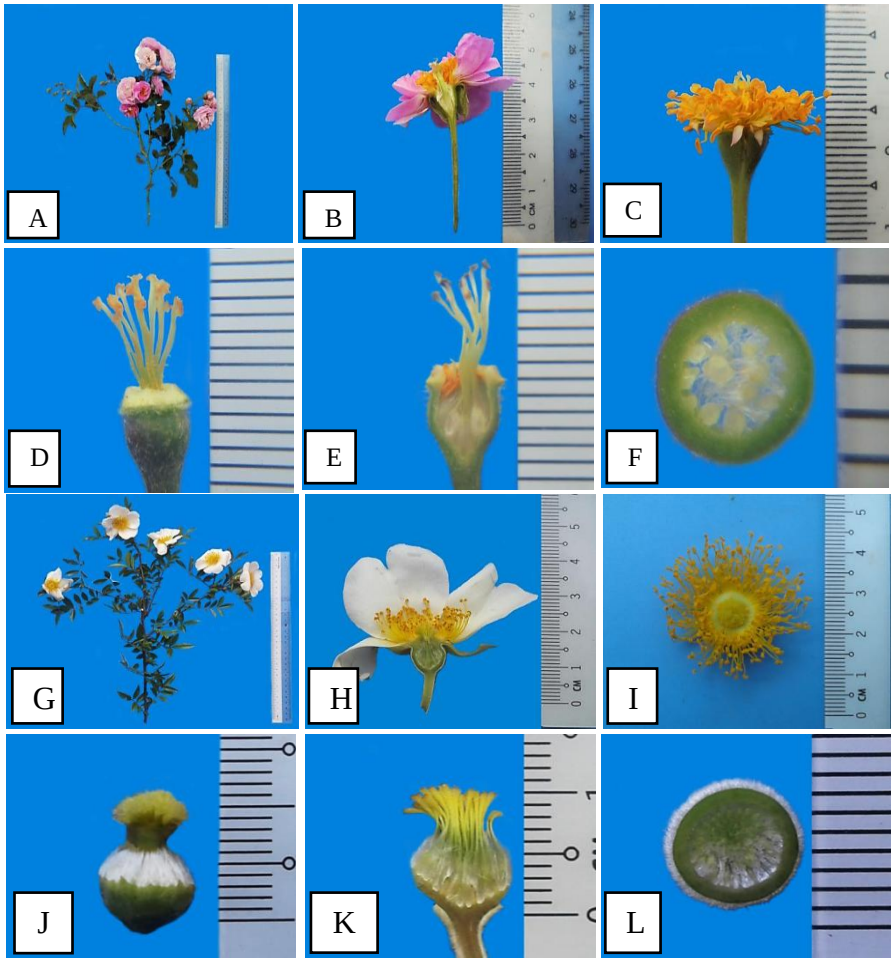


Figure 4 *Rosa chinensis* Jacq., **A**.- Inflorescence, **B**.- L.S of flower, **C**.- Stamens, **D**.Pistil, **E**.- L.S of hypanthium, **F**.- T.S of hypanthium, showing ovaries; ***Rosa clinophylla* Redout & Thory, G**.- Inflorescence, **H**.- L.S of flower, **I**.- Stamens, **J**.- Pistil, **K**.- L.S of hypanthium, **L**.- T.S of hypanthium showing ovaries

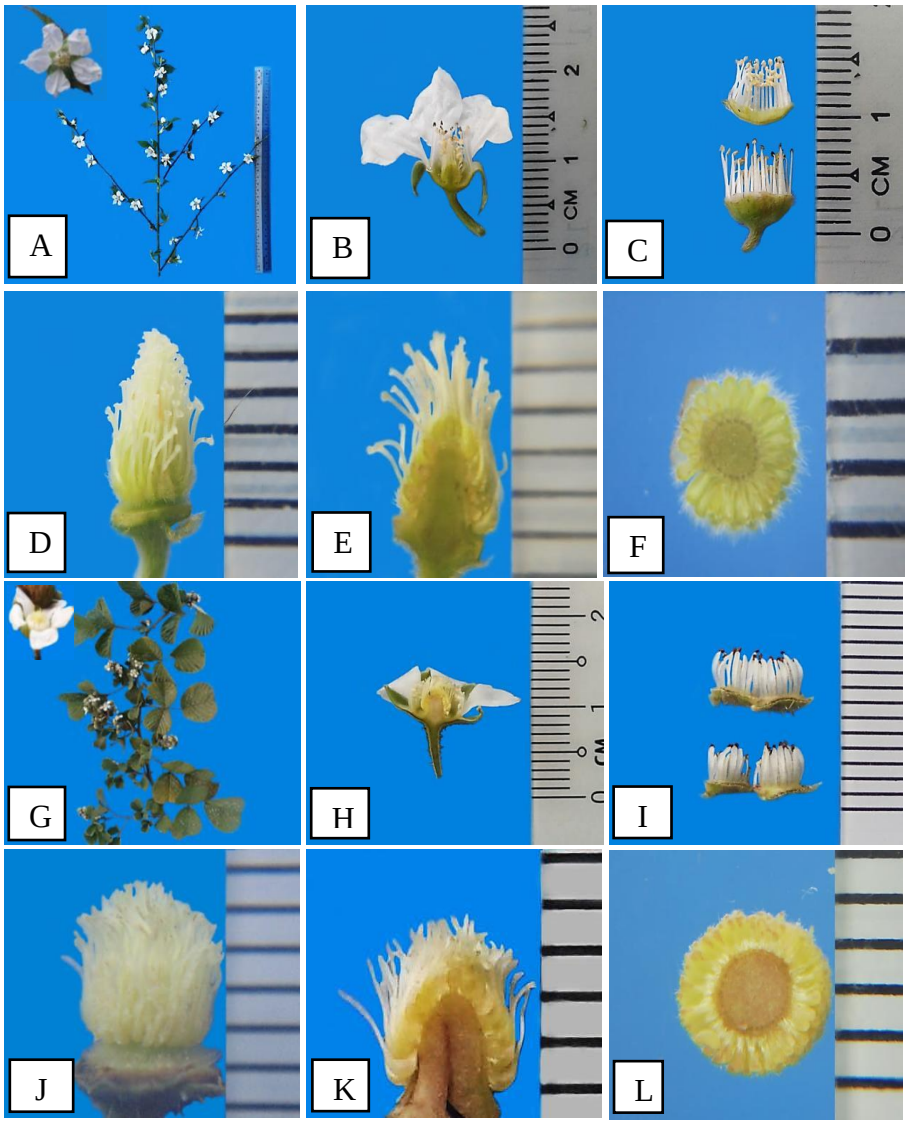
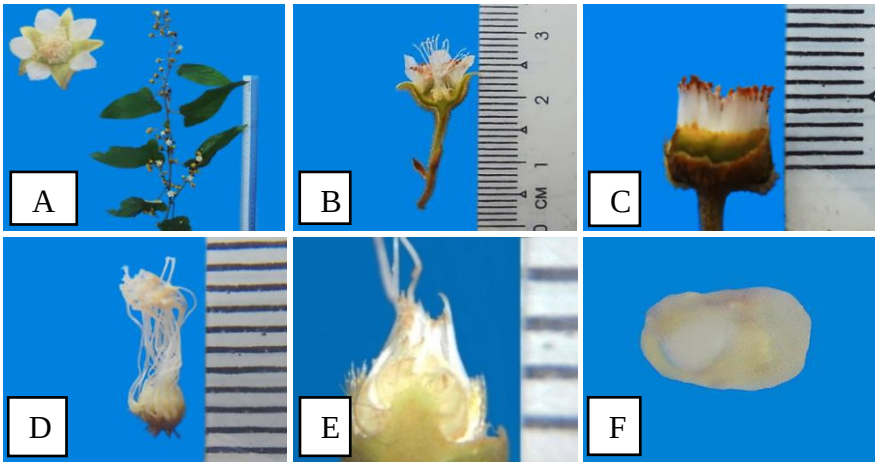


Figure 5 *Rubus corchorifolius* L.f., **A**.- Inflorescence, **B**.- L.S of flower, **C**.- Stamens, **D**.- Pistil, **E**.- L.S of ovaries on thalamus, **F**.- T.S of ovaries on thalamus; ***Rubus ellipticus* var. *obcordatus* Focke, G**.- Inflorescence, **H**.- L.S of flower, **I**.Stamens, **J**.- Pistil, **K**.- L.S of ovaries on thalamus, **L**.- T.S of ovary attaching on thalamus



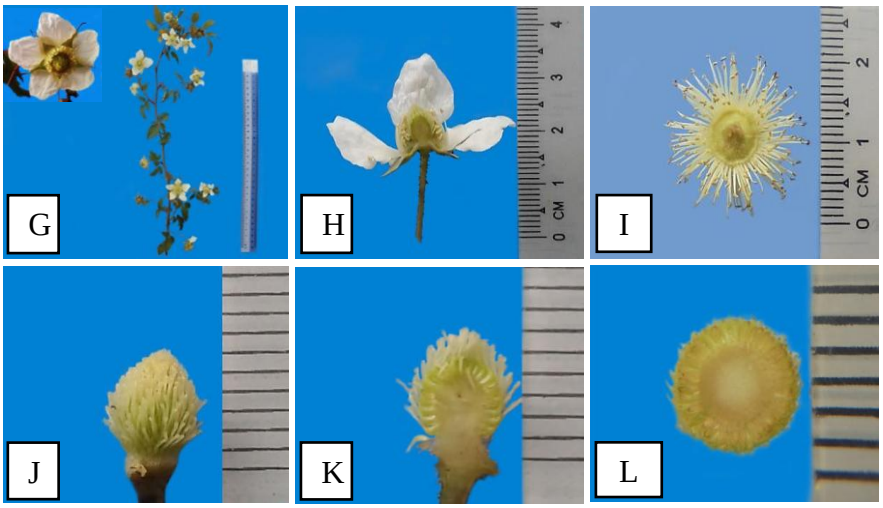


Figure 6 *Rubus reflexus* Ker Gawl., **A**.- Inflorescence, **B**.- L.S of flower, **C**.- Stamens, **D**.- Pistil, **E**.- L.S of ovaries on thalamus, **F**.- T.S of ovaries on thalamus; ***Rubus rosifolius*** **Sm., G**.- Inflorescence, **H**.- L.S of flower, **I**.- Stamens, **J**.- Pistil, **K**.- L.S of ovaries on thalamus, **L**.- T.S of ovaries on thalamus

Discussion and Conclusion

The present research deals with the taxonomic study on some member of rosaceous plants growing in Waingmaw Township is located in east bank of Ayeyarwaddy river. It has been found that the totally 12 species belonging to 6 genera of Rosaceae were distributed in study area.

The growing habits of the rosaceous plants vary in the studied area. The 4 species such as *Prunus cerasoides* D.Don, *Prunus mume* (Siebold) Siebold & Zucc., *Prunus persica* (L.) Batsch, and *Pyrus pyrifolia* (Burm.f.) Nakai were growing as tree, other species such as *Chaenomeles speciosa* (Sweet) Nakai, *Rosa clinophylla* Redout & Thory., *Rubus corchorifolius* L.f., *Rubus ellipticus* var. *obcordatus*, were growing shrub and *Neillia sinensis* Oliv. Icon, *Rosa chinensis* Jacq., *Rubus reflexus* Ker. Gawl., and *Rubus rosifolius* Sm. were growing climbing shrub.

All species were bisexual, actinomorphic, pentamerous, perigynous flowers with hypanthium. The leaves aestivation types of some rosaceous plants were simple and imparipinnate compounds. The inflorescences type of rosaceous plants were axillary cymose, terminal racemose, terminal cymose and terminal paniculate cymose. The various colour of flower such as pink and white were found in some member of rosaceous plants. The types of rosaceous fruits were varying such as aggregate, drupe, follicles, hip and pome.

Chaenomeles speciosa (Sweet) Nakai, *Pyrus pyrifolia* (Burm.f.) Nakai, *Rosa chinensis* Jacq., and *Rosa clinophylla* Redout & Thory., carpels were within the hypanthium, *Neillia sinensis* Oliv. Icon, *Prunus cerasoides* D.Don, *Prunus mume* (Siebold) Siebold & Zucc. and *Prunus persica* (L.) Batsch were one carpel and connate to on cupular receptacle and *Rubus corchorifolius* L.f., *Rubus ellipticus* var. *obcordatus*, *Rubus reflexus* Ker. Gawl. and *Rubus rosifolius* Sm. carpels were free and adnate to inner surface of cupular receptacle.

Qi-ming and De-lin, (2008) mentioned that the fruits of *Prunus mume, Prunus persica, Rubus reflexus* and *Pyrus pyrifolia* are edible. The plants of medicinal value were *Prunus mume,*

Prunus persica, *Rosa chinensis*, *Rubus reflexus* and *Rubus rosifolius* and *Prunus mume* and *Rosa chinensis* were the ornamental plants.

Among them, *Chaenomeles speciosa* (Sweet) Nakai, *Neillia sinensis* Oliv. Icon., *Pyrus pyrifolia* (Burm.f.) Nakai., *Rosa chinensis* Jacq., *Rubus reflexus* Ker. Gawl. and *Rubus rosifolius* Sm. are not recorded in previous list of Myanmar. These species are very valuable for compilation of Flora of Myanmar.

In the research work, many valuable species not only can be recorded but also various forest products can be found. Taxonomy was essential in theoretical and applied biology. Nevertheless, these natural plants resources will also be applied for future researchers. Therefore, the threatened and endangered species were needed to conserve. It was hope that this research work of floristic study on angiosperms of Waingmaw Township in Kachin state will provide valuable taxonomic information, affinities and distribution of plants for further researchers.

Acknowledgements

We would like to express my sincere appreciation and deep gratitude to Dr. Khin Win Naing, Professor and Head, Department of Botany, University of Myitkyina for permission to do this research work and for valuable guidance. Our sincere thanks are also due to Dr. Seine Nyoe Nyoe Ko, Professor, Department of Botany, University of Myitkyina, for her valuable suggestions. We are especially grateful to Daw Bauk Ja, Retired Professor, for her kind help for our field trip throughout our research work.

References

Andrew, L. & E. David (2005). Plant biology, second edition. Ttlor & Francis Group, UK.

Byng, J.W. & *et al.* (2016). An update of the Angiosperm phylogeny group classification for the orders and families of flowering plants: APG IV. The Linnean Society of London, Botanical Journal of the Linnean Society, 2016, 181, 1-20.

Dassanayake, M.D. (1981). A revised handbook to the Flora of Ceylon. Vol. III. University of Peradeniya, Department of Agriculture, Peradeniya, Sri Lanka.

Geesink, R. & *et. al.* (1981). Thonner's analytical key to the families of flowering plants. Centre for agricultural publishing and documentation, PUDOC.

Heywood, V.H. (2007). Flowering plants of the world. Oxford University Press, London.

Hooker, J.D. (1879). Flora of British India. Vol. II. L.Reeve & Co.Ltd. The Oast house. Book. NR. Rest, England.

Hundley, H.G. & Chit Ko Ko. (1987). List of trees, shrubs, herbs and principle climbers; etc., fourth revised edition, Shwe Daw Oo Press, Mayangon, Yangon, Myanmar.

Hutchinson, J. (1959). The families of the flowering plants. Vol. I. Dicotyledons, 2nd ed. Clarendon Press Oxford.

Kress, W. J., R. A. Defilipps. E. Farr & Yin Yin Kyi (2003). A checklist of the trees, shrubs, herbs and principle climbers of Myanmar. United States National Herbarium vol.45: 1-590.

Lingdi, L. & G. Cuizhi, L. Chaoluan, C. Alexander, B. Bartholomew and A.R. Brach. (2003). Flora of China. Vol. IX. Herbarium, Institute of botany, China Academy of Sciences, Beijing 100093, People's Republic of China.

Qi - ming, HU, WU De-lin & XIA Nian-he. (2008). Flora of Hong Kong. Vol. II. Agriculture, Fisheries and and Conservation Department, Government of the Hong Kong.

Simpson, M.G. (2006). Plant systematics. 30 Corporate Drive, Suite 400, Burlington, MA 01803, USA.

Singh, G. (2010). Plant systematics, An Integrated Approach. 234 May Street, Post Office Box 699, Enfield, New Hampshire 03748, USA.

MONTHLY VARIATION OF GREEN ALGAE (CHLOROPHYCEAE) AND THEIR CORRELATED WITH PHYSICOCHEMICAL PARAMETERS OF SEDAWGYI DAM, MANDALAY REGION

Phyo Phyo Khaing[1], Hla Min Thein[2]

Abstract

Monthly variations of green algae (Chlorophyceae) and water quality of Sedawgyi Dam, Madaya Township, Mandalay Region were studied from March to December 2018. Algal specimens and water samples were collected from three stations of Sedawgyi Dam. Physicochemical parameters of water samples such as pH, temperature, alkalinity, total hardness, nitrite nitrogen, phosphate, biological oxygen demand and dissolved oxygen were also analyzed. In the present study, a total of 14 genera and 37 species of green algae (Chlorophyceae) were recorded. The green algae specimens of Sedawgyi Dam consisted of *Chlorococcum* (1 species), *Tetradon* (1 species), *Selenastrum* (1 species), *Kirchneriella* (1 species), *Coelatrum* (2 species), *Scenedesmus* (5 species), *Pediastram* (10 species), *Closterium* (4 species), *Cosmarium* (3 species), *Spondylosium* (2 species), *Arthrodesmus* (1 species), *Staurastrum* (4 species), *Stauradesmus* (1 species) and *Eurastrum* (1 species). The algal specimens were found to be more abundant in April and May and less abundant in July to September. The physio-chemical data analysed in Sedawgyi Dam indicates that the lake is at present free from pollution.

Keywords: Green algae, monthly variations, Physico-chemical parameters, Sedawgyi Dam

Introduction

Rivers, lakes, dams, canals and etc. are important resources of water for various purposes. Standing or running water is important sources of community water supply and irrigation and therefore their quality has to be monitored regularly. The quality of water can be monitored by the types of organisms present in water (Kumar *et al*. 2009).

The physical and chemical characteristics of water bodies affect the species composition, abundance, productivity and physiological conditions of aquatic organisms. These stressed systems support an extraordinarily high proportion of the world's biodiversity.The phytoplankton in a reservoir is an important indicator of the water quality. Phytoplanktons are recognized worldwide as bioindicators in the aquatic environment (Yakubu *et al*. 2000). Phytoplankton is one of the most essential characteristics of the aquatic ecosystem for maintaining its stability and a means of coping with any environmental change (Jayaraman *et al*. 2003 and Tiwari *et al*. 2004). Water maintains an ecological balance between various groups of living organisms and their environment (Kumar *et al*. 2009).

Most freshwater algae are highly sensitive to pollutants. Any physical or chemical changes in aquatic systems, naturally or by entry of pollutants, will bring about changes individual organisms, populations and communities. The most important sources of organic matters in water are the disposal of municipal sewage, industrial waste water, urban and run-off. The source of toxic pollutants to take is usually material derived human activities (Goel 2006).

According to Goel (1997), the abundance, species composition and condition of aquatic organisms remain largely dependent upon the quality of water. Any changes in water quality by pollution will affect the aquatic communities. The number and kind of microorganisms in water depended largely upon the available supply of nutrients, organic matter, presence of other organisms and the environmental conditions.

[1] Assistant Lecturer, Department of Botany, University of Myitkyina
[2] Associate Professor, Department of Botany, Kyaing Tong University

The abundance of species composition and condition of aquatic organisms remain largely upon the available supply of nutrients, organisms matter, presence of other organisms and the environmental conditions. Some species of algae are more sensitive to change in water quality (Goel 2006).

Chlorophyceae the free living phytoplankton, is mostly limited to shallow waters and attached to the submerged plants or found in moist soil. Chlorophyta are rarely the predominating organisms in the phytoplankton of ponds and lakes, the number of species in the fresh water plankton is very large (Smith 1950). Green algae are ecologically important as major producers of biomass in freshwater systems, either as planktonic (standing water) or attached (running waters) organisms (Bellinger & Sigee 2010).

Water in dams is important source of community water supply and irrigation. Sedawgyi dam serve as source of water for irrigation, drinking and recreational purposes. Sedawgyi dam is situated in Madaya Township, Pyin Oo Lwin District, Mandalay Region. It was constructed in 1976 and built in 1987. It is situated between North Latitude 22˚19" and 22˚24", between East Longitude 96˚19" and 96˚25" at 131.37 meters above sea level.

The water in Sedawgyi dam flows into the ditches and canals along the villages, agricultural fields and fishery ponds. So the quality of water based on environmental factors such as occurrence of algae, chemical properties of water, anthropogenic activities and other sources.

The aim and objectives of this research was to reveal the green algae growing in Sedawgyi dam, to study the monthly variations of green algae occurrence and to analyze the physico-chemical parameters of water and their effects on algal population.

Materials and Methods

Sedawgyi Dam is located in Madaya Township, Pyin Oo Lwin District, Mandalay Region.Three sampling sites were chosen to examine the algal composition, the monthly variation of phytoplanktons and water analysis of Sedawgyi Dam. Sampling site I is northern part of Sedawgyi Dam, sampling site II is middle part of Sedawgyi Dam and sampling site III is southern part of Sedawgyi Dam.

Algal specimens and water samples were collected monthly intervals between March, 2018 and December, 2018. Physico-chemical parameters of water samples such as temperature, pH, total hardness, phosphate, nitrite nitrogen, biological oxygen demand and dissolved oxygen were analyzed at freshwater aquaculture research, water and soil examination laboratory, Yangon.

The number of algae belonging to different genera were determined and counted under the microscope using a haemacytometer and are calculated with the following formula used by Lavens and Sorgeloos (1996) and then calculated with relative abundance (%) by Boyd (1982).

Number of cell m-1 = $(n_1 + n_2) / (2 + 80) + 80 + 10^3 + d$

= $(n_1 + n_2) / 2 + 10^3 + d$

n_1 = number of cells counted in upper rafter

n_2 = number of cells counted in lower rafter

d = dilution factor

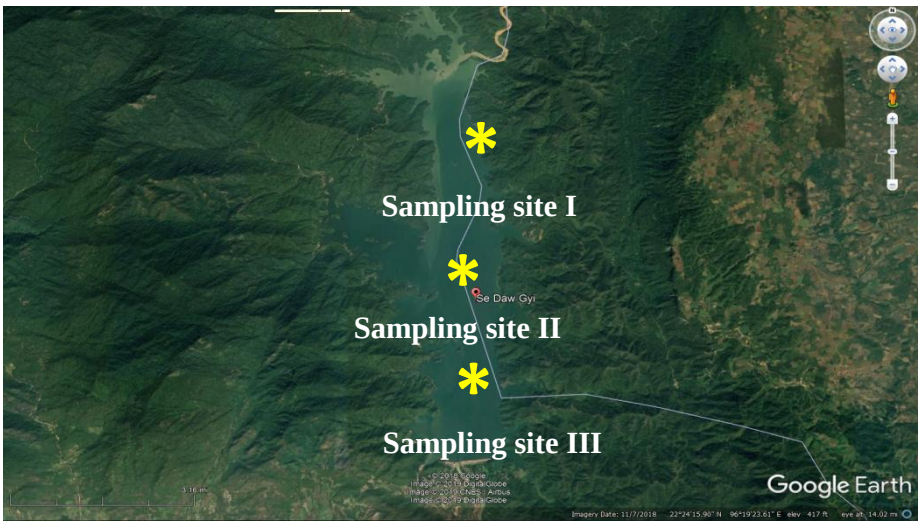


Figure 1 Location Map of Study Area

Results

Totally 37 algal species belong to 14 genera were collected and recorded from the study areas. The green algae specimens of Sedawgyi Dam consisted of *Chlorococcum* (1 species), *Tetradon* (1 species), *Selenastrum* (1 species), *Kirchneriella* (1 species), *Coelatrum* (2 species), *Scenedesmus* (5 species), *Pediastram* (10 species), *Closterium* (4 species), *Cosmarium* (3 species), *Spondylosium* (2 species), *Arthrodesmus* (1 species), *Staurastrum* (4 species), *Stauradesmus* (1 species) and *Eurastrum* (1 species). The list of the algal species was stated in Table 1. Water analysis parameter found in study area from March to December, 2018 was shown in Table (2 - 7) and Figure (6- 10).

Table 1 List of Green Algae Recorded in Sedawgyi Dam

1	*Chlorococcum humicola*	20	*Pediastrum simplex* var. *radians*
2	*Tetradron trigonum*	21	*Pediastrum simplex* var. *sturmii*
3	*Selenastrum bibraianum*	22	*Closterium intermedium*
4	*Kirchneriella lunaris*	23	*Closterium moniliferum*
5	*Coelastrum indicum*	24	*Closterium praelongum*
6	*C. microporum*	25	*Closterium pseudolunula*
7	*Scenedesmus acutus*	26	*Cosmarium contractum* var. *minutum*
8	*Scenedesmus baculiformis*	27	*Cosmarium quadrum*
9	*Scenedesmus acuminatus*	28	*Cosmarium lundelli* var. *ellipticum*
10	*Scenedesmus quandricauda*	29	*Spondylosium pulchrum* var. *constrictum*
11	*Scenedesmus quadricauda* var. *westii*	30	*Spondylosium pulchellum*
12	*Pediastrumbiradiatum* var. *langecornutum*	31	*Arthodesmus convergens*
13	*Pediastrum boryanum*	32	*Staurastrum arachne* var. *curvatum*
14	*Pediastrum boryanum* var. *langecurne*	33	*Staurastrum bibrachiatum*
15	*Pediastrum duplex* var. *clathratum*	34	*Staurastrum natator*
16	*Pediastrum duplex* var. *genuinum*	35	*Staurastrum proboscideum*
17	*Pediastrum duplex* var. *reticulatum*	36	*Stauradesmus cuspidatus*
18	*Pediastrum simplex*	37	*Eurastrum spinulosum*
19	*Pediastrum simplex* var. *duodenarium*		

1. Monthly Variation of Genera in Three Sampling Sites

Relative abundant (%) of 14 genera of Chlorophyceae: *Coelastrum, Scenedesmus, Pediastrum, Closterium, Cosmarium, Arthodesmus* and *Staurastrum* found in study area were given in graphs for monthly variations in Figure (2 - 5). In sampling site I, the maximum population of *Coelastrum* observed in April and the minimum population was occurred in September. In sampling site II, the maximum growth recorded in March and the maximum growth observed in May. The population of *Coelastrum* was not occurred in July. The maximum population recorded in May and the minimum population occurred in June at sampling site III. The three sites as one unit, the maximum population recorded in April and the minimum population was in December.

The maximum growth of *Scenedesmus* recorded in April and the minimum growth was in July at sampling site I. The maximum population occurred in May at sampling site II and III and the minimum population was observed in August at sampling site II and December at sampling site III. The three sites as one unit, the maximum population occurred in May and the minimum was in August.

The *Pediastrum* population existed nearly same population throughout the study period at sampling site I. In sampling site II, the maximum population was occurred in May and the minimum population was in July. In sampling site III, the maximum population was in April to June and the other months observed nearly same population. The three sites as one unit, the maximum population was in May and the minimum was in July.

In sampling site I, the maximum population of desmids was recorded in May and November. The minimum population was observed in December. In sampling site II, the maximum growth of desmids was observed in May and the minimum growth was in August. In sampling site III, the minimum population was in July and the other months observed nearly same population. The three sites as one unit, the maximum population was in May and the minimum population was in July.

2. Physico-chemical Parameters of Water Samples of Three Sampling Sites

All the results of monthly variation in Physico-chemical parameters were showed in Table (2 - 7) and Figure (6 - 10). In this research, the temperature ranges during study period was observed from 27.5°C to 30.5°C.

The high temperature of water (30°C - 3.5°C) was observed in May at three sampling sites. The low temperature of water (27°C-27.5°C) was recorded in December at three sampling sites.

The lowest value of pH of water was (6.5 mg/L) in May at sampling site I and II. The highest value (7.5mg/L) was occurred in April and November at sampling site III.

The lowest value of total alkalinity was (30 mg/L) in September at sampling site III. The highest value of (120 mg/L) in April at sampling site I. The value of total alkalinity in summer was higher than those in other seasons.

The highest value of hardness was (140mg/L) in July at sampling site I. The smallest value of hardness was (35 mg/L) in September and (38 mg/L) in August at sampling site I.

The maximum value (0.55 mg/L) of phosphate was in May (summer) at sampling site I and the minimum value (0.001 mg/L) was in June at sampling site I. The phosphate content ranged from (0.001 mg/L – 0.55 mg/L).

The value of nitrite-nitrogen was (0 mg/L) in June at the three sampling sites. The highest and lowest value of nitrite-nitrogen concentration were 0.411 mg/L and 0.006 mg/L. The maximum value (0.411 mg/L) was observed in August (rainy season) at sampling site I and the minimum value (0.006 mg/L) was recorded in March (summer) at sampling site II.

The maximum value (3.5 mg/L) of biological oxygen demand was in May (summer) at sampling site II and the minimum value (0.2 mg/L) was in December (winter) at sampling site III.

In this research, dissolved oxygen contents in the water samples were found in the range of 1.5 mg/L-6.0mg/L. The maximum value (6.0 mg/L) of dissolved oxygen in September at sampling site I and the lowest value (1.5 mg/L) was in June at sampling site II and III.

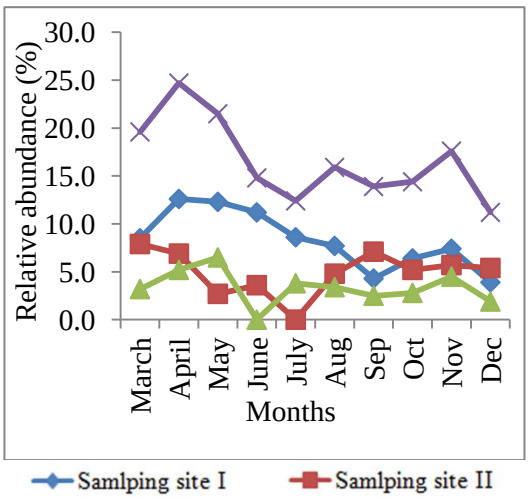


Figure 2 Monthly variation of *Coelastrum* found in individual sampling site

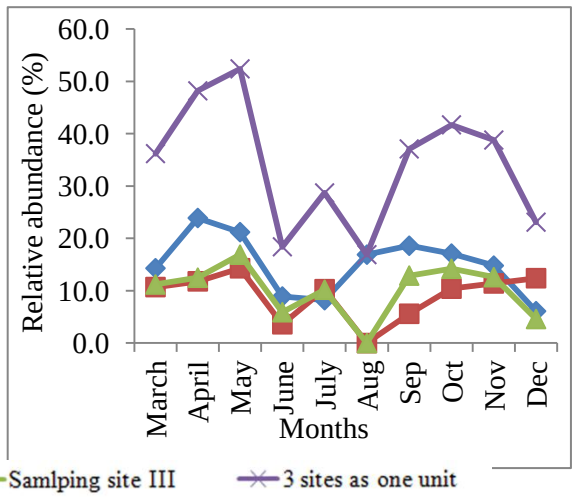


Figure 3 Monthly variation of *Scenedesmus* found in individual sampling site

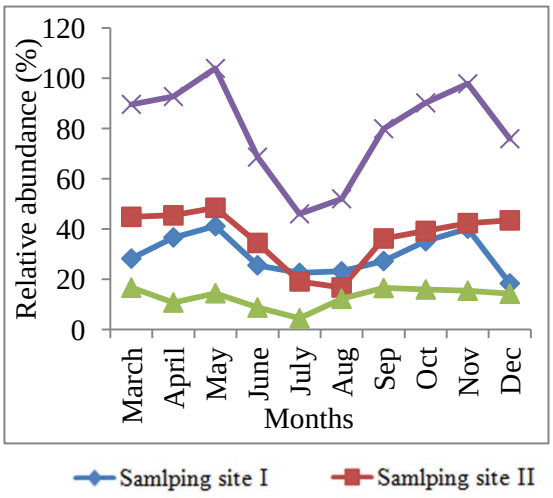


Figure 4 Monthly variation of *Pediastrum* found in individual sampling site

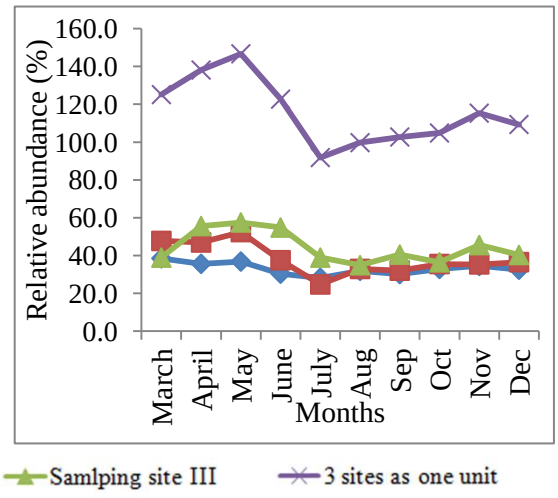


Figure 5 Monthly variation of desmids found in individual sampling site

Table 2 Monthly variation of temperature in water samples of three sampling sites (March- December, 2018)

Site No.	Temperature									
	March	April	May	June	July	August	September	October	November	December
I	29	29	30	28.9	28	29.7	29.5	29.5	28.9	27
II	29.5	29	30.5	28.7	28.2	29.7	29.5	29.5	28.9	27.5
III	29.5	29	30.2	28.5	28	29.8	28.9	29.7	28.6	27.5

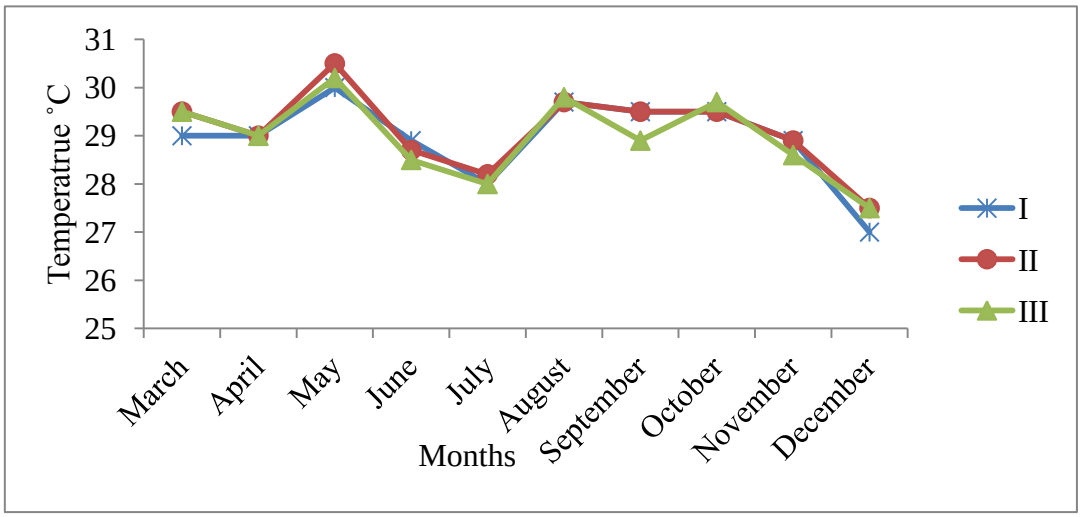


Figure 6 Monthly variation of temperature in water samples of three sampling sites (March- December, 2018)

Table 3 Monthly variation of pH in water samples of three sampling sites (March- December, 2018)

Site No.	pH									
	March	April	May	June	July	August	September	October	November	December
I	6.8	6.9	6.5	6.9	6.9	6.7	6.6	6.9	7.2	7.1
II	7.2	7.4	6.5	6.9	7.3	7.4	7.2	7.3	7.3	7.1
III	7.2	7.5	6.7	7.2	7.2	7.2	7.1	7.2	7.5	7.2

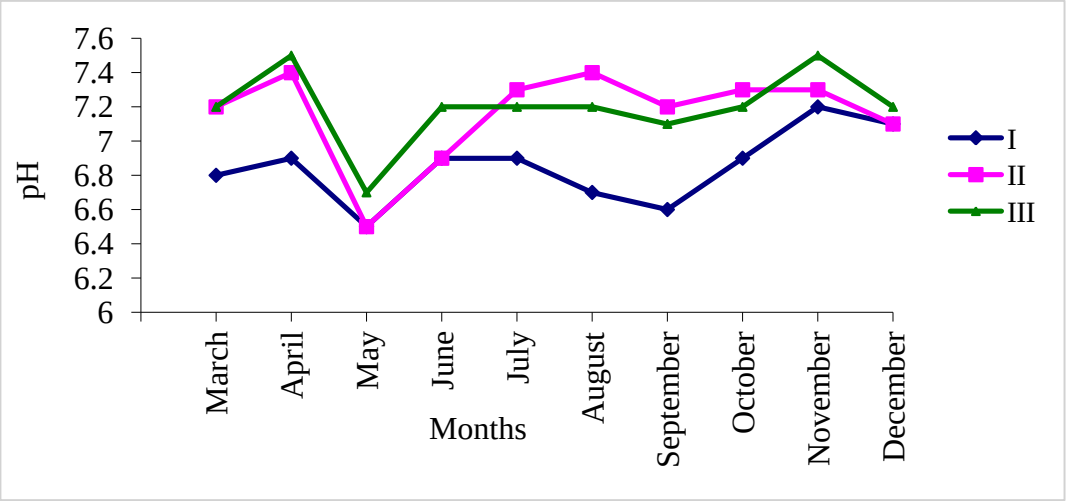


Figure 7 Monthly variation of pH in water samples of three sampling sites (March- December, 2018)

Table 4 Monthly variation of Alkalinity in water samples of three sampling sites (March- December, 2018)

	Alkalinity									
Site No.	**March**	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**
I	100	120	80	50	50	80	75	85	100	90
II	80	100	90	50	70	60	55	78	90	80
III	60	80	100	60	80	40	30	55	70	60

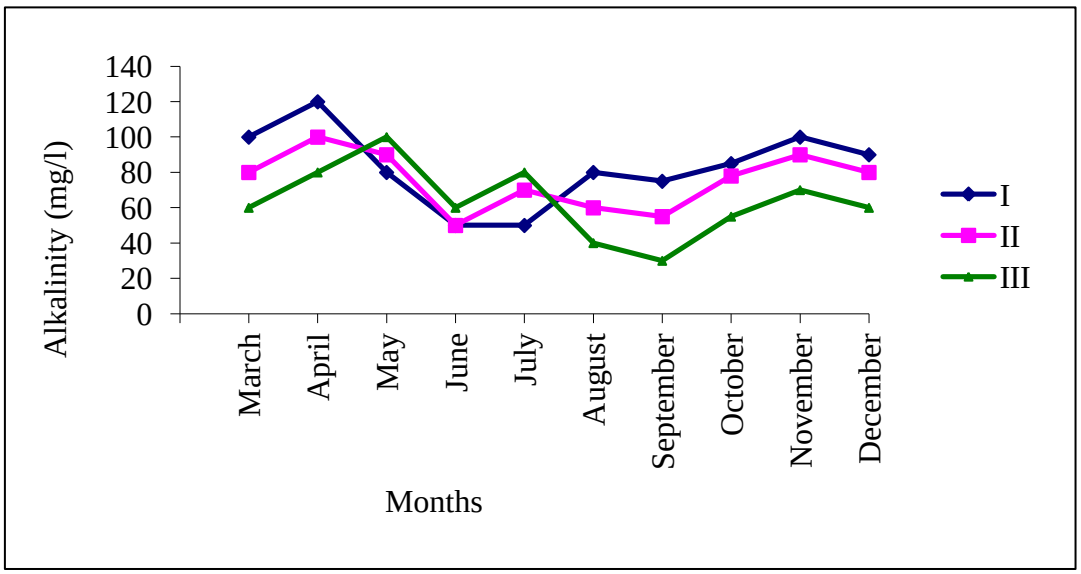


Figure 8 Monthly variation of Alkalinity in water samples of three sampling sites (March- December, 2018)

Table 5 Monthly variation of Hardness in water samples of three sampling sites (March- December 2018)

	Hardness									
Site No.	**March**	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**
I	80	85	70	80	140	38	35	50	60	61
II	65	74	60	90	120	62	60	80	70	74
III	60	80	40	100	110	64	62	82	80	75

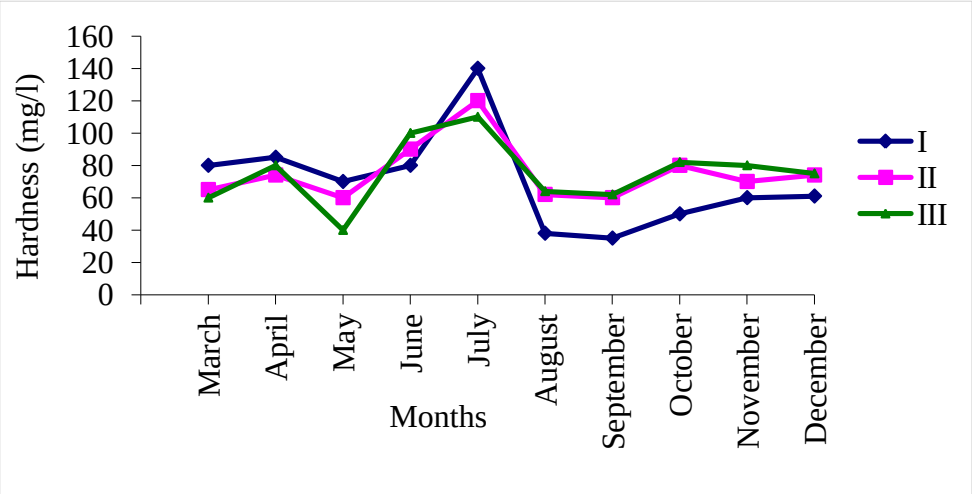


Figure 9 Monthly variation of Hardness in water samples of three sampling sites (March- December, 2018)

Table 6 Monthly variation of DO, BOD, Phosphate and Nitrite nitrogen in water samples of three sampling sites (March- July, 2018)

	March			April			May			June			July		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
DO (mg/L)	2.5	3.5	4	1.5	2.5	3	3.5	4	4	2	1.5	1.5	3	3.5	4
BOD (mg/L)	1	1.5	1.5	2	2.5	2.5	2	3.5	3	0.5	0.5	0.5	0.5	0.5	0.5
Phosphate	0.06	0.05	0.06	0.07	0.05	0.07	0.55	0.5	0.5	0	0.02	0.04	0	0.01	0.02
Nitrite nitrogen	0.072	0.01	0.03	0.12	0.1	0.11	0.11	0.06	0.14	0	0	0	0.17	0.1	0.09

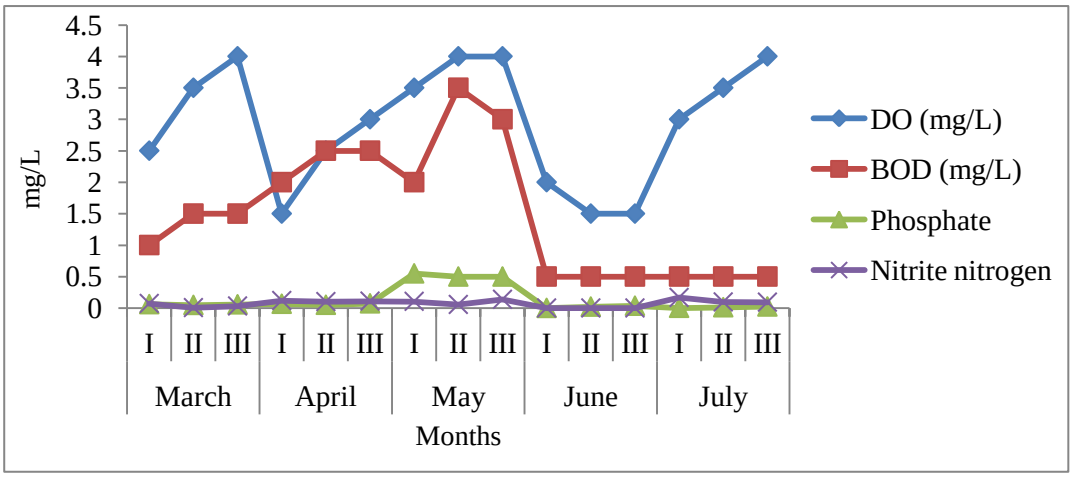


Figure 10 Monthly variation of DO, BOD, Phosphate and Nitrite nitrogen in water samples of three sampling sites (March-July, 2018)

Table 7 Monthly variation of DO, BOD, Phosphate and Nitrite nitrogen in water samples of three sampling sites (August- December, 2018)

	August			**September**			**October**			**November**			**December**		
	I	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**
DO (mg/L)	5	4	4.5	6	5	5.5	5	4	4.5	5	5.5	5.5	4.5	5	4.5
BOD (mg/L)	1	0.5	0.5	0.5	1	1	1	1.5	1	0.5	0.5	0.5	0.3	0.3	0.2
Phosphate	0.2	0.24	0.29	0.33	0.39	0.31	0.4	0.32	0.39	0.02	0.01	0.02	0.01	0.01	0.02
Nitrite nitrogen	0.4	0.231	0.204	0.276	0.1	0.102	0.1	0.09	0.1	0.05	0.009	0.086	0.04	0.005	0.082

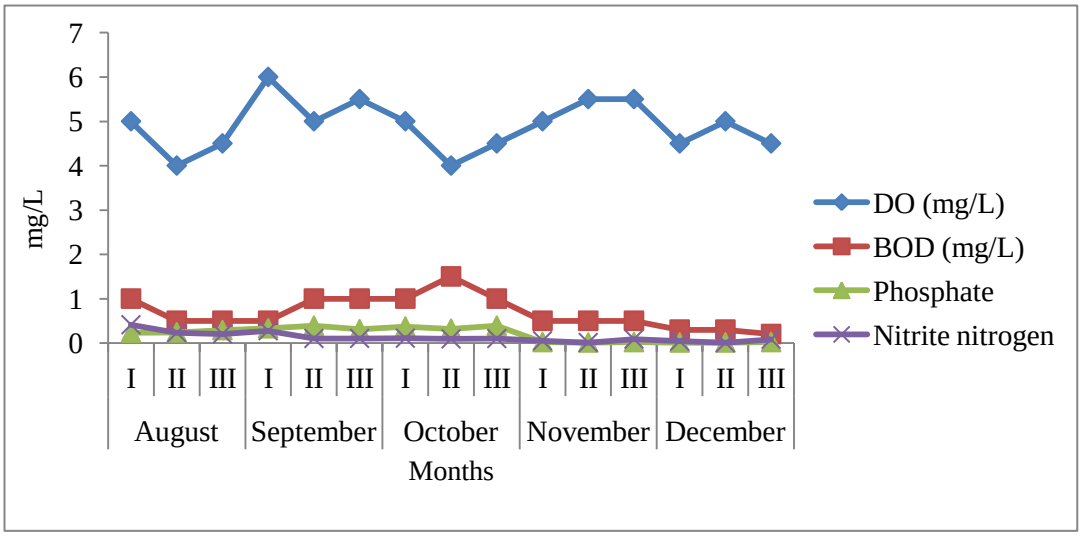


Figure 11 Monthly variation of DO, BOD, Phosphate and Nitrite nitrogen in water samples of three sampling sites (August- December, 2018**)**

Discussion and Conclusion

In this research, the physico-chemical parameters of water and the monthly variations of green algae found in Sedawgyi Dam, Madaya Township, Pyin Oo Lwin District, Mandalay Region were studied from March, 2018 to December, 2018. A total of 14 genera and 37 species of algae were recorded from three sampling sites. In Sedawgyi Dam, 32 species in sampling site I, 35 species in sampling site II and 37 species in sampling site III were collected from study period. Chlorophyceae was dominated by species of *Pediastrum, Cosmarium, Closterium, Scenedesmus* and *Staurastrum* and it was found in three sampling sites during the study period (Figure 2- 5). The species of *Chlorococcum humicola, Tetradon trigonum, Selenastrum bibraianum, Kirchneriella lunaris, Spondylosium pulchrum, Arthrodesmus archne, Stauradesmus cuspidatus* and *Eurastrum spinulosum* were less abundant in study period. It was found that there are fluctuations both in the total algal population and in the relative abundance of different genera from month to month, and from site to site, though they were not far from each other.

Monthly variations in members of Chlorophyceae showed maxima in April and May (summer) and minima in July, August and September (rainy season) at all the sampling sites. In the present investication, it was observed that high temperature and pH are favourable for rapid development of Chlorophyceae. This observations agreed with Misra *et al*. (2001) stated that High Chlorophycean count registered during summer months may be due to low DO and bicarbonate level prevailing during these periods, which favors its quick growth. The minimum population of Chlorophyceae recorded in July to September (rainy season) at all sampling sites. This findings agreed with Krishnan *et al*. (1999) stated that groups the minimum density of chlorophyceae in monsoon may be attributed to the dilution effect due to the rains as well as drifting of algae along with the water.

In the present study, the dominance of Chlorophyceae in April and May (summer season) and November and December (winter season) were recorded in three sampling sites. The range of temperature was 30˚C-30.5˚C in May (summer). Shukla *et al.* (2013) stated that the dominance of Chlorophyceae in the summer season which indicates that the temperature of these months played an important role in increasing the population of Chlorophyceae and bright sunlight have been reported favourable factors for Chlorophyceae. Minimum population in rainy season due to the minimum transparency, cloudy weather and heavy flood decline the phytoplankton density. This findings agreed with Shukla *et al.* (2013). Aktar *et al.* (2007) stated that abundance of Chlorophyceae indicate absence of pollution. Therefore, it may be suggestd that the water in Sedawgyi Dam indicate absence of water pollution.

The species composition of Chlorophyceae with total 37 species and 14 genera of Sedawgyi Dam. These genera were *Chlorococcum, Tetradon, Selenastrum, Kirchneriella, Coelastrum, Scenedesmus, Pediastrum, Closterium, Cosmarium, Spondylosium, Arthodesmus, Staurastrum, Staurasdesmus* and *Eurastrum.* Of these *Closterium, Staurastrum* and *Cosmerium* are considered as desmids which indicate good quality of water and absence of desmids is an indication of heavy pollution of water Hosmani *et al.* (2002). According to Hutchinson (1967) desmids (e.g. *Cosmarium)* are associated with oligotrophic freshwater and in these, they may form an important food source for herbivore fish. The food chain relations of endemic and endangered species of fishes may include specific phytoplankton species. Moreover, the dominance of Desmids over Chlorococcales (e.g. *Pediastrum*) a group indicative of eutrophication. According to Munawar (1974), the species of *Coelastrum, Oocystis, Scendesmus,*

Zygnema, *Chlamydomonas*, *Chlorella*, *Spirogyra*, *Tribonema* and *Closterium* are found in polluted waters. Of these only *Coelastrum, Scendesmus* and *Closterium* were found in study area of Sedawgyi Dam in low density.

During the study period desmid population was declined during rainy season and the lowest values. This is an agreement with Sukumaran and Das (2001). In Sedawgyi Dam, the maximum population of desmids was recorded in May and December. The higher concentration of phosphate 0.50-0.55 mg/L was in May. This findings agreed with Venkateswarlu (1986) stated that the higher concentration of phosphate favour the abundance of desmids. The pH value above 7 was in April, November and December. This findings generally agreed with Gonzalves and Joshi (1946) who stated that pH was above 7 which contained fairly good number of desmids indicating that the alkaline water supports the desmid population.

The pH of water is important to the chemical reactions that take place within water, and pH values that are too high or low can inhibit the growth of microorganisms. Most natural water has a pH range of 6-8 (Goel 2006). In this research, water in Sedawgyi Dam has a pH range of 6.5-7.5. This finding agreed with Umavathi *et al.* (2007) who stated that pH in ranged of 5-8.5 is the best for plankton growth but harmful when more than 8.8.

The pH value ranged between 6.5 mg/L to 7.5 mg/L. Maximum value 7.5 mg/L was observed in April to November and minimum value 6.5 mg/L occurred in May. The pH values of water in Sedawgyi Dam water were within the acceptable limit of WHO (2000) standard which was 6.5-9.5 mg/L.

The range of alkalinity of water in Sedawgyi Dam was between 30 mg/L and 120 mg/L. The highest total alkalinity in April in sampling sites II and III and the lowest value in June in all sampling sites. This findings were greater than WHO standard which was < 25 mg/L.

Hardness of the water is due to presence of calcium and magnesium. Shilpa *et al.* (2011) stated that the hardness concentrations up to 60 mg/L are called soft water and those containing 120-180 mg/L as hard water. In this study, the range of hardness of water in Sedawgyi Dam was between 35 mg/L and 120 mg/L. According to Shilpa *et al.* (2011), the water in Sedawgyi dam may be soft water.

The concentration of phosphate ranged from between 0.001mg/L and 0.55mg/L. The highest phosphate value was obtained in May. The lowest value was found in June, July, November and December. This value was not acceptable limit for WHO (2000).

Biological oxygen demand is used as a measurement of pollutants in natural water. Biological oxygen demand is an indicator of organic load of water. BOD values ranged between 0.2 mg/L and 3.5 mg/L. The highest BOD value was occurred in April and May. The value BOD in the water of Sedawgyi Dam was lesser than WHO level (<6.0 mg/L).

In Sedawgyi Dam, 37 species and 14 genera of Chlorophyceae were recorded. The maximum growth of Chlorophyceae were recorded in April and May (Summer) and the minimum growth was in July to September (rainy season). These genera of *Scenedesmus, Pediastrum, Closterium, Cosmarium and Staurastrum* were dominant in study period. Of these *Closterium, Staurastrum* and *Cosmerium* are considered as desmids which indicate good quality of water and absence of desmids is an indication of heavy pollution of water. The physio-chemical data analysed in Sedawgyi Dam indicates that the lake is at present free from pollution.

Acknowledgements

We would especially like to Dr. Hla Min Thein, Associate Professor, Kyaing Tong University, for his valuable suggestion and enthusiastic encouragement and over all supervision of my research work. I am thankful to my sincere appreciation and deep gratitude to Dr. Khin Win Naing, Professor and Head, Department of Botany, University of Myitkyina for permission to do this research. My sincere thanks are also due to Dr. Seine Nyoe Nyoe Ko, Professor, Department of Botany, University of Myitkyina, for her valuable suggestions.

References

Akhtar R, M. K. N Jyoti, Sawhney and Rajnder Singh (2007). "Studied on Population Dynamics of Cladocerans and Copepodsin Sarkoot Pond, Dist, Doda, Jammu and Kashmir". J. Aqa. Biol.; 22 (2): 15-18.

Boyd, C. E. and C. S. Tucker, (1998). Pond Aquaculture Water Quality Management, Kluwer Academic Publisher London.

Goel, P.K. (2006).Water Pollution. Causes effects and control, Department of Pollution Studies, Y.C. Collage of Science Vidyanagar, Karod, Maharashta.

Goel, P.K. (1997). Water pollution: Causes, Effects and Control, Department of Pollution Studies Y.C.College of Science, maharastra, NewDelhi.

Gonzalves, E.A. and D.B. Joshi. (1946). Freshwater algae near Bombay. I. The seasonal succession of the algae in a tank at Bandra. J. Bomb. Nat. Hist. Soc., 46: 154-176.

Hosmani, S. P., P. Nagarathna, and J. Mahadev. (2002). IJEP, 22 (8):852-857.

Hutchinson, G. E. (1967). A treatise on Limnology (ii) Introduction to Lake Biology and the limnoplankton. John Wiley and Sons, Inc. New York, London.

Jayaraman, P.R., T. Ganga Devi, and T. Vasudevan Nayar. 2003. Water quality studies on Karamana River, Thiruvananthapuram district, South Kerala. Ind. Pollut. Res. 22: 89-100.

Krishnan, K. H., S.Thomas, S. George, R. P. Murugan, S. Mundayoor, and M. R. Das. (1999). Pollution Research, 18: 261-269.

Kumar, A. L.L. Sharma, and N.C. Aery. (2009). Physico-chemical characteristics and diatom diversity of Jawahar Sagar Lake, Bundi – A Wet land of Rajasthan. Sarovar Saurabh Vol. 5(1): 8-14.

Lavens, P. and P. Sorgeloos, (1996). Manual on the production of live food for aquaculture, FAO Fisheries Technical Paper, University of Ghent, Belgium.

Misra, P.K., R.K. Mehrotra, Jai Prakash and A.K. Shrivastava, (2001). Fresh water green algae from Basti district, Uttar Pradesh, Geophytology, 31(1&2):1-7

Munawar, M. (1974). Hydrobiologia, 45(1): 1-32.

Shilpa, G., C.S. Goroba, J.A. Suhas & P.D. Raut. (2011). Study of physico-chemical and biological characteristics of lake from Shivaji University Campus, Kolhapur, Maharashtra, Department of Environmental Science, Shivaju University, Kilhapur.

Sigee, D.C. (2010). Freshwater microbiology, biodiversity and dynamic interactions of microorganisms in the aquatic environment, John Wiley and Sons ltd, University of Manchester, UK.

Smith, G.M. (1950). Freshwater Algae of the United States 2nded, the MC Grouo Hill

Shukla P., Preti and Singh A. (2013). A seasonal variation of plankton population of Maheshara lake in Gorakhpur, Word journal of Zoology, India.

Sukumaran, P.K. and A.K. Das. (2001). Distribution of plankton in some freshwater reservoirs of Karnataka. *J. Inland. Fish. Soc. India.* 33: 29-36.

Tiwari, S., S. Dixit, and S.K. Gupta. (2004). An evaluation of various physico-chemical parameters in surface waters of Shahpura Lake, Bhopal. Pollut. Res. 23: 829-832.

Umavathi, S., Longakumar & K. Subhashini. (2007). Studies on the nutrient content of sulphur pond in coimbator, Tamil Nadu. J. Ecol. Env. & Cons.

Venkateswarlu, V. (1986). Ecological studies on the rivers of Andhra Pradesh with special reference to water quality and pollution. Proc. Indian Acad. Sci. Plant Sci., 96: 495-508.

World Health Organization (WHO) (2000). Health guidelines for the use of wastewater in agricultural and aquaculture. Report of a WHO Science Group. Technical Report Series No. 778, Genera, Switzerland.

Yakubu, A.F., Sikoki F.D., Abowei J.F.N., and Hart S.A. (2000). A comparative study of phytoplankton communities of some rivers, creeks and burrow pits in the Niger delta area. J.Applied sci. Environ Manage. 4: 41-46.

STUDY ON SOME HEAVY METAL CONTENTS, NUTRITIONAL VALUES AND ANTIMICROBIAL ACTIVITY OF RHIZOME OF *COSTUS SPECIOSUS* (KOEN.) SM.

Ohn Mar[1]

Abstract

Costus speciosus (Koen.) Sm. is locally known as Phalan-taung-hmwe and belongs to the family Costaceae. In this research, the plant was collected from Hpa-an Township, Kayin State during June to October, 2017. In this study, atomic absorption spectrophotometer (AAS) analysis, nutritional values and antimicrobial activity of rhizome of *Costus speciosus* (Koen.) Sm. were undertaken. The content of heavy metals was analyzed by using Atomic Absorption Spectrophotometer(AAS). In according to the results of atomic absorption spectrophotometer, only cadmium (0.004 ppm) is found among heavy metals. Therefore, the level of cadmium in *Costus speciosus* (Koen.) Sm. was below the standard limit considered safe for human consumption and did not give harmful effect on human health. The experiment for the nutritional values of *Costus speciosus* (Koen) Sm. was carried out at the Food Industries Development Supporting Laboratory (FIDSL), Yangon. According to the results, the carbohydrate content in rhizome was higher percentage than others. In antimicrobial activities, the different solvent extracts were investigated with six types of microorganisms by using agar well diffusion method. According to this experiment, ethyl acetate extract showed most significant antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis* and *Candida albicans.*

Keywords: *Costus speciosus* (Koen.) Sm., Heavy metal contents, nutritional values and antimicrobial activity.

Introduction

The plant *Costus speciosus* (Koen.) Sm. belongs to the family Costaceae. The native of plant is Indo-Malayan region, occurring from India to New Guinea (Rodriguez, 2005). *Costus speciosus* (Koen.) Sm., locally known as Phalan-taung-hmwe in Myanmar and Indian spiral ginger in English (Hundley and Chit Ko Ko, 1987; Kress *et al.*, 2003).

A large number of plants have been used as traditional medicine in many parts of the world. Among them, *Costus speciosus* (Koen.) Sm. plays an important role as an herbal medicine for the treatment of various health ailments. In general, plant based medicines are safe and there are no side effects compared to other drugs and more effective treatment of health disorders (Malabadi *et al*., 2016).

Costus speciosus (Koen.) Sm. is used in traditional medicine in the treatment of fever, expectorant and cough (Ashin Nagathein, 1968). Leaves and young stems are used internally for diarrhea, eye and ear infections. Decoction of stem is used to control fever and dysentery (Duraipandiyan, *et al*., 2012). In Myanmar, the juice of stem is used for ear infection and otorrhoea (San Hla, 1960). The roots are peeled and placed on the infected tooth for an hour to provide relief from toothache (WHO, 2009). The roots are used to treat cough, dyspepsia, skin disease, worms, snake bite, aphrodisiac, purgative, anthelmintic, febrifuge, expectorant, and catarrhal fever (Malabadi *et al.,* 2016).

[1] Dr, Associate Professor, Department of Botany, Dawei University.

The rhizome of *Costus speciosus* (Koen.) Sm. may be beneficial in protection and alleviation of diabetic complications. The rhizomes are bitter, astringent, cooling, aphrodisiac, purgative, anthelmintic, expectorant, tonic and useful in burning sensation, constipation, worm infection, skin diseases, fever, asthma, bronchitis, inflammations, anemia, improve digestion, pneumonia, urinary diseases and jaundice (Verma and Khosa, 2012).

The human body requires a number of minerals for their growth and other activities which are obtained from plants, since plants absorb and accumulate minerals from the environment which is necessary for its growth. Plants can also accumulate metals from the environment. Environment, pollution, atmosphere, soil, harvesting and handling are some of the factors, which play a major role in contamination of medicinal plants by metals and also by microbial growth. Therefore, it is necessary to measure and establish the levels of metallic elements in the herbal plants as these elements when consumed at higher levels become toxic (Gajalakshmi, *et al*., 2012)

Nutrition is a science that studies all the reactions that occur between living organisms and food. Food includes plant and animal products that when consumed, can yield energy and provide nutrients needed to maintain life and allow growth and reproduction (Grosvenor and Smolin, 2002). The human body requires substantial amounts of some nutrients, particularly those that will provide energy and support growth and development of the body tissues, namely carbohydrates, fat, and protein, as well as water (Williams, 1999).

Microorganisms are living things so small that they can be seen only with the aid of microscope. They are widely distributed in nature and are responsible for many physical and chemical changes importance to the life of plants, of animals and of human beings (Sarles, *et al*., 1956).

The aim and objectives of this research are to analyze the content of some heavy metals in powdered drug, to investigate the nutritional values, and to examine the antimicrobial activities of the different solvent extracts from rhizomes of *Costus speciosus* (Koen.) Sm.

Materials and Methods

Collection and preparation of powdered samples of *Costus speciosus* (Koen.) Sm.

The specimens of *Costus speciosus* (Koen.) Sm. was collected from Hpa-an Township, Kayin State during June to October, 2017. The collected samples of rhizomes were thoroughly washed with water to remove impurities. After washing the samples, they were cut into small pieces then air dried at room temperature for several days. When constant weight was obtained, the dried samples were pulverized by grinding machine to get powder and stored in airtight containers for further studies.

Determination of some heavy metals by Atomic Absorption Spectrophotometer

The ash samples were used to study the content of heavy metals and analyzed by using Atomic Absorption Spectrophotometer according to the method of Levinson (1974) at the Applied Geology Department, University of Yangon.

Preparation of sample for Atomic Absorption Spectrophotometer (AAS)

In this study, Perkin Elmer Analyst 800 spectrophotometer was used. Ten grams of powdered sample was placed in a weighed crucible and heated in a Muffle furnace at 300ºC to

achieve complete ash. About 0.5 g of ash was filtered with 80 meshes and digested in 5 ml of HNO_3: HCl (1: 4) concentrated acid mixture. The solution was evaporated overnight to dryness in air. The residue was leached on a water-bath treated with 10 ml of HNO_3 weak acid mixture at a temperature of about 70ºC for 30 minutes and then 10 ml of deionized water were added. The solution was stirred by using vortex mixer. The resultant solution (10 ml) was pipetted accurately and made up to 100 ml with deionized water again. The solution was stand for overnight and then aspirated on an atomic absorption spectrophotometer.

Nutritional values from the rhizome of *Costus speciosus* (Koen.) Sm.

The analysis for the nutritional values of rhizomes of *Costus speciosus* (Koen.) Sm. was carried out at the Food Industries Development Supporting Laboratory (FIDSL), Yangon. The nutritional value had been investigated according to the method AOAC (Horwitz, 1980).

Antimicrobial activities of different solvent extracts from rhizomes of *Costus speciosus* (Koen.) Sm.

Antimicrobial activities of different solvent extract from rhizomes of *Costus speciosus* (Koen.) Sm.were tested on six pathogenic microorganisms by using agar well diffusion method at the Central Research and Development Center (CRDC), Yangon.

Preparation of crude extracts from rhizomes of *Costus speciosus* (Koen.) Sm.

The dried powdered rhizomes (5g) of *Costus speciosus* (Koen.) Sm. was extracted with 50ml of petroleum ether, chloroform, ethyl-acetate, acetone, ethanol, methanol and distilled water respectively for seven days and then filtrated by using filter paper. The filtrate solvents were evaporated on a water bath. All these extracts were used for the determination of antimicrobial activities.

Test organisms

The test organisms used were *Bacillus subtilis, Bacillus pumalis, Candida albican, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus. These* microorganisms are listed in Table 1.

Table 1 Test organisms, their respective code numbers and diseases

Test organisms	Code number	Diseases
Bacillus subtilis	N.C.I.B-8982	Food spoilage and fever
Bacillus pumalis	N.C.T.C-8236	Eye infection, soft tissue infection
Candida albican	IFO-1060	Pathogenic, vaginal candidiasis, alimentary tract infection, cardiac infection, skin infection, intestinal tract infection and vagina mucosa, sinus irritation, intense itching, sores and inflammation
Staphylococcus aureus	N.C.P.C-6371	Food poisoning, boils, abscesses, headache, skin infection, wound sepsis, burns, muscle cramping, nausea, vomiting, illness,
Escherichia coli	N.C.I.B-8134	Diarrhoea and vomiting, dysentery, urinary-tract infections,
Pseudomonas aeruginosa	N.C.T.C-6749	Urinary-tract infections, surgical wound respiratory infection, soft tissue infection, bone and joint infections, central nervous system infection, gastrointestinal infection, chronic lung, burn infection, ear infection, eye infection, bacteremia and septicemia.

Preparation of sample for testing antimicrobial activity

Screening of Antimicrobial activity of crude extracts has been done by agar-well diffusion method. Nutrient agar was prepared according to the method described by Cruickshank (1975). Nutrient agar was boiled and 20-25 ml of the medium was poured into each conical flask, plugged with cotton wool and autoclaved at 121 °C for 15 minutes. Then the conical flask was cooled down to 40-45 °C and poured into sterilized petridishes and each of 0.1-0.2 ml of test organisms were also added into the dishes. The agar was allowed to set for 2-3 hours. After this, 10 mm agar-well was punched with the help of sterilized agar-well borer. Then, about 0.2 ml of sample was introduced into each agar- well and incubated at 37 °C for 24 hours. The inhibition zone appeared around the agar-well, indicates the presence of antimicrobial activity. Then the diameter of inhibitory zone was measured with the help of a transparent ruler. At the same time, the controlled experiments using solvent only were prepared for the comparison with rhizomes extracts.

Results

Determination of some heavy metals by Atomic Absorption Spectrophotometer

The content of heavy metals (As, Hg, Pb and Cd) were analyzed in the powdered rhizome samples by using AAS, measured in the unit of ppm. According to the results of AAS, the contents of elements cadmium (Cd) was found as 0.004 ppm but arsenic (As), mercury (Hg) and lead (Pb) were not found in the rhizome. The results were shown in Table 2.

Table 2 Some heavy metals in *Costus speciosus* (Koen.) Sm. rhizome.

No.	Elements	ppm
1.	Arsenic (As)	ND
2.	Mercury (Hg)	ND
3.	Lead (Pb)	ND
4.	Cadmium (Cd)	0.004

Nutritional values from the Rhizome of *Costus speciosus* (Koen.) Sm.

The nutritional values of *Costus speciosus* (Koen.) Sm had been undertaken according to the method AOAC (Horwitz, 1980). In this experiment, the amount of protein content 3.47%, crude fiber content 20.06%, crude fat content 1.60% and carbohydrate content 51.71% were observed in *Costus speciosus* (Koen.) Sm. rhizomes. Therefore, the carbohydrate content in rhizome was higher percentage than others. The results were shown in Table 3 and Figure 1.

Table 3 Nutritional values from the rhizome of *Costus speciosus* (Koen.) Sm.

No.	Types of Nutrients	Content (%)
1.	Protein	3.47
2.	Crude Fiber	20.06
3.	Crude Fat	1.60
4.	Carbohydrate	51.71

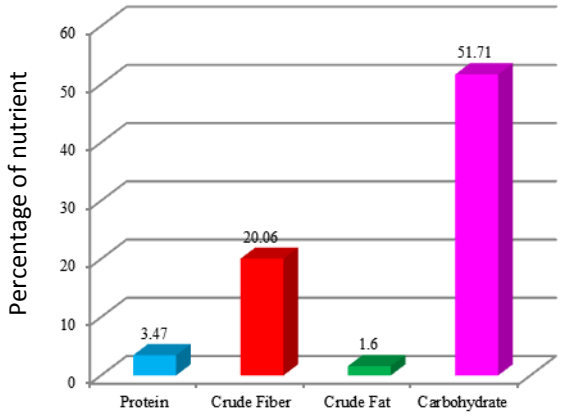


Figure 1 Nutritional values from the rhizome of *Costus speciosus* (Koen.) Sm.

Antimicrobial activities of different solvent extracts from the rhizomes of *Costus speciosus* (Koen.) Sm. by using agar well diffusion method

The antimicrobial activities were tested on six types of pathogenic microorganisms by using agar-well diffusion method. According to this experiment, ethyl acetate extract of rhizomes showed the most significant antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis* and *Candida albican but* non against *Escherichia coli.* Chloroform extract of rhizomes indicated moderate antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus,* weak *against Escherichia coli* and *Pseudomonas aeruginosa.* Methanol and ethanol extracts showed moderate antimicrobial activity against *Pseudomonas aeruginosa*, weak activity against *Bacillus subtilis* and *Staphylococcus aureus.* Acetone extract showed weak antimicrobial activity against *Bacillus subtilis, Pseudomonas aeruginosa* and *Bacillus pumalis.* Petroleum ether extracts exhibited weak antimicrobial activity against *Bacillus subtilis* and aqueous extracts showed moderate against *Staphylococcus aureus* but none against other microbes. The results were shown in Table 4 and Figures 2 to 8.

Table 4 Antimicrobial activities of different solvent extracts from the rhizomes of *Costus speciosus* (Koen.) Sm.

Solvents	***Bacillus subtilis***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Bacillus pumalis***	***Candida albican***	***Escherichia coli***
Petroleum Ether	14 mm	-	-	-	-	-
Chloroform	15 mm	15 mm	11 mm	-	-	13 mm
Ethyl acetate	45 mm	40 mm	38 mm	44 mm	20 mm	-
Acetone	12 mm	-	12 mm	11 mm	-	-
Methanol	13 mm	11 mm	19 mm	-	-	-
Ethanol	12 mm	11 mm	19 mm	-	-	-
Distilled water	-	17 mm	-		-	-

Agar well~ 10 mm

10 mm~14 mm (weak activity), 15 mm~19 mm (moderate activity), 20 mm~above (high activity)

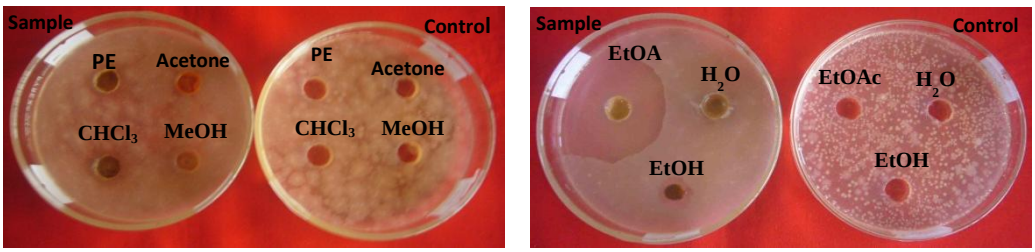


Figure 2 Antimicrobial activities of rhizomes of *Costus speciosus* (Koen.) Sm. againsts *Bacillus subtilis*

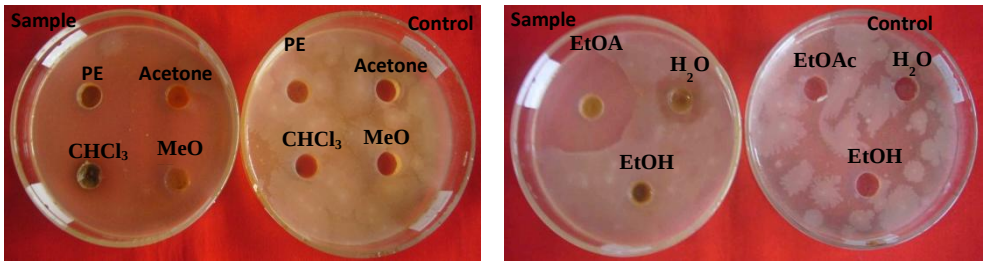


Figure 3 Antimicrobial activities of rhizomes of *Costus speciosus* (Koen.) Sm. againsts *Staphylococcus aureu*

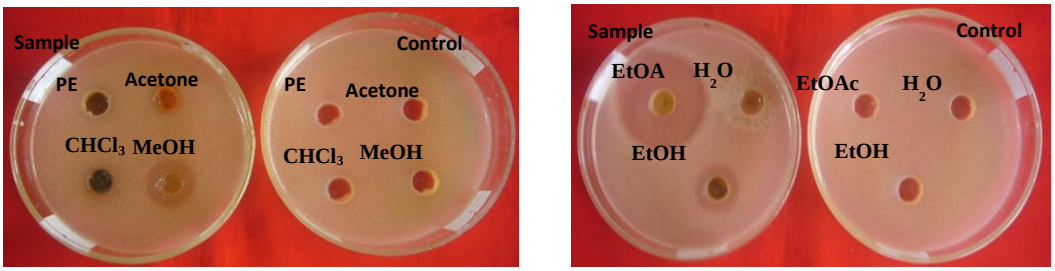


Figure 4 Antimicrobial activities of rhizomes of *Costus speciosus* (Koen.) Sm. againsts *Pseudomonas aeruginosa*

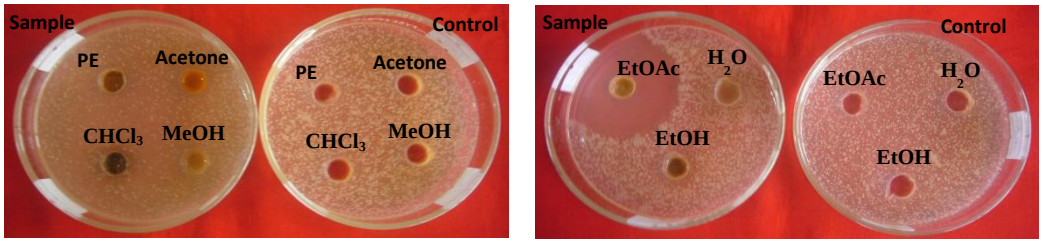


Figure 5 Antimicrobial activities of rhizomes of *Costus speciosus* (Koen.) Sm. againsts *Bacillus pumalis*

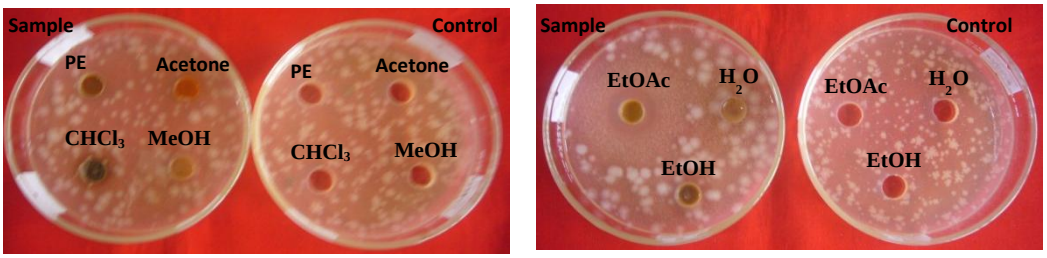


Figure 6 Antimicrobial activities of rhizomes of *Costus speciosus* (Koen.) Sm. againsts *Candida albican*

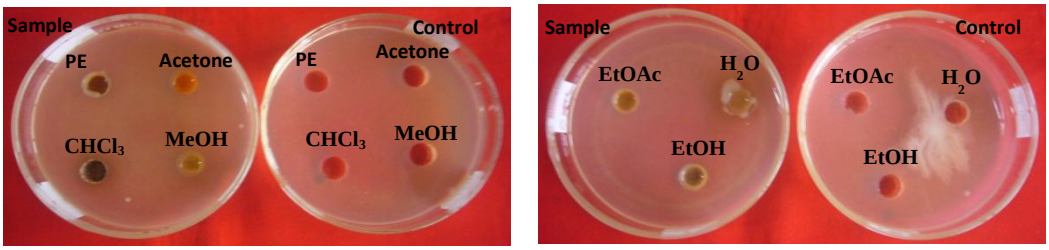


Figure 7 Antimicrobial activities of rhizomes of *Costus speciosus* (Koen.) Sm. againsts *Escherichia coli*

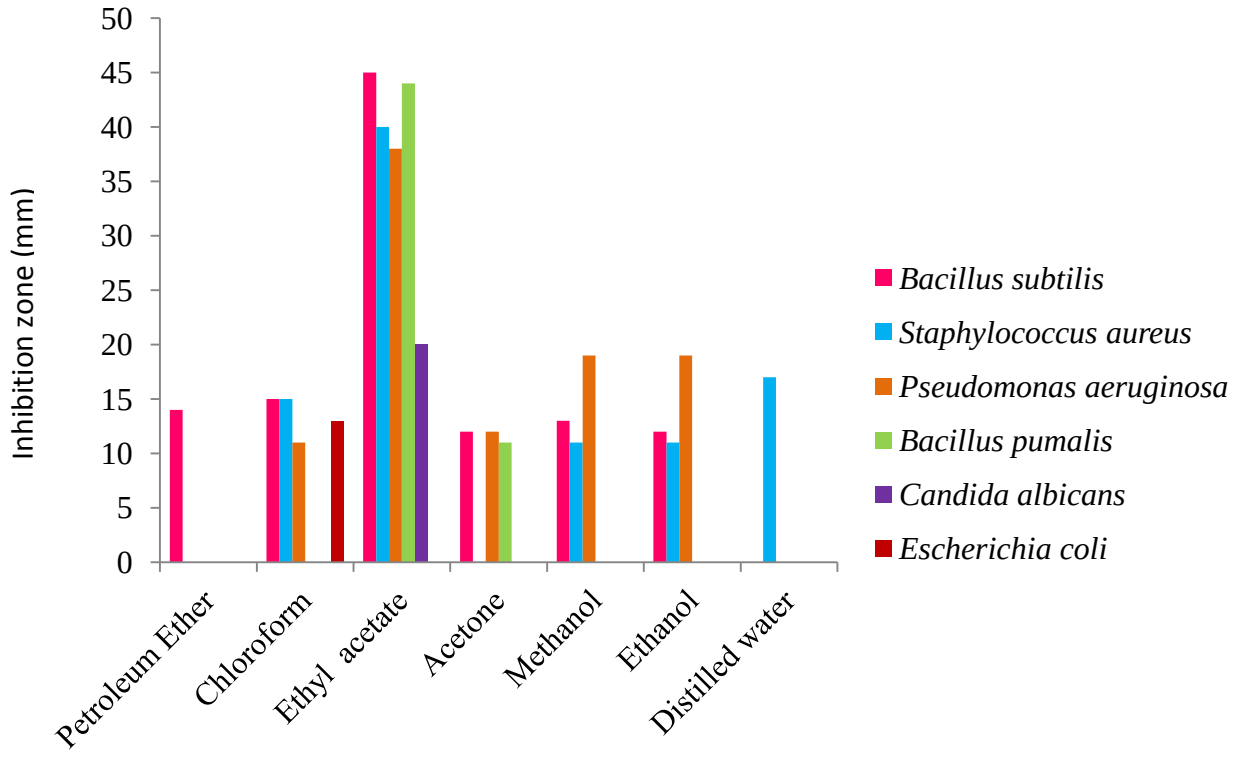


Figure 8 Antimicrobial activities from the rhizome of *Costus speciosus* (Koen.) Sm.

Discussion and Conclusion

In this research, some heavy metal contents, nutritional values and antimicrobial activity of rhizome of *Costus speciosus* (Koen.) Sm. were investigated.

According to the results of (AAS), the contents of elements cadmium (Cd) was found to be 0.004 ppm but arsenic (As), mercury (Hg) and lead (Pb) were not found in the rhizome of *Costus speciosus* (Koen.) Sm.

The ingestion of cadmium exceeding 15 mg kg^{-1} body weight (bw) may give rise to gastrointestinal symptoms such as vomiting, abdominal cramps and diarrhoea, whereas doses of 20-30 mg kg^{-1} bw have caused human fatalities. The lowest emetic dose reported is 0.07 mg kg^{-1} bw (Bull, 2010). The Food and Drug Administration limits the amount of cadmium in food colors to 15 parts of cadmium per million parts of food color (15 ppm) (http://extoxnet.orst.edu/faqs/foodcon/ cadmium.htm). According to Bull (2010) and WHO (http://extoxnet.orst.edu/faqs/foodcon/ cadmium.htm), the level of cadmium in *Costus speciosus* (Koen.) Sm. was below the standard limit (<0.07mg/kg body weight and 15 ppm respectively) considered safe for human consumption and would not have harmful effect on human health. WHO (2007) recommended permissible limit for cadmium in medicinal plant is 0.3 mg/kg. In this study, cadmium in rhizome of *Costus speciosus* (Koen.) Sm. was below WHO permissible limit for cadmium. So, the rhizome of the plant cannot give harmful effect on human health.

According to the result of nutritional values, the amount of protein content 3.47%, crude fiber content 20.06%, crude fat content 1.60% and carbohydrate content 51.71% were found to be present in *Costus speciosus* (Koen.) Sm. rhizomes. The carbohydrate was present as major

constituent than others. Therefore, *Costus speciosus* (Koen.) Sm. rhizomes possess a greater amount of carbohydrate content.

Carbohydrates are the main source of energy for the body. Human body can store only limited amounts of carbohydrates. Excess carbohydrates are converted and stored as fat. The major function of carbohydrates is to provide energy for bodily functions. This energy is needed to carry on body processes such as breathing, maintaining body temperature, and contraction and relaxation of the heart and muscles (Meeks *et al.,* 2009).

In the results of antimicrobial activities, all solvent extracts such as petroleum ether, chloroform, acetone, ethyl-acetate, ethanol, methanol and aqueous extracts of *Costus speciosus* (Koen.) Sm. rhizomes showed different antimicrobial activities on six test microorganisms (*Bacillus substilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albicans* and *Escherichia coli*).

Duraipandiyan *et al*., (2012) stated that chloroform, ethyl acetate and methanol extracts from the rhizomes of *Costus speciosus* (Koen.) Sm. showed antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis* and *Candida albicans* but according to the results of this research, ethyl acetate extract of rhizomes showed the most significant antimicrobial activity against *Pseudomonas aeruginosa, Bacillus pumalis* in addition to *Bacillus subtilis, Staphylococcus aureus*, and *Candida albicans*. Chloroform extract of rhizomes indicated moderate antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus*, weak effect against *Escherichia coli* and *Pseudomonas aeruginosa*. Methanol and ethanol extracts showed moderate antimicrobial activity against *Pseudomonas aeruginosa*, weak effect against *Bacillus subtilis* and *Staphylococcus aureus*. Acetone extract showed weak antimicrobial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Bacillus pumalis*. Peteroleum ether extract exhibited weak antimicrobial activity against *Bacillus subtilis* and aqueous extract showed moderate effect against *Staphylococcus aureus*. Therefore, ethyl acetate extract showed more significant antimicrobial activity than other extracts on six test organisms.

In conclusion, the rhizome of *Costus speciosus* (Koen.) Sm. could be used for the treatment of urinary tract infection, respiratory system infection, gastrointestinal infection, asthma, fever, ear infection, eye infection, skin infection, worm infection, inflammation, sores and burn infection and illness which are caused by microorganisms.

Acknowledgements

I wish to express my deep gratitude to Dr Mi San Mar Lar, Professor and Head and Dr Thandar Soe, Professor, Department of Botany, Dawei University for giving their permission to present this paper.

References

AShin Nagathein. (1968). **Pon-pya –say A- bidan**.Vol II. Mingala Press. Yangon.Columbia University Press, New York.

Bull, S. (2010). **CadmiumToxicological Overview**. Health Protection Agency.

Cruickshank, R. J. P. (1975). **Medicinal Microbiology**. Living Stone Ltd., London.

Duraipandiyan, V., N. A. Al-harbi, S. Ignacimuthu and C. Muthukumar. (2012). **Antimicrobial activity of sesquiterpene lactones isolated from traditional medicinal plant, *Costus speciosus* (Koen.) Sm**. BMC Complementary and Alternative Medicine.

Gajalakshmi, S., V. Iswarya, R. Ashwini, G. Divya, S. Mythili and A. S. Sathiavelu (2012)**. Evaluation of heavy metals in medicinal plants growing in Vellore District.** Pelagia Research Library. European Journal of Experimental Biology, 2012, 2 (5):1457-1461

Grosvenor, M. B. and L. A. Smolin. (2002). **Nutrition from Science to Life**. Harcourt College Publishers. United State of America.

Horwitz, W. (1980). **Official Methods of Analysis of the Association of Official Analytical Chemists**. (13th ed.). PO BOX 540, Benjamin Franklin Station, Washington DC.

Hundley, H. G. and Chit Ko Ko. (1987). **List of Tree, Shrubs, Herbs and Principle Climbers etc**. Govt. Printing and Stationery. Rangoon.

Kress, W. J., R. A. Defilipps, Ellen Farr and Yin Yin Kyi. (2003). **A Checklist of the trees, Shrubs, Herbs and Climbers of Myanmar**, Department of Systematic Biology. Botany, National Museum of Natural History, Washington, DC.

Levinson, A. A. (1974). **Introduction to Exploration Geochemistry**, Applied Publishing Ltd. Calgary.

Malabadi, R. B., R. K. Chalannavar, N. T Meti, R. S. Gani, R. S. Gani, S. Vijayakumar, G. S. Mulgund, S. Masti, R. Chougale, B. Odhav, K. Sowmyashree, S. Supriya, B. R. Nityasree and M. S. Divakar. (2016). **Insulin Plant, *Costus speciosus***: Ethnobotany and Pharmacological Updates. International Journal of Current.

Meeks, L., P. Heit and R. Page. (2009). **Comprehens School Health Eduction**. Sixth Edition. Publish by McGraw-Hill, an imprint of The McGraw-Hill Companies, Inc., 1221 Avenue of the America, New York.

Rodriguez, P. A. (2005). **Monocotyledons and Gymnosperms of Puerto Rico and the Virgin Islands**, Volume 52:1-415, Department of Botany National Museum of Natural History, Washington, D.C.

San Hla. (1960). **Handbook of Natural food**s. The Nananaya Publishing House 180, Botataung Pagoda Road, Rangoon.

Sarles, W. B. (1956). **An introduction to the microorganisms**. Department of bacteriology, University of Wisconsin, New York.

Verma, N. and R. L. Khosa. (2012). **Development of standardization parameters of *Costus speciosus* rhizomes with special reference to its pharmacognostical and HPTLC studies**. India.

Williams, M. H. (1999). **Nutrition for Health, Fitness and Sport**. Fifth Edition. The McGraw - Hill Companies, Inc. Library of Congress Cataloging - in - Publication Data. United State of America.

World Health Organization. (2007). **WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues**. WHO Library Cataloguing-in-Publication Data. Printed in Spain.

World Health Organization. (2009). **Medicinal Plants in Papua New Guinea**. WHO Library Cataloguing in Publication Data.

Websites

http://extoxnet.orst.edu/faqs/foodcon/ cadmium.htm

SEPARATION OF FATTY ACID BY FUNCTIONAL SBA-15, AG/SBA- 15 AND CHARACTERIZATION OF AG- NANOPARTICLE

Thwe Thwe Oo[1], Bo Chen[2] , Zhifeng Zheng[2*]

Abstracts

Rubber (*Hevea brasiliensis* L.) seed oil was collected and tested at the International joint research center , Southwest Forestry University in Kummin from Yunnan Province in China ,in 2017-2018. These oil extract fatty acid methyl ester (FAME) samples were separated the derivatives of fatty acid by using two catalytic reaction. The samples catalyst of Santa Barbara Amorphous -15 (SBA -15) and silver Santa Barbara Amorphous -15(Ag /SBA- 15) were characterized by X-ray diffraction (XRD), Scanning electron micrograph (SEM), transmission electron microscopy (TEM), N_2 adsorption – desorption isotherm for the characterization of Ag nanoparticle. Ag nanoparticle was found pore size and pore volume 8.628nm and 0.989755 cm^3/g. Moreover this FAME are mixed with SBA-15, Ag/SBA -15 catalysis were produced fatty acid derivatives. These mixing of fatty acid methyl ester (FAME) were conducted for characterization and identification by checking with FTIR and GC/Mass. Ten fatty acids was converted from the fatty acid methyl ester.

Keywords: Sample seed oil, Calcination, Nanoparticle of silver

Introduction

Many scientists prepared SBA-15 functionalized with $(CH_3O)_3$ $Si(CH2)_3$ $N(CH3)_3Cl$ (TPTAC) and further synthesized metal nanoparticles by anion exchange between grafted SBA-15 and metal precursors inside the channels as well as upon reduction of precursors. The amount of metal loading as well as the morphology of metal in host SBA-15 can be rationally controlled through repeating ion-exchange/reduction cycles in the TPTAC-SBA-15 silica host. Bui Thi Thanh ,*etal* (2012).

Mesoporous silica materials such as SBA-15 are preferred to be an ideal host for the deposition of metal nanoparticles as they possess a high surface area and pore volume, greater hydrothermal stability, and a hexagonal structure with tunable pore diameter (5–30 nm) with minimum hindrance, thus allowing easy diffusion of the reactants.

It is accepted that the unique architecture of SBA-15 can contain nanoparticles within mesopores with improved dispersal leading to the enhancement of their catalytic efficiency. Liu Yue, *etal*.(2017).

SBA-15 is a silica-based mesoporous material with uniform hexagonal channels ranging from 3 to 30 nm with a narrow pore size distribution. It is also one of the attractive supports with respect to high hydrothermal stability and larger surface areas, of 600–1000 m^2 g-1. Kim Na Young, *etal* .(2015). Huan Ma *etal*. (2016).

[1] Lecturer,Department of Botany, Dawei University, Myanmar

[2,2*] Professor,National Joint Engineering Research Center for Highly-Efficient Utilization Technology of Forest Biomass Resources; Yunnan Provincial International Joint Research Center for Bioenergy; Yunnan Provincial Engineering Laboratory for Highly-Efficient Utilization of Biomass; Yunnan Provincial University Key Laboratory

Mesoporous silica SBA-15 having surface area (500-1500 m^2 g^{-1}), large pore size (50-100 Å) with narrow pore size distribution and thermal stability has been incorporated with sulfated zirconia on their surface to generate solid acid catalyst via direct synthesis or post synthesis route. It exhibited high activity for esterification reactions such as reaction of **fatty acid** with methanol for biodiesel. **Hermida** Lilis**, *etal*.(2010).**

Several patents have been granted for different types of adsorbents and techniques to separate fatty acids. Fatty acid methyl esters can be used as alternatives to fatty acids in the production of many oleochemicals (fatty alcohols, alkanol amides, a-sulfonated methyl esters, sucrose esters, and other fatty esters). Methyl esters are preferable to fatty acids as they yield higher purity finished products and require milder conditions during synthesis. Methyl esters are obtained by methanolysis of fats and oils in the presence of an alkaline catalyst, usually sodium methoxide, or splitting the fat by energy- and capital-intensive Colgate–Emery process followed by esterification of the resulting fatty acids with methanol, Udaya,*etal*.(2005).

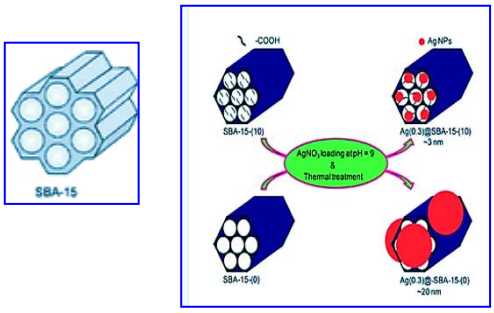
The catalyst of various amount ranged from 3 to 7 wt% was dispersed in methanol at temperature ranged from 50 to 70°C for a period of time prior to contact with the preheated feed-stock, providing a robust transesterification catalyst system. Jolius Gimbun ,(2012).

The use of these homogenous catalysts reduces the reaction temperature and controls product selectivity .However, they are associated with problems such as their sensitivity to free **fatty acids** and water present in the oil feedstocks and alcohol. The formation of soap resulting from reaction between free **fatty acids** and basic catalysts complicates the glycerol separation process thereby significantly affecting the yield of methyl esters. The employment of a heterogeneous catalytic system involving an environment friendly solid support can answer the problems arising due to the use of homogenous catalysts. The advantages of heterogeneous catalytic system include ease of regeneration, recycling of catalyst, ease in handling and scale-up. **Narayanan** Guru Krupa **,*etal*. (2012).**

Researcher obtained fine sized Ag particles whose size distribution was between 15 and 36 nm by polyol reduction. Silver powder of modified surfaces with controlled morphology can be prepared by several methods including the reduction of silver nitrate by reducing agents. An easy synthetic route for silver nanoparticles by hydrazine hydrate as reducing agent. The nanoparticles were characterized by various techniques. The silver particles' size show that the nanoparticles size is 92± 30 nm. Rahmani Behrad Mosavar ,*etal*.(2016). Ethanol in the solution phases reduced silver ions into silver nanoparticles. The linoleic acid caps the silver nanoparticles along with the reduction process thereby stabilizes the nanoparticles. Sliver nanoparticles having average diameter (size) of 12nm with the size range 7 – 15nm. The particles range in size from 8 to 50nm with mean diameter 24nm. Landage,*etal*.(2014).

The main aims and objectives of the study was to evaluate the performance of SBA-15 and Ag-SBA/15, to inform the mass production of catalysts for commercial sources to investigate the fatty acid derivatives from the FAME of selected rubber seed oil by using SBA-15and Ag/ SBA-15 catalysis reaction and to investigate the Ag- nanoparticle by the use of electronic spectroscopy.

The schematic illustration of the synthesis of Ag/ SBA 15catalysts showed by the Sadjadi .S and M.M.Heravi .(2017).



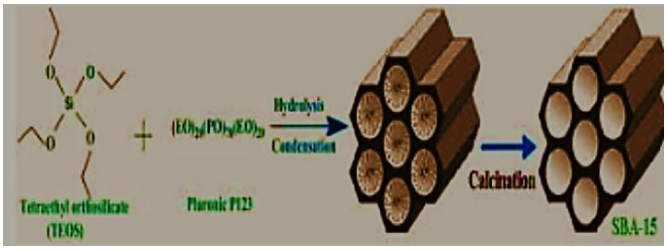
Materials and Methods

Rubber (*Hevea brasiliensis* L.) seed oil was collected at the International joint research center , Southwest Forestry University in Kummin from Yunnan province in China in 2017-2018.

Poly (ethylene glycol) ,propyleneglycol),(ethylene glycol),TEOS,$AgNO_3$, hydrazine hydrate,Potassium hydroxide, concentrated Sulphuric acid, Sodium carbonate (anhydrous), Hexane , Distilled water .Ethanol , Methanol ,Hydrochloric acid .

Preparation of SBA-15 and Ag/SBA -15Catalysts

According to the synthesis of mesoporous SBA- 15 figure showed by the literature survey Chaudhary Vasu And Sweta Sharma (2016).



The SBA catalysts were prepared as follows, 2 g of P123 was dissolved in 15 mL of distilled water at 40 °C, then 55 mL of 2 M hydrochloric acid was added slowly from the dripping funnel with vigorous agitation. The mixture was stirred for 3 h at 40 °C, then phosphotungstic acid (the loading amount of SBA was 10, 15, 20, 25, and 30%) was dissolved in water and added into the reaction. Subsequently TEOS was added slowly with vigorous stirring, resulting in a clear solution. The solution was transferred to a water-heated Teflon reactor and kept for 24 h at 100 °C, then filtered under vacuum and washed with deionized water and ethanol. After drying at 90 °C for 12 h, it was calcinated for 5 h at 550 °C in air, resulting inSBA ⁻15 catalysts. (Min Li ,(2008), **Narayanan** Guru Krupa ***,etal.,*** Bui Thi Thanh Ha,*etal*..(2012)Young Kim Na ,(2015) , Huan Ma , Rahmani Behrad Mosavar etal,(2016), Liu Yue,*etal* (2017).

In a typical experiment, the SBA-15 (0.5 g) was added to a solution of $AgNO_3$(0.04 g) in water (5 mL) and stirred for 24 h at 50 ˚C. The mixture was filtrated and washed three times with 5 mL water, and then dried in vacuum. Afterward, the dried sample was stirred with 5 mL of 1 M hydrazine hydrate at 60 ˚C for 3 min. The mixture was then filtrated, washed three times with 10 mL water, and dried at 100 ˚C at 5 mins an ovan. Finally, a white gray powder (Ag/SBA-15) was obtained. Similarly steps of Liu Yue *etal.* (2017).

Production of Fatty Acid Methyl ester

The two step of hydrolysis first step -1.5% of concentration of sulphuric acid, 40% of water and rubber seed oil l ,100g were placed to the three neck of round bottom flask at water bath 95 ˚ C for ten hours , then using separation funnel ,until separately two layers. the upper layer collected to do the next step.The upper layer, 1% of concentrated sulphuric acid and 40% of water were added again for eight hours using similar step. Fatty acid methyl ester were obtained, which components of fatty acid derivatives.

Characterization techniques

FTIR, SEM, TEM, and XRD investigation of the SBA -15 and Ag/SBA -15 samples were carried out. The mixing of catalytic FAME were subjected to FTIR (Nicolet IS 50 FTIR spectrophotometer in the range 500 - 4000 Spectrometry). The FTIR spectrum was recorded between 4500-500 cm^{-1} using the KBr pellet mode. The FAME sample was also analyzed using GC-MS (GC- MS analyzer of GC–2010 plus, SHIMADZU.GC-MS analysis were performed on a HP3050 gas chromatograph equipped).

The morphology of the SBA-15 and Ag /SBA-15 was characterized using field emission scanning electron microscopy (JSM 6701F, JEOL, Japan). transmission electron micrographs(TEM) were obtained using a field emission transmission electron microscope (JEM 2100F, JEOL, Japan) after dispersing the sample on a copper grid. The crystalline phases of the catalyst were identified using SBA-15and Ag /SBA -15 mesoporous silica was characterized by a combination of physical techniques. X-ray diffraction (XRD) patterns were recorded using a SIMENS, XRD 5005 powder diffractometer system with CuKα_ radiation (Kα =1.54056 ˚A) with 0.2 step size and 1 s step time over the range 0 < 2 < 10 Cu-Kα radiation to record the XRD pattern. N_2 adsorption – desorption isotherm 77K were obtained ASAP 2020 equipment from micromatritic.BET (Brunauer,Emmett and Teller) specific surface area of the SBA-15 and Ag /SBA -15 was recorded using BET technique (ASAP 2020, Micromeritics, USA).

Results and Discussion

Characterization of the catalyst: The mesoporous SBA-15 and Ag /SBA 15catalyst was synthesized by using a modified thermal process and the catalyst was characterized by electron microscopy. Figure (5) shows the scanning and transmission electron micrographs of the SBA-15 and Ag /SBA- 15 which reveals the presence of highly ordered hexagonal pores with tubular morphology.

Infra-red spectral data

The FTIR spectra of the investigated SBA-15 and Ag/SBA -15 sample and mixing of FAME samples analysis of the synthesized have showed in Fig (1-2). The IR spectrum data was recording by using Nicolet IS 50 FTIR spectrophotometer in the range 500 - 4500 cm^{-1} with the FTIR spectrophotometer. The determination of FTIR spectrum was obtained from the following data. The main peaks and their assignment to functional groups of SBA-15 and Ag/ SBA-15, the rubber seed oil is given in Table (1-2). The results showed characteristic strong absorption bands at 3431.79, -OH stretching in alcohol, 2970 ,2998 cm^{-1}, -CH stretching for CH_3 group ,1742 cm^{-1} for the ester carbonyl (C=O) functional groups, 1624.19 for -C=O stretching for keto-enol system and at 1446$cm^{-1}$for a double bond, respectively. The functional groups present in rubber seed oil is similar to the literature of Seal Soma, (2012) and Yousif Emad (2013).

Table 1 FTIR spectral Data the catalyst of SBA-15 and Ag /SBA-15 functional Group assignments

No	Frequency (cm^{-1})	Functional group
1	3431.79	- OH stretching in alcohol
2	2898.57	-CH stretching for CH_3 group
3	1624.19	-C=O stretching for keto-enol system
4	1417.12	-C=C stretching
5	1076.76	C-O stretching in alcohol
6	993.93	CH-bending (out of plane)
7	779.93	C -C bending (out of plane)

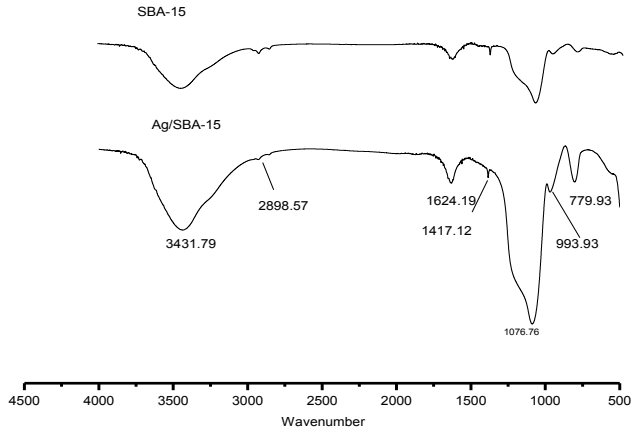


Figure 1 FTIR spectral Data of catalyst SBA-15 and Ag /SBA-15

Table 2 FTIR spectral Data the mixing of catalyst SBA-15 and Ag /SBA-15 fatty acid methyl ester functional Group assignments of Rubber seed oil.

No	Frequency (cm^{-1})	Functional group
1	3327.81	-OH stretching in alcohol
2	2898.57 - 2794.60	-CH stretching for CH_3 group
3	1728.27	- C = O stretching for acid system
4	1461.56	-C=C stretching
5	1121.29	C-O -C stretching (sym)
6	1017.21	C-O stretching in alcohol
7	718.73	C -C bending (out of plane)

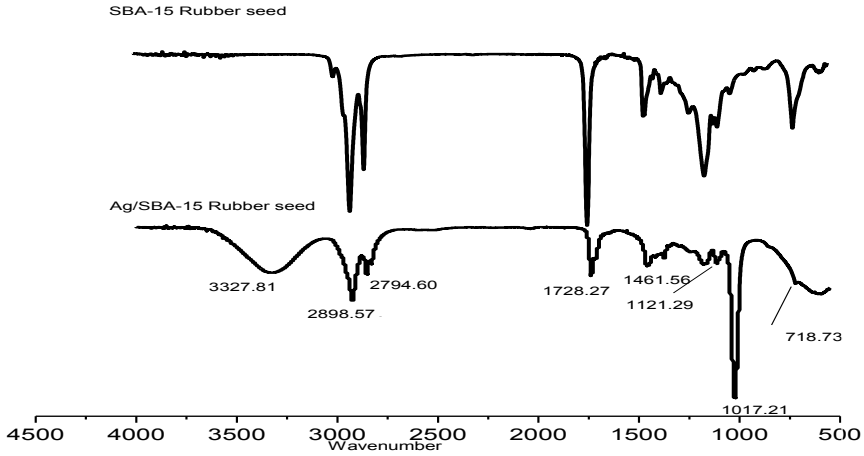


Figure 2 FTIR spectral Data of mixing FAME (Rubber seed oil) and catalyst SBA-15, Ag/SBA- 15

According to the GC/Mass data were shown in Table (3 to 4) Hexadecanoic acid, ethylester (16.48%),Linoleic acid ethyl ester (37.54%),(E)-9-Octadececanoic acid ethyl ester (35.45%) ,Octadecanoic acid ethyl ester (7.05%) by using catalyst of SBA-15. Hexadecanoic acid,ethyl ester (15.59%),Linoleic acid ethyl ester (36.11%) and (E)-9-Octadecenoic acid ,ethyl ester(34.94%),Octadecanoic acid ethyl ester were more yield percent than other fatty acids obtained by using Ag/SBA-15. FAME was showed separation effectively more activity by using two catalysts reaction.

Table 3 The derivatives of fatty acid from FAME (Rubber seed oil) by using catalystic of SBA -15.

No	Type of fatty acids	Retention time	Percentage %
1	Ethyl 9-hexadecenoate	37.618	1.39
2	Hexadecanoic acid, ethyl ester	37.964	16.48
3	Linoleic acid ethyl ester	39.223	0.31
4	9-Octadecenoic acid, methyl ester, (E)-	39.306	0.2
5	9,12-Octadecadienoic acid (Z,Z)-	39.74	0.49
6	Oleic Acid	39.826	0.95
7	Linoleic acid ethyl ester	40.073	37.54
8	(E)-9-Octadecenoic acid ethyl ester	40.155	35.45
9	Octadecanoic acid, ethyl ester	40.394	7.09
10	Cyclononasiloxane, octadecamethyl-	90.946	0.09

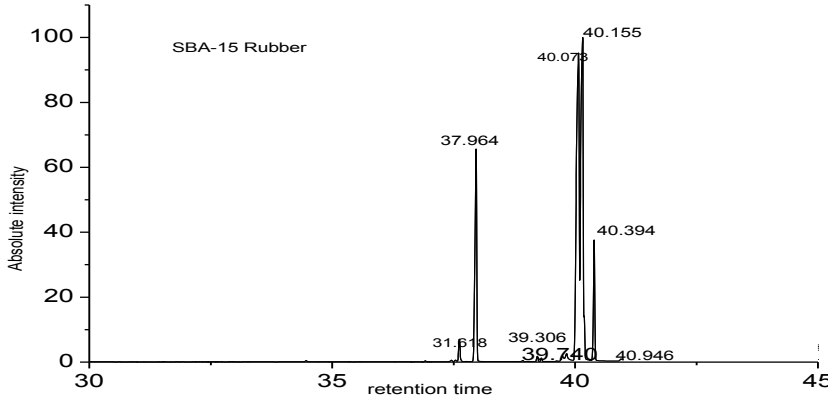


Figure 3 GC/Mass Data of mixing FAME (Rubber) and catalyst SBA-15

Table 4 The derivatives of fatty acid from FAME (Rubber seed oil) by using catalytic of Ag/SBA.

No	**Types of fatty acids**	**Retention time**	**Percentage(%)**
1	Ethyl 9-hexadecenoate	19.039	1.32
2	Hexadecanoic acid, ethyl ester	19.383	15.59
3	9,12-Octadecadienoic acid (Z,Z)-	21.514	0.75
4	Oleic Acid	21.629	0.93
5	Linoleic acid ethyl ester	21.989	36.11
6	(E)-9-Octadecenoic acid ethyl ester	22.109	34.94
No	Types of fatty acids	Retention time	Percentage(%)
7	Octadecanoic acid, ethyl ester	22.428	7.05
8	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	27.350	0.52
9	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	30.692	1.13
10	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	30.806	1.66

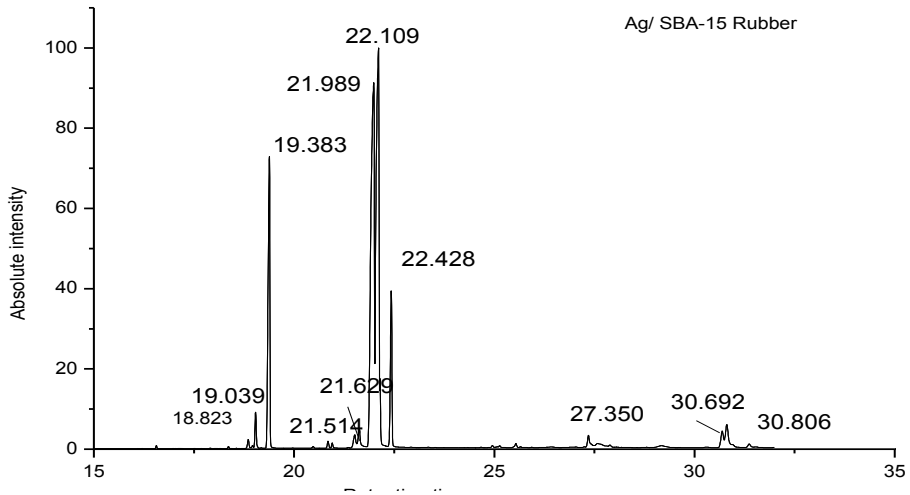


Figure 4 GC/Mass Data of mixing FAME (Rubber) and catalyst Ag/SBA-15.

The X- ray differentiation pattern of SBA- 15 and Ag/ SBA- 15 obtained from scheme of figure (5). the diffraction peak of Ag /SBA-15 was as like as the SBA-15. It is clear that the well-organized mesoporous structure of the support still remained after loaded with Ag. The defraction space d_{110} and d_{200} data were showed as table (5).

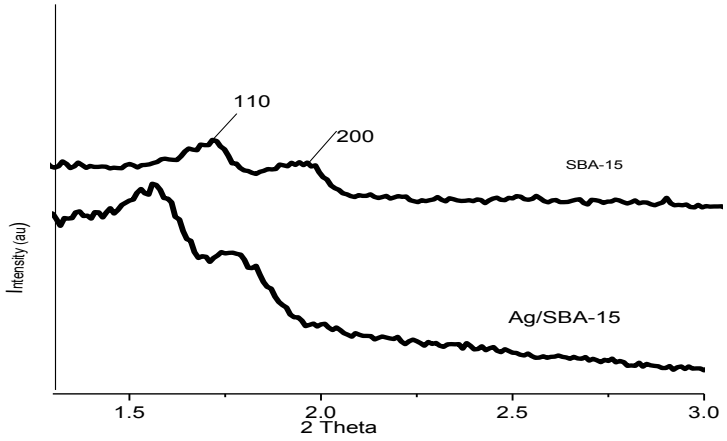


Figure 5 X- ray differentiation pattern of SBA- 15 and Ag/ SBA- 15

Table 5 XRD parameters of sample catalysts.

Sample	**d_{110}(nm)**	**d_{200} (nm)**	**Cell parameter (nm)**	
			d_{110}	d_{200}
SBA-15	5.13	4.53	7.25	9.06
Ag/SBA-15	5.56	4.93	7.91	9.86

The result from the SEM and TEM analysis data of SBA-15 and Ag / SBA -15 are showed in figure (5). The mesoporous structure of Ag/SBA-15were found at unifoundly distributed in the present inside or deposited on the outside of the channel of SBA-15.are agreed with those mentions by Rahamani (2016) and similarly research of Liu Yue (2017). Ag silver particles were found more than outside area because the pore size is a little large than SBA-15 pore channel.

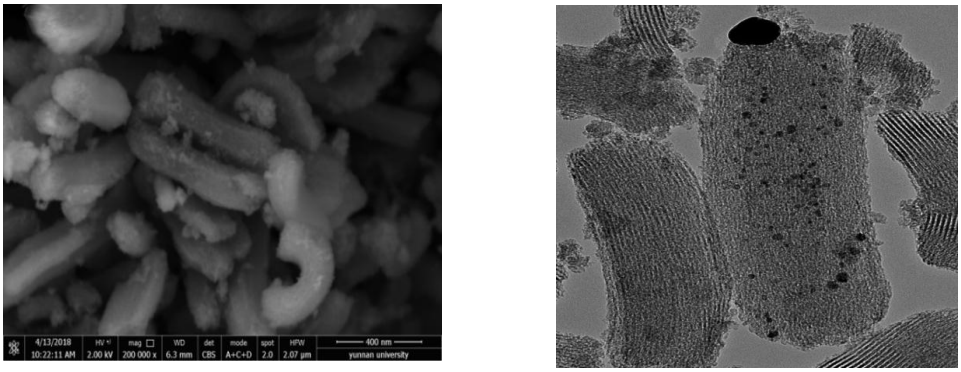


Figure 5 SEM image and TEM image of Ag/SBA-15

Table (6). BET-nitrogen absorption and desorption analysis about the surface area of SBA-15 (655 m^2/g) is larger than the surface area of the Ag /SBA -15, (461 m^2/g). The pore size of SBA-15 (6.5042 nm) and Ag/SBA-15 (8.6528 nm) and then the pore volume 0.923082 and 0.989774 m^2/g., Ag /SBA -15 surface area was small but pore size and pore volume were large. The average SBA-15 nanoparticle size 9.1468 nm and Ag -nanoparticle size 12.9871nm were obtained. The specific surface area, pore size, and pore volume decrease or increase with the dependent on the amount of silver content. Ag SBA/15 hexagonal shape mesoporous structure had shown to high separately total ester conversion rate and more adsorption and absorption rate.

Table 6 The result data of BET analysis of the SBA-15 and Ag/SBA-15

	SBA-15	**Ag/SBA-15**
Specific surface area,	655.9661m^2/g	461.9955m^2/g
Pore size	6.5042 nm	8.6528 nm
Pore volume	0.91468cm^3/g	0.989755cm^3/g
Porosity	90%	90%

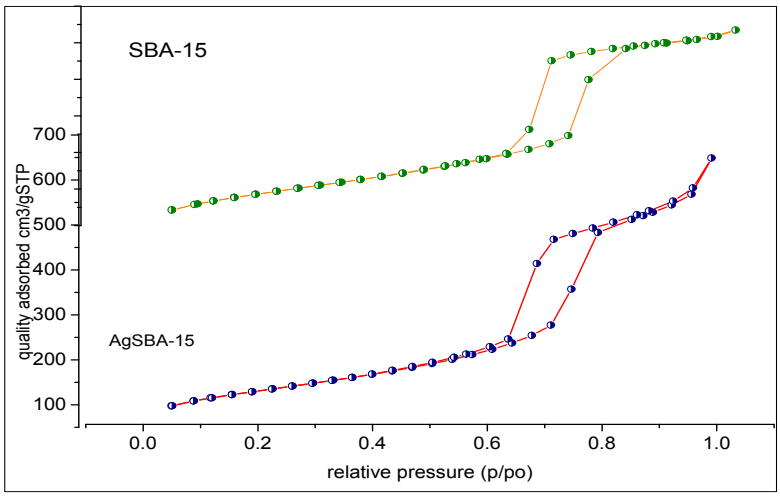


Figure 6 N_2 absorption and desorption isotherm of SBA-15 and Ag/SBA-15.

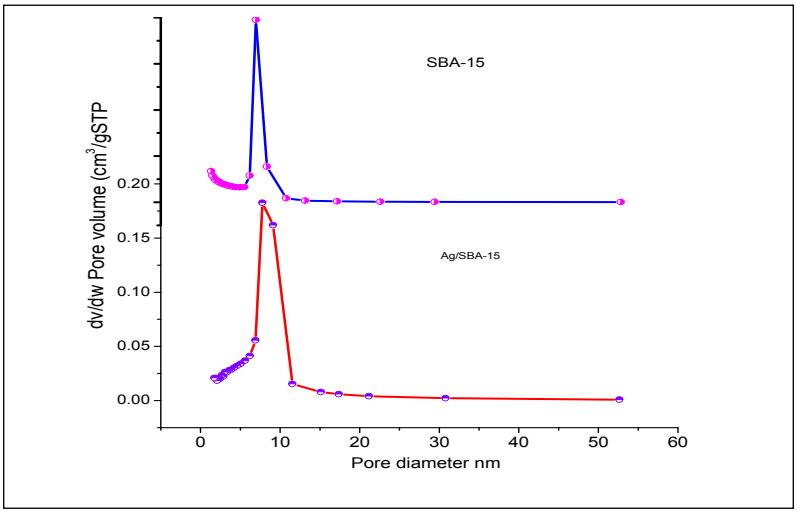


Figure 7 Barrett-Joyner- Halenda (BJH)Pore distribution curve of SBA-15 and Ag/SBA-15.

Conclusion

The derivatives of fatty acid were shown above data by using two catalytic reaction that it was obtained high yield percentage of fatty acids such as Oleic acid, linoleic acid etc. Ten fatty acids were successfully converted from the fatty acid methyl ester. And then silver nanoparticles confined outside of the channel of SBA-15 mesoporous channel like structure or deposits on the external surface of SBA-15. The meso porous structures were showed high surface areas, and tunable pore sizes of SBA-15 simplifies its used for sensor preparation and sensing application. Ag nanoparticles are one of the most attractive nanomaterials for commercialization uses. Silver nanoparticles are an important role of nanoscience and technology and nanomedicine. The large pore size supported the highest adsorptive quality and fastest kinetic activities.SBA-15 and Ag/SBA-15 were found to be an effective catalyst for the analysis of fatty acid from plants oil.

Acknowledgements

This research was completely supported and financed by the National Key Research & Development Program (No. 2016YFD0600802), National Natural Science Foundation of China (No. 31200452), and Special Fund of Renewable Energy Development of Yunnan Province (No. Yuncai Industry [2015]86).

References

Bui Thi Thanh Ha, *etal.*(2012).Silver Nanoparticles Con_ned in SBA-15 Mesoporous Silica and the Application as a Catalyst for Glucose Oxidation,Faculty of Chemistry, Hanoi University of Science, 334 Nguyen Trai Road, Thanh Xuan, Hanoi, Vietnam

Chaudhary Vasu And Sweta Sharma (2016) overview of ordered mesoporous material SBA 15:synthesis, functionalization and application in oxidation reactions

Hermida Lilis, *etal.* (2010).Mohaned Post Synthetically Functionalized SBA-15 with Organosulfonic Acid and Sulfated Zirconia for Esterification of Glycerol to Monoglyceride ,Journal of Applied Sciences, 10: 3199-3206.

Huan Ma, *etal* .(2016). Phosphotungstic Acid Based Mesoporous Silica Catalysts for the Preparation of Soybean Oil-Based Polyols , © Springer Science+Business Media New York 2017.

Jolius Gimbun, *etal*. (2012). Biodiesel Production from Rubber Seed Oil Using A Limestone Based Catalyst, *Advances in Materials Physics and Chemistry*, 2012, 2, 138-141 oi:10.4236/ampc.2012.24B036 (http://www.SciRP.org/journal/ampc), Malaysia

Kim Na Young, *etal*. (2015). Synthesis and characterization of Al-modified SBA-15 for Fischer–Tropsch synthesis (FTS) reaction, South Korea.

Landage S.M.*etal* .(2014). Synthesis of Nanosilver Using Chemical Reduction Methods., D.K.T.E. Society's, Textile & Engineering Institute, Ichalkaranji, India.

Liu Yue, *etal.* (2017) .The selective hydrogenation of rosin to hydroabietic content using Pd/SBA-15 as catalysts,China.

Min Li, *etal*. (2008). SBA-15-supported ionic liquid compounds containing silver salts: Novel mesoporous p-complexing sorbents for separating polyunsaturated fatty acid methyl esters, Department of Chemistry, Box 9573, Mississippi State University, Mississippi State, MS 39762, USA

Narayanan Gueu Krupa *etal.*(2012), SBA-15 as a Nanostructured Catalyst for Preparation of Biodiesel from Rice Bran Oil, Asian Journal of Scientific Research, 5: 196-206.DOI:

Rahmani Behrad Mosavar and Hamid Reza Ghorbani horbani,(2016). Fabrication of anosized Ag Colloids by Hydrazine Hydrate ,http://dx.doi.org/10.13005/ojc/320152, Iran

Sadjadi .S and M.M.Heravi .(2017) Current advances in the utility of functionalized SBA mesoporous silica for developing encapsulated nano catalysts; state of the art. Alzahra University. Iran.

Seal Soma, *etal*.(2012). Vapor of Volatile Oils from *Litsea cubeba* Seed Induces Apoptosis and Causes Cell Cycle Arrest in Lung Cancer Cells, Published onlineOctober 16. doi: 10.1371/journal.phon.0047014,PLoS One. 2012; 7(10): e47014.

Udaya N. Wanasundara, P. K. J. P. D. Wanasundara, and Fereidoon Shahidi.(2005).Novel SeparationTechniques for Isolation and Purification of Fatty Acids and Oil By-Products ,Bailey's Industrial Oil and Fat Products, Sixth Edition, Six Volume Set. Edited by Fereidoon Shahidi. Copyright # 2005 John Wiley & Sons, Inc. Canada.

Young Kim Na, *etal*. (2015).Synthesis and characterization of Al-modified SBA-15 for Fischer–Tropsch synthesis (FTS) reaction, Received: 10 July 2015 / Accepted: 22 October 2015 / Published online: 12 November 2015_ Springer Science+Business Media Dordrecht 2015.

Yousif Emad, Bashar Abdullah, Hanan Ibraheem, Jumat Salimon and Nadia salih, (2013). Rubber Seed Oil Properties, Authentication and Quality Assessment Using (Chloroform: Methanol) as Solvent, Journal of Al-Nahrain University Vol.16 (3), Malaysia.

MORPHOLOGICAL AND HISTOLOGICAL STUDIES OF *GNETUM GNEMON* L. (TANYIN-YWE) FROM YE-PHYU TOWNSHIP

Thi Khaing Htay[1] , Swe Swe Aye[2]

Abstract

The *Gnetum gnemon* L. is an indigenous plant from Thaninthayi Region. The specimens were collected from Ye-Phyu Township, during July 2018 to January 2019. Its aerial parts such as leaves, strobili and seeds are used by local people for food. The main purpose of this study was to examine the morphological and histology of the leaves, stems, roots and seeds of *Gnetum gnemon* L. The morphological features were described. The specimens were evergreen dioecious small trees, the leaves were simple, opposite, lanceolate to elliptic, and exstipulate. The male strobilus has 5-9 involucral collars, each collar contains 3-6 male flowers, stamen 1, adnate to the base of perianth, anther bifid. The female strobilus was 5-9 collars, each collars with 3-6 female flowers, without perianth, orthotropous ovule, pendulous placentation. The seeds were large and drupe. The distinguished histological characters of *Gnetum gnemon* L. revealed paracytic type of stomata in lower surface view of lamina only, pitted non lignified tracheary elements occurred in both surface view of lamina, cortex region of petiole, midrib and stem as well as in roots. Asterosclereids are commonly found in the whole plant parts except T.S of lamina. The vascular bundles in T.S of stem are collateral and close type. In addition, starch containing parenchymatous cells is found only in phelloderm region of mature roots. Moreover, the seed coat comprises sarcotesta, sclerotesta and inner endotesta regions. Underneath the seed coat is the endosperm containing starch grains and proteinaceous globules.

Keywords: strobilus, orthotropus ovule, asterosclereids, sarcosta,*Gnetum gnemon* L.

Introduction

Gnetum is the sole genus within the family Gnetaceae and the order Gnetales of the gymnosperm. This genus consists of about 40 species and widely distributed in South America, West Africa, tropical and subtropical Asia (Fell *et al*., 2015). According to Kress *et al*., 2003, there are 7 species of genus *Gnetum* were found in Kachin State, Taninthayi and Sagaing Regions in Myanmar. The selected sample, *Gnetum gnemon* L. belongs to the family Gnetaceae. It is known as Hyinbyin, Tanyin-ywe in Myanmar, "Melinjo" in Indonesia and Japan, Bago in Phillippines, Mejhergut in India. Local people from Ye-Phyu Township has been used the leaves and seeds of *G. gnemon* L. as vegetable crop. *G. gnemon* L. is a dioecious evergreen tree. The trunk is smooth and cylindrical, the branches are opposite and symmetrical branches. Leaves are simple and opposite, elliptic, lanceolate or oblong-oval. As a gymnosperm, *Gnetum gnemon L.* does not have true flower; the cones or strobili are presented at the tip of a slender stem or axis. It has yellow single-seeded fruits (Fell *et al*, 2015).

Gnetum gnemon L. widely cultivated in Southeast Asia. The fruit, seed, leaves and flowers of this plant can be consumed. This plant is the stable food in some places. *Gnetum gnemon* L. rich in resveratrol. The plant of *Gnetum gnemon* L. is used to reduce the level of sugar in the blood, anti-inflammatory activity an induce apoptosis in colon cancer (Hafidz *et al*., 2017) .The seed extract of *Gnetum gnemon* L. is sold as a nutritional supplement in Japan (Narayanan *et al*., 2015). It is reported that the leaves of *Gnetum gnemon* L. contain bioactive compounds such as saponins, stilbenoids, isovitexin and gnetins. However, the scientific investigations on *Gnetum gnemon* L. are still lacking in Myanmar. Therefore, the aim of the present study is to identify the morphological characters of *Gnetum gnemon* L. and to investigate the histological

[1] Assistant Lecturer, Department of Botany, East Yangon University
[2] Lecturer, Department of Botany, University of Yangon

studies of leaves, stems, roots and seeds of *Gnetum gnemon* L.from Ye-phyu Township, Taninthayi Region.

Materials and Methods

I. Morphological study of *Gnetum gnemon L.*

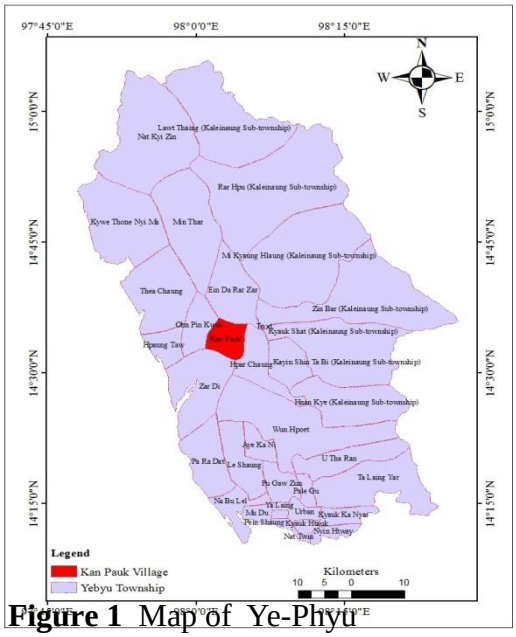


Figure 1 Map of Ye-Phyu

Collection, Classification and Identification

The plants use in this research were collected from Ye-Phyu Township (North Latitudes 14° 35′ 49.8″ and East Latitude 98° 02′ 14.4″), Taninthayi Region during July 2018 to January 2019. Fresh specimens were used for classification and identification with the help of standard literatures. Collected specimens were examined and described according to standard method used in Department of Botany, University of Yangon.

14° 35′ 49.8″ N
98° 02′ 14.4″ E

Characteristics of Gymnosperm (Flora of Hong Kong, Vol I., 2007)

Trees or shrubs, rarely woody vines: mostly evergreen: vascular bundles arranged in circles, with cambium, secondary xylem consists of tracheids, rarely with vessels. Leaves usually linear, needle-shaped or scale-like. Cones generally unisexual, mostly dioecious, globose, conical to cylindrical; ovules naked, not enclosed in an ovary. Seeds with endosperm, embryo straight: cotyledon 2 to many.

Key to the Families (Flora of Hong Kong, Vol I., 2007)

1a. Leaves large, partitioned, fascicled on top of stem.

2a. Pinnae with midrib……..……...…… Cycadaceae

2b. Pinnae without midrib, with many longitudinal veins……..…….…….. Zamiaceae

1b. Leaves small, not partitioned.

3a. Leaves fan-shaped, veins dichotomous……...……. Ginkgoaceae

3b. Leaves not fan-shaped, veins not dichotomous.

4a. Woody vines: leaves simple, opposite, veins reticulated: flowers with pseudoperianth……..……...…...……. Gnetaceae

4b. Trees.

Key based on male plant (Flora of China, Vol II., 1995)

1a. Shrubs or small trees; leaves drying yellowish; spikes often very lax visible between involucral collars……..…….……..…..*G. gnemon*

1b. Vines; leaves drying dark green to brown or black; spikes always very dense with axis concealed by involucral collars and flowers.

Key based on female plant (Flora of China, Vol II., 1995)

1a. Shrubs or small trees; leaves drying yellowish; seed coat usually velvety, rarely glabrous…….……..……...…….*G.gnemon*

1b. Vines; leaves drying dark green to brown or black; seed coat glabrous or with minute, silvery scales.

II. Histological study of *Gnetum gnemon* L.

Microscopical study of leaves, stems, roots and seeds were also examined by using free hand sections. The free hand sections were cleared in chloral hydrate solution on a glass slide, stained with safranin and temporary mounted by glycerin and then observed under a light microscope (Esau, 1964; Biswas and Johri, 1997 and Vasishta *et al*., 1939).

Determination of Stomatal index

The average number of stomata per square millimeter of epidermis is termed the stomatal number. The percentage proportion of the ultimate divisions of the epidermis of a leaf, which have been converted into stomata, is termed the stomatal index (Trease and Evans, 1978).

$$S.I = \frac{S}{E+S} \times 100$$

S.I = stomatal index
S = number of stomata per unit area
E = number of ordinary epidermal cells in the same unit area

Determination of palisade ratio

The average number of palisade cells beneath each epidermal cell is termed the palisade ratio. Pieces of leaf about 2 mm square or powder are cleared by boiling with chloral hydrate solution, mounted and examined with a 4 mm objective. First a number of each group of four epidermal cells is traced and their outlines inked in to make them more conspicuous. The palisade cells lying beneath each epidermal cell are focused and traced. The palisade cells in each group are counted, those being included in the count which are more than half-covered by the epidermal cells, the figure obtained divided by 4, gives the palisade ratio of the group (Trease and Evans, 1978).

Results

I. Morphological characters of *Gnetum gnemon* L.

Scientific Name - *Gnetum gnemon* L.
Local Name - Hyinbyin, Tanyin-ywe
English Name - Melinjo, belinjo, bago, maninjau, voe, khalel,peedae, phak,gam cay,bet, mejherguti, letera
Family - Gnetaceae
Flowering and Fruiting period - October to February

Evergreen dioecious small trees (2-30 m) in high; bark grayish (Fig.2 and Fig.9); branches opposite and decussate, green or yellowish green. Leaves simple, opposite, 10cm-22cm long, 3cm-7cm wide, petiole 0.8cm-1.5cm, lanceolate in young leaves and elliptic in mature leaves, 8.3cm-14.8cm long, 2.7cm-5cm wide, the tips acuminate, the margins entire, the bases acute, reticulate vernation, exstipulate (Fig. 4-5) and (Fig.10-11). Male spikes axillary or terminal, 4.5cm-7.3cm (Fig.6), involucral collars clearly separated, one male spike contain 5-9 involucral collars, to 1cm apart, each collar contains 3-6 male flowers and a single ring of 3-9 globose sterile female flowers (Fig.8), male flowers with a claw-shaped, 0.2cm-0.3cm, perianth narrowly clavate, 0.1cm, entire, stamen one, stamen adnate to the base of the perianth, 0.1cm, synangia (anther) bifid (Fig.7). Female spikes axillary or terminal, 4.5cm-9.0cm (Fig. 10), one female spike contains 5-9 collars, to 1 cm apart, each collars with 3-9 female flowers (Fig. 13), female flower are globose (Fig.14), without perianth, each containing of a single orthotropous ovule, with three integuments, the inner one produced into a fimbriate mouth, placentation pendulous (Fig.15-16). Seed large, drupe-like, ellipsoid, 1.5cm-1.8cm, longitudinally ribbed (Fig.20), green in young (Fig.17) and yellowish-red when mature (Fig. 1), consisting of the fleshy coats, enclosing the hard seed. External seed coat green, thick, internal seed coat brown, thin, ovule large and white.

Figure 2 A male plant in nature habit

Figure 3 Male strobilus with nature plant

Figure 4 Upper surface of

Figure 5 Lower surface of leaves

Figure 6 Male strobilus

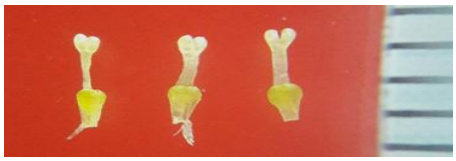


Figure 7 Male flowers

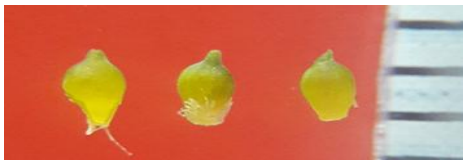


Figure 8 Globose abortive ovules

Figure 9 A female plant in nature habit

Figure 10 Female strobilus with nature plant

Figure 11 Upper surface of leaves

Figure 12 Lower surface of leaves

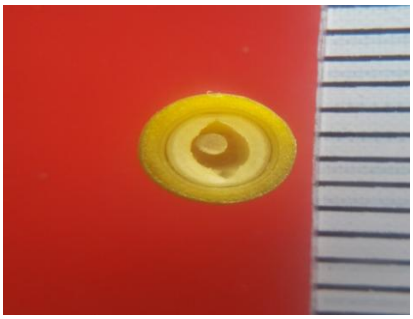


Figure 15 T.S of ovary

Figure 16 L.S of ovary

Figure 17 Cluster of seeds

Figure 18 Cluster of ripen seeds

Figure 19 Ripen seeds

Figure 20 Dry seeds

II. Histological characters of *Gnetum gnemon* L.

Lamina

In surface view of lamina, paracytic type of stomata are present only in lower surface (Fig. 22). The subsidiary cells in lower surface views are waiver than the upper surfaces. The stomatal index is 15.0-18.5 and palisade ratio is 3.0-10.5. The vascular bundles in T.S of lamina is collateral and closed type (Fig.23). In T.S of lamina, the upper and lower epidermal cells are covered by thick cuticle, a single layer of palisade mesophyll cells lie beneach the upper epidermal cells, 6-8 layers of spongy mesophyll cells lie internal to the palisade layer, the cells are thin wall and loosely arranged with intercellular spaces. Pitted non lignified tracheary elements are scattered in mesophyll layers.

Midribs

In Tranverse section, the midrib is slightly convex in adaxial region and concave in abaxial region, a single layer of epidermis is covered by cuticle.The cortex is composed of 10-13 layers of parechymatous cells in adaxial side and the cells are more or less rounded, where as 16-19 layers in abaxial side (Fig. 24). There are 4-5 bundles lie in the center of midrib and the arrangement of bundle is collateral and close type (Fig.25).

Petioles

The petiole of *Gnetum gnemon* L. is slightly flattened with two wings at the upper region and semicircular-shaped in lower region a single layer of epidermis is covered by cuticle,the cortex is made up of 15-18 layers of parenchymatous cells in adaxial region and 15-20 layers in abaxial region. The arrangement of vascular bundles are cresent- shaped, xylem lying inward and

the phloem lying outward. Astrosclereids are found in hypodermis regions of midribs as well as petioles (Figure 26).

Stems

In transverse section, the stem, is cylindrical-shaped, the cortex region is composed of outer collenchymatous, middle parenchymatous and inner sclerenchymatous region. The pitted non lignified tracheary elements scattered in cortex region of stem. The vascular bundles are collateral and closed type. The xylem is composed of tracheids and vessels whereas phloem is made up of sieve tube and phloem parenchyma cells (Fig.27).

Roots

In transverse region of root, the periderm region is composed of phellogen or cork cambium, phellem or cork cells and phelloderm. The circular pitted non lignified tracheary elements are scattered in phelloderm region of root. The endodermis is not distinct. Vascular bundles occur as protostele type and concentric type (Fig. 28).

Seeds

In T.S of seeds, the seed coat is made up of outermost sarcotesta, middle sclerotesta and inner endotesta. The sarcotesta has cutinized epidermis and group of asterosclereids region (Fig. 29). The sclerotesta forms the protective layer of the seed. It is made up of upper sclerenchymatous region and inner membranous region (Fig.30). The endotesta is composed of more or less rounded parenchymatous cell and small vascular bundles are also occur in this region. The endosperm comprises parenchymatous cells filled with starch grains and proteinaceous granules (Fig.31-32).

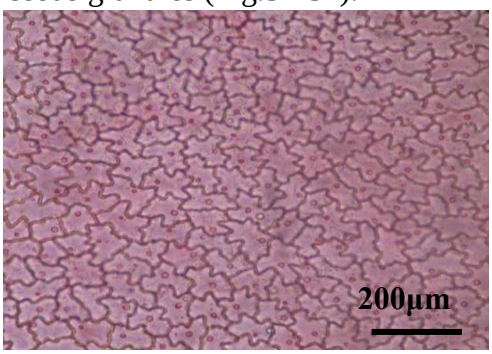


Figure 21 Upper surface view of lamina

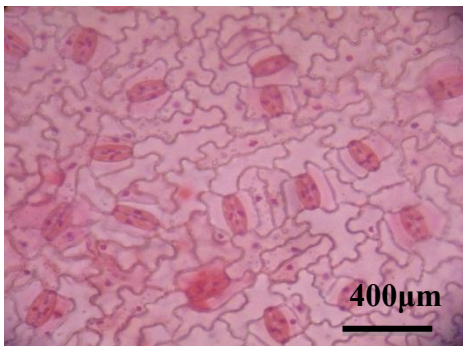


Figure 22 Lower surface view of lamina

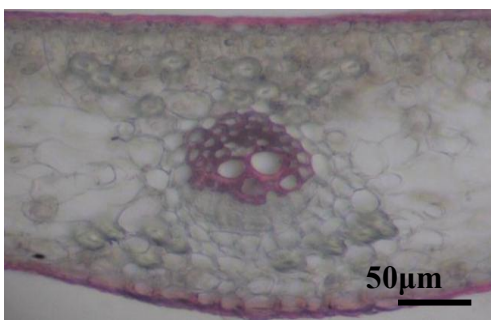


Figure 23 Close up view of lamina showing vascular bundle

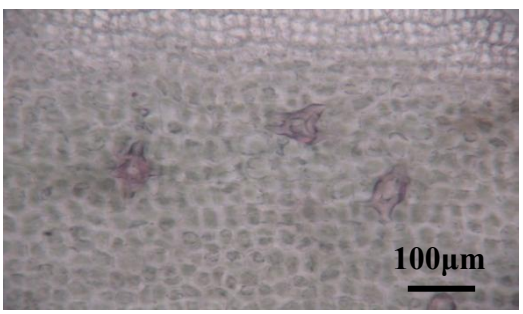


Figure 24 Asterosclereids are found in surface view of midrib

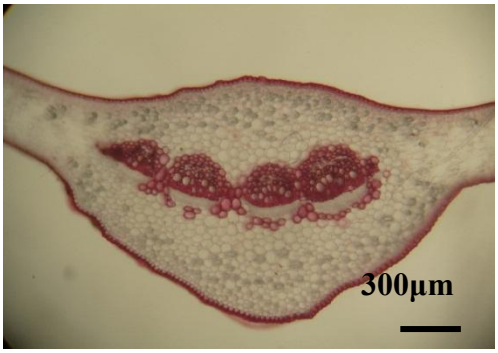


Figure 25 T.S of midrib

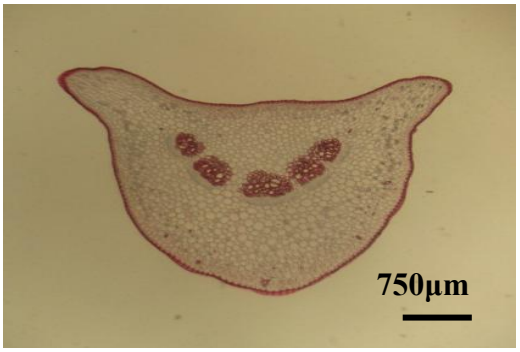


Figure 26 T.S of petiole

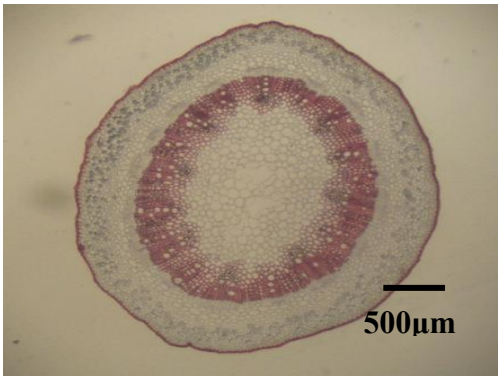


Figure 27 T.S of stem outline

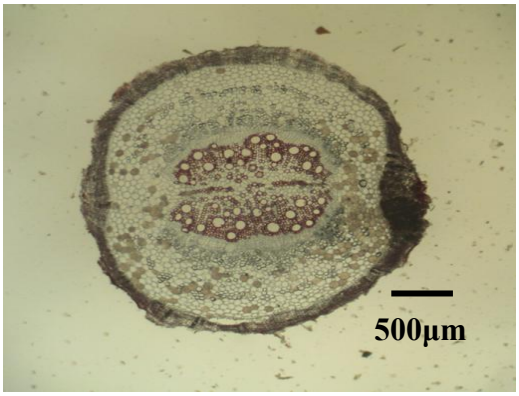


Figure 28 T.S of root outline

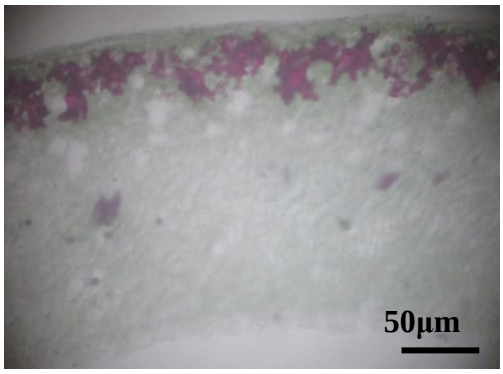


Figure 29 T.S of seed showing Asterosclereids

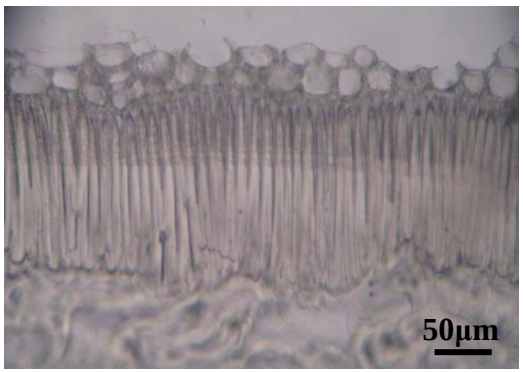


Figure 30 T.S of seed coat showing middle sclerotesta

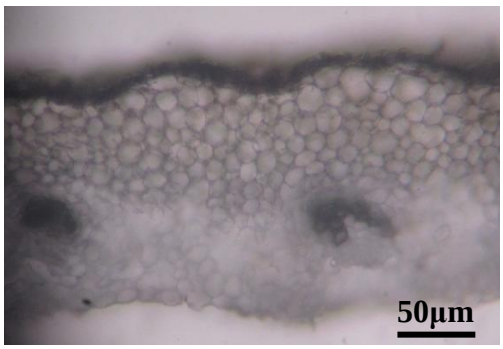


Figure 31 T.S of seeds showing endotesta

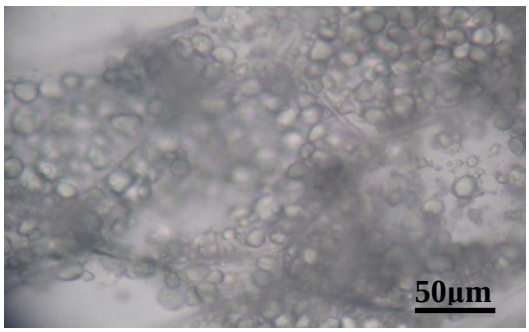


Figure 32 Starch grains and proteinaceous globules found endosperm region

Discussion and Conclusion

In the present research, the morphological characters on the vegetative parts, reproductive parts and histological characters of leaves, stems, roots and seeds have been undertaken. Thus, the specimen used in this research was identified as *Gnetum gnemon* L. and it belongs to the family Gnetaceae.

Gnetum gnemon L., *G. latifolium* Blue, *G. macrostachyum* Hook.f., *G. montanum* Markgraf., *G. scansens* were listed in "A checklist of the trees, shrubs, herbs and climbers of Myanmar" by Kress *et al*. (2003).

Moreover, Kress *et al.* (2003) reported that the species *Gnetum gnemon* L. was found Thaninthyi Region only. In this research, the species, *Gnetum gnemon* L. was found as a wild plant in Ye-phyu Township, Launglon Township and Dawei Township of Thaninthyi Region.

Barua (2015) described that the young leaves, tender tips and inflorescences of *Gnetum gnemon* L. were harvested from the wild for consumption, mostly by Karbi tribe in Indonesia. In this study, local people from selected area said that the young shoot of this plant could be used as vegetable crop.

In morphological study, plant *Gnetum gnemon* L. are dioecious evergreen trees, the leaves are simple, opposite, reticulate vernation, exstipulate, 5-9 rings of male flowers develop basipetally above each collar, 6-9 rings of female flowers and the seeds are drupe. These characters are agreement with Larsen (1975) and Zhu *et al*. (1995). The spikes are axillary or terminal. These characters are agreed with Hooker (1885) and Kirtikar and Basu (1973). Male spikes contain male flowers and a single ring sterile female flowers, male flowers are claw-shaped, stamen one. These characters are in agreements with Larsen (1975).

The lamina of Gnetum has a well-marked cuticle on epidermal cells and short palisade cells and a well-developed spongy tissue. Stellately branched sclereids occur near the lower epidermis These characters are agreement with those given by Biswas and Johri (1997).

In transverse section, the stems are circular outline, cuticle is thick and the outer walls of the epidermal cells are also very thick. The cortex layer which is differentiated into an outer chlorenchymatous, a middle parenchymatous and an inner sclerenchymatous region. Outer chlorenchymatous cells contain chloroplast and intercellular spaces. Middle parenchymatous cells in cortex region that consists of few layers of thin-walled cells. The endodermis and pericycle layers are not distinct.

In this study, the vascular bundles of the seed coat are collateral and closed type, asterosclereids are foud stems and roots as well as in seed coat. Rodin (1966) reported that the asterosclereids, which is very common in petiole, midrib and larger veins of *Gnetum gnemon* L.

In present study, pitted non-lignified tracheary elements occur in lamina,cortex region of petiole, midrib, stems and roots of *Gnetum gnemon* L.. Carlguist and Goose (1995) suggested that circular bordered pits of tracheary element of *Gnetum* represents a clearly gymnosperm feature rather than on transitional to angiosperms.

In this research, the histology of seed coat comprises the parenchymatous sarcotesta region, sclerenchymatous sclerotesta and starch containing endotesta region. These characters are in aggrement with those stated by Biswas and Johri (1997) and Vasishta *et al.* (1939). In

conclusion, this study has highlighted detailed descriptive information of *Gnetum gnemon* L. The presence of asterosclereids and pitted non lignified tracheary elements distribution in the whole plant could be useful in providing taxonomic information and could also serve as a database for future references of the species. In conclusion, this study has highlighted detailed descriptive information of *Gnetum gnemon* L.

Acknowledgements

The authors acknowledge to Dr Aye Pe, Professor and Head, Department of Botany, University of Yangon, for providing the departmental facilities.

References

Barua, C.C., P, Haloi and I. C. Barua. (2015). ***Gnetum gnemon* Linn. A compresensive review on its biological, pharmacological journal of pharmacognosy and phytochemical.** Research 2015; 7(3); 531-539

Biswas, C & B. M. Johri, (1997). **The Gymnosperms.** Chhaya Princinal Gargi Collage, University of Delhi South Cempus, New Deihi, India.

Carlguist & Goose (1995). **Wood, Bark, and Stem Anatomy of Gnetales**: A Summary. Plant Sci.157 (6 Suppl.): S58-S76

Esau, K., (1964). **Plant Anatomy**. John AWielay and Son, New York, London. Family. Species profiles for Pacific Island Agroforestry. April 2006 ver. I. I.

Fell, D.G., D.J. Stanton, D. Williams, F. Loban, F. Nona, T. Stow, J. Wigness, E. Manas, and G. Uiduldam, (2015). **First record of the Gnetales in Australia *Gnetum gnemon* L. (Gnetaceae) on Badu and Mua island, Torres strait, Queensland.**

Hafidz, K. A., N. Puspitasari, A. Azminah, Y. Artha and A. Mnium, (2017). **HMG-CoA Reductase Inhibitory Activity of *Gnetum gnemon* Seed Extract and Identification of Potential Inhibitors for Lowering Cholesterol Level**, J young pharm, 9(4): 559-565

Hooker, J. D., (1885). **The Flora of British India**. (Vol. V). Reeve and Company Ltd., London.

Kirtikar, K. R. & B. D. Basu, (1973). **Indian Medicinal Plants**. (Vol.III), (2nd edition). Lalit Mohan Basu. No. 49, Leader Road, Allahabad, India.

Kress, W.J., Robert, A. D. E. farr and Daw Yin Yin Kyi, (2003). A **Chicklist of the Tree, Shrubs, Herbs and Climbers of Myanmar**. Washington, D.C. Department of Systematic Biology- Botany, National Museum of Natural History

Larsen, K., (1975). **Flora of Thailand**. Vol II. Applied scientific research corporation of Thailand Bangkok. Page 204-210

Narayanan, N. K, K. Kunimasa, Y. Yamori, M. Mori, H. Mori, K. Nakamura, G. Miller, U. Manne, K. Amit, Tiwari and B. Narayanan, (2015). **Antitumor Activity of Melinjo (*Gnetum gnemon* L.) Seed Extract in Human and Murine Tumor Models in Vitro and in a Colon-26 tumor-bearing Mouse Model in vivo**. Cancer Medicine, 4(11): 1767-1780

Qi-Ming, HU., & WU. De-Lin, (2007). **Flora of Hong Kong.** (Vol. 1), p.1, 14-15. Hong Kong Herbarium and South China Botanical Garden. Agriculture, Fisheries and Conservation Department, Government of the Hong Kong Special Administrative Region, Hong Kong.

Rodin. R., (1996). **Leaf Structure and Evolution in American species of Gnetum. Phytomorology** 16: 56-68.

Trease & Evans., (1978). **Pharmacognosy**. 15th Ed. Edinburgh London, New York.

Vasishta, P.C, Sinha, A.K. and A. Kumar, (1939). **Botany for degree students Gymnosperms**.

Zhu. G-Ling., R. Harald and K. Rudolf, (1995). **Flora of China**. Vol 4. Page 105

TAXONOMIC STUDIES OF WILD MUSHROOMS DISTRIBUTED IN BANMAUK AND INNTAW TOWNSHIPS

Chaw Thiri Mon[1], Soe Myint Aye[2]

Abstract

The taxonomic studies of wild mushrooms distributed in Banmauk and Inntaw Townships were undertaken. Those mushrooms were collected from June to October during the year 2018 and identified in Department of Botany, Monywa University. Fifteen species belonging to 10 genera of 9 families were resulted. The identified species were under the genera *Amanita, Termitomyces, Tricholoma, Auricularia, Scleroderma, Cantharellus, Phallus, Lentinus, Lactarius* and *Russula.* The volva were present in *Amanita fulva* Fr. and *Phallus indusiatus* Vent. while these were not possessed in remaining species. The stipe was spongy in *Phallus indusiatus* Vent. Among the 15 species, only the members of the genus *Lactarius* have exude of milky latex. According to the literature nine species were edible and six species were inedible. These finding will be one of the valuable information for the future researchers and compilation on mushroom flora of Myanmar.

Keywords: Mushrooms Taxonomy, Banmauk and Inntaw, Myanmar

Introduction

Mushroom is the term applied to the fleshy fruiting bodies of fungi. Under natural conditions, the mycelium lies buried in the soil or in the substratum. When conditions are favourable, it forms the reproductive structure, the fruiting bodies which are fleshy and this structure is generally called the mushroom (Nair 1990). Lacking chlorophyll, mushrooms must obtain their food by absorption from the surrounding medium (usually soil or decaying wood) in which they grow (Krieger & Schaffer 1967).

Weather conditions, especially warmth and moisture, have their usual influence on the production of fruit-bodies, and more than one crop can occur in a year, or, on the other hand, no growth may appear above ground (Ramsbottom 1923).

Equally important is the role of mushrooms and related fungi in nutrient recycling, whereby they make food available for many organisms. Some mushrooms are a primary food source for animals of diverse kinds and others are a preferred food of many animals, including man (McKnight & McKnight 1987). Most fungi will grow between 0º and 35ºC, but optimum temperatures lies in the range of 20-30ºC. The ability of many fungi withstand extremely low temperatures (as low as -195ºC.) (Alexopoulos 1962).

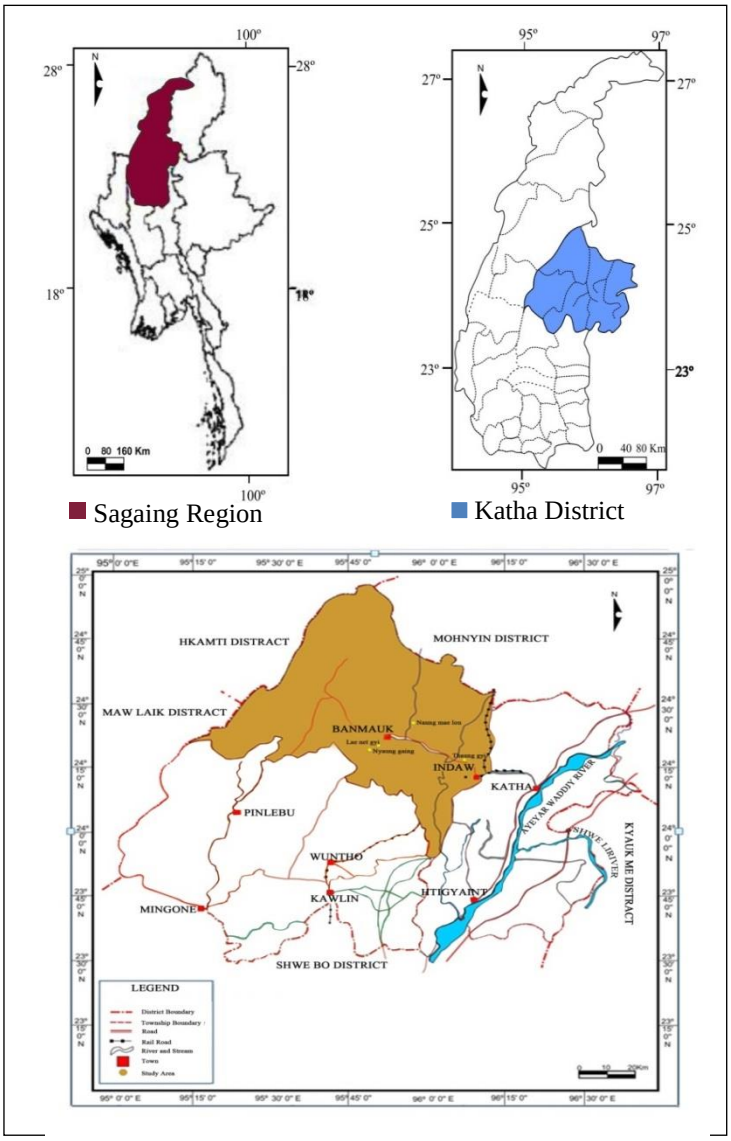
Mushrooms generally grow in the rainy season and prefer to high humidity and moisture level. They usually grow on the ground, organic matter, dead woods, living trees and grass land. The study area is located in Katha District, Sagaing Division. The climatic condition of the study area is the subtropical zone and it has plenty of vegetation. But because of the natural disaster and human impact, the area becoming deforestation and climate change is also affecting on the type of forest. The resources of mushrooms are important information for the study area in the future. Therefore it is needed to be explored and recorded on the wild mushrooms in various area of Myanmar.

The aim and objectives of the present work are to identify and classify the naturally growing mushrooms, to characterize the mushrooms systematically and to provide the systematic information to others researchers.

[1] Lecturer, Department of Botany, Monywa University
[2] Dr, Pro-Rector, University of Myitkyina

Materials and Methods

The wild mushrooms were collected from the month of June to October during in the year of 2018. The collection and the spore print techniques were followed by Krieger & Schaffer (1967) and Kuo & Methven (2014). To make a spore print, cut the stipe off the mushroom and place the cap with gills or pores downward on a piece of paper or glass slide. And then be covered with a glass petridish to keep air currents from blowing the spores. The spores were measured under the microscope by using stage, ocular micrometer and the objective lens 40 x. The collected specimens were preserved in Formalin-Acetic acid-Alcohol (FAA) by the ratio of 5: 5: 90. The classification and identification of collected specimens were done by referring the literature such as Thomas (1948), Krieger & Schaffer (1967), McKnight & McKnight (1987), Nair (1990), Roger (2006), Davis *et al*. (2012) and Kuo & Methven (2014).



Source: Land Records Department, Katha District, Sagaing Region

Figure 1 Location map of study area in Katha District

Results

According to the morphological and spores characters, 15 species of 10 genera belonging to 9 families and 7 orders were classified and identified. The resulting species were stated in Table 1 and the comparable morphological characteristics were shown in Table 2.

Table 1 List of the collected species

Order	Family	No.	Scientific name
Agaricales	Amanitaceae	1.	*Amanita fulva* Fr.
	Lyophallaceae	2.	*Termitomyces albuminosus* (Berk.) R. Heim.
	Tricholomataceae	3.	*Tricholoma irinum* (Fr.) P. Kumm.
Auriculariales	Auriculariaceae	4.	*Auricularia auricular*-Juade (Bull.) Quel.
Boletales	Sclerodermataceae	5.	*Scleroderma aerolatum* Ehrenb.
		6.	*S. verrucosum* (Bull.) Pers.
Cantharellales	Cantharellaceae	7.	*Cantharellus cibarius* Fr.
		8.	*C. infundibuliformis* (Pers.) P. Kumm.
Phallales	Phallaceae	9.	*Phallus indusiatus* Vent.
Polyporales	Polyporaceae	10.	*Lentinus arcularius* (Batsch) Fr.
Russulales	Russulaceae	11.	*Lactarius camphoratus* (Bull.) Fr.
		12.	*L. piperatus* (L.) Pers.
		13.	*L. rubidus* (Hesler & A. H. Sm.) Methven
		14.	*Russula foetens* Pers.
		15.	*R. roseipes* Scer. ex Bres.

An Artificial Key to the Studied Species

1. Volva present……..…….…… 2
1. Volva absent……..……. 3
   2. Cap conical-shaped; stipe with spongy; spores oblong……. .....9. *Phallus indusiatus*
   2. Cap convex; stipe without spongy; spores globose..1. *Amanita fulva*
3. Umbo present…….…..……*2. Termitomyces albuminosus*
3. Umbo absent…….……...……..…… 4
   4. Stipe absent……...…….4. *Auricularia auricular*-Juade.
   4. Stipe present……..…….. 5
5. Latex present…… 6
5. Latex absent…….…… 8
   6. Gills decurrent; spores white, smooth……12. *Lactarius piperatus*
   6. Gills adnate; spores pale cream or pale yellow, echinulate…… 7
7. Cap red brown; gills yellowish brown; stipe reddish brown…….. ……..…….11. *Lactarius camphoratus*
7. Cap yellow; gills pale yellow; stipe yellow…….13. *Lactarius rubidus*

8. Stipe hollow……..7. *Cantharellus cibarius*
8. Stipe solid……..…… 9

9. Cap globosed……..…… 10
9. Cap depressed or flattened…….. 11

10.Fruiting body yellow patches; spores brown.....
.....5. *Scleroderma aerolatum*
10.Fruiting body brown; spores dark brown..6. *Scleroderma verrucosum*

11. Stipe tapered at the base…… 12

11. Stipe equal……..…….. 13

12.Cap flattened; gills sinuate; spores smooth, elliptic.....
….....3. *Tricholoma irinum*

12.Cap depressed; gills adnate; spores echinulate, globose.....
.....…15. *Russula roseipes*

13. Spores echinulate……...14. *Russula foetens*

13. Spores smooth…… 14

14. Gills present; stipe pale yellow; spores globose……
……..8.*Cantharanthus infundibuliformis*

14. Gills absent; stipe brown; spores oblong……10. *Lentinus arcularius*

1. *Amanita fulva* Fr., Ovserv. mycol. 1:2 (1815) (Figure 2, 3. A)

Cap 3.0-5.0 cm broad, thin, oval at first, then convex, sticky when moist, smooth, margin prominently streaked, yellowish brown. Gills free, white. Stipe 5.5-6.5 cm long, 0.5-0.8 cm thick, equal, hollow, pale cream. Ring absent. Volva present, thin, white. Spores white, smooth, globose, 10.0-12.5 μm.

Habitat : Growing on soil, solitary.

Specimen examined: Inntaw Township, Thaung gyi village; N. 24°21' 06.37", E. 96° 01' 36.36"; 14 August, 2018; Chaw Thiri Mon, collection no. 117.

2. *Termitomyces albuminosus* (Berk.) R. Heim, Mem. Acad., Sci., Paris 44: 72 (1941) (Figure 2, 3. B)

Agaricus albuminosus Berk. 1847.

Cap 6.0-7.5 cm broad, thick, plano-umbonate at first, becoming flattened, margin split when mature, pale brown. Gills free, white and then pale pink as spore mature. Stipe 6.5-14.0 cm long, 1.7-2.0 cm thick, central, equal to tapering at the base, solid, fibrous, pale gray. Ring absent. Spores pink, elliptic, smooth, 7.5-12.5 x 5.0-7.5 μm.

Habitat : Growing on sandy soil and solitary

Specimen examined: Banmauk Township; Nyaung gaing, N. 24°26' 50.20", E. 95° 52' 21.56"; 22 June, 2018; Chaw Thiri Mon, collection no. 49.

3. *Tricholoma irinum* (Fr.) P. Kumn., Fuhr. Pilzk. (Zerbst): 132 1871. (Figure 2, 3. C)

Agaricus irinus Fr. 1838.

Cap 13.5-18.5 cm broad, thick, flattened, smooth, margin incurved, pale brown. Gills sinuate, pale cream. Stipe 20.0-22.0 cm long, 2.0-2.5 cm thick, central, cylindrical, solid, smooth,

tapered at the base, pale cream. Ring absent. Spores pale cream, elliptic, smooth, 7.5-12.5 x 5.0 μm.

Habitat : Growing on soil and solitary

Specimen examined: Banmauk Township, Lae net gyi village; N. 24°23' 18.28", E. 95° 49' 40.89"; 22 June, 2018; Chaw Thiri Mon, collection no. 53.

4. *Auricularia auricula*-judae *(*Bull.) Quel., fung. 207 (1886) (Figure 2, 3. D)

Tremella auricula-judae Bull. 1789

Cap 2.5-6.0 cm broad, thin, usually hanging downward from a point of attachment, cup or ear shaped, then expanded, texture gelatinous when moist, hard when dry, reddish brown. Gills absent. Stipe absent. Ring absent. Spores white, smooth, elliptic, 15.0-17.5 x 10.0-12.5 μm.

Habitat : Growing on decay wood and living tree, clustered

Specimen examined: Banmauk Township, Naung mae lon hill; N. 24°27' 55.50", E. 95° 52' 23.45"; 23 June, 2018; Chaw Thiri Mon, collection no. 60.

5. *Scleroderma areolatum* Ehrenb., Sylv. mycol. Berol. (Berlin): 27 (1818) (Figure 2, 3. E)

Fruiting body 2.0-3.5 cm across, subglobose, covered with yellow patches, thin wall, brown. Gleba pale brown. Stipe 2.0-3.0 cm long, 0.5-1.2 cm thick, central, equal, solid, yellowish brown. Ring absent. Spores brown, globose, echinulate, 10.0-17.5 μm.

Habitat : Growing on soil and solitary

Specimen examined: Bamauk Township, Naung mae lon village; N. 24°26' 00.42", E. 95° 52' 23.38"; 24 June, 2018; Chaw Thiri Mon, collection no. 69.

6. *Scleroderma verrucosum* (Bull.) Pers., Syn. meth. fung. (Gottingen) 1: 154 (1801) (Figure 2, 3. F)

Lycoperdon verrucosum Bull. 1791

Fruiting body 1.0-2.5 cm across, subglobose, covered with brown scales, thin wall, brown. Gleba brown. Stipe 0.5-0.7 cm long, 0.3-0.5 cm thick, central, equal, solid, brown. Ring absent. Spores dark brown, globose, echinulate, 15.0-20.0 μm.

Habitat : Growing on soil and grouped

Specimen examined: Banmauk Township, Naung mae lon hill; N. 24°27' 55.50", E. 95° 52' 23.45"; 23 June, 2018; Chaw Thiri Mon, collection no. 65.

7. *Cantharellus cibarius* Fr., Syst. Mycol. 1:318(1821) (Figure 2, 3. G)

Cap 1.5-2.0 cm broad, thick, slightly depressed, funnel-shaped, margin incurved and wavy, orange. Gills decurrent, pale yellow. Stipe 2.0-3.5 cm long, 0.3-0.6 cm thick, central, equal, hollow, pale yellow. Ring absent. Spores pale yellow, smooth, oblong, 10.0-15.5 x 7.5-10.0 μm.

Habitat : Growing on soil, grouped

Specimen examined: Inntaw Township, Thaung gyi village; N. 24°21' 06.37", E. 96° 01' 36.36"; 13 August, 2018; Chaw Thiri Mon, collection no. 108.

8. *Cantharellus infundibuliformis* (Scop.) Fr., Epicr. Syst. Mycol. (Upsaliae): 366 (1838) (Figure 2, 3. H)

Merulius infundibuliformis Scop. 1772

Cap 2.5-6.0 cm broad, thin, convex when young, becoming funnel-shaped, centrally depressed, pale cream at the margin, pinkish to the center. Gills decurrent, excentric, pale yellow.

Stipe 4.5-6.0 cm long, 0.5-0.7cm thick, equal, stuffed with fibrous, pale yellow. Ring absent. Spores pale cream, smooth, globose, 7.5 x 12.5 μm.

Habitat : Growing on soil, clustered

Specimen examined: Banmauk Township, Lae net gyi village; N. 24°23' 18.28", E. 95° 49' 40.89"; 22 June, 2018; Chaw Thiri Mon, collection no. 58.

9. *Phallus indusiatus* Vent., Mem. Inst. nat. Sci. Arts 1:520 (1798) (Figure 2, 3. I)

Cap 2.5-2.8 x 2.5-3.0 cm, conical-shaped, white circlet surrounding the open pore at the top of the stem, prominent net-like veil, greenish brown. Gills absent. Stipe 17.5-19.0 cm long, 2.0-2.5 cm thick, hollow, spongy-like structure, tapered at the tip, white. Ring absent. Volva present, thin. Spores greenish brown, oblong, smooth, 5.0-7.5 x 2.5 μm.

Habitat : Growing on dirty places and solitary

Specimen examined: Banmauk Township, Lae net gyi village; N. 24°38' 14.09", E. 95° 49' 35.62"; 25 June, 2018; Chaw Thiri Mon, collection no. 79.

10. *Lentinus arcularius* (Batsch) Fr. Zmitr., 12 (1): 88 (2010) (Figure 2, 3. J)

Boletus arcularius Batsch 1783.

Cap 2.5-4.0 cm broad, thin, convex with shallowly depressed, finely scaly, margin fringed with tiny hairs, brown. Gills absent. Pores running down the stem, white. Stipe 2.5-3.5 cm long, 0.2-0.3 cm thick, central, equal, solid, slightly scaly, brown. Rings absent. Spores white, oblong, smooth, 7.5-10.0 x 3.7 μm.

Habitat : Growing on decay wood and grouped

Specimen examined: Banmauk Township, Naung gaing village; N. 24°21' 07.97", E. 95° 48' 45.59"; 24 June, 2018; Chaw Thiri Mon, collection no. 76.

11. *Lactarius camphoratus* (Bull.) Fr., Epicr. syst. mycol. 346 (1838)

(Figure 2, 3. K)

Agaricus camphoratus Bull. 1793

Cap 4.0-7.0 cm broad, slightly thick, firm, flattened and slightly depressed, latex present, red-brown. Gills adnate, yellowish brown. Stipe 3.5-4.5 cm long, 1.2-1.7 cm thick, acentric, solid, smooth, cylindrical, slightly tapered at the base, reddish-brown. Ring absent. Spores pale cream, subglobose, echinulate, 12.5-17.5 x 10.0-15.0 μm.

Habitat : Growing on soil and grouped

Specimen examined: Inntaw Township, Thaung gyi village; N. 24°21' 06.37", E. 96° 01' 36.36"; 13 August, 2018; Chaw Thiri Mon, collection no. 107.

12. *Lactarius piperatus* (L.) Pers., Tent. disp. meth. fung: 64 (1797)

(Figure 2, 3. L)

Agaricus piperatus L. 1753.

Cap 4.5-7.0 cm broad, thick, convex and then expanded, center depressed, milky latex present, white at first, becoming yellow spotted in age. Gills decurrent, white at first, then pale yellow. Stipe 4.5-6.0 cm long, 1.2-1.4 cm thick, acentric, solid, tapering towards the base, white. Ring absent. Spores white, subglobosed, smooth, 10.0-15.0 x 7.5-10.0 μm.

Habitat : Growing on soil and grouped

Specimen examined: Inntaw Township, Thaung gyi village; N. 24°21' 06.37", E. 95° 00' 36.36"; 13 August, 2018; Chaw Thiri Mon, collection no. 109.

13. *Lactarius rubidus* (Hesler & A.H. Sm.) Methven, Russulaceae II. Lactarius 67 (1997) (Figure 2, 3. M)

Lactarius fragilis var. *rubidus* Hesler & A.H. Sm. 1979.

Cap 4.5-6.0 cm broad, slightly thick, firm, flattened and shallowly depressed, latex present, yellow. Gills adnate, closed, pale yellow. Stipe 4.5-8.0 cm long, 1.0-1.3 cm thick, acentric, equal, solid, slightly curved, yellow. Ring absent. Spores pale yellow, subglobose, echinulate, 15.0-20.0 x 12.5-17.5 μm.

Habitat : Growing on soil and solitary

Specimen examined: Inntaw Township, Thaung gyi village; N. 24°21' 06.47", E. 96° 00' 25.40"; 13 August, 2018; Chaw Thiri Mon, collection no. 110.

14. *Russula foetens* Pers., Observ. Mycol. 1: 102 (1796) (Figure 2, 3. N)

Cap 3.5-4.0 cm broad, thick, firm, at first nearly globose, then flattened, slightly depressed, yellowish brown. Gills adnexed, forked at the margin, pale cream. Stipe 2.5-3.5 cm long, 1.2-1.4 cm thick, central, equal, stuffed, pale yellow. Ring absent. Spores pale cream, subglobose, echinulate, 15.0-20.0 x 7.5-10.0 μm.

Habitat : Growing on soil and solitary

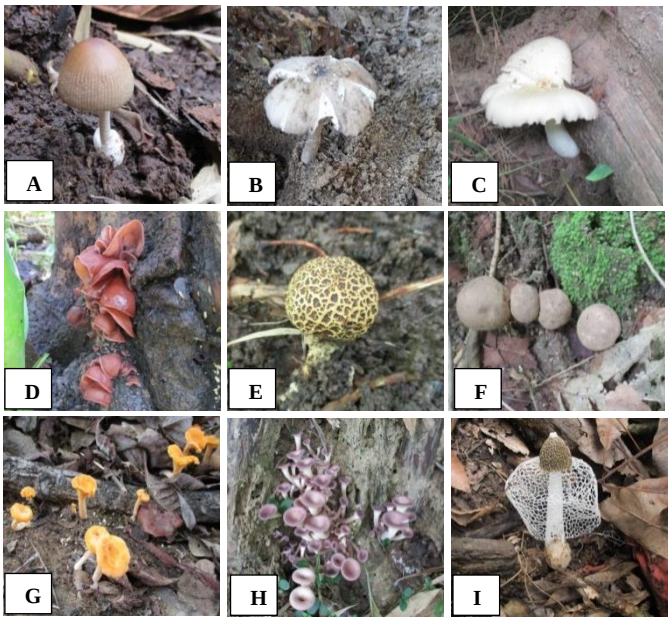
Specimen examined: Inntaw Township, Thaung gyi village; N. 24°20' 50.44", E. 96° 01' 32.69"; 14 August, 2018; Chaw Thiri Mon, collection no. 114.

15. *Russula roseipes* Secr. ex Bres., Fung. trident. 1(3): 37 (1883) (Figure 2, 3. O)

Cap 3.0-3.5 cm broad, slightly thin, convex then flattened, slightly depressed, rosy pink. Gills adnate, thin, white. Stipe 3.0-4.0 cm long, 0.7-1.0 cm thick, central, tapered at the base, stuffed, smooth, rosy-pink. Ring absent. Spores white, globose, echinulate, 10.0-15.0 μm.

Habitat : Growing on soil and grouped

Specimen examined: Inntaw Township, Thaung gyi village; N. 24°21' 06.18", E. 96° 00' 24.87"; 13 August, 2018; Chaw Thiri Mon, collection no. 96.



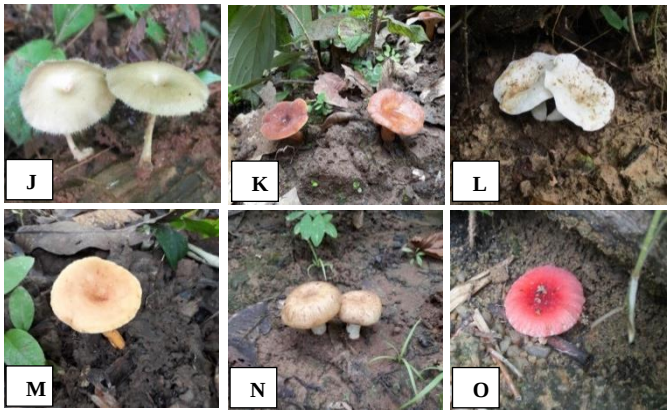
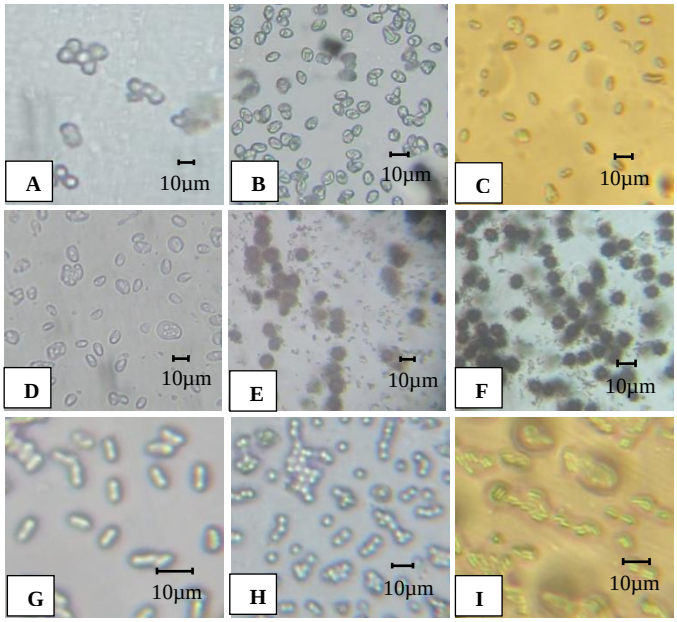


Figure 2 Natural habitat of mushrooms

A *Amanita fulva* Fr.
B *Termitomyces albuminosus* (Berk.) R. Heim.
C *Tricholoma irinum* (Fr.) P. Kumm
D *Auricularia auricular*-Juade (Bull.) Quel.
E *Scleroderma aerolatum* Ehrenb.
F *S. verrucosum* (Bull.) Pers.
G *Cantharellus cibarius* Fr.
H *C. infundibuliformis* (Pers.) P. Kumm.
I *Phallus indusiatus* Vent.
J *Lentinus arcularius* (Batsch) Fr.
K *Lactarius camphoratus* (Bull.) Fr.
L *L. piperatus* (L.) Pers.
M *L. rubidus* (Hesler & A. H. Sm.) Methven
N *Russula foetens* Pers.
O *R. roseipes* Scer. ex Bres.



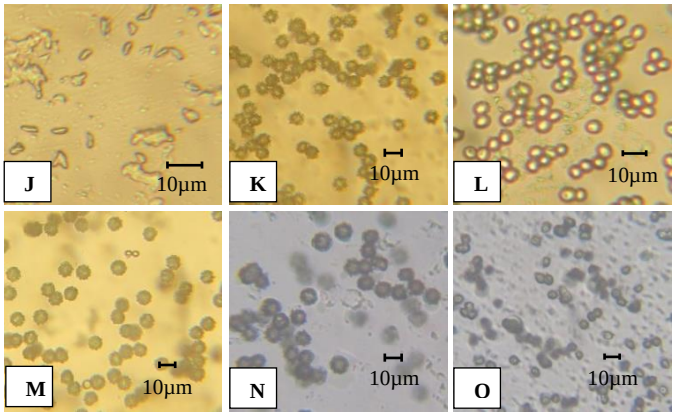


Figure 3 Spores of mushrooms

A *Amanita fulva* Fr.
B *Termitomyces albuminosus* (Berk.) R. Heim.
C *Tricholoma irinum* (Fr.) P. Kumm
D *Auricularia auricular*-Juade (Bull.) Quel.
E *Scleroderma aerolatum* Ehrenb.
F *S. verrucosum* (Bull.) Pers.
G *Cantharellus cibarius* Fr.
H *C. infundibuliformis* (Pers.) P. Kumm.
I *Phallus indusiatus* Vent.
J *Lentinus arcularius* (Batsch) Fr.
K *Lactarius camphoratus* (Bull.) Fr.
L *L. piperatus* (L.) Pers.
M *L. rubidus* (Hesler & A. H. Sm.) Methven
N *Russula foetens* Pers.
O *R. roseipes* Scer. ex Bres.

Table 2 Comparable Morphological Characteristics of Mushrooms from the Study Area

No.	Scientific name	Growing habitat	Edible/ Inedible	Cap		
				Colour	Shape	Um
1.	*Amanita fulva* Fr.	soil	inedible	yellowish brown	convex	abs
2.	*Termitomyces albuminosus* (Berk.) R. Heim.	soil	edible	pale brown	flattened	pres
3.	*Tricholoma irinum* (Fr.) P. Kumm.	soil	edible	pale brown	flattened	abs
4.	*Auricularia auricular*-Juade (Bull.) Quel.	decay wood	edible	reddish brown	cup or ear shaped	abs
5.	*Scleroderma aerolatum* Ehrenb.	soil	inedible	brownish yellow	globose	abs
6.	*S. verrucosum* (Bull.) Pers.	soil	inedible	brown with scales	globose	abs
7.	*Cantharellus cibarius* Fr.	soil	edible	orange	depressed	abs
8.	*C. infundibuliformis* (Pers.) P. Kumm.	soil	edible	pale cream	depressed	abs
9.	*Phallus indusiatus* Vent.	soil	inedible	greenish brown	conical	abs
10.	*Lentinus arcularius* (Batsch) Fr.	decay wood	inedible	brown	depressed	abs
11.	*Lactarius camphoratus* (Bull.) Fr.	soil	edible	red brown	depressed	abs
12.	*L. piperatus* (L.) Pers.	soil	edible	white	depressed	abs
13.	*L. rubidus* (Hesler & A. H. Sm.) Methven	soil	edible	yellow	depressed	abs
14.	*Russula foetens* Pers.	soil	inedible	yellowish brown	depressed	abs
15.	*R. roseipes* Scer. ex Bres.	soil	edible	rosy pink	depressed	abs

No.	Scientific name	Stipe			Spores	
		Colour	**Shape**	**Hollow/ solid**	**Colour**	**Shape**
1.	*Amanita fulva* Fr.	pale cream	equal	hollow	white	globose
2.	*Termitomyces albuminosus* (Berk.) R. Heim.	pale gray	tapered at the base	solid	pink	elliptic
3.	*Tricholoma irinum* (Fr.) P. Kumm.	pale cream	tapered at the base	solid	pale cream	elliptic
4.	*Auricularia auricular*-Juade (Bull.) Quel.	-	-	-	white	elliptic
5.	*Scleroderma aerolatum* Ehrenb.	yellowish brown	equal	solid	brown	globose
6.	*S. verrucosum* (Bull.) Pers.	brown	equal	solid	dark brown	globose
7.	*Cantharellus cibarius* Fr.	pale yellow	equal	hollow	pale yellow	oblong
8.	*C. infundibuliformis* (Pers.) P. Kumm.	pale yellow	equal	solid	pale cream	globose
9.	*Phallus indusiatus* Vent.	white	tapered at the tip	hollow	greenish brown	oblong
10.	*Lentinus arcularius* (Batsch) Fr.	brown	equal	solid	white	oblong
11.	*Lactarius camphoratus* (Bull.) Fr.	reddish brown	tapered at the base	solid	pale cream	subglobose
12.	*L. piperatus* (L.) Pers.	white	tapered at the base	solid	white	subglobose
13.	*L. rubidus* (Hesler & A. H. Sm.) Methven	yellow	equal	solid	pale yellow	subglobose
14.	*Russula foetens* Pers.	pale yellow	equal	solid	pale cream	subglobose
15.	*R. roseipes* Scer. ex Bres.	rosy pink	tapered at the base	solid	white	globose

Table 2 Continued

Discussion and Conclusion

The taxonomic studies of wild mushrooms distributed in Banmauk and Inntaw Townships were carried out from June to August during the year 2018. Fifteen species of wild mushrooms belonging to 10 genera and 9 families were collected and identified. Eight species were found in Banmauk Township and 7 species were collected in Inntaw Township. The identified species are *Amanita fulva* Fr., *Termitomyces albuminosus* (Berk.) R. Heim., *Tricholoma irinum* (Fr.) P. Kumm., *Auricularia auricular*-Juade (Bull.) Quel., *Scleroderma aerolatum* Ehrenb., *S. verrucosum* (Bull.) Pers., *Cantharellus cibarius* Fr., *C. infundibuliformis* (Pers.) P. Kumm., *Phallus indusiatus* Vent., *Lentinus arcularius* (Batsch) Fr., *Lactarius camphoratus* (Bull.) Fr. *L. piperatus* (L.) Pers., *L. rubidus* (Hesler & A. H. Sm.) Methven, *Russula foetens* Pers. and *R. roseipes* Scer. ex Br.

According to the present study, *Auricularia auricular*-Juade (Bull.) Quel. and *Lentinus arcularius* (Batsch) Fr. were growing on the decay woods while the other species were growing on the soil. The cap of 8 species are depressed, 4 species are expended, 2 species are globose and 1 species is conical in shape. Among the 15 species, *Termitomyces albuminosus* (Berk.) R. Heim. was plano-umbonate on the cap.

The attachment of gills were free in 2 species, adnate in 3 species, decurrent in 3 species, sinuate in 1 species and adnexed in 1 species. In this study, the stipe shape of 8 species were equal. In *Termitomyces albuminosus* (Berk.) R. Heim., *Tricholoma irinum* (Fr.) P. Kumm., *Lactarius camphoratus* (Bull.) Fr. *L. piperatus* (L.) Pers. and *Russula roseipes* Scer. ex Br., the stipes were tapered at the base and *Phallus indusiatus* Vent. was tapered at the tip. In this study, the spongy stipe was found in *Phallus indusiatus* Vent. The volva were present in *Amanita fulva* Fr. and *Phallus indusiatus* Vent. while the other 13 species were not present.

According to the present study, the most common family was Russulaceae. The common species are *Lactarius camphoratus* (Bull.) Fr., *L. piperatus* (L.) Pers., *L. rubidus* (Hesler & A.H. Sm.) Methven, *Russula foetens* Pers. and *R. roseipes* Scer. ex Bres. All members of the genus *Lactarius* have the milky latex that exudes from all parts of the fruiting body when cut or broken.

Of these 9 species were edible and 6 species were inedible. The edible mushrooms were commonly eaten by local people. Some of the mushrooms are inedible or deadly poisonous that are confused with edible mushrooms. Some edible mushrooms have a pleasant smell and inedible or poisonous have strongly smell. In this study, *Lactarius piperatus* (L.) Pers. have a strongly acrid taste and *L. camphoratus* (Bull.) Fr. and *L. rubidus* (Hesler & A. H. Sm.) Methven have a mild taste. In the present study, the pink spores was found in *Termitomyces albuminosus* (Berk.) R. Heim. which can be eaten without harm by local people. Although Bessey (1960) stated that many of the pink-spored fungi are poisonous.

The study area is in subtropical area and possessing a good condition for the growing of mushrooms in natural. Therefore, it is hoped that there is still many species of wild mushrooms in Banmauk and Inntaw Townships. The study work will provide the valuable taxonomic information and knowledge for future researchers who are interested in mushroom for applied researches. Finally, the resulting data will partially provide for the compilation of Flora of Mushroom in Myanmar.

Acknowledgements

I would like to express our gratitude to Dr Nu Nu Yee, Professor and Head, Department of Botany, University of Mandalay, for her permission and encouragement for this research work. I am grateful to Dr Swe Swe, Professor and Head and Dr Theingi Htay, Professor, Department of Botany, Monywa University, for their permission and invaluable advice in this research.

References

Alexopoulos, C. J. (1962) . Introductory mycology, Second Edition. John Wiley and Sons, Inc., New York and London.

Bessey, E. A. (1960). Morphology and taxonomy of fungi. University Microfilms, Inc., Ann Arbor, London.

Davis, R. M., R. Sommer & J. A. Menge. (2012). Field guide to mushrooms of Western North America. University of California Press, Ltd. London, England.

Krieger, L. C. C. & R. L. Schaffer. (1967). The mushroom handbook. Dover Publications, Inc., New York.

Kuo, M. & A. S. Methven. (2014). Mushrooms of the Midwest. University of Illinois Press. United States of America.

McKnight, K. H. & V. B. McKnight. (1987). A field guide to mushrooms of North America. Houghton Mifflin Company, United States of America.

Nair, M.C. (1990). Mushrooms. Kerala Agricultural University Press, Mannuthy, Kerala.

Ramsbottom, J.(1923). A handbook of the larger British Fungi. Printed by Oder of The Trustees of the British Museum, London.

Roger, P. (2006). Mushrooms. Pan Macmillan, 20 New Wharf Road, London.

Thomas, W. S. (1948). Field book of common mushrooms. New and enlarged Third Edition, G.P. Putnam's Sons, New York and London.

JOURNAL OF THE MYANMAR ACADEMY OF ARTS AND SCIENCE

Botany

Vol. XVIII, No.4A, July, 2020

Myanmar Academy of Arts and Science

Journal of the Myanmar Academy of Arts and Science

Vol. XVIII, No.4A

Contents

Botany

Edition

2020, July, 500 Copies

Published by

Dr Aung Min (00322) Chairman, Journal Publication Committee,
Myanmar Academy of Arts and Science

ISSN 2520-0186
LCCN 2003-323143

Printed by

U Win Aung (00171), Manager,
Universities Press, Yangon, Myanmar