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Editorial Board

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Botany (II)

Preliminary Phytochemical Investigation into *Dolichandrone spathacea* (L.f.) K. Schum Leaves

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Abstract

The traditional medicinal plant, *Dolichandrone spathacea* (L.f.) K. Schum. belongs to the family Bignoniaceae, and locally known as Tha-Khut is widely distributed halophytic tree and as an economically value plants grown in Myanmar. It was collected from Hlegu Township, Yangon Region and is also commonly known as mangrove trumpet tree in Myanmar. The leaves of this plant has effected in nervous diseases, thrush and flatulence. Morphology, Taxonomy of the vegetative and reproductive parts of this plant were studied for classification and identification according to the available literature. The morphological and anatomical characters of leaves were described with relevant photographs and scientific name, Myanmar name, English name and part uses were also mentioned in this paper. The fresh specimens were cut by free hand sections and examined under microscope. Investigation into preliminary phytochemical tests of the powdered samples of the leaves were examined for the presence or absence of primary and secondary metabolites.

Keyword: Morphological and histological characters, phytochemical test

Introduction

Medicinal plants have a long history of use in most communities throughout the world. It has been confirmed by the WHO that herbal medicines serve the health needs about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products. The history of plants being used for medicinal purpose is probably as old as the history of mankind. (http://en wikipedia, pharmacognosy)

Bignoniaeceae is a family of about 120 genera and 650 species mainly tropical trees, shrubs or lianas; particularly abundant in Brazil, most parts of tree are used indigenously and commercial fruit extract is used in skin-care products. Myanmar traditional medicine is widely practically and

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well accepted to many Myanmar people, partly as a supplement and partly as an attractive to modern medicine. In modern medicine, various active compounds isolated from plants as well as synthetic active substances are used. Medicinal plants constitute an important natural wealth of a country. Binoniaceae family is pantropical and subtropical with a few generate in temperate part of North America, Asia and the Southern hemisphere. (Kirtikar and Basu, 1987).

Dolichandrone spathacea (L.f.) K. Schum named mangrove trumpet tree is a common smooth tree growing wild in river banks and mangroves of the Asia-pacific area. The leaves of this medicinal plants are used in different countries to treat thrush (as mouthwash), flatulence and bronchitis (juice of leaves). The sole report on the plant found in the literature mentioned the detection of flavonoids, triterpenes and tannins (http://www.download/bignoniaceae.htm).

In Indonesia, the leaves of *Dolichandrone spathacea* are used to treat thrush. In Philippines, this plant is used to treat nervous diseases and flatulence. The pharmacological potentials of this interesting plant remain unexplored. This traditional plant is widely used in Asia and India as antiseptic, for bronchitis and thrush treatment and the methanolic extract has been shown to possess antibacterial activity against methicillin-resistant *staphylococcus aureus* and anti-infectious uses of the leaves of *Dolichandrone spathacea* (http://www.plant.journal).

This family is econormically important because of its predominantly woody members and many large trees and climbers. Some of the members are also medicinally useful. The species is widespread at location on the banks of sea about 200 meters. *Dolichandrone spathacea* is an attractive, evergreen tree with intensely fragrant flowers, growing up to 20 meters tall. The plant is harvested from the wild for local use as a food, medicine and source of wood (http://www.plant.journal).

Phytochemical investigation of *Dolichandrone spathacea* (L.f.) K. Schum leaves have been reported as the presence of numerous chemicals. Plant extract contains various secondary metabolites such as tannins, alkaloids, saponins, glycosides and flavonoids. Therefore, the plants are selected on the basic of the presence of secondary metabolites (Khare, 2007).

In this research paper, the morphology, the taxonomy of vegetative and reproductive parts, the anatomy of leaves and preliminary

phytochemical tests of *Dolichandrone spathacea* (L.f.) K. Schum leaves have been studied.

Materials and Methods

Botanical studies

The specimens of *Dolichandrone spathacea* (L.f.) K. Schum were collected from Hlegu Township, Yangon Region from April to July, 2019. Immediately, after collecting some of the specimens were pressed, dried and preserved for study and storage.

The fresh specimens of vegetative and reproductive parts have been used for species identification that were under taken with the help of available literature of Hooker (1885), Backer (1965) and HU-QI-ming (2008).

The anatomical studies of the plant were also examined by free hand sections according to the methods given by Trease and Evans (2002) and identification of the leaves anatomy studied by the literature of Metcalfe and Chalk (1950), Esau (1953) and Pandey (1988).

The specimens were used for cleaning section cutting to examine chloral hydrate solution and the oil globules were confirmed by using Sudan III and IV.

Chemical Studies

Preliminary Phytochemical Test

Preliminary phytochemical investigation of leaves of *Dolichandrone spathacea* (L.f.) K. Schum was carried out to examine the plant constituents.

According to the methods of Marini Bettolo, *et al.*, (1981), Central Council for Research in Unani Medicine (1989) and Trease and Evans (2002) were applied for the investigation of phytochemical studies. The results were as shown in Figure 1 and Table 1.

Figure 1. Phytochemical test

Test for Alkaloids

Dried powder sample 1g was taken in a test tube and 2ml of 10% acetic acid was added together with 2 drops of 95% ethanol. The test tube was warmed in water-bath for 10 minutes and cooled and filtered. The filtrate was divided into two portions and tested with Mayer's reagent and Dragendroff's reagent. The precipitate formed on addition the reagent indicates the presence of alkaloids (Marini-Bettolo G.B. et al ., 1981).

Test for α -Amino Acids

Dried powder sample 1g was boiled with 25ml of distilled water for 10 minutes, cooled and filtered. A few drops of each filtrate were spotted on a filter paper using a capillary tube (micropipette), allowed to dry and spray with ninhydrin reagent. The filter paper was dried at room temperature and then kept in an oven at 110°C for five minutes after which the purple colour appears due to the presence of œ-amino acid (Trease and Evans, 1978).

Test for Carbohydrates

Dried powder sample 1g was boiled with 25ml distilled water, cooled and filtered. The filtrate was introduced into a test tube and a few drops of 10%-naphthol were added and shaken. The test tube was then inclined at an angle of 45°C and concentrated sulphuric acid was added slowly along the side of the test tube. A red ring was formed between the two layers, showing the presence of carbohydrates (Trease and Evans, 1978).

Test for Starch

About 1g of dried powder sample was boiled 25ml of distilled water for about 20 minutes. It was then filtered and two drops of iodine, potassium iodide solution was added to the filtrate. Dark blue precipitate was formed which indicate the presence of starch (Marini-Bettolo G.B. *et al*., 1981).

Test for Reducing Sugar

Dried powder sample 1g was boiled with dilute sulphuric acid and 5N sodium hydroxide for 10 minutes and cooled and filtered. The filtrate was then treated with Benedict's solution; it furnished brick-red precipitates, indicating the presence of a reducing sugar (Trease and Evans, 1978).

Test for Glycosides

Dried powder sample 1g was heated in a glass test tube with 25ml distilled water on the water-bath for 10 minutes. The mixture was filtered and 10% basic lead acetate solution was added drop-wise to the filtrate. White precipitate was observed which showed the presence of glycosides (Central Council for Research in Unani Medicine, 1987).

Test for Phenolic Compounds

Dried powder sample 1g was boiled with 25ml distilled water, cooled and filtered. When the filtrate was treated with neutral 3% ferric chloride solution, it gave deep brown colouration, indicating the presence of phenolic compound (Marini-Bettolo G.B. *et al*., 1981).

Test for Saponins

1g powdered plant materials were introduced in test tube and shaken vigorously with 25ml distilled water for a few minutes. Marked frothing which lasted for about half an hour took place, indicating the presence of saponins (Marini-Bettolo G.B. *et al*., 1981).

Test for Tannins

Dried powder sample 1g were boiled with 25ml distilled water for about 10 minutes, cooled and filtered. The filtrate was treated with a few drops of 1 % ferric chloride solution. If a deep blue colour was produced indicating the presence of tannins (Central Council for Research in Unani Medicine, 1987).

Test for Flavonoids

Dried powder sample 1g was extracted with methanol and filtered. When the methanolic extract was treated with 5-10 drops of dilute hydrochloric acid was added, followed by a small piece of zinc or magnesium. The solution was boiled for few minutes. The appearance of pink or brown colour indicates the presence of flavonoids (Central Council for Research in Unani Medicine, 1987).

Results

Morphological characters of *Dolichandrone spathacea* (L.f.) K.Schum

Scientific name	:	*Dolichandrone spathacea* (L.f.) K. Schum
Myanmar name	:	Tha-Khut
English name	:	Mangrove trumpet tree
Family	:	Bignoniaceae
Part Used	:	Leaves
Flowering and Fruiting period	:	from April to July

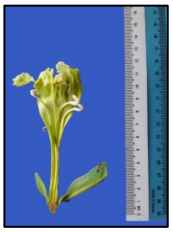
Dry deciduous perennial tree. Bark greyish brown with a crooked trunk. Leaves opposite, imparipinnate compound. Leaflets 5-9, opposite, ovate, base oblique or obtuse, apex acute, glabrous. Petiole short and petiolule stout, exstipulate. Inflorescence terminal or axillary corymb with 3-4 flowers. Raceme erect, widely branched. Flower, bracts lanceolate and bracteolate inconspicuous, pedicel short. Complete, bisexual, zygomorphic, hypogynous. Sepals (5), Synsepalous, sometime deciduous, spathaceous, tubular, petals (5), synpetalous, campanulate, white, corolla long, cylindrical. Androecium, stamens 4, epipetalous, didynamous. Filament slender, staminode small. Anthers dithecous, disc annular, dorsifixed, splitting by longitudinal dehiscence. Gynoecium, carpels (2), bicarpellary, syncarpous, bilocular, axile placentation, many ovules in each locule. Style long and slender. Stigma 2-lobed, disc present, superior. Fruit dehiscent capsule, purplish – brown 2-valved septicidal, long-linear, cylindrical. Seed suborbicular, non-endosperm, white seeds overlapping in many rows, corky-winged. Flowering and fruitine time is from April to July.

Habit

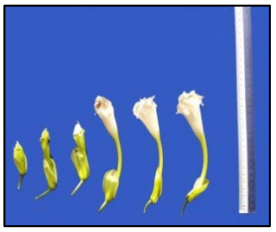
Compound Leaves

Imparipinnate

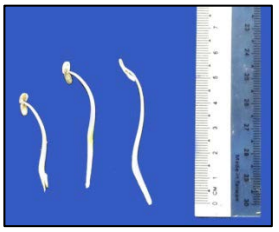
Inflorescence

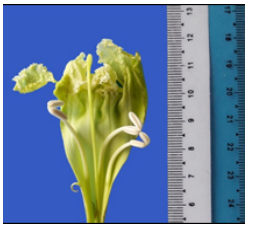


L. S of flower

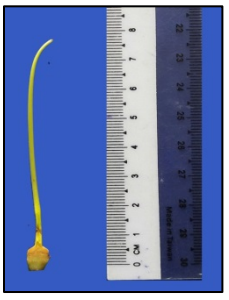


Flowers





Stamen



Carpel

T. S of ovary

Histological characters of *Dolichandrone spathacea* (L.f.) K. Schum

In lamina, surface view of upper epidermis are irregular cells, anticlinal walls thin and wavy, peltate trichomes and oil globules are present. Lower epidermis are also irregular cells, anticlinal walls are more wavy than upper. Anomocytic stomata and peltate trichomes are present, both surfaces are striated cuticle. In transverse section, striated cuticle layer and epidermis one layered on both sides. Palisade parenchyma one to two layers and a lot of chlorophyll. Spongy parenchyma 3-4 layers with

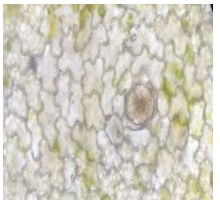
abundance of oil globules. Peltate trichomes, unicellular trichome and multicellular trichome were present.

Midrib

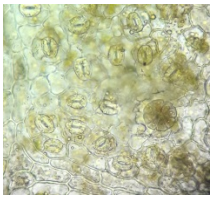
In midrib, surface view of epidermal cells are thick walled, rectangular shaped. Oil globules and peltate trichome are present. In transverse section, epidermal cells are rounded to oval shaped parenchymatous cells. A heart-shaped vascular bundle, bicollateral and opened. Six to eight layers of collenchymatous cells and two to three layers of parenchymatous cells are found in lower portion. Peltate trichomes, unicellular trichomes and pigment are present.

Petiole

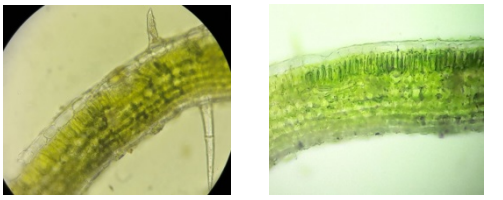
In petiole, surface view of epidermal cells are rectangular shaped and elongated along the axis. Numerous peltate trichomes are present. In transverse section, oval-shaped in outline, winged petiole. Single layer of epidermal cells are present. Five to seven layer of collenchymatous cells and two to three layers of parenchymatous cells are present. Vascular bundles are heart-shaped, bicollateral and opened. Unicellular trichomes and pigment present.



Surface view of upper epidermis peltate trichomes (X400)



Surface view of lower epidermis with anomocytic stomata and peltate trichomes (X100)



Transverse section of lamina with peltate trichome, unicellular trichome and multicellular trichome (X100)

Surface view of midrib (X400)

Transverse section of midrib with peltate and unicellular trichomes (X100)

Surface view of petiole with peltate trichome: (X100)

Transverse section of petiole peltate and unicellular trichomes (X100)

Phytochemical Test of *Dolichandrone spathacea* (L.f) K Schum leaves

The phytochemical tests were carried out in order to know presence or absence of alkaloid, α-amino acid, carbohydrate, starch, reducing sugar, glycoside, phenolic compound, saponin, tannin and flavonoid in the leaves. Among them, starch was small amount and reducing sugar was absent in the leaves of *Dolichandrone spathacea* (L.f.) K. Shcum. The results are shown in Table 1.

Table. 1. Phytochemical Test of *Dolichandrone spathacea* (L.f) K Schum leaves

No.	Test	Extract	Test Reagents	Observation	Result
1.	Alkaloid	10% Acetic acid + EtOH	Mayer's Reagent Dragendroff's reagent	White ppt Orange ppt	+ + + +
2.	α-amino acid	H_2O	Ninhydrin solution	Purple spot	+ + +
3.	Carbohydrate	H_2O	10% α -naphthol + conc-H_2SO_4	Red ring	+ + +
4.	Starch	H_2O	I_2KI solution	Blue ppt	+

No.	Test	Extract	Test Reagents	Observation	Result
5.	Reducing sugar	Dil H_2SO_4+5N NaOH	Benedict's solution	Brick red ppt	-
6.	Glycoside	H_2O	10% lead acetate solution	White ppt	+ + +
7.	Phenolic compound	H_2O	Ferric chloride solution	Deep blue color	+ + +
8.	Saponin	H_2O	Distilled water	Frothing	+ +
9.	Tannin	H_2O	Ferric chloride solution	Deep blue color	+ + +
10.	Flavonoid	Methanol	(1) Mg turning (2) Conc HCl acid	Pink color	+ +

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

Discussion and Conclusion

In this study, the morphological characters of *Dolichandrone spathacea* (L.f.) K.Schum is in agreement with those described by Hooker (1885), Backer (1965), HU Qi-ming (2008), Heywood (2007) and Khare (2007).

In this paper, the plant is dry deciduous perennial tree and the bark is greyish brown with a crooked trunk. Leaves opposite, imparipinnate compound and leaflets 3-4, opposite, petiole short and exstipulate. Inflorescence terminal or axillary corymb with 3-4 flowers. Flowers bisexual, pentamerous, hypogynous. Calyx tubular, spathaceous and sometime deciduous. Corolla white, long, synpetalous and campanulate. Stamens 4, epipetalous, didynamous, filaments slender and anther dithecous, dorsifixed and disc annular. Ovaries 2, bicarpellary, axile placentation and many ovules in each locule. Style long, stigma 2-lobed and superior. Fruit dehiscent capsule, 2-valved septicidal and long-linear. Seed suborbicular, non-endoperm and corky-winged.

In microscopical studies, the upper epidermal cells are irregular, the anticlinal wall of lower epidermis are more wavy than upper epidermis.

Anomocytic stomata types are present on the lower surface of lamina only. Oil globules, peltate trichomes, unicellular trichomes and multicellular trichomes are present on both surfaces. Heart-shaped vascular bundles, bicollateral and opened types are found in midrib and petiole. These characters are in agreement with Esau (1953), Metcalfe and Chalk (1950) and Pandey (1988).

In this study, investigation of preliminary phytochemical test on the leaves of *Dolichandrone spathacea* (L.f.) K Schum showed that alkaloid, α-amino acid, carbohydrate, glycoside, phenolic compound, saponin, tannin and flavonoid were especially abundant present but starch was small amount and reducing sugar was absent in the leaves of *Dolichandrone spathacea* (L.f.) K. Schum.

Glycosides play many important roels in living organisms and numerous plant-produced glycosides. This characters are in agreement with Bettolo, et.al., (1981) and Central Council for Research in Unani Medicine (1987).

Herbs are commonly used by people across the globe, especially in developing countries through traditional medicine. Thus, phytochemical screening studies are still relevant. In the present study, the phytochemical constituents in different species of *Dolichandrone spathacea* was evaluated. The results showed that these plants are rich repository of secondary metabolites which import various pharmacological potentials and development of promising treatment modalities (http://www.plant.jounal).

Acknowledgements

I would like to express my sincere gratitude to Dr. Myat Myat Moe, Professor and Head, Department of Botany, Dagon University, for the permission to use various department facilities during the study period and her kind understanding throughout this research.

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http://www.plants.journal.com.

Effects of Seed Dormancy Breaking Treatments on Germinability of *Psophocarpus tetragonolobus* (L.) DC

Mang Uap*

Abstract

The research was conducted using the seeds of winged bean, (*Psophocarpus tetragonolobus* (L.) DC (Winged bean) at the Department of Botany, Dagon University. The study was carried out with three experiments from July 2019 to August 2019.In this experiment, germination is carried out and six treatments with 24 replicates were set up in Completely Randomized Design (CRD). The present study was conducted to assess the loss of viability and find out methods of increasing germination of the stored seed of *Psophocarpus tetragonolobus* (L.) DC. Maximum seed germination of T2 4% was observed where the seed were treated in hot water 60˚ C and T3 3% in cold water.10% germination was observed where it was treated in T2 20% in HCL.

Keywords*:* *Psophocarpus tetragonolobus*, winged bean

Introduction

The winged bean is a tropical herbaceous legume. It produces edible pods, leaves, flowers, tuberous roots and edible seed oil which are rich in protein. Winged bean is a self-pollinated crop. *Psophocarpus* is a genus with nine species. Among them, *P. tetragonolobus* and *P.palustris* are used for food. Other species have never been under cultivation (Arnon, 1975)

Winged bean *P. tetragonolobus* (L.) D.C plays an important role in widespread malnutrition. Except stems and roots, all the other parts are edible, palatable and highly nutritious. The young, tender pods may be eaten raw, sliced and chopped. They may be used in salads, soups and curries and may be boiled in water or coconut milk or in oil. Pods are good for blood and is also useful for curing diabetes (Arnon, 1975).

The mature dry seeds are the most nutritious part of winged bean. It also contains edible oil (15-20 %). The seeds can be steamed, boiled, fried, roasted, fermented or made into milk. These seeds also cleanse the blood and are used in the treatment of venereal diseases. The tubers can be boiled,

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steamed, fried or baked. The brown skin peels off readily after 40 minutes of boiling, exposing cream - colored firm flesh. Tubers are boiled in sugar lumps and forms a treatment for thrush. Usually the top three sets of leaflets are eaten since they are tender and tastes sweet. Tender leaves have higher protein content than mature leaves. Leaves are used for eye and ear infections. Juices from young shoots and leaves are used for teeth and morning dyspepsia, respectively. Other uses of winged bean are : it can be grown as a cover crop, or fallow restorative crop, due to its nodulation system of roots which fixes nitrogen and increases the fertility status of poor soils. Beside all these, it is sometimes used as animal feed (Arnon, 1975).

Winged bean (*P. tetragonolobus* (L.) D.C. which is known by many names in various languages is a multipurpose leguminous plant. It has been grown widely throughout South East Asia, Africa and Papua New Guinea for centuries as a home or market garden crop. The worldwide interest in winged bean was drawn in 1975, when the National Academy of Science identified this under-utilized crop as a promising source of protein and oil for developing countries. It is grown in humid tropical countries like Indonesia. Malaysia, Thailand, Philippines, India, Bangladesh, Myanmar and Srilanka (Arnon, 1975).

Dormancy is temporary failure of a seed to complete germination under favourable conditions. It allows for the dispersal of seeds in space and time. There are several types of dormancy, which include physical, mechanical, or chemical inhibition by the covering layers of the embryo, the inability to germinate because of an undifferentiated or immature embryo and the repression of germination by metabolic restraints. The breaking of dormancy is governed by environmental factors, including temperature, light, nitrate, and some smoke components. This allows seedlings establishment during suitable conditions to maximize survival. one of the major problems in achieving rapid propagation of this wonder crop is the low rate of germination of the stored seeds. There are many controversial reports regarding the germinability of the seeds of winged bean on storage. (Baskin, 2004).

A dormant seed is one that is unable to germinate in a specified period of time under a combination of environmental factors that are normally suitable for the germination of the non-dormant seed. Dormancy is a mechanism to prevent germination during unsuitable ecological

conditions, when the probability of seedling survival is low (Bewley and Black, 2004).

Another form of delayed seed germination occurs when a seed fails to germinate because the external environmental conditions are too dry or warm or cold for germination. The seeds from many species of plants have delayed germination for many months or years, and some seeds can remain in the soil as seed for more than 50 years before germination (Randolph and Schmid, 2008). Regarding to the above facts, the study is aimed to record the germination rate, to observe the seed dormancy rate and to examine the agents used breaking seed dormancy and its effects on seed germination.

Materials and Methods

The pod experiment of winged bean seeds, *Psophocarpus tetragonolobus* (L.) DC. was carried out from July 2019 to August 2019. The seeds were collected from the Marlarmyaing Agricultural Service, Kamayut Township, Yangon Region.

Figure 1. *Psophocarpus tetragonolobus* (L.) DC. Seeds.

Soil Preparation

Before sowing seeds, collected soil were sieved to remove all sticks, rocks, large objects and other unwanted materials in the soil. Then the soil was put into the black polyethylene bags, each bag with 1kg of soil. At the base of the bags were perforated for easy dispensing and to allow excess water to drain out. In this experiment, 25 cm x 30 cm size polyethylene bag was used for seed germination. The medium used in the germination was soil mixed with sand.

Figure 2. Soil Preparation for experiment

Study 1

In this experiment, six treatments with 24 replicates were set up in Completely Randomized Design (CRD). The distance between individual bag was 2.5 cm and between replication was 1 cm. The treatments are as follows:

T_0 = control

T_1 = immersed in water overnight (24 hours)

T_2 = immersed in hot water at 60°C, for 10 minutes

T_3 = immersed in cold water for 10 minutes

T_4 = immersed in 40 % HCl for 10 minutes

T_5 = immersed in 40 % H_2S0_4 for 10 minutes

Evaluation for acid in seed germination using H_2S0_4 was not successful because not even one seed get germinated. So H_2S0_4 was eliminated for germination of winged bean. Using 40 % H_2S0_4 gave seed digestion. 40 % HCl gave 2 % of germination, thus, reduction in HCl was tested again and the embryo of seed was examined under dissecting microscope to obtain the proper result.

Germination of winged bean seeds treated with 10%, 20%, 30% and 40% HCl

In this experiment, five treatments with 20 replicates were set up in completely Ramdomized Design (CRD). The distance between individual bags was 2.5 cm and between replications was 1 cm. The treatments are as follows:

T_0 = control

T_1 = immersed in 10 % HCl for 10 mins

T_2 = immersed in 20 % HCl for 10 mins

T_3 = immersed in 30 % HCl for 10 mins

T_4 = immersed in 40 % HCl for 10 mins

Data Collection

The experimental data were daily recorded. The germination survived and dead plants were recorded. The germination was using the formula of Soupe (2009). The recorded data were analyzed using the following the formula:

$$\text{Germination (\%)} = \frac{\text{Germinated Seeds}}{\text{Total Sown Seeds}} \text{ x } 100$$

Results

Seed germination treated with hot water, cold water, HCl and H_2SO_4

The germination of *Psophocarpus tetragonolobus* (L.)DC was set up in Dagon University. The mean temperature of the place was 25°C and relative humidity of 80 % during germination.

The germination of T_0 (control) started at 7 days occur in ToR_3, ToR_4 and the germination rate is 2%. In treatment T_1(immersed in water overnight) occur no germination. The germination of T_2 (Hot water at 60°C) started at 7 days occur in T_2 R_3, T_2 R_4 and at 9 days occur in T_2R_1. The germination rate is 4% . The germination of T_3 (cold water) started at 7 days occur in T_3 R_4. At 14days occur in T_3R_1 and T_3R_2 and the germination rate is 3%. The germination of T_4 (40 %HCl) started at 7days occur in T_4 R_4 and at 9 days occur in T_4R_3 .The germination rate is 2%.In treatment T_5 occur no germination.

Table 1. Showing results of different treatments and germination percentages of winged bean seeds

Treatment	Germinated seedling (Number)									Germination (%)
	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14	Total	
T_0 (control)	2	-	-	-	-	-	-	-	2	2 %
T_1(immersed in water)	-	-	-	-	-	-	-	-	-	0 %
T_2(hot water 60 ˚C)	2	-	2	-	-	-	-	-	4	4 %
T_3(cold water)	1	-	-	-	-	-	-	2	3	3%
T_4(40% HCl)	1	-	1	-	-	-	-	-	2	2%
T_5 (40%H_2SO_4)	-	-	-	-	-	-	-	-	-	0%

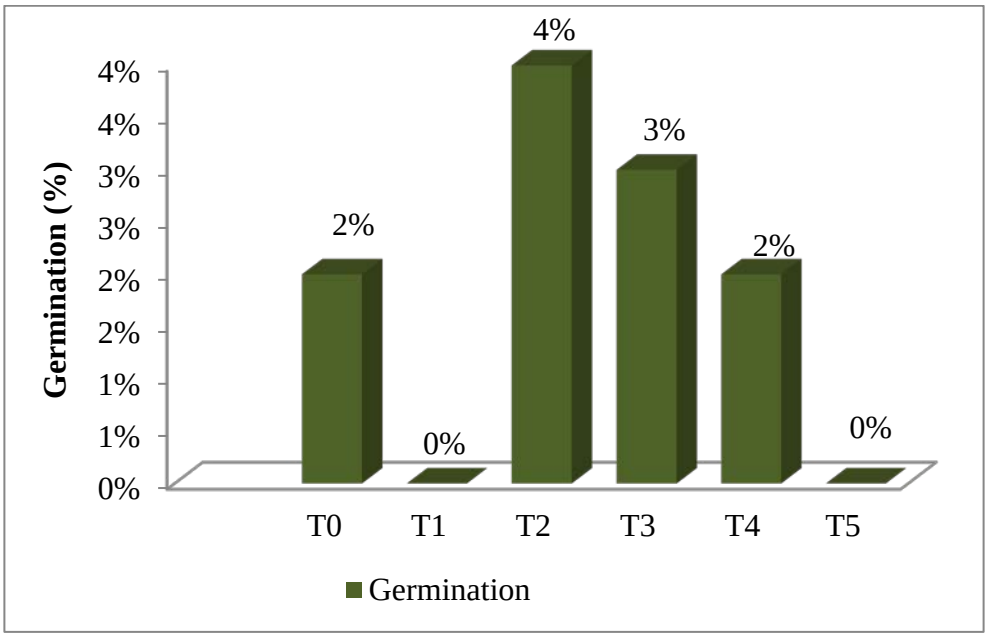

Figure 3. The germination percentages of winged bean seeds in different treatments

Seed germination treated with 10%, 20%, 30% and 40% HCl.

The germination of T_2 (20 % HCl) started at 7 days occur in T_2R_3 and the germination rate is 10 %. In treatment T_0, T_1, T_3 and T_4 occur no germination.

Table 2. Showing results of HCl treatments and germination percentages of winged bean seeds.

Treatment	Germinated seedling (Number)									Germination (%)
	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14	Total	
T_0(control)	-	-	-	-	-	-	-	-	0	0 %
T_1(10%HCl)	-	-	-	-	-	-	-	-	0	0 %
T_2(20%HCl)	1	-	-	-	-	-	-	-	1	10 %
T_3(30%HCl)	-	-	-	-	-	-	-	-	0	0 %
T_4(40%HCl)	-	-	-	-	-	-	-	-	0	0 %

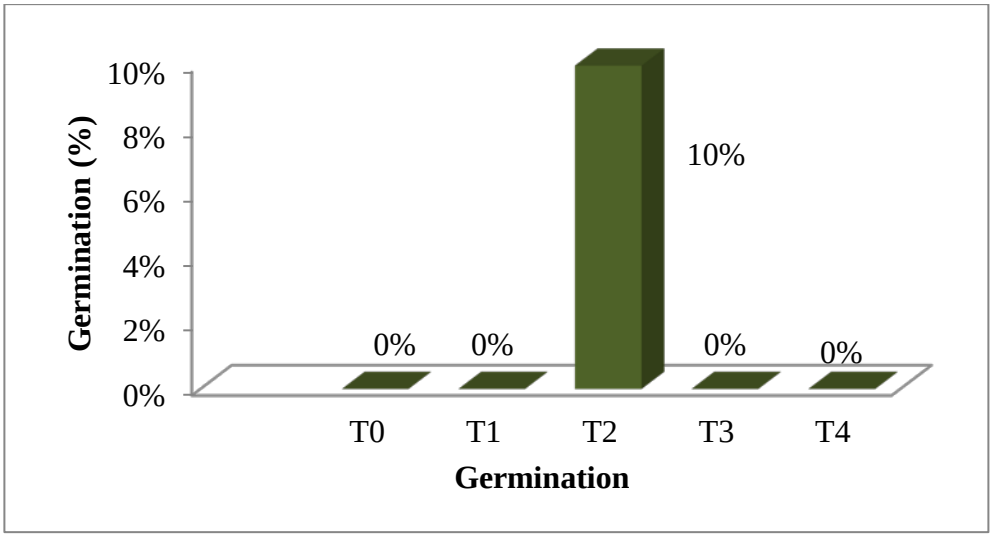

Figure 4.The germination percentages of winged bean seeds in HCl treatments

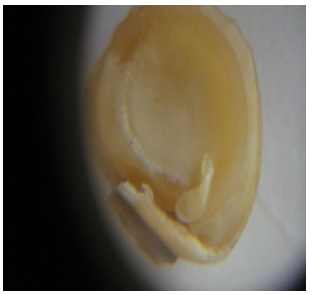

Figure 5. *Psophocarpus tetragonolobus* (L.) DC embryo in Seed

Discussion and conclusions

The local variety winged bean, *Psophocarpus tetrogonolobus* (L.) DC. was germinated in the polyethylene bag containing prepared soil with sand medium. It was observed that germination was found in the sand medium. The medium preparation was consistent with Agrawal (1995) who reported that the seeds were germinated in the seedbeds containing sand.

Among the treatment of hot water, cold water, HCl and H_2SO_4, the highest germination percentage was observed in T_2 (hot water at 60° C for 10 minutes) which gave the germination percentage of 4 % and T_3 (cold water) which gave the germination percentage of 3 % in this experiment. This finding was in agreement with Agboola and Adedire (1998) who reported that a sudden dip of dry seeds in boiling or hot water may lead to the rapture of the seed coat tissues causing physiological changes and subsequent germination of the embryo. These results also agreed with Leopold and Kreidman (1975) who reported that plants that pass through their rest period at low temperature, may have their rest period broken by warm water bath.

Treatment with 10 %, 20 %, 30 % and 40 % HCl was showed that the seeds soaked in 20 % HCl for 10 minutes gave the germination percentage of 10 % in this experiment. This results agreed with Nikoleave (1977) who reported that the immersion of seeds in HCl acid disrupts the seed coat and the fact that it gave 10% of germination percentage within 14 days after sowing. The untreated seeds (control) did not get germination 7 days after sowing which gave a germination percentage is 0 %.

The embryo under dissecting microscope revealed that the presence of mature embryo found in 14 seeds among the 20 seeds. The results did not

agree with Emery (1987) who reported that the dormancy causing factors is immature embryo present in a given seed. In this research embryo is present but the germination rate is very low and cannot germinate in some treatment. The results agreed with Krugman *et al*. (1974) who reported that many seeds have failed to germinate after processing and placement in favorable growing conditions.

In this experiments, germination of winged bean seeds treated with hot water gave 4 % of germination and cold water gave 3 % of germination percentage. The treatments with HCL gave 10 % of germination percentage. The result of various treatment in this study confirms that seed of *Psophocarpus tetragonolobus* (L.)DC, exhibit dormancy. So, further study of *Psophocarpus tetragonolobus* (L.)DC should be carried out on how to improve the variety and seed dormancy inhibition because this plant is a multipurpose plant apart from its medicinal uses and it also has the ability to fix atmospheric nitrogen in soil and the seeds are reported to retain viability for a very long time.

Acknowledgements

The author gratefully acknowledges Dr. Thanda Aye, Professor, Department of Botany, University of Yangon for her comments and editing on this paper.

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Study on the Morphology, Anatomy and Medicinal Uses of Two Monocotyledonous Species in Taungoo Township

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Abstract

Two monocot species from different genus and different families such as Amaryllidaceae and Pontederiaceae were collected in Taungoo Township, Bago Region. The collected specimens were studied in Department of Botany, Taungoo University. Macroscopical characters of plants and microscopical characters of leaves were also conducted in this paper. Morphological characters of vegetative parts and reproductive parts were investigated to ascertain their correct identification according to Bakey (1963), Dassanayake (1985), and Hooker (1879). The anatomical characters of leaf of these two different species were examined according to Esau (1953) and Trees and Evans (2002) and prepared the mount specimens.

keywords: Amaryllidaceae, Pontederiaceae, Macroscopical characters, Macroscopical characters

Introduction

The Amaryllidaceae are mainly terrestrial flowering plants that are herbaceous or succulent geophytes that are perennial, with the exception of four species. Most genera grow from bulbs, but a few such as *Agapanthus*, *Clivia* and *Scadoxus* develop from rhizomes. *Crinum asiaticum* L. is a main plant subtropical and tropical regions of Asia and Australasia, growing mainly near the coast (Website 3).

Pontederiaceae with 6 genera and 28 species is distributed in the tropical and subtropical zones. *Eichhornia crassipes* (Mart.) Solms., (water hyacinth) is originated in tropical South America, but is now naturalized in Africa, Australia, India and many other countries. It is a native of South America, a major freshwater weed in most of the frost-free regions of the world and is generally regarded as the most troublesome aquatic plant. It has been widely planted as water ornamental around the world because of

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its striking flowers. Wherever it has encountered suitable environmental conditions, it has spread with phenomenal rapidity to form vast monotypic stands in lakes, rivers and rice paddy fields.

Then it adversely affects human activities (fishing, water transport) and biodiversity. It is impossible to eradicate, and often only an integrated management strategy, inclusive of biological control, can provide a long-term solution to this pest (Website 4).

The monocotyledons as a group show greater diversity of specialized leaf types. The leaves of this group are not made up of stipules, petiole and leaf blade. In general monocotyledonous leaves are parallel-veined. Most of monocotyledonous leaves are nearly erect and more or less both surfaces usually receive equal amount of sunlight directly. Such leaves are called isobilateral. The internal structure of such leaves is more or less similar to both the upper and the lower halves. The epidermis on either sides contains the stomata and the mesophyll is usually not differentiated into palisade and spongy parenchyma, but consists only of parenchyma cells, having chloroplasts and intercellular spaces among them (Pandey, Dr. B.P., 2011)**.** To investigate the morphological characters (vegetative and reproductive parts) of the two monocot species, to identify these selected monocot species, to examine the leaf anatomy of the monocot plant and to share the medicinal uses of the two plant.

Materials and Methods

These two plant specimens were collected from various parts of the Taungoo Township. Fresh specimens of the vegetative and reproductive parts were collected at the time of flowering periods. The collected plants were identified by Bakey (1963), Dassanayake (1985), and Hooker (1879).

For anatomical studies of the leaves were made on both fresh and preserved specimens. Freehand sections were prepared for microscopic study. Hand sections were cleared by using chloral hydrate solution as a clearing agent. The sections were stained with safranin. The specimens were permanently mounted in Canada Balsam.

Result

Tables 1. Two selected species of Monocot Family in Taungoo Environ

Sr.No	Scientific Name	Myanmar Name	Common Name	Family	Collected Places
1	*Crinum asiaticum* L.	Ko-yan-gyi	Poison bulb	Amaryllidaceae	Near the Kan Taw Gyi
2	*Eichhornia crassipes* (Mart.) Solms.	Be-da	Water hyacinth	Pontederiaceae	Near the Kaung Mu Taw Pagoda

Scientific Name : *Crinum asiaticum* L.

Myanmar Name : Ko-yan-gyi

Common Name : Poison bulb

Family : Amaryllidaceae

Habit; herbs and tall usually with subterranean bulbs. Leaves; liner, radical, generally distichous and elongate sheathing at base, sessile. Inflorescence; umbel like, subtended by an involucres of bracts. Flowers; white, large, umbelled, bracterate broad and membrous, bracteolate, subsessile or shortly pedicelled, bisexual, irregular, zygomorphic, and epigynous. Perianth; salver-form, long and narrow tube, lobes which is longer than the filaments, linear to oblong. Stamens; six, reddish on the throat of the perianth tube; filament free, filiform, shorter than the lobes of the perianth, and white. Anthers; yellow turning purple, linears and versatile. Gynoecium; (three), syncarpous, trilocular, axile placentation; style long, filiform; stigma minute and subcapitates with white papillae. Fruit; capsule irregularly subglobose. Seeds; few, large and rounded.

Medicinal uses; leaves are used in anthracia and swelling toxicity, adenolymphitis, laryngopharyngitis, headache, arthralgia spasm and numbness, falls and bruises, fractures, venomous snake bites.

Squamous bulb: superficial infections, swelling sores, sarcoptidosis, mammary abscess, laryngalgia, toothache, pain of rheumatic joints, injuries caused by falls, fractures, venomous snake bites.


Habit

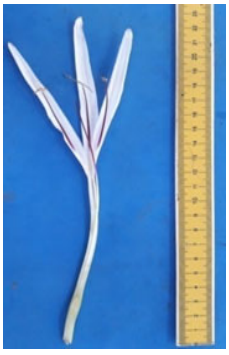

Upper surface of leaves


Lower surface of leaves

Figure 1. Habit and leaves of *Crinum asiaticum* L.


Inflorescence


Flower


L.S of Flower


T.S ovary

Figure 2. Inflorescence, flower and T.S ovary of *Crinum asiaticum* L

Microscopical Characters of *Crinum asiaticum* L.

Lamina

In surface view, the epidermal cell of both surfaces are parenchymatous thin wall and polygonal shape. The anticlinal wall of both surfaces are straight. There are stomata on both surfaces and more abundant in the lower surface. Some stomata are paracytic type, ellipsoidal in outline. The guard cells are reniform of elliptic with abundant chloroplast.

In transverse section, the cuticle is thick and conspicuous on both surfaces. Both upper and lower epidermal cells are rectangular shape and compactly arranged. Mesophyll cells consist of palisade and spongy

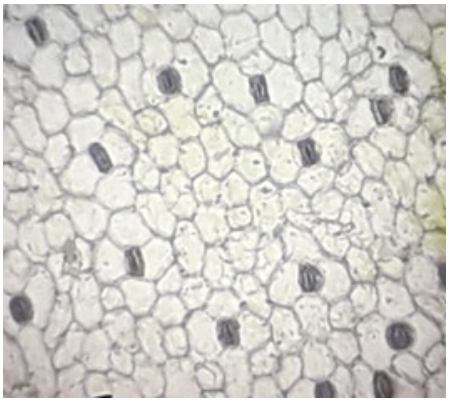
parenchyma. Only one layer palisade cells are found below the upper epidermis. The spongy mesophylls are between palisade and lower epidermis, oval or irregular in shape. Six to eight layers of parenchyma cell and a few numbers of large intercellular spaces are between them.

The numerous vascular bundles are embedded in the mesophyll tissue. Their arrangement is radial and open type. The phloem tissue consists of sieve tube and companion cell. Xylem tissue consists of vessel elements, tracheids , fiber tracheids, fiber and xylem parenchyma.

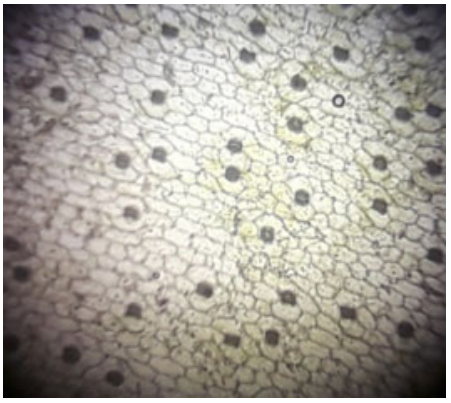
Midrib

In the midrib region, abaxial and adaxial surface are straight. Both surfaces of epidermal cells are thin wall parenchymatous, rectangular in shape. Stomata are in both surfaces. Air chambers are extending from the lower to upper epidermis.

The large vascular bundle is situated in center and radial types. The phloem tissue consists of sieve tube and companion cells. The xylem tissue consists of vessel elements, tracheids, fibre tracheid fiber and xylem parenchyma



Upper epidermis



Lower epidermis

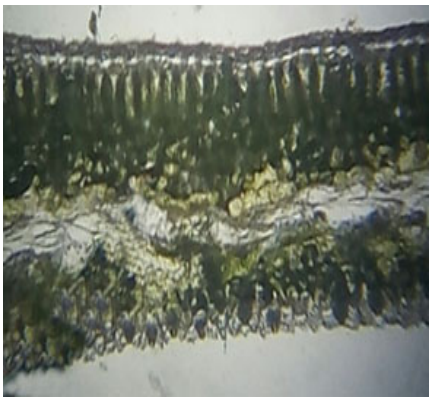
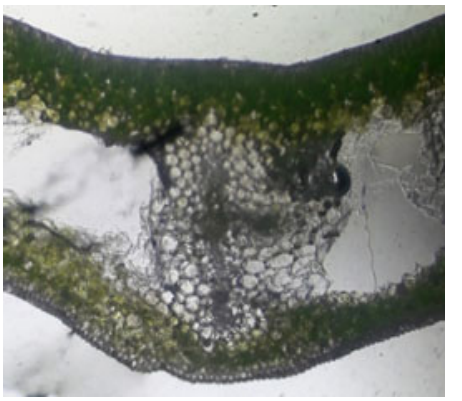



Figure 3. Anatomical characters of leaf (*Crinum asiaticum* L.)

Scientific Name : *Eichhornia crassipes* (Mart.) Solms.

Myanmar Name : Be-da

Common Name : Water Hyacinth

Family : Pontederiacea

Habit; free floating aquatic perennial with pendent roots. Stem; very short producing a cluster of fibrous roots, and stolons which can produce new plants at their apices. Leaves; rosettes and simple, whorl, thick, exstipulate, inflated spongy petiolate, thick and leathery. Inflorescence; pseudoraceme, with 6-12 flowers are all opening together bract-broadly sheathing with translucent. Flowers; arranged in a panicle, ebracteate, ebracteolate, sessile, complete, bisexual, irregular, zygomorphic, cyclic, hypogynous, showy lilac in colour. Perianth (3+3), syntepalous, tube long and curved; lobe pale mauve with a brightly yellow blotch at center surrounded by blue patch. Stamens; 3+3, apostemonous, tepalostemonous, filament; three long three short, adnate to the perianth tube at different level, anther; dithecous, introrse, basifixed, longitudinal dehiscence and inferior. Gynoeciu; (three), syncarpous, trilocular, axile placentation, many ovules in each locule, style; long, white and glardular hairy, stigma; densely hairy, superior. Fruit; dry, membranous, loculicidally capsule. Seeds; small and ovoid.

Medicinal use; the decoction of the whole plant is used to cure blood disorders, emaciation, and weakness. The decoction is used to treat goiter. However, along with this decoction, a poultice is prepared from the crushed plant and applied onto the enlarged part of the neck

Habit

Figure 5. Habit of *Eichhornia crassipes* (Mart.) Solms.

Habit

Upper surface of leaves

Lower surface of leaves

Figure 6. Habit of *Eichhornia crassipes* (Mart.) Solms.

Inflorescence

Flower

L.S of Flower

T.S ovary

Figure 7. Inflorescences, Flowers and T.S ovary of *Eichhornia crassipes* (Mart.) Solms.

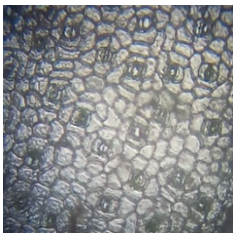
Microscopical Characters of Leaf of *Eichhornia crassipe* (Mart.) Solms.

Lamina

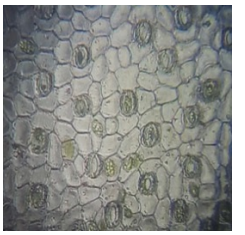
In surface view, the epidermal cells of both surfaces were parenchymatous, compact, thin wall and irregular in shape. The anticlinal walls of both surfaces are straight. The stomata are in both surfaces, more abundant on the lower surface. The stomata are tetradiacytic type. Chloroplasts are abundant in both surfaces.

In transverse section; epidermis layers are found in both surfaces and cutical are present. The stomata are confined to both epidermis layers. The sub stomata chambers are seen in vertical section. The mesophyll tissue is clearly differentiated in palisade and spongy. Numerous intercellular spaces are present. Large air spaces are present.

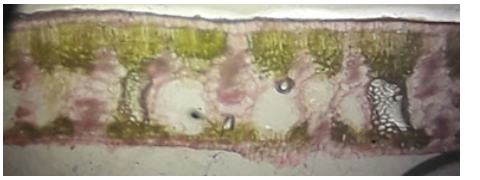
The vascular bundles are collateral and close type. Xylem occurs toward upper surface and phloem toward lower surface. Each bundle remains surrounded by a bundle sheath consisting of thin wall parenchyma cell.



Upper epidermis



Lower epidermis



T.S of lamina

Figure 8. Anatomical characters of leaf *Eichhornia crassipes* (Mart.) Solms

Discussion and Conclusion

The genus *Crinum* is about 120 species, tropical and subtropical, widely dispersed in dry and maritime regions. Some species are cultivated for ornament. Flowering more or less throughout the year, flowers usually open in the evening with strongly fragrant (Dassanayake, M.D. 1985). *Crinum asiaticum* L. is an evergreen, perennial plant producing a rosette of fleshy leaves growing from an underground bulb. The plant is harvested from the wild for local medicinal use. It is commonly cultivated as an ornamental plant in tropical areas, being valued especially for its showy flowers (Website 3).

Water hyacinth (*Eichhornia crassipes* (Mart.) Solms.) is a free-floating perennial aquatic plant (or hydrophyte) native to tropical and sub-tropical in South America. It is vigorous growers and mats can double in size in two weeks (Website 4). According to Dassanayake, M.D. (1985), the most distinctive features of this plant are its greatly inflated spongy petioles which allow it to float on the surface of water and perianth lobes pale mauve with bright yellow blotch at the centre surrounded by blue patch, marked with distinct oblique veins.

In the two specimens of different monocot family, epidermal cells of both surfaces are parenchymatous, compact, straight wall and regular. The shape of upper epidermal cells are polygonal and compactly arranged in

Crinum asiaticum L., but in *Eichhornia crassipe* (Mart.) Solms.is irregular in shape. Stomata of the two species are present in both surfaces but abundant on the upper surface and rare in lower surface. Types of stomata are paracytic in *Crinum* while tetradiacytic in *Eichhonia*. In transverse section, cuticles are present in both surfaces of the two plants. Types of vascular bundle are collateral and close types in *Eichhornia crassipe* (Mart.) Solms although *Crinum asiaticum* L. has radial and open typed. These anatomical characters agree with the reported by Metcalfe C.R and Chalk 1950, Pandey S.N. 2004 , Trees and Evans 2002 and Katherine Esau 1953.

The distinguishing morphological characters which include leaf, inflorescence and flowers are found to be useful in the identification. These plants are in the treatment ofanthracia and swelling toxicity, adenolymphitis, laryngopharyngitis, headache, arthralgia spasm and numbness, falls and bruises, fractures, venomous snake bites.swelling sores, sarcoptidosis, mammary abscess, laryngalgia, toothache, pain of rheumatic joints, injuries caused by falls, fractures, venomous snake bites.blood disorders, emaciation,and weakness.

It is hoped that the results of this paper will be of some value in future research.

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We would like to express our deepest gratitude to Dr. Myat Myat Moe (Professor and Head, Department of Botany, Dagon University) for her permission to use the departmental facilities during our research. It is an honour to express a word of thank to Dr. Than Than Nu (Professor and Head, Department if Botany, Taungoo University) for her kind permission to study our research paper and for their valuable suggestion.

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Botanical and Phytochemical Studies of *Boerhavia diffusa* L.

Yin Yin Khaing[1], Yin Yin Sint[2] & Myat Myat Moe[3]

Abstract

This paper is about the botanical study on *Boerhavia diffusa* L. belonging to family Nyctaginaceae naturally grown wild throughout Myanmar, especially at the sandy beach of streams. The plants were collected from Kamayut and Tamwe Townships, in Yangon Region. The collected plants were studied, classified, and identified to ascertain their correct identification. In morphological study, the plants were perennial herbs, erect, ascending and woody stem. Microscopical characters were also conducted by free hand section method according to Pandey. In microscopical study, the cells of upper and lower epidermal cells of leaves were wavy to polygonal in shape. Stomata were present on both surfaces and anomocytic type. The vascular bundles of midrib and petiole were collateral and closed types. The preliminary phytochemical tests were also investigated from the powdered leaves of *Boerhavia diffusa* L. according to the methods Marini-Bettelo. The presence of alkaloids, glycosides, saponins, tannins, flavonoids and terpenes were mostly found in phytochemical study.

Keywords: *Boerhaavia diffusa*, creeping, phytochemical

Introduction

Myanmar has a long traditional of medicinal practice using indigenous plants since historical time in taking care of health of the nation. In this research, the plants *Boerhavia diffusa* L. were studied. The plant *Boerhavia diffusa* L. is known in English as “Hog-weed” or “Horse Purslane”, and in Bengali as “Punarnaba” or “Sweet Panarbaha” or “Gadha purna”. The genus Boerhavia belongs to the tribe Boerhavieae of the family Nyctaginaceae (Nilar, 1995).

Nyctaginaceae family has 28 genera and about 250 species, distributed mostly in tropic and subtropic of both hemispheres but only 3 genera indigenous to the old world (Lawrence, 1964).

Boerhavia diffusa L. is found in all warm or tropical countries, possibly originating from the old world tropics. Three *Boerhavia* species

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occur widely in Malesia: *Boerhavia diffusa* L. and *Boerhavia ereta* L. are pantropical, occurring between 35°N and 40°S, while *Boerhavia chinensis* (L.) Rottb. occurs only in the old world tropic. In Malaysia, *Boerhavia diffusa* L. is used medicinally on a small scale, mainly as a diuretic. In India, the whole herb is a very popular medicine, called "Punarnava" and the root has entered the Indian Pharmacopoeia. *Boerhavia diffusa* L. is applied as a stomachic, cardiotonic, hepatoprotective, laxative, diuretic, anthelmintic, febrifuge, expectorant and in higher doses, as an emetic and purgative (Bunyapraphatsara and Valkenburg, 2002).

A number of pharmacological actions had been demonstrated for plant extracts including inhibition of increased serum aminotransferase activity in arthritis animals and an increase in liver ATP phosphorhydrolase activity (Trease & Evans, 1978).

The leaves are cooked and eaten as vegetable. The root and leaves are considered to have an expectorant action to be emetic and diuretic in large doses and are used in the treatment of asthma (Bharathi, 2014).

In this paper, morphological and microscopial characters of fresh specimens, preliminary phytochemcial tests of the dried powdered leaves of *Boerhavia diffusa* L., were carried out. The aim of this paper was the present study to identify the plant of *Boerhavia diffusa* L., to examine the microscopical characters of leaves and to investigate the phytochemical tests of the leaves.

Materials and Methods

Botanical Studies

1. Collection and Identification

For Botanical Studies, the specimens of *Boerhavia diffusa* L. were collected from Kamayut and Tamwe Townships, in Yangon Region. After collection, the specimens were identified with the help of available literatures Hooker (1885), Kirtikar and Basu (1935), Dutta (1979), Dassanayake (1999), Pandey (2004). Both the vegetative and reproductive parts of the fresh specimens were used for the morphological and microscopical studies.

For microscopical studies, lamina, midrib and petiole were examined by preparing free hand sections from the fresh specimens,

according to the methods by Metcalfe and Chalk (1950), Esau (1965), Trease and Evans (1978) and Pandey (2004).

The samples were washed and dried at room temperature and then crushed into powder to study the powdered characteristics. Chloral hydrate solution was used as a clearing reagent. The presence of calcium oxalate crystals and prisms were tested by 80% sulphuric acid, acetic acid (BP) was used to distinguish between calcium carbonate and calcium oxalate crystals. Solution of phloroglucinol with concentrated hydrochloric acid was tested for lignin.

2. Chemical Studies

For Chemical Studies, the preliminary phytochemical study on the leaves of *Boerhavia diffusa* L. has been undertaken. The experiment was carried out to determine the presence or absence of alkaloid, α -amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, starch, steroids, terpenoids and tannin, according to the method of British Pharmacopoeia (1968) and Marini Bettalo et.al, (1981).

Preliminary phytochemical test

For preliminary phytochemical investigation, the collected plant parts were washed repeatedly with tap water and finally washed with distilled water. Then, they were shade dried and powdered with help of grinder and stored in air tight container for chemical analysis.

Extraction

3 gm of dried powdered leaves of *Boerhavia diffusa* L. was extracted with 100 ml of two different solvents ethanol and aqueous respectively.

Results

Botanical Studies

Morphological characters of *Boerhavia diffusa* L. (Figure 1, A - F)

Scientific name : *Boerhavia diffusa* L.

Myanmar name : Payan-nawa

English name : Hogweed, Pigweed

Family : Nyctaginaceae

Part used : Leaves

Flowering and fruiting period : August- December

Distribution : Wildly grows throughout Myanmar, especially at the sandy beaches of streams.

Perennial herbs; stems woody, branched, spreading on the ground, nodes swollen, greenish purple, fracture tough and hollow in the centre. Leaves simple, opposite, the opposite pair unequal at each node, exstipulate, petiolate, the upper surface puberulent, green, the lower surface glabrous, slivery white. Inflorescence terminal and axillary, umbelloid cymes, dichasial cymes, the cymule 1- 3 flowered. Flower very small, purple in colour, bracteates, ebracteolate, pedicellate, complete, bisexual, actinomorphic, regular, hypogynous. Perianth lobe fused, campanulate and purple in colour. Stamens 2, filament long, anthers dithecous. Ovary globose, 1- carpelled, 1- loculed, the ovule solitary and basal placentation, the style inserted, purple, the stigma capitate, superior; Fruit an achene, obscurely obovoid, 5 prominent ridges and grooves on the surface, globose viscid glandular hairs dense in furrows.

Microscopical Characters of leaves of *Boerhavia diffusa* L.

Petiole (Figure 2, A & B)

In surface view, the epidermal cells were parenchymatous, thin-walled and mostly rectangular in shape and elongated along the length of the petiole. Trichomes were abundant and stomata were absent.

In transverse section, the petiole was cordate in outline and a deeply “v” shaped notch on the upper side and prominently rounded on the lower side. The cuticle layer was thick. The epidermal cells were rounded in shape. Hairs consisting of thick or thin-walled multicellular of trichomes were present. The cortex was made up of two different types of tissues, collenchymatous and parenchymatous tissues. The collenchymatous type tissues consisted of 1 to 2 layers in thickness. The parenchymatous tissues composed of 6 to 8 layers in thickness. The parenchyma cells were oval to isodiametric in shape. Prismatic and solitary crystals of the calcium oxalate were present in the cells.

The vascular bundles were more or less rounded in outline and embedded in the parenchymatous tissues. Vascular bundles were arranged in crescent shaped, collateral and surrounded by a bundle sheath.

Lamina (Figure 2, C - E)

In surface view, the epidermal cells of both surfaces were parenchymatous cells, thin-walled and compactly arranged. Anticlinal walls of the lower surface were wavier than the upper ones to polygonal in shape. Stomata were slightly present on upper surface and abundant in lower surface. They were anomocytic type. Stomata were elliptic in shape with very small pores; guard cells were reniform shape with chloroplast. Multicellular trichomes were present on both surfaces. In transverse section, the lamina was dorsiventral smooth cuticle present on both surfaces. The upper epidermal cell was one layer on both sides, rectangular in shape and the lower epidermal cells were barrel shaped. The mesophyll composed of palisade and spongy parenchyma. The palisade mesophyll was made up of single layer of vertically elongated cylindrical cells, which were closely packed with one another compactly arranged. The spongy mesophyll consisted of 4-5 layers of cells, irregular to isodiametric shape and loosely arranged. Solitary and prismatic crystals of calcium oxalate were present in mesophyll cells.

The vascular bundle of lateral veins consisted of xylem always lying towards the inner side and phloem always towards the outer. This arrangement was collateral and closed type. The phloem cells were very small.

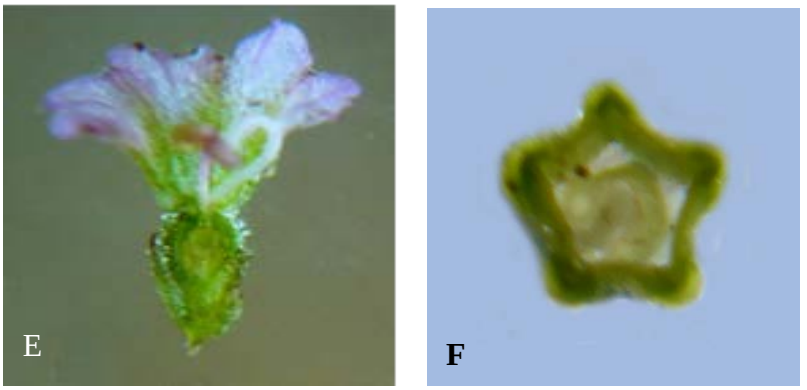
Midrib (Figure 2, F & G)

In surface view, the epidermal cells were parenchymatous and compactly arranged and irregular in shape. Multicellular trichomes were present.

In transverse section, convex at both sides covered with thick cuticle. Both epidermal cells were rounded shaped. Below the epidermis, the cortex was differentiated into collenchyma and thin- walled parenchyma cells. The collenchymatous cells were 2-3 layers in thickness towards the upper surface and 3-4 layers in thickness towards the lower surface. They were rounded to isodiametric in shape. The parenchyma cells were 3 to 4

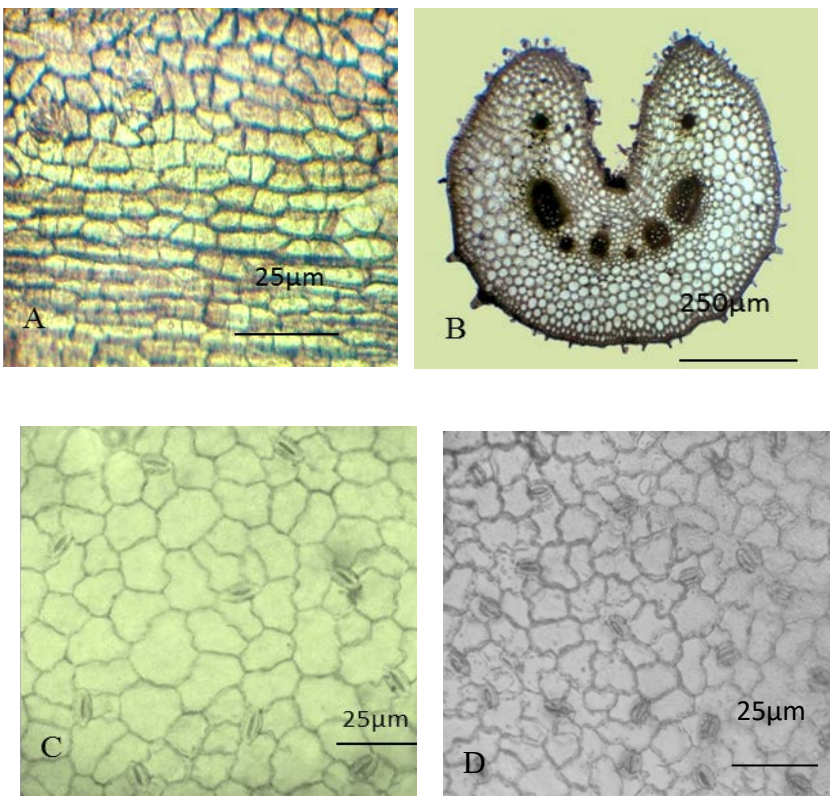
layers in thickness above the vascular bundle and 5 to 6 layers in thickness below the vascular bundle. They were thin- walled and irregularly rounded or oval in shape. Intercellular spaces were numerous, solitary and prismatic crystals of calcium oxalate were present in both parenchymatous cells.

The vascular bundle was rounded or oval in outline, collateral and closed type. The four vascular bundles are embedded with an arc of centrally and the smaller one is located at its upper side of midrib.



(A) Flowers
(B) Various size of Leaves
(C) Inflorescence
(D) Flowers
(E) L.S of Flower
(F) T.S of Ovary

Figure 1. Morphological characters of *Boerhavia diffusa* L.



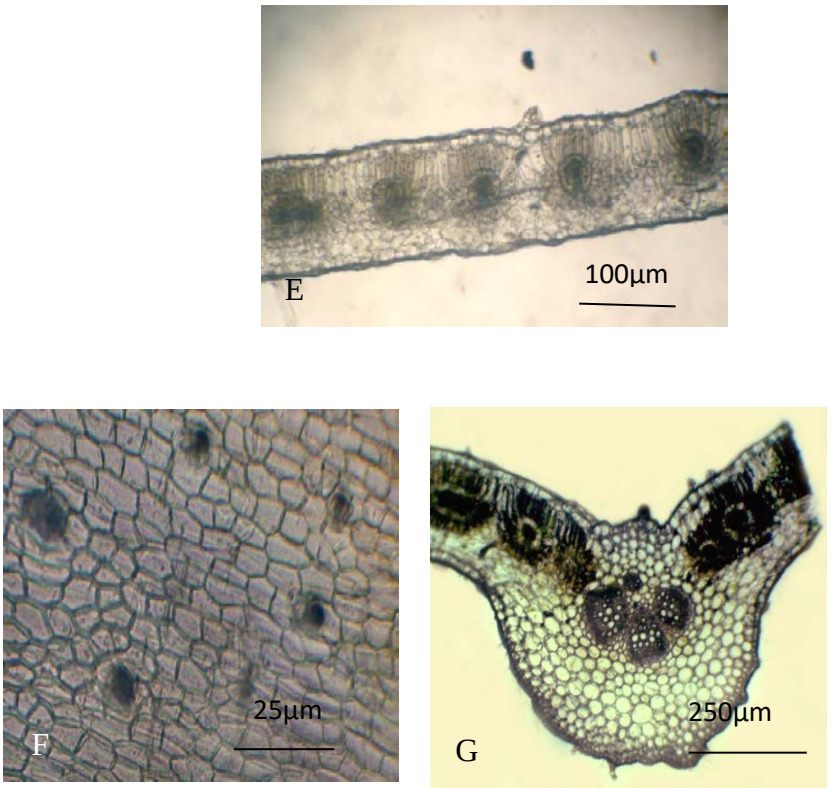


Figure 2. Microscopical characters of leaves of *Boerhavia diffusa* L.

(A) Surface view of Petiole
(B) T.S of Petiole
(C) Upper surface view of Lamina
(D) Lower surface view of Lamina
(E) T.S of Lamina
(F) Surface view of Midrib
(G) T.S of Midrib

Chemical Studies

Preliminary phytochemical test of leaves of *Boerhavia diffusa* L.

The preliminary phytochemical test of the leaves of *Boerhavia diffusa* L. indicated the presence of alkaloid, glycosides, saponins, terpenoids and steroids are present in ethanol extract and starch, reducing sugar, α-amino acids, carbohydrate and phenolic compound are absent in both extracts (Table 1).

Table 1. Results of Preliminary phytochemical test

Tests	Extracts	Reagent	Observation	Results
Alkaloids	D/W	Mayer's reagent	Foaming	-
	Ethanol		White ppt	+
	D/W	Dragendroff's reagent	Foaming	-
	Ethanol		White ppt	+
α-amino acid	D/W	Ninhydrin reagent	Foaming	-
	Ethanol		Nochange	-
Carbohydrate	D/W	10 %, α-napthol +H2SO4 (conc:)	Foaming	-
	Ethanol		Dark green	-
Phenolic compounds	D/W	4%$FeCl_3$, solution	Brown	-
	Ethanol		Dark brown	-
Reducing sugars	D/W	Benedict's solution	Foaming	-
	Ethanol		White ppt	-
Starch	D/W	I_2 solution	Foaming	-
	Ethanol		Light green	-
Steroids/ Terpenoids	D/W	H_2SO_4	Brown ppt	-
	Ethanol		Blue green	+
Flavonoids	D/W	Mg/HCl(conc:)	Brown	+
	Ethanol		Blue green	-
Glycosides	D/W	10% lead acetate solution	Brown	+
	Ethanol		Yellow ppt	+
Saponins	D/W	Distilled water	Foaming	+
	Ethanol		Green	-
Tannins	D/W	1%$FeCl_3$ solution	Foaming	-
	Ethanol		Yellowish ppt	+

(+) = present ; (-) = absent

Discussion and Conclusion

In this paper, the morphological studies on both vegetative and reproductive parts of the plants, the microscopical studies of leaves and phytochemical study of *Boerhavia diffusa* L. have been undertaken.

In morphological study, the plants of *Boerhavia diffusa* L. are perennial herbs, creeping or climbing herb branched; stem woody, and spreading on the ground. The leaves are simple, opposite pair unequal at each node, exstipulate, the upper surface puberulent, and the lower surface glabrous. The inflorescences are axillary, umbelloid cymes. The flowers are small, purple in color, bracteates, ebracteolate, actinomorphic, bisexual and pedicellate. Perianth lobe is fused, campanulate and purple in color. The stamens are two, filament unequal and anther dithecous. Ovary is superior, ovary globose, 1-carpelled, 1- loculed, the ovule solitary and basal, purple, the stigma capitates. Fruits are small, ovoid, one seeded nut, achene and viscidly glandular. These characters are in agreement with those mentioned by Hooker (1885), Kirtikar and Basu (1935), Hutchinson (1967) and Dassanayake (1999).

In the microscopical study, multicellular trichomes are present on both surfaces of the leaves. Stomata are rare on upper surface and abundant on the lower one. They are anomocytic type. In transverse section of the lamina, cluster small of crystals are found in the upper ends of the palisade cells. In transverse section of petiole, the vascular bundles are arranged as a curved line. These characters are in agreement with those stated by Metcalfe and Chalk (1950) and Pandey (2004).

Preliminary phytochemical tests of the plants from two extracts such as Distilled water and Ethanol indicated that the leaves of *Boerhavia diffusa* L. contain alkaloids, flavoid, saponin, glycosides, tannins, steroid and terpenoid. These characters are in agreement with those described by Kirtikar and Basu (1935) and Mohammed (1994).

Boerhavia diffusa L. plant is diuretic, useful in nephritic syndrome chronic oedema and liver diseases. The plant is used as a maintenance drug in abdominal tumors and cancer. The leaves are diuretic, used in oedema and dropsy and in cases of ascites especially due to liver, peritoneal and kidney condition. The leaves are diuretic, used in oedema and dropsy and in cases of ascites especially due to liver, peritoneal and kidney conditions Mohammed (1994). In conclusion, the species of *Boerhavia* include many

chemical constituents which are used for medicinal purposes. Furthermore, *Boerhavia diffusa* L. is used as vegetables in the world. Hence, the members of the family Nyctaginaceae are not only the medicinal plants but also the economic ones. Therefore, it is sincerely hoped that this present study can be beneficial for the future researchers.

Acknowledgements

I would like to express my sincere gratitude to Dr. Thar Htun Maung, Rector, Dagon University for kindly allowing me to make use of research facilities. I would like to express deepest gratitude to Professor Dr. Seine Nyoe Nyoe Ko, Professor and Head, Department of Botany, Dagon University, for reviewing the manuscript.

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Study on Inoculation of *Pleurotus ostreatus in vitro* (Ngwe-moe-Hmo)

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Abstract

Oyster mushroom is one of the most widely grown mushrooms worldwide. Oyster mushroom was collected from Htukyan Township, Yangon Division. This research focuses on the optimization of agar medium (using the conventional potato dextrose agar medium PDA). Tissue culture was raised separately from different growth stages 6days (button stage), 9days (stipe with small pileus), 12days (stipe with well-differentiated pileus), and 15days (mature fruiting body) of Ngwe-moe-Hmo (*Pleurotus ostreatus*) and their effects on growth and yield were investigated. After two days, the growth of mycelium is started to check for 3 weeks. Potato dextrose agar media records a value of 9 days in stipe of small pileus.

Keywords: *Pleurotus ostreatus*, PDA media

Introduction

Oyster mushrooms (*Pleurotus* sp.) belongs to the class Basidiomycetes and family Pleurotaceae. The oyster mushroom is widespreads in many temperate and subtropical are as throughout the world. The earliest record for *Pleurotus* cultivation in India appears to be the Bano and Srivastava (1962). *Pleurotus* is one of edible mushrooms which can be cultivated in the tropics. *Pleurotus ostreatus* is the second most cultivated edible mushroom worldwide after *Agaricus bisporus*. (Chang and Mile 2004). *Pleurotus ostreatus* is sometimes referred to as the tree oyster mushroom or the grey oyster mushroom to differentiate it from other species in the genus. *Pleurotus ostreatus* is an edible species, it requires a short growth time in comparison to another edible mushroom. The common name “oyster mushroom” comes from the white shell-like appearance of the fruiting body. The fruiting bodies of this mushroom are distinctly shell or spatula-shaped with different shades of white, cream, grey, yellow, pink, or light brown depending upon the species. *Pleurotus ostreatus* is a rich source

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of proteins, minerals (Ca, P, K, Fe, and Na) and vitamin C, B-complex (thiamine, riboflavin, folic acid, and niacin) (Caglarismak, 2007). *Pleurotus ostreatus* contain high potassium to sodium ratio, which makes mushroom an ideal food for patients suffering from hypertension and heart disease. Growing oyster mushrooms *Pleurotus ostreatus* are less expensive productive technology and simple as well. Tissue culture is a simple method for obtaining the mycelial culture and considered important as a mushroom clone. There are different methods but basic methods for removing sterilely a cap, stem, or a piece of mushroom and place in an agar plate. PDA (potato dextrose agar) is the simplest and the most popular medium for growing mycelia of most cultivated mushrooms. Culturing of tissue and production of spawn are initial steps for the production of mushroom. It essentially involves the preparation of pure culture of mushroom from tissue that is generally maintained on an agar medium, followed by culturing on sterilized multiple grains. The aims and objectives are to study the mycelial growth of the edible mushrooms on different stages, to apply in commercial production of mushrooms from the mycelium for further research.

Materials and Methods

Collection of mushroom

Oyster mushrooms were collected from Htukyan Township, Yangon division. After collecting the mushrooms, they were identified and classified according to Krleger and Shaffer (1967) and Svrcek (1998).

Selection of mushrooms for culture

6days = button
9days = stipe with small pileus
12 days = stipe with well differentiated pileus
15 days = Mature fruiting body

Figure 1. Different stage of Oyster mushroom

Culture media preparation by the method of Singh *et al*., 2004

Material required 39 g of commercial PDA powder and 1000 ml distilled water, stirred, beakers, aluminum pot, test tubes, cotton wool, paper, rubber bands, and autoclave. All the solutions were continuously stirred by a porcelain stirrer with regular speed until completely dissolved. The hot solutions were poured into cleaned test tubes about one-fourth of each test tube. Each test tube was plugged with cotton wool and covered with a piece of paper (3 cm in diameter). The plug has to be plugged tightly to keep the cotton stuffing in place and to prevent wetting. After covering, the bottles were tied together by the rubber bands. Then, the bottles were placed in the up position in an autoclave at 15 psi and 121^{o}C for 15 minutes to ensure complete sterilization. After sterilizes had cooled down without opening the lid for the surface area of the medium, the solution should come close to the neck but must not touch the cotton plug. After the PDA medium had a slant position, it was left over to solidify.

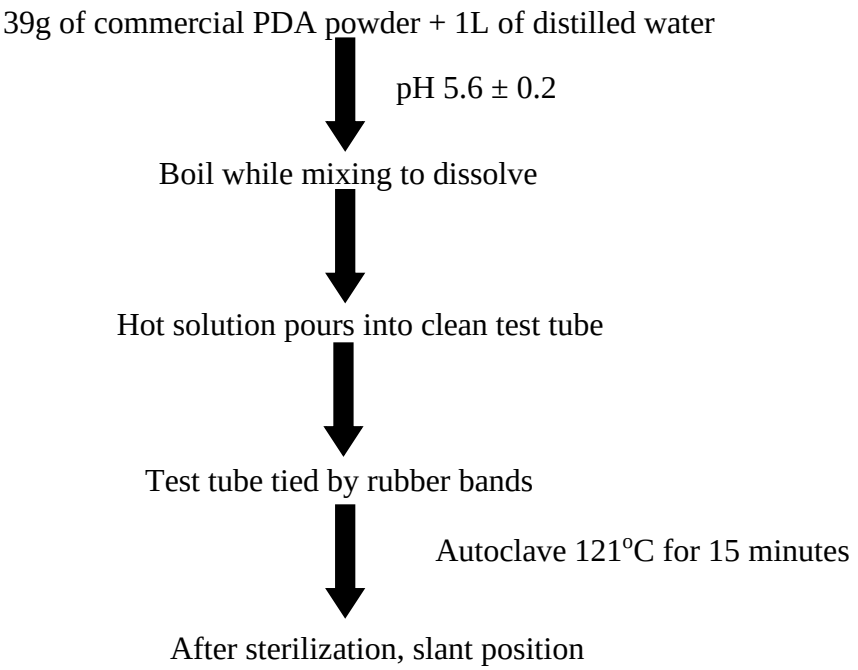

Figure 2. Flow Chart of PDA medium

Figure 3. Culture media preparation

Procedure of mushroom tissue culture

The Fruit body of the mushroom (6 days, 9 days, 12 days, 15 days) was collected. The mushroom was surface sterilization with 70% alcohol for about one minute. The selected mushroom was cut by a sterilized knife and a small piece of internal tissue the between the cap and the stalk of the mushroom was removed. They are PDA media. After the inoculation, the mouth of the test tubes was immediately flamed and plugged with cotton wool. After the tissue had been placed in the agar medium, the bottle was placed in an incubator at 27-31^{o}C for mycelium growth. During this time, the mycelium growth rate was recorded every day.

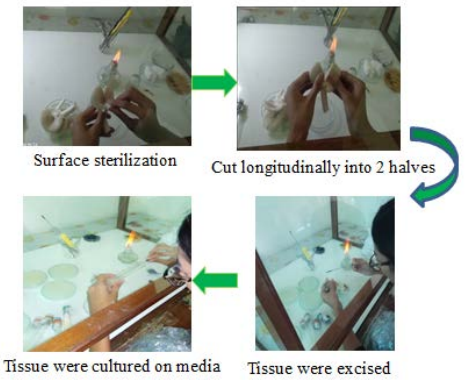

Figure 4. Producer of mushroom tissue culture

Culture conditions

All culture were inoculated at (20 ± 30°C) in a dark room and (65 – 70%) relative humidity (Varshey, 2007).

Recording mycelium growth

In case of solid media, the growth was recorded at different intervals depending on the rate of growth of the fungus. The growth was measured by taking average of linear growth of the colony in two directions at right angle and the morphological characters were also recorded.

Result

Morphological study

After collecting of the mushroom, they were identified according to Krieger and Shaffer (1967) and Svrcek (1998).

Classification of Oyster mushroom

Kingdom	:	Fungi
Division	:	Basidiomycota
Sub-division	:	Eumycota
Class	:	Agaricomycetes

Order	:	Agaricales
Family	:	Pleurotaceae
Genus	:	*Pleurotus*
Species	:	*Pleurotus ostreatus* (Jacq.) P. Kumm
Common name	:	Oyster mushroom
Local name	:	Ngwe-moe-hmo

Morphological characters of Oyster mushroom

Habit	:	Grows solitary or tufted
Pileus	:	convex at first, then becoming plane or uplifted with age, broadly depressed, top view orbicular to flabelliform, surface dull, moist, smooth, glabrous, grayish when immature, then discolored to pale grey, flash white, thin, taste pleasant.
Gill	:	Short or medium, decurrently forked and branched, whitish to grayish; margin and face smooth.
Stipe	:	lateral, occasionally eccentric or central, surface whitish, smooth; flesh solid, white, and fibrous.

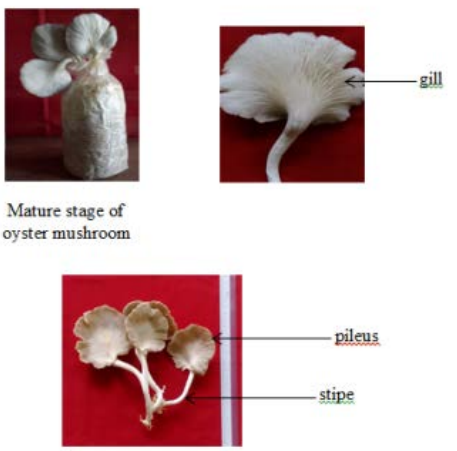

Figure 5. Morphological character of oyster mushroom

Effect of Potato Dextrose Agar (PDA) medium on mycelium growth

In Potato Dextrose Agar (PDA) medium, mycelium started on the entire inoculated tissue, 6 days (button stage), 9 days (stipe with small pileus), 12 days (stipe with well differentiated pileus) and 15 days (mature fruiting body).

In two, days, button stage is 0.10cm, stipe with small pileus is 0.50cm, stipe with well differentiated pileus is 0.10cm, and mature fruiting body is 0. In three days, button stage is 0.13cm, stipe with small pileus is 1.89cm, stipe with well differentiated pileus is 0.20cm, and mature fruiting body is 0.42cm. In four days button stage is 0.19cm, stipe with small pileus is 2.34cm, stipe with well differentiated pileus is 1.89cm, and mature fruiting body is 1.79cm. In five days button stage is 0.26cm, stipe with small pileus is 3.56cm, stipe with well differentiated pileus is 2.67cm, and mature fruiting body is 2.76cm. In six days button stage is 0.56cm, stipe with small pileus is 3.90cm, stipe with well differentiated pileus is 3.34cm, and mature fruiting body is 3.00. In seven days button stage is 1.50cm, stipe with small pileus is 4.50cm, stipe with well differentiated pileus is 3.99cm, and mature fruiting body is 3.89. In eight days button stage is 2.66cm, stipe with small pileus is 5.56cm, stipe with well differentiated pileus is 4.19cm, and mature fruiting body is 4.07cm. In nine days button stage is 3.83cm, stipe with small pileus is 6.05cm, stipe with well differentiated pileus is 5.79cm, and mature fruiting body is 4.87cm. In ten days button stage is 4.34cm, stipe with small pileus is 6.93cm, stipe with well differentiated pileus is 6.02cm, and mature fruiting body is 5.63cm. In eleven days button stage is 4.50cm, stipe with small pileus is 7.35cm, stipe with well differentiated pileus is 6.99cm, and mature fruiting body is 5.98cm. In twelve days button stage is 4.90cm, stipe with small pileus is 7.90cm, stipe with well differentiated pileus is 7.54cm, and mature fruiting body is 6.09cm. In thirteen days button stage is 5.68cm, stipe with small pileus is 8.35cm, stipe with well differentiated pileus is 8.31cm, and mature fruiting body is 6.85cm. In fourteen-day, button stage is 6.30cm, stipe with small pileus is 9.82cm, stipe with well differentiated pileus is 8.92cm, and mature fruiting body is 7.23cm. In fifteen-day, button stage is 7.24cm, stipe with small pileus is 10.00 cm, stipe with well differentiated pileus is 9.01cm, and mature fruiting body is 7.89cm. In sixteen days button stage is 8.15cm, stipe with well differentiated pileus is 9.31cm, and mature fruiting body is 8.00cm. In seventeen days button stage is 8.56cm, stipe with well differentiated pileus is 9.85cm, and mature fruiting body is 8.86cm. In

eighteen days button stage is 9.01cm, stipe with well differentiated pileus is 10.00cm, and mature fruiting body is 9.42cm. In nineteen days button stage is 9.58cm, mature fruiting body is 9.83cm. In twenty days button is 10.00cm, mature fruiting body is 10.00cm.

In these experiments, stipe with small pileus (9 days) results in the full colonization within a shorter period.

Table 1. Average mycelium growth of the different stage of mushrooms in Potato Dextrose Agar media (cm)

Days	Button stage	stipe with small pileus	stipe with well differentated pileus	maturing fruiting body
	(cm)	(cm)	(cm)	(cm)
1	0	0	0	0
2	0.1	0.5	0.1	0
3	0.13	1.89	0.2	0.42
4	0.19	2.34	1.89	1.79
5	0.26	3.56	2.67	2.76
6	0.56	3.9	3.34	3
7	1.5	4.5	3.99	3.89
8	2.66	5.56	4.19	4.07
9	3.83	6.05	5.79	4.87
10	4.34	6.93	6.02	5.63
11	4.5	7.35	6.99	5.98
12	4.9	7.9	7.54	6.09
13	5.68	8.35	8.31	6.85
14	6.3	9.82	8.92	7.23
15	7.24	**10**	9.01	7.89
16	8.15		9.31	8

Days	Button stage	stipe with small pileus	stipe with well differentated pileus	maturing fruiting body
17	8.56		9.85	8.86
18	9.01		**10**	9.42
19	9.58			9.83
20	**10**			**10**

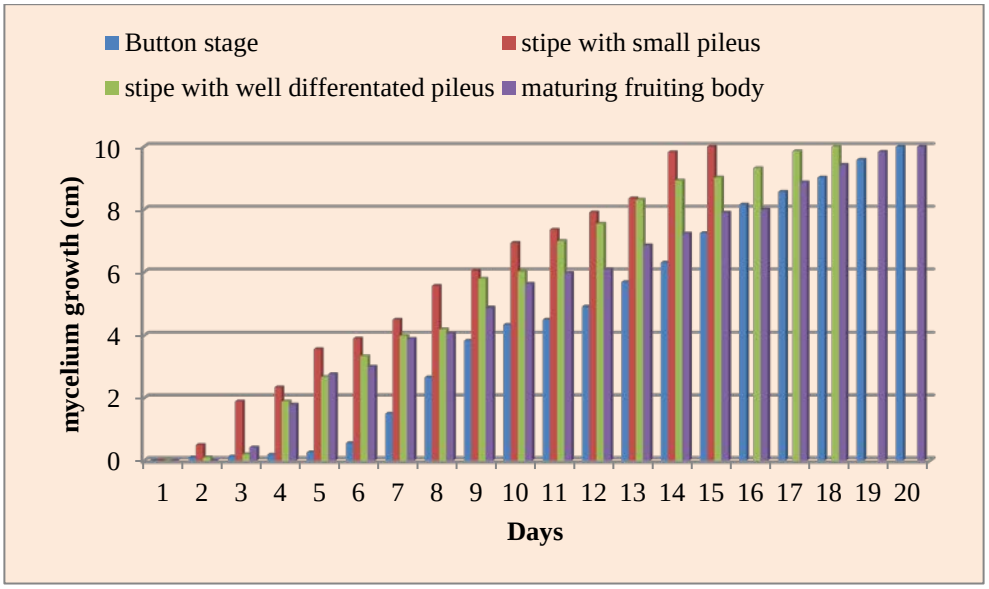

Figure 6. Average mycelium growth of the different stage of mushrooms in Potato Dextrose Agar media

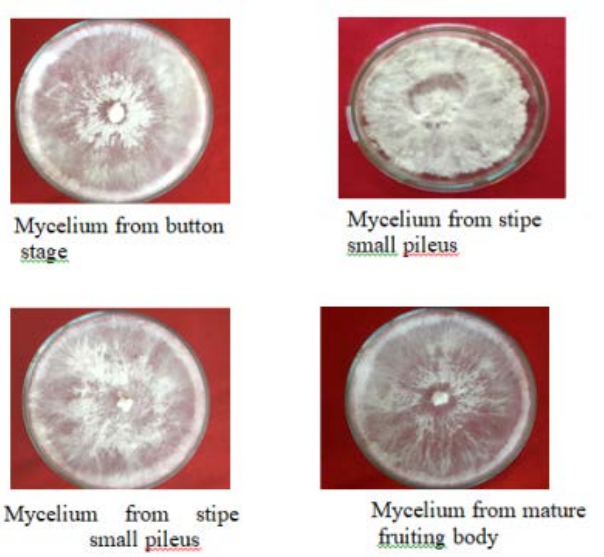

Figure 7. Different stages of mushrooms in Potato Dextrose Agar media (Three weeks old culture)

Discussion and Conclusion

In this study, Oyster (*Pleurotus ostreatus*) mushroom was identified. Oyster mushrooms are grown in tufts or cluster on wood and grown in solitary or in shelf-like clusters. The pileus is convex to broadly, depressed when young or fan-shaped. It agreed with Krieger and Shaffer (1967) and Svrcek (1998). The tissue of these mushrooms was inoculated by pure culture for the growth of mycelium on Potato Dextrose Agar (PDA) medium. For many years, PDA is considered as a standard medium for fungal cell cultivation and usually considered as the first choice for mushroom cell cultivation to support high cell growth.

In this investigation, a stipe with small pileus (9days) in PDA medium showed that mycelium growth of oyster mushroom was the fastest one among the culture of button stage (6days), stipe with well-differentiated pileus (12 days) and mature fruiting body (15days). This finding agrees with Pani (2016) who reported that the higher linear growth was achieved in response to the tissue culture obtained from the mushroom which consisted of the stipe with small pileus in PDA media.

Mushroom cultivation is one of the efficient ways by which residues can be recycled. It may also offer these economic residues as valuable resources and develop new enterprises to use them to produce nutritious mushroom products. Therefore, mushroom cultivation may become one of the most profitable agribusinesses that could produce food products from different substrates and help to dispose of them in an environment-friendly manner.

Acknowledgement

We wish to express my deep gratitude to Rector and Pro-rector from Dagon University for allowing us to use all the facilities available for this research in Dagon University.

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Study on the Isolation and Identification of *penicillium fernandesiae* Against on *Bacillus subtilis*

Naw Nwe Nwe Soe[1] & Moe Pa Pa[2]

Abstract

The soil samples were collected from five different places of Hinthada Township, Ayeyarwaddy Region. These soil samples were used for the isolation of fungi. The isolation of microorganisms is carried out by serial dilution method. Sixteen fungi were isolated from five different soil samples. According to the results of antimicrobial activities test, the fungi NSF-07 showed more active on *Bacillus subtilis* IFO 90571 than other stains. In the screening program of antibacterial metabolite against *Bacillus subtilis*, fungus NSF-10 was selected for further investigations. Based on its morphological-microscopical characters and references key, this fungus NSF-10 was identified as *Penicillium fernandesiae* Barbosa, Motta, Oliveira & Houbraken 2018.

Keywords: Isolation, antimicrobial activity, identification.

Introduction

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis (Kingston, 2008).

Most identification systems for the microorganisms which are based on the morphological, physiological and molecular data have been developed to provide such as a species level identification. Identification of organisms is a critical step in understanding and analyzing biological processes. The ability to recognize and to name the organisms is the important key (Watanabe, 2002 & 2010).

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Materials and Methods

Isolation of fungi from five different soil samples

The five different soil samples were collected from the places of Shwe taung thaya village, Ohm pin su village, Thin ganai village, Taman Oo village and Kokkosu village, Hinthada Township. These soil samples were used for the isolation of microorganisms. The isolation of microorganisms was carried out by serial dilution method. Low carbon agar medium (LCA) and potato glucose agar medium (PGA) were used for the isolation of soil fungi in this study.

Screening for antimicrobial activities of soil fungi by paper disc diffusion assay(Hokkaido Uni., 1988)

The isolated fungi were inoculated on seed medium and incubated at room temperature for 3 days. Twenty mL of seed culture was transferred into the fermentation medium and incubated at room temperature for 5 days. Twenty µL of fermented broth was put on paper disc (8mm) and placed on assay plate containing test organisms. Paper disc having eight millimeter diameter (Advantec, Roshi Kaisha Co. Ltd., Japan) were utilized for antimicrobial assays.

The assay medium (Glucose 10 g, Polypepton 30 g, KNO_3 10 g, Agar 18 g, Distilled water 1000 mL) was used for the antimicrobial activity test. One or two drops of test organism was added to assay medium and then poured into plates. After solidification paper disc impregnated with fermented broth were applied on the agar plates and the plates were incubated for 24-36 hours at room temperature to examine the inhibitory zones.

The test organisms were used in paper disc diffusion assay such as *Agrobacterium tumefaciens* NITE 09678, *Aspergillus paraciticus* IFO 5123, *Bacillus subtilis* IFO 90571, *Candida albicans* NITE 09542 , *E. coli* AHU 5436, *Micrococcus luteus* NITE 83297, *Pseudomonas fluorescens* IFO 94307, *Saccharomyces cerevisiae* NITE 52847, *Salmonella typhi* AHU 7943 and *Staphylococcus aureus* AHU 8465. These test organisms were obtained from NITE (National Institute of Technology and Evaluation, Kisarazu, Japan). **Seed medium** (composition per liter)

Glucose 20 g, Polypepton 3 g, KNO_3 1 g, K_2HPO_4 1 g, pH 6.5

Fermentation medium (composition per liter)

Glucose 20 g, Yeast extract 8 g, K_2HPO_4 1 g, $MgSO_4$ 1 g, $CaCO_3$ 1 g, pH 6.5

Investigation on identification of antibacterial metabolites producing fungus NSF-10

The morphological and microscopical characters of fungus NSF-10 were observed by the methods of Samon and Pitt. 2000 Watanabe, 2002; Dilip, 2003; Asan, 2004; Ando, 2006; Misra *et al.*, 2012 & 2014;Visagie *et al.*, 2013, 2014, 2016 and Leeuwenhoek, 2018.

Microscopical characters of fungus NSF-10 were studied by Camera attached Olympus microscope. Comparison of these characters with reference keys was undertaken to identify according to Watanabe, 2002; Dilip, 2003; Ando, 2006; Misra *et al.*, 2012 & 2014 and Leeuwenhoek, 2018.

For the study of macroscopical characters, PGA, YMEA, GAN, LCA, YSA, MEA, PCA, BMEA and CMA agar media the uses of carbon and nitrogen sources were employed according to Watanabe, 2002; Visagie *et al.*, 2013, 2013, 2014, 2016 and Leeuwenhoek, 2018.

Each plate was inoculated and incubated for 7 days at 15°C, 25°C, 30°C, 35°C and 40°C. Identification for key to species was supported by Prof. Dr. Keiko Natsuaki, Vice-President of Tokyo Agricultural University, Prof. Dr. Kyoko Watanabe, Tamagawa University, Tokyo and Prof. Dr. Nyunt Phay, DG, DM&E (Education) according to Prof. Dr. Tetsuno Watanabe, 2002 & 2010.

Results

Isolation of fungi from five different soil samples

Five different soil samples were collected from Hinthada Township, Ayeyarwaddy Region. The soil texture of N0.1 and No.5 were clay loam. The soil No.2 and 4 were sandy clay loam and No.3 soil was loam soil type. These soil samples were employed for the isolation of microorganism. In this study, sixteen fungi were collected from five soil samples by using serial dilution method. Fungi NSF-01 to NSF-05 were

isolated from soil N0.1. Fungi NSF-06 and NSF-07 from soil N0.2, fungi NSF-08 to NSF-10 from soil N0.3, fungi NSF-11 to NSF-13 were isolated from soil N0.4.Fungi NSF-14 to NSF-16 were isolated from soil N0.5.

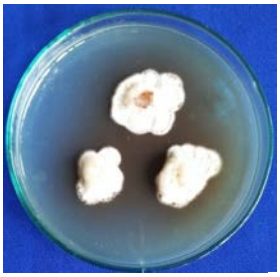
Table 1. Isolated soil fungi from five different soil samples

Soil No.	**Soil type**	**Soil pH**	**Isolated strains**
S-1	Clay loam	5.10	NSF-01,02,03,04,05
S-2	Sandy clay loam	5.89	NSF-06,07
S-3	Loam	5.42	NSF-08,09,10
S-4	Sandy clay loam	5.71	NSF-11,12,13
S-5	Clay loam	5.44	NSF-14,15,16

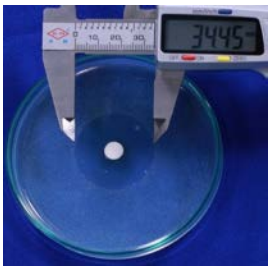
Preliminary study for antimicrobial activities of soil fungi by paper disc diffusion assay (Hokkaido Uni., 1988)

Sixteen fungi were isolated from five different soil samples of Hinthada Township, Ayeyarwaddy. In this study, sixteen fungi were used the antimicrobial activities by using the paper disc diffusion assay.

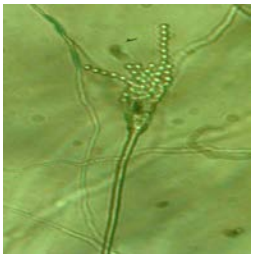
According to the results of antimicrobial activities test, sixteen fungi showed the antimicrobial activities on some test organisms. NSF-01 to NSF-16 more showed the antimicrobial activity against *Bacillus subtilis, Candida albicans* and *E.coli.* Among them, the soil fungi(NSF-10) selected for the identification based on the results of the antibacterial activity for the identification based on the results of the antibacterial activity especially against *Bacillus subtilis.*



NSF-10



Clear zone-34.45 mm on *Bacillus subtilis*



Photomicrograph (X200)

Figure 1. Morphological and Photomicrograph (X200) of fungus NSF-10

Morphological Characters (Colony characters) of fungus NSF-10

PGA, 25°C, 7 days: Colonies cotton like, 1.3-1.6 cm diam, moderately deep, gently radially sulcate; margins entire; mycelium white; colony texture velvety; soluble pigment in shades of red or pink; reverse orange.

MEA, 25°C, 7 days: Colonies cotton like, 1.3-1.6cm diam, convex; margins entire; mycelium white, colony texture floccose; red soluble pigment in shades of pink; reverse orange.

YSA, 25 °C, 7 days: Colonies cotton like, 1.1-1.4cm diam, flat, margins irregular; mycelium white; soluble pigment in shades of red; reverse orange.

Micromorphology: Conidiophores strictly monoverticillate. Stipes smooth walled, 7.5-20 × 1.5-2.0 µm, non-vesiculate. Phialides 4-7 per stipe, ampulliform, 6-11 × 2.0-3.0 µm. Conidia smooth walled, globose, 2-3 µm. Sclerotia or ascomata not observed,

KEY TO GENUS

(Luangsa *et al*., 2011; Perdomo *et a*l., 2013 & 2017; Dan *et al,* 2015)

1.Conidium lacking septum…. ……. …….....Ameroconidium

1.Conidium with1 septum …….....Didymoconidium.

3.Conidium with more than 1 septum and only transverse septa ……

…….....Phragmoconidium

1. Conidium body subdivided by intersecting septa in more than one plane …..…….Dictyoconidium

Ameroconidium

A. Conidiophore not produced

B. Conidiophore not produced or not clear

C. Conidiophores with or without septa develop single, not branched

1. Conidia holoblastic

2. Conidia enteroblastic

i. Phialo-conidium

ii. Multi-phialides with parallel arrangement

- *Paecilomyces*

- *Penicillium*

- *Aspergillus*

- *Purpureocillium*

According to references key,fungus NSF-10 may be the genus of *Paecilomyces, Penicillium, Aspergillus,* and *Purpureocillium*

Table 2. Comparison of Microscopical characters of *Paecilomyces, Penicillium, Aspergillus, Purpureocillium* and NSF-10 fungus

Genus of Fungi	**Distinct Characters**
Paecilomyces *	Phialides are basically swollen, taper towards their apices are slightly apart each other
***Penicillium* ***	**Phialides have thicker apices**
Aspergillus *	Vesicle present
Purpureocillium *	Phialides are basically swollen, taper

Genus of Fungi	Distinct Characters
	towards their apices are slightly apart each other
NSF-10	**Phialides have thicker apices**

KEYS TO SPECIES (Domsch *et al.*, 1993 & 2007; Samon and Pitt. 2000 Watanabe, 2002; Dilip, 2003; Asan, 2004; Ando, 2006; Misra *et al.*, 2012 & 2014; Visagie *et al.*, 2013, 2013, 2014, 2016 and Leeuwenhoek, 2018)

1 Cleistothecia or sclerotia present …….....2

Cleistothecia or sclerotia absent……4

2(1) Cleistothecia soft, covered by a few to several layers of loose hyphae, ripening within 1-2week…… *Talaromyces*

Cleistothecia firm, of pseudoparenchymatous sclerotioid structure or only sterile sclerotia present…….. 3

3(2) Sclerotia on all media abundantly produced, flesh to pink, not embedded among orange- red hyphae, never developing asci; conidiophores simple, bearing loose conidial columns ……*P.thomii*

Sclerotia sooner or later developing asci in the center; conidiophores simple, one-stage branched or two-stage branched, divaricate …… … ……*Eupenicillium*

4(1) Conidiophores strictly unbranched, exceptionally with an additional branch…….....5

-Conidiophores at least one-stage branched.....12

12(4) Phialides lanceolate, in compact whorls on metulae, usually without additional branches*;* colonies often intermixed with yellow or red vegetative hyphae; reverse bright coloured; conidial chains tangled 39

- Phialides not lanceolate, usually with a short but distinct, or a

long and conspicuous neck, rarely without a neck; additional branches often present ……..……..13

13(12) Phialides less than 6µm long, short cylindrical, usually without a distinct neck; conidiophores two to four-stage branched with divergent branches; colonies grey, loosely synnematous ……... ……*P. griseofulvum*

-Phialides longer than 6µm, with distinct neck……..14

14(13) Phialides 15-28µm long, cylindrical, with an abruptly tapering neck; conidia ellipsoidal to cylindrical, commonly 5- 8 x 4-6µm and larger; colonies olivaceous on MEA, very restricted and thin on CZA; causing green citrus rot….. …… ……..*P. digitatum*

-Not combining the above characters…….15

15(14**) Phialides cylindrical, tapering abruptly to a conspicuous, fairly long narrow conidium-bearing neck; conidia chains tangled; conidia ellipsoidal or sometimes subglobose in older cultures ....……...16**

-Phialides not as above …….....17

16(15)Colonies with glaucous conidial areas, often intermixed with bright coloured vegetative mycelium; reverse usually red, yellow or purple occasionally colourless; conidiophores smooth-walled or roughened, irregularly divergently branched; conidia smooth- walled or finely roughened,3.0-3.5µm long.....*P. janthinellum*

- Colonies usually pale blue-green; or white reverse colourless or slightly yellow; conidiophores coarsely roughened or monoverticillate, penicillium commonly consisting of a terminal verticil of divergent metulae; branches occasionally present; conidia finely echinulate, globose, 2.0-3.0 or 2.5-3.0 µm long..... *P. simplicissimum, P. spionulosum*

P. thomii, P. apimei, P. fernandesiae

Table 3(a). Comparison of *P. simplicissimum, P. spinulosum, P. thomii, P.apine, P.fernandesiae* and fungus NSF-10

Fungi	**Colony**		**Conidiophore and Conidia**
	On YSA	**On MEA**	
P. simplicissimum *	Olivaceous Colony (no diffusible pigment)	Olivaceous Colony (no diffusible pigment)	Oval to ellipsoidal, (1.5-2.8) x (1.3-2.5)mm, conidia spiny
P. spinulosum *	Olivaceous Colony (no diffusible pigment)	Olivaceous Colony (no diffusible pigment)	Oval to ellipsoidal, (1.5-2.8) x (1.3-2.5)mm, conidia spiny
P. thomii *	Olivaceous Colony (Yellow diffusible pigment)	Olivaceous Colony (Yellow diffusible pigment)	Subglobose to limoniform (2.0-3.0) x (1.5-3.0) mm
Fungus NSF-10	**Cotton like white colony (Red soluble pigment; reverse orange)**	**Cotton like white colony (Red soluble pigment; reverse orange)**	**Conidia smooth walled, globose, 2–3 µm**

Table 3(b). Comparison of *Penicillium simplicissimum, P.spinulosum, P. thomii, P. apime,* and fungus NSF-10

Fungi	**Colony**		**Conidia**
	On **YSA**	On **MEA**	
P. apimei *	Cotton like Olivaceous Colony (no diffusible pigment)	Cotton like Olivaceous Colony (no diffusible pigment)	Oval to ellipsoidal, (1.5-2.8) x (1.3-2.5)mm,
P. fernandesiae	**Cotton like white colony**	**Cotton like white colony**	**Conidia** smooth

Fungi	Colony		Conidia
	On **YSA**	On **MEA**	
	(Red soluble pigment; reverse orange)	(Red soluble pigment; reverse orange)	walled, **globose**, 2–3 µm
Fungus NSF-10	**Cotton like white colony** (Red soluble pigment; reverse orange)	**Cotton like white colony** (Red soluble pigment; reverse orange)	**Conidia** smooth walled, **globose**, 2–3 µm

*****Samon and Pitt. 2000 Watanabe, 2002; Dilip, 2003; Asan, 2004; Ando, 2006; Misra *et al*., 2012 & 2014; Visagie *et al.*, 2013, 2013, 2014, 2016 and Leeuwenhoek, 2018.

Table 4 . Comparison of Colonies' Sizes between NSF-10 and literatures on PGA for 7 days at 25 ºC

Culture Media	Leeuwenhoek, 2018	NSF-10
PGA, 25°C, 7 days	- Colonies cotton like, - 1.2-1.6 cm diam, - mycelium white; - colony texture velvety; - soluble pigment in shades of red or pink; - reverse orange.	- Colonies cotton like, - 1.3-1.6 cm diam, - mycelium white; - colony texture velvety; - soluble pigment in shades of red or pink; - reverse orange.

Based on the morphological - microscopical characters and according to the literature references keys (Visagie *et al.*, 2013, 2013, 2014, 2016 and Leeuwenhoek, 2018), the fungus **NSF-10** was identified *Penicillium fernandesiae* Barbosa, Motta, Oliveira & Houbraken 2018.

Kingdom: *Fungi*

Phylum: *Ascomycota*

Class *Eurotiomycetes*

Order: *Eurotiales*

Family: *Trichocomaceae*

Genus: *Penicillium*

Species *P. fernandesiae*

Discussion and Conclusion

In the screening program of antibacterial metabolite against *Bacillus subtilis*, fungus NSF-10 was selected due to the highest antibacterial activity for further investigations. Pink soluble pigment was also observed on PGA, YMEA, GAN and PCA. Colonies are cotton like, 1.3-1.6 cm diam, moderately deep, gently radially sulcate; margins entire; mycelium white; colony texture velvety; soluble pigment in shades of red or pink; reverse orange on PGA for 7 days old culture at 25 ºC. Colonies are cotton like, 1.3-1.6cm diam, convex; margins entire; mycelium white, colony texture floccose; red soluble pigment in shades of pink; reverse orange on MEA for 7 days old culture at 25ºC. It was also found on YSA, 25 °C, 7 days, colonies are cotton like, 1.1-1.4cm diam, flat, margins irregular; mycelium white; soluble pigment in shades of red; reverse orange.

In the study of microscopical characters, it was observed that conidiophores strictly monoverticillate. 7.5-20 × 1.5-2.0 µm, non-vesiculate. Phialides are ampulliform, 6-11 × 2.0-3.0 µm. Conidia smooth walled, globose, 2-3 µm. According to its morphological-microscopical characters, it was considered that fungus NSF-10 may be *Paecilomyces*, *Penicillium*, *Aspergillus,* and *Purpureocillium*.

However fungus NSF-10 was resemble to *Penicillium* according to microscopical characters based on the literatures (Asan, 2004; Dan *et al.*, 2015; Luangsa *et al.*, 2011; Misra *et al.*, 2011; Visagie *et al.*, 2013, 2013, 2014 & 2016). The references key of Leeuwenhoek, 2018, the fungus NSF-10 was similar to *P. simplicissimum, P. spinulosum, P. thomii, P. apimei* and *P. fernandesiae*. In the comparison of these *Penicillium* species and fungus NSF-10, it was found that the morphological-microscopical characters of fungus NSF-10 was the most resemble to those of *P. fernandesiae*. Therefore, fungus NSF-10 was identified as *Penicillium fernandesiae* Barbosa, Motta, Oliveira & Houbraken 2018.

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Taxonomic Studies of Some Wild Mushroom Found in Chaung Oo Township

Zaw Lwin Oo[1], San Nyunt Nwe[2] & Aye Aye Maw[3]

Abstract

The taxonomic studies of wild mushrooms from Chaung Oo Township were undertaken during 2019. In this study, 12 species of 9 genera belonging to 5 families were collected, preserved, identified and classified. Among them, 8 species are edible and 4 species are inedible. All the collected species were fully described with photographs. Moreover, an artificial key to the species of all the collected specimens were constructed.

Keywords: Taxonomic, Mushrooms, artificial key

Introduction

Fungi are important organisms that serve many vital functions in forest ecosystems including decomposition nutrient cycling, symbiotic relationships with trees and other plants. Mushrooms are sources of food for wildlife, and fungi that cause decay in living trees are beneficial to many species of birds and mammals (Michael *et al.*, 2010).

Mushrooms grow normally after rain because of the higher humidity and temperature in a short period. Therefore, it can be collected within 2 or 3 days after rain. The fruiting body of terrestrial mushrooms can only be seen within a day started from initial growth. The epiphytic mushroom can survive at least 3 days and some species can be decayed wood even for many months.

Mushrooms have a great nutritional value, they are quite rich in protein, with an important content of essential amino acids and fiber. Edible mushrooms also provide a nutritionally significant content of vitamins, many different nutraceuticals such as unsaturated fatty acids, ascorbic acid and carotenoids. Thus, they might be used directly in diet and promote

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health, taking advantage of the additive and synergistic effects of all the bioactive compounds present (Pereira *et al*., 2012).

The aims and objectives of this research were to study the nature of mushrooms from Chaung Oo Township, to identify and classify and to achieve a valuable information of mushrooms distributed in the study area.

Materials and Methods

The naturally growing wild mushrooms were collected from Chaung Oo Township in Monywa District during 2019. All the collected specimens were recorded with digital camera to get their natural habit and noted the visual fruiting characteristics. To prepare the spore print, the fleshy mature specimens were selected. The stipe was removed by cutting it off as close as possible to the point of attachment of the cap. It is obtained by placing a cap with the hymenium facing down on a sheet of white and black paper or a piece of glass-slide. After a few hours, a layer of the spores was deposited.

The spores were taken from the spore print. First, the spores were dropped on the glass-slide. Then the spores were covered with cover-slip and sealed with Canada balsam. Identification and classification of the collected specimens were done by referring to Thomas (1948), Pacioni (1981) and Keizer (1998).

Results

In the present study, altogether 12 species of 9 genera belonging to 5 families were collected from Chaung Oo Township, Sagaing Region. According to morphological and spores characters, these species were classified and identified. The list of collected species were shown in Table1.

Table 1. the list of collected species from Chaung Oo Township

Order	Family	No.	Scientific name
Agaricales	Agaricaceae	1	*Lepiota cristata*(Fr.) Kummer.
		2	*Psalliota haemorrhoidaria* (Schulz.) Quel.
		3	*Psalliota rodmani* (Pk.) Kauffm
		4	*Termitomyces schimperi* (Pat.) Heim.
	Hygrophoraceae	5	*Hygrophorus cantharellus* (Schw.) Fr.

Order	Family	No.	Scientific name
	Pluteaceae	6	*Pluteus cervinus* (Schaeff.) Quel.
		7	*Volvariella gloiocephala* (Schaeff) Quel
		8	*Volvariella negerodisca* (Schaeff) Que.
	Tricholomataceae	9	*Clitocybe caespitosa* Pk.
		10	*Tricholoma personatum* (Fr.) Quel.
		11	*Tricholoma ustaloide* (Fr.) Quel
Aphyllopoorales	Ganodermataceae	12	*Ganoderma lucidum* Leyss. ex Fr.

1. Scientific name : *Lepiota cristata* (Fr.) Kummer. (Figure 1)

Local name : Unknown

Family : Agaricaceae

Description

Cap 2.0 - 4.0 cm in broad, thin, campanulate at first, then expanded, white, fibrillose, orange scales cover the disc consistently. Gills close, free, crowded, white. Stipe 4.0 - 10.0 cm long, 0.5 -1.0 cm thick, equal, hollow at maturity, fibrillose, white. Ring small, membernous and short-lived. Spores white, smooth, elliptic, 5.0 - 8.0 × 2.0 - 4.0 µ.

Edibility : Inedible Habitat : Soil

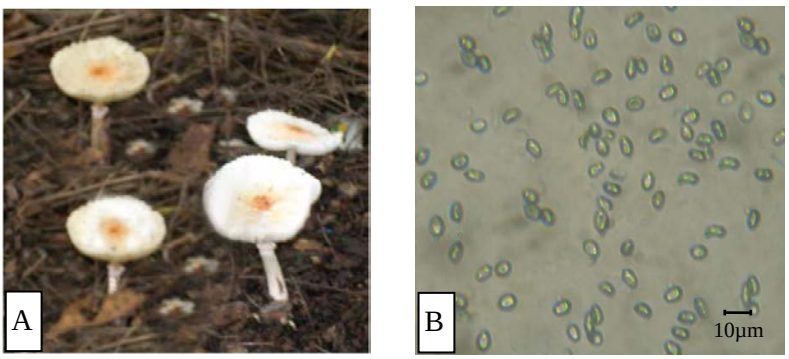

Figure 1. Habit of *Lepiota cristata* (Fr.) Kummer.
A. Growing Habi **B.** spore

2. Scientific name : *Psalliota haemorrhoidaria* (Schulz.) Quel. (Figure 2)

Local name : Unknown

Family : Agaricaceae

Description

Cap 3.0 - 6.0 cm broad, at first ovate, becoming convex, umbo present, with yellowish scales at the center, white. Gills thin, narrow, free, at first pink, then brown. Stipe 3.0 - 5.0 cm long, 0.5 - 1.0 cm thick, equal or slightly tapering upward, hollow, white. Ring membranaceous, persistent, white. Spores brown, smooth, elliptic, 6.5 – 8.0 × 4.0 - 5.0 µ.

Edibility : Edible Habitat : Soil

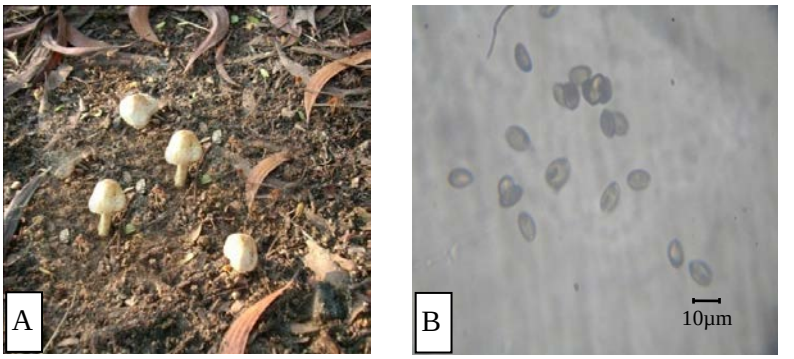

Figure 2. *Psalliota haemorrhoidaria* (Schulz.) Quel.
A. Growing Habit **B.** spore

3. Scientific name : *Psalliota rodmani* (Pk.) Kauffm. (Figure 3)

Local name : Unknown

Family : Agaricaceae

Description

Cap 3.0 - 10.0 cm broad, at first ovate, becoming convex or nearly expanded, umbo absent, white with brown scales. Gills thin, narrow, free, pink. Stipe 2.5 - 5.0 cm long, 0.5 - 0.8 cm thick, equal or slightly tapering upwards, hollow, white. Ring membranous, persistent, white. Spores pale-brown, smooth, elliptic, 3.5 - 4.5 × 2.5-4.0 µ.

Edibility : Inedible Habitat : Soil

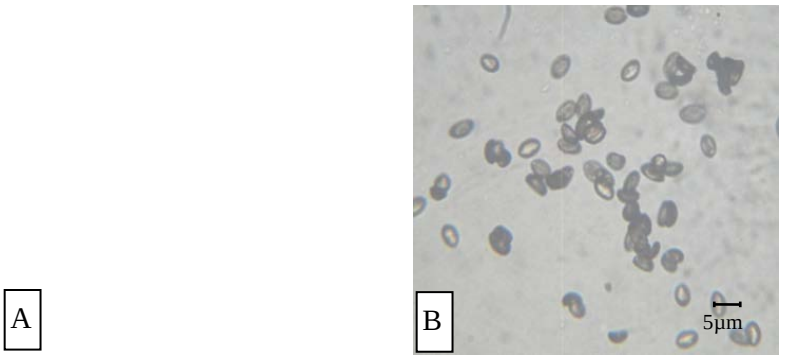

Figure 3. *Psalliota rodmani* (Pk.) Kauffm
A. Growing Habit **B.** spore

4. Scientific name : *Termitomyces schimperi* (Pat.) Heim. (Figure 4)

Local name : Taungbohmo
Family : Agaricaceae

Description

Cap 6.5 – 8.0 cm broad, at first convex, then expanded, umbonate, silky, darker at the centre, white, margin slightly wavy and split. Gills crowded, free, white. Stipe 12.0 - 20.0 cm long, 1.0 - 1.5 cm thick, equal, slightly tapering upwards near the cap, stuffed and fibrillose above the ring, hollow, upper part white, lower part greyish. Ring double conspicuous, persistent, whitish. Spores pink, smooth, elliptic, 2.0 - 6.0 × 3.0 - 5.0 µ.

Edibility : Edible Habitat : Soil

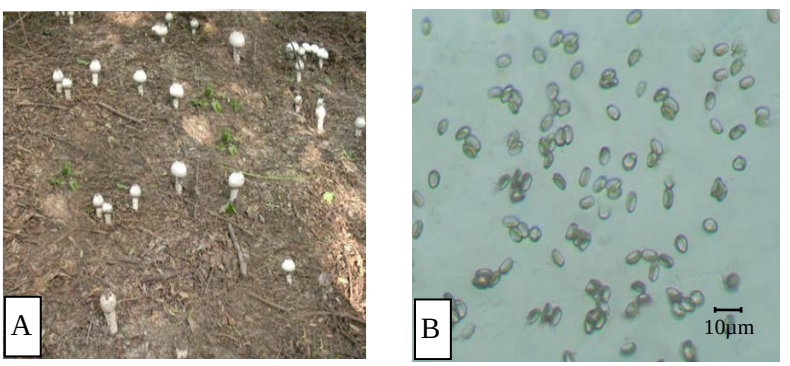

Figure 4. *Termitomyces schimperi* (Pat.) Heim.
A. Growing Habit **B.** spore

5. Scientific name : *Hygrophorus cantharellus* (Schw.) Fr. (Figure 5)

Local name : Unknown

Family : Hygrophoraceae

Description

Cap 1.5 – 3.0 cm broad, thin, at first convex, then funnel-shaped, smooth pale yellow. Gills broad, thin, decurrent, pale-yellow. Stipe 1.0 - 2.5 cm long, 0.1 - 0.3 cm thick, equal, fragile, solid, reddish-brown. Ring absent. Spores pale yellow, smooth, elliptic, 4.4 - 7.6 × 3.6 – 5.0 µ.

Edibility : Edible Habitat : Soil

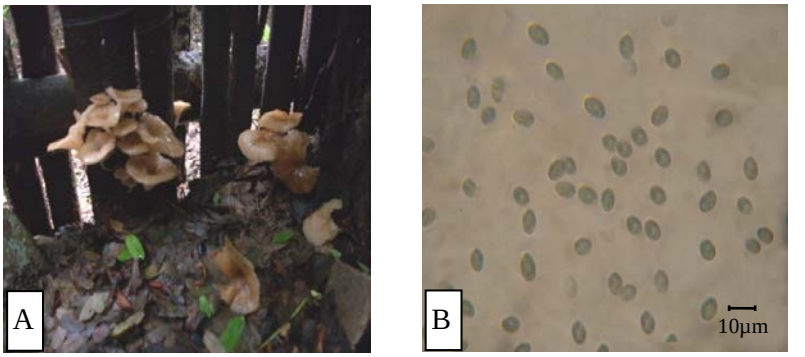

Figure 5. *Hygrophorus cantharellus* (Schw.) Fr.
A. Growing Habit **B.** spore

6. Scientific name : *Pluteus cervinus* (Schaeff.) Quel. (Figure 6)

Local name : Unknown

Family : Pluteaceae

Description

Cap 5.0 - 10.0 cm broad, at first convex, then expanded, umbonate, centrally brown scales, white. Gills close, thick, free, white. Stipe 5.0 - 15.0 cm long, 0.5 - 1.5 cm thick, equal, hollow, white with brown scales. Ring absent. Spores white, smooth, elliptic, 5.0 -7.0 × 3.0 - 5.0 µ.

Edibility : Inedible; Habitat : Soil

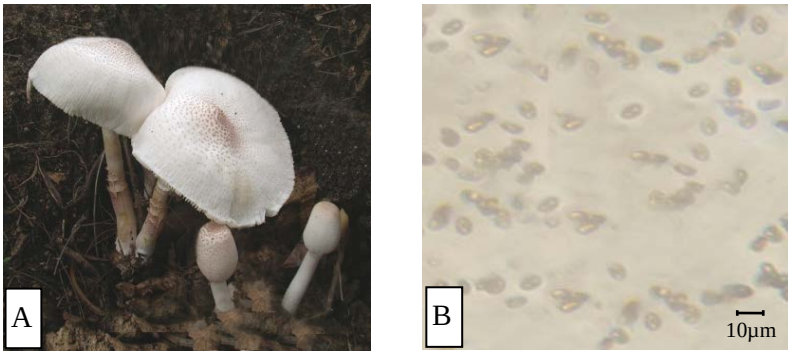

Figure 6. *Pluteus cervinus* (Schaeff.) Quel.
A. Growing Habit **B.** spore

7. Scientific name : *Volvariella gloiocephala* (Schaeff.) Quel. (Figure 7)

Local name : Hgnet pyaw hmo

Family : Pluteaceae

Description

Cap 5.0 - 10.0 cm broad, globose when young then campanulate, sometime slightly umbonate, greyish-white with darker at the center. Gills close, broad, free, pink. Stipe 3.0 - 8.0 cm long, 0.5 - 1.0 cm thick, equal, solid, greyish-white. Ring absent. Volva large, soft, white. Spores pink, smooth, elliptic, 7.0 - 9.0 × 4.5 - 6.0 µ.

Edibility : Edible Habitat : Soil

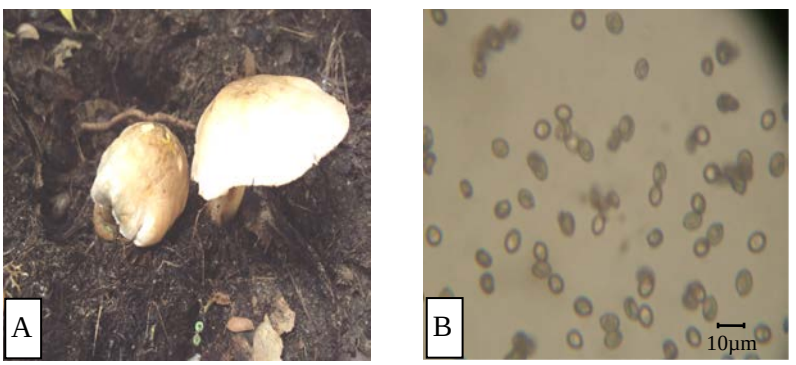

Figure 7. *Volvariella gloiocephala* (Schaeff.) Quel.
A. Growing Habit **B.** spore

8. Scientific name :*Volvariella negerodisca* (Schaeff.) Quel. (Figure 8)

Local name : Sagwehmo

Family : Pluteaceae

Description

Cap 1.0 - 2.0 cm broad, campanulate, umbonate, covered with fine, silky, white with greyish at the center. Gills close, free, white. Stipe 3.0 - 6.0 cm long, 0.2 - 0.3 cm thick, equal, solid, white. Ring absent. Volva scale-like. Spores pink, smooth, elliptic, 6.5 - 8.0 × 5.0 - 6.0 µ.

Edibility : Edible; Habitat : Soil

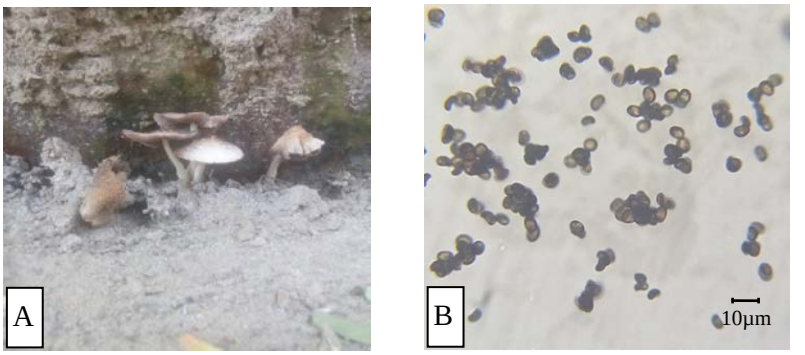

Figure 8. *Volvariella negerodisca* (Schaeff.) Quel.
A. Growing Habit **B.** spore

9. Scientific name : *Clitocybe caespitosa* Pk. (Figure 9)

Local name : Thayethmo

Family : Tricholomataceae

Description

Cap 1.0 - 4.5 cm broad, thin, hard, funnel-shaped, white. Gills narrow, close, decurrent, white. Stipe 2.0 - 3.0 cm long, 0.5 - 1.0 cm thick, equal or slightly tapering upward with hairs, solid, white. Ring absent. Spores white, smooth, elliptic, 5.0 - 6.0 x 2.0 - 3.0 µ.

Edibility : Edible; Habitat : Decayed wood

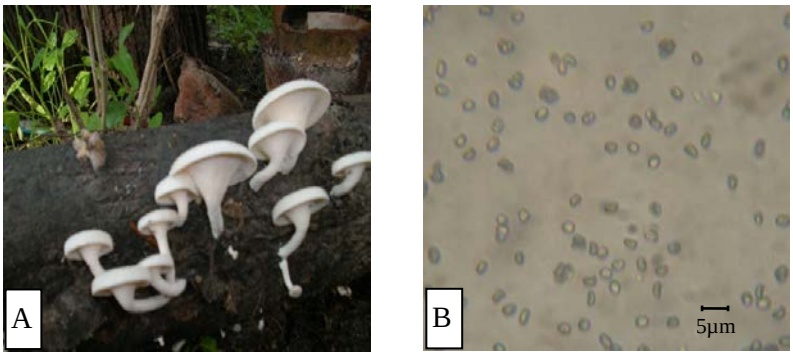

Figure 9. *Clitocybe caespitosa* Pk.
A. Growing Habit **B.** spore

10. Scientific name : *Tricholoma personatum* (Fr.) Quel. (Figure 10)

Local name : Hmoohnnat

Family name : Tricholomataceae

Description

Cap 6.0 - 10.0 cm broad, convex at first, then expanded with age, umbonate, viscid, sooty brown in the center and paler to the margin, Gills broad close, crowded, sinuate, white. Stipe 10.0 - 15.0 cm long, 1.0 - 1.5 cm thick, equal, solid, fibrillose, white. Ring absent. Spores, sordid- white, smooth, sub-elliptic, 7.0 - 9.0 × 4.5 -6.0 µ.

Edibility : Edible Habitat : Soil

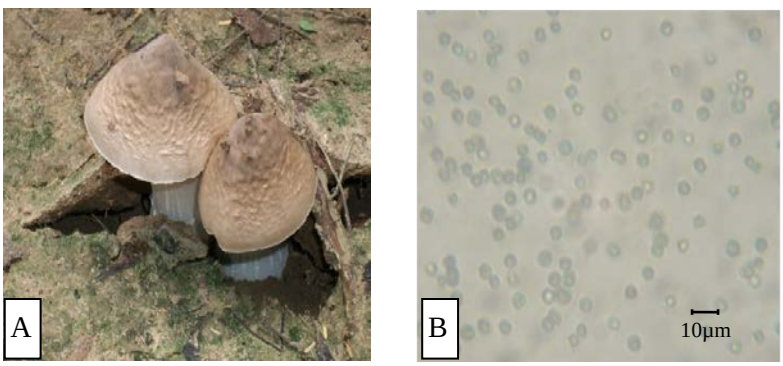

Figure 10. *Tricholoma personatum* (Fr.) Quel.
A. Growing Habit **B.** spore

11. Scientific name : *Tricholoma ustaloide* (Fr.) Quel. (Figure 11)

Local name : Unknown

Family : Tricholomataceae

Description

Cap 5.0 - 10.0 cm broad, at first convex, then expanded, umbonate, bright brown, shiny, viscid then dry. Gills close, not very crowded, adnate, white with highlights, and reddish sport when mature. Stipe 6.5 - 10.0 cm long, 1.0 -1.5 cm thick, fusiform, cylindrical at base, solid, white at top. Ring absent. Spores white, smooth, elliptic, 6.0 - 7.0 × 4.2 -5.0 µ.

Edibility : Edible Habitat : Soil

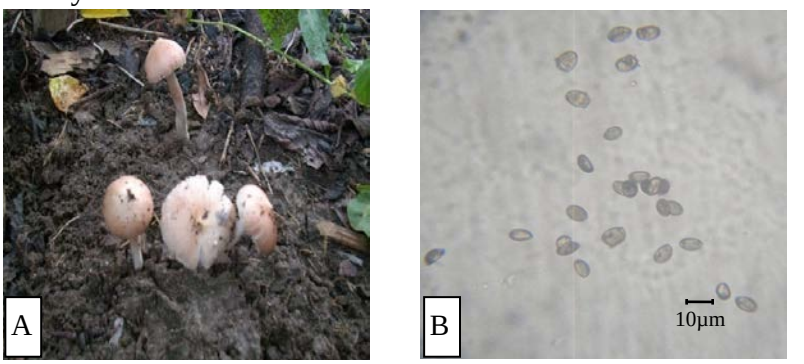

Figure 11. *Tricholoma ustaloide* (Fr.) Quel.
A. Growing Habit **B.** spore

12. Scientific name: *Ganoderma lucidum* Leyses.ex.Fr. P. Karst. (Figure 12)

Local name : Lingzhi

Family : Ganodermataceae

Description

Cap 5.0 - 28.0 cm broad, circular or kidney-form, covered with a shiny crust, cap zoned from yellow to dark red and margin white or yellow. Pores small and round, white then cinnamon-colored, flesh whitish spongy. Stipe 4.0 - 6.0 cm long, 1.0 - 5.0 cm thick, usually eccentric-lateral, smooth, shiny, dark brown-red. Spores brown, rough, elliptic, 8.0 -10.0 × 5.0-8.0 µ.

Edibility : Edible; Habitat : Decayd wood

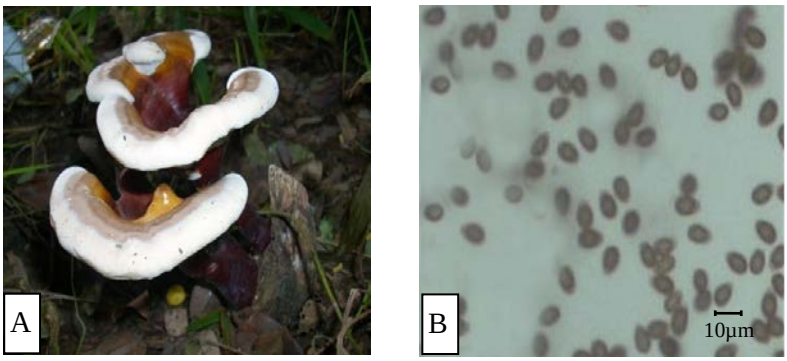

Figure 12. *Ganoderma lucidum* Leyses.ex. Fr.P. Karst.
A. Growing Habit **B.** spore

An Artificial key to the studied species

1. Cap with umbo ---------- 2
1. Cap without umbo ---------- 9
2. Stipe hollow ---------- 3
2. Stipe solid ---------- 6
3. Ring or annulus absent ---------- 6. *Pluteus cervinus*
3. Ring or annulus present ---------- 4
4. Gills brown; spore brown ---------- 2. *Psalliota haemorrhoidaria*
4. Gills white; spore white or pink ----------5
5. Cap white with orange scales; stipe without double ring; spore white------ ----------1. *Lepiota cristata*
5. Cap white without orange scales, stipe with double ring; spore pink ------- ----------4. *Termitomyces schimperi*
6. Spores white or sordid white ---------- 7
6. Spore pink ---------- 8
7. Cap reddish brown; gill adnate; stipe fusiform - 11. *Tricholoma ustaloide*
7. Cap sooty brown; gill sinuate; stipe equal ---- 10. *Tricholoma personatum*

8. Gills white, cap white ---------- 8. *Volvariella negerodisca*

8. Gills pink; cap grayish white ---------- 7. *Volvariella gloiocephala*

9. Cap expanded ---------- 3. *Psalliota rodmani*

9. Cap kidney or funnel-shaped ---------- 10

10. Gills white ---------- 5. *Hygrophorus cantharellus*

10. Gills dull yellow or pale yellow ---------- 11

11. Cap kidney shape ---------- 12. *Gandoderma lucidum*

11. Cap funnel shape ---------- 9. *Clitocybe caespitosa*

Discussion and Conclusion

In the present study, 9 genera and 5 families were recorded. Among them, 11 species are gill mushrooms and one species, *Ganoderma lucidum* Leyss. ex. Fr. was pore mushroom.

Among the collected species, *Lepiota cristata*(Fr.) Kummer., *Psalliota haemorrhoidaria* (Schulz.) Quel., *Psalliota rodmani* (Pk.) Kauffm, *Termitomyces schimperi* (Pat.) Heim., *Pluteus cervinus* (Schaeff.) Quel., *Volvariella gloiocephala* (Schaeff) Quel, *Volvariella negerodisca* (Schaeff) Que., *Tricholoma personatum* (Fr.) Quel. and *Tricholoma ustaloide* (Fr.) Quel were grown in soil. *Gandoderma lucidum* Leyss. ex Fr., *Hygroposus cantharellus* (Schw.) Fr., and *Clitocybe caespitosa* Pk. were grow on decayed wood.

In the present study, 9 species are edible and 3 species are inedible. The edible species were *Psalliota haemorrhoidaria* (Schulz.) Quel., *Termitomyces schimperi* (Pat.) Heim., *Hygrophorus cantharellus* (Schw.) Fr., *Volvariella gloiocephala* (Schaeff) Quel, *Volvariella negerodisca* (Schaeff) Que., *Clitocybe caespitosa* Pk., *Tricholoma personatum* (Fr.) Quel., *Tricholoma ustaloide* (Fr.) Quel and *Ganoderma lucidum* Leyss. ex Fr. These species are commonly eaten by local people. The inedible species were *Lepiota cristata*(Fr.) Kummer., *Psalliota rodmani* (Pk.) Kauffm and *Pluteus cervinus* (Schaeff.) Quel.

The present study provides general information of classification, identification and key characteristics of collected wild mushrooms. The interested taxonomic and morphological characteristics of mushrooms can

also be used a guide for interested researchers and that will be partially accomplished the flora of mushrooms.

Acknowledgements

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Qualitative and Quantitative Analysis of Leaves and Flowers of *Artemisia vulgaris* L. and its Antimicrobial Activities

Nwe Oo[*]

Abstract

In this research, the qualitative, quantitative and antimicrobial determinations of leaves and flowers of *Artemisia vulgaris* L. were studied. The samples were collected from Taunggyi Township, Southern Shan State. In qualitative analysis, alkaloids, α-amino acids, carbohydrates, starch, reducing sugars, glycosides, phenolic compounds, saponins, tannins, steroids/terpenoids and flavonoids except cyanogenic glycosides were detected in both samples. In quantitative analysis, the soluble contents in aqueous and methanol are higher than other extracts in both samples. The concentration of potassium is highest in both samples according to X-ray Fluorescence Spectrometer (XRF) analysis. Toxic metals, As, Hg, Pb were not detected by Atomic Absorption Spectroscopy (AAS) analysis except Cd in both samples. The presence of fiber, fat, carbohydrate and energy values are higher in flowers than in leaves except protein content. In antimicrobial assay of leaves, chloroform, ethyl acetate, acetone and methanol extracts showed activities on all test microorganisms. In flowers, chloroform, ethyl acetate and acetone extracts showed activities on all test microorganisms. Ethyl acetate extracts of both samples showed the highest activities especially on *Pseudomonas aeruginosa*.

Keywords: *Artemisia vulgaris* L., qualitative, quantitative, antimicrobial activities

Introduction

Evaluation of drug means confirmation of its identity and determination of its quality and purity and detection of nature of adulteration. The detection of active principles in medicinal plants plays a strategic role in the phytochemical investigation of crude plant extracts and is very important with regard to their potential pharmacological effects (Pascual *et al*. 2002). The compounds that are responsible for therapeutic effect are usually secondary metabolite. (Kokate, 1994).

Many plants have been used because of their antimicrobial traits, which are due to phytochemicals synthesized in the secondary metabolism

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of the plant (Romero *et al*. 2005). Besides being used for the treatment of diseases, the medicinal plants are also used as dietary supplements once they are found to be rich in one or more elements. Elemental content in medicinal plants can vary in a wide range, depending on factors such as soil geochemical characteristics, atmospheric deposition and the ability of each plant species to selectively accumulate some of them (Łozak *et al*. 2002).

Artemisia vulgaris L. is commonly known as Mel de dote in Myanmar and mugwort and wormwood in English (Hundley and Chit Ko Ko, 1987 and Padua, 1999). Mugwort leaves have been used as an insecticide, mosquito repellency, and hemostatic by local people in this collected area, Taunggyi Township, Southern Shan State.

In folk medicine, mugwort is also employed as a choleretic and formerly as an anthelmintic, as well as for amenorrhea and dysmenorrhea (Norman, 2001 and Blagojevic, 2006). The objectives of this research are to perform the qualitative, quantitative and antimicrobial determinations of leaves and flowers of *A. vulgaris* L.

Materials and Methods

The present study was carried out in 2014. The leaves and flowers samples were collected from Taunggyi Township, Southern Shan State. The samples were cleaned, air-dried, pulverized and stored in air-tight containers to be used in this study.

Qualitative analysis

Qualitative analysis was carried out at Department of Botany, University of Yangon. Tests for alkaloids, α-amino acids, carbohydrates, starch, reducing sugars, cyanogenic glycosides, glycosides, phenolic compounds, saponins, tannins, steroids/ terpenoids and flavonoids were performed by Grainer (1968), Marrini Bettolo *et al*. (1981) and Trease and Evans (2002).

Quantitative analysis

Quantitative analysis was performed at Universities Research Center, University of Yangon. Determination of moisture content, total ash content, acid-insoluble ash content, water-soluble ash content, soluble matter was carried out according to Grainer (1968) and WHO (1998).

X-ray Fluorescence Spectrometer (XRF) analysis

The elemental analysis was determined by XRF spectrometer at Department of Physics, University of Mandalay by the FP- Pellets-121997ne1 method.

Atomic Absorption Spectroscopy (AAS) analysis

Atomic Absorption Spectroscopy (AAS) analysis was carried out at Applied Geology Department. The analytical precisions were determined from duplicate analysis. Procedure for the determination of concentrated acid extractable metal is the same as used by Levinson (1974). 0.5g sample (-80 mesh) was weighed into a dry clean 18×150 mm Pyrex test-tube. 5ml of HNO_3: HCL (1:3) concentrated acid mixture was added. The solution was evaporated to dryness overnight on an air bath. The residue was leached on a sand tray with 10 ml of 1 M HNO_3 weak acid mixture at a temperature of about 70ºC for 30 minutes. The solution was stirred by using vortex mixture. The solutions were stand for overnight and aspirated on an atomic absorption spectroscopy (Varian Textron Model A.A.S).

Determination of nutritional values

The study for nutritive values were performed at Myanmar Food Processors and Exporters Association (MFPEA), Yangon by Association of Official Analytical Chemist (AOAC) method (Horwitz, 1980).

Determination of antimicrobial activities

Antimicrobial activities of various solvent extracts from leaves and flowers were tested on six pathogenic microorganisms by agar well diffusion method (Cruickshank, 1975) at Central Research and Development Center (CRDC), Yangon. Nutrient agar was boiled and 20 - 25 ml of the medium was poured into a test tube and plugged with cotton wool and autoclaved at 121º C for 15 minutes. Then, the test tubes were

cooled down to 30 - 35º C and poured into sterilized petridishes and 0.1 - 0.2 ml of test microorganisms were also added into dishes. Then, about 0.2 ml of sample was introduced into the agar-well and incubated at 37º C for 24 hours. The inhibition zone appeared around the agar-well, indicating the presence of antimicrobial activity. The extents of antimicrobial activities were measured from the diameter zone of inhibition.

Results

Outstanding characters

Scientific Name - *Artemisia vulgaris* L.

English Name - Mugwort, Wormwood

Myanmar Name - Mel de dote

Family - Asteraceae

Aromatic perennial herb; leaves alternate, deeply pinnatisect, dark green on adaxial surface, slightly hairy silvery grey on abaxial surface with silvery-white wooly hairs; head consists of marginal female florets and central disc florets. The results were shown in Figure 1.

Figure 1. *Artemisia vulgaris* L.

A. Habit

B. Leaves

C. Flowers

Qualitative analysis

The results confirmed the presence of alkaloids, α-amino acids, carbohydrates, starch, reducing sugars, glycosides, phenolic compounds, saponins, tannins, steroids/terpenoids and flavonoids except the cyanogenic glycosides in leaves and flowers of *A. vulgaris* L. The results were shown in Table 1.

Table 1. Results of qualitative analysis

No.	Tests	Extr-acts	Test Reagents	Observations	Results	
					Leaves	Flowers
1.	Alkaloids	1% HCL	Dragendroff's reagent	White ppt.	+	+
			Wagner's reagent	Reddish brown ppt.	+	+
2.	α-amino acids	H_2O	Ninhydrin reagent	Pink spot	+	+
3.	Carbohyd-rates	H_2O	10 % α-napthol and conc:H_2SO_4	Red ring	+	+
4.	Starch	H_2O	Iodine solution	Blue black ppt.	+	+
5.	Reducing sugars	H_2O	Benedicts solution	Brick red ppt.	+	+
6.	Cyanogenic glycosides	H_2O	Sodium picrate paper	No color	–	–
7.	Glycosides	H_2O	10 % lead acetate solution	Yellow ppt.	+	+
8.	Phenolic compounds	HCL	3 % $FeCl_3$ solution	Yellow ppt.	+	+
9.	Saponins	H_2O	Distilled water	Frothing	+	+
10.	Tannins	H_2O	1 % $FeCl_3$ solution	Greenish brown ppt.	+	+
11.	Steroids/ Terpenoids	Pet-ether	Acetic anhydride and conc: H_2SO_4	Greenish/ pink ppt.	+	+
12.	Flavonoids	EtOH	HCL/Mg	Pink	+	+

+ = present, - = absent, ppt. = precipitation

Quantitative analysis

A. vulgaris L. leaves were highly soluble in distilled water and methanol. Flowers are more soluble in methanol and distilled water than other solvents. These results were shown in Table 2.

Table 2. Results of quantitative analysis

No.	Quantitative Determination	(Average %)	
		Leaves	Flowers
1.	Moisture content	13.35	9.75
2	Total ash content	10.21	6.56
3.	Acid-insoluble ash content	29.45	32.11
4.	Water-soluble ash content	7.81	6.54
5.	Petroleum ether soluble content	2.6	4.1
6.	Chloroform soluble content	4.8	1.4
7.	Ethyl acetate soluble content	7.2	7.0
8.	Acetone soluble content	5.0	7.4
9.	Methanol soluble content	11.5	16.8
10.	Ethanol soluble content	7.4	13.5
11.	Distilled water soluble content	11.9	14.9

X-ray Fluorescence Spectrometer (XRF) analysis

Among the elements, potassium was the highest in both samples. The contents of potassium, calcium, chlorine, manganese and zinc were higher in leaves than in flowers. The percentage of iron, copper, mercury, lead and arsenic were higher in flowers than in leaves. The results were shown in Table 3.

Table 3. Results of XRF analysis

No.	Elements	Concentration %	
		Leaves	Flowers
1.	Potassium	3.182	2.021
2.	Calcium	1.236	0.8639

No.	Elements	Concentration %	
		Leaves	Flowers
3.	Chlorine	1.500	1.202
4.	Manganese	0.01316	0.01178
5.	Iron	0.02237	0.09010
6.	Zinc	0.00430	0.00331
7.	Copper	0.00168	0.00170
8.	Mercury	0.00015	0.00019
9.	Lead	0.00007	0.00009
10.	Arsenic	0.00006	0.00014

Atomic Absorption Spectroscopy (AAS) analysis

Toxic metals, As, Hg, Pb were not detected by AAS analysis except Cd in both samples. The cadmium content was higher in leaves than in flowers. The results were shown in Table 4.

Table 4. Results of Atomic Absorption Spectroscopy (AAS) analysis

Elements	Leaves (ppm)	Flowers (ppm)
Arsenic (As)	-	-
Mercury (Hg)	-	-
Lead (Pb)	-	-
Cadmium (Cd)	0.03	0.006

Determination of nutritional values

The protein content in leaves was higher than in flowers. The fiber, fat, carbohydrate and energy values were higher in flowers than in leaves. The results were shown in Table 5.

Table 5. Results of nutritional values

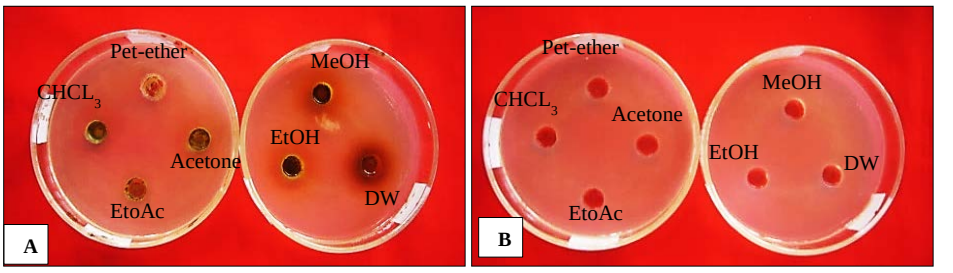
No.	Test of Parameter	Results %	
		Leaves	**Flowers**
1.	Protein	21.86	12.57
2.	Crude Fiber	13.01	20.05
3.	Crude Fat	0.64	0.72
4.	Carbohydrate	36.86	46.75
5.	Energy Value (Kcal/100g)	245	249

Antimicrobial activities

Chloroform, ethyl acetate and acetone extracts of leaves and flowers showed activities on all the six pathogenic microorganisms. Methanolic leaves and flowers extracts showed high activities against on most of all test organisms.

Ethyl acetate extracts of flowers showed the highest activity against on *P. aeruginosa* (40 mm) and very high activity on *E. coli* (38 mm) and *B. pumalis* (35 mm). Ethyl acetate extracts of leaves showed very high activities especially on *P. aeruginosa* (28 mm), *E. coli* (27 mm) and followed by *B. pumalis* (26 mm).

Distilled water extracts of leaves did not show activities on *B. subtilis*, *P. aeruginosa* and *Candida albicans.* Distilled water extracts of flowers showed no activities on almost all test organisms and weak activity only on *B. subtilis*. The results were shown in Figures 2 - 4 and Table 6.



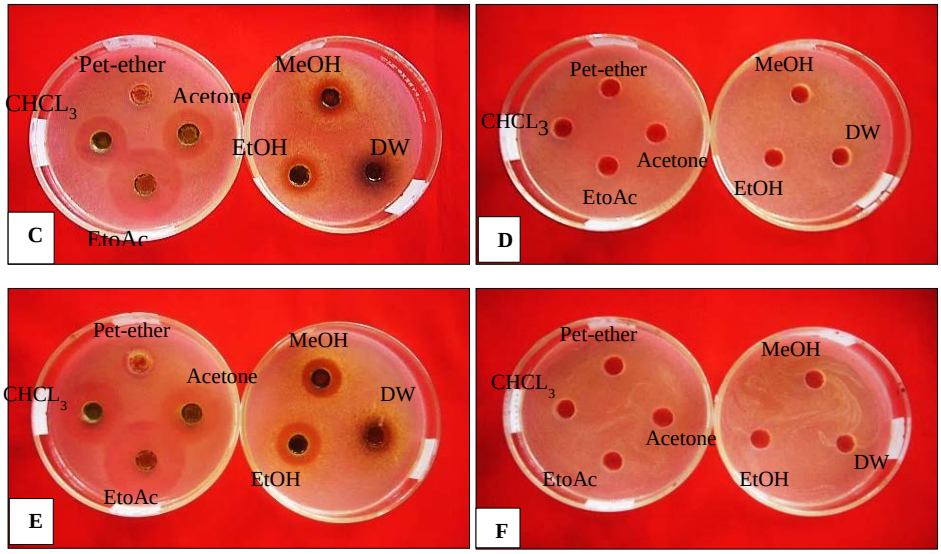


Figure 2. Antimicrobial activities of *Artemisia vulgaris* L. leaves against on test microorganisms

A. Antimicrobial activities against on *Bacillus pumalis*

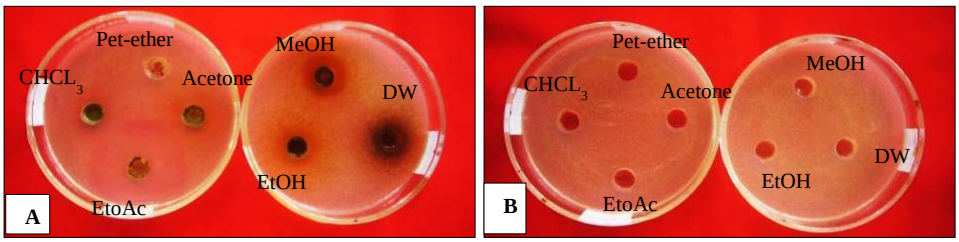
B. Control against on *Bacillus pumalis*

C. Antimicrobial activities against on *Bacillus subtilis*

D. Control against on *Bacillus subtilis*

E. Antimicrobial activities against on *Escherichia coli*

F. Control against on *Escherichia coli*



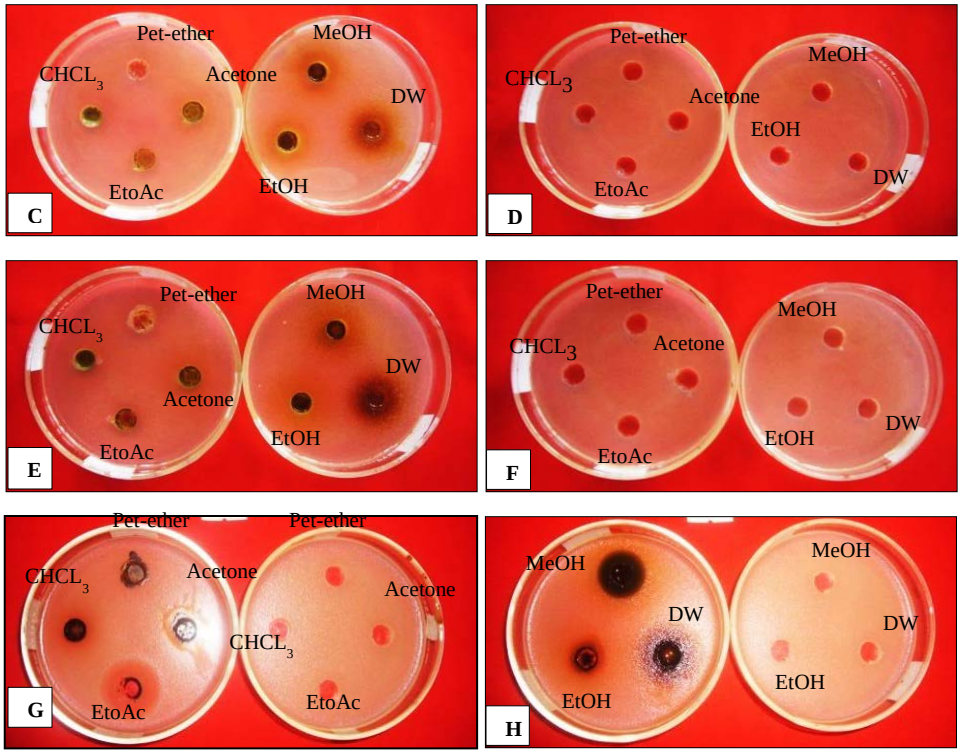


Figure 3. Antimicrobial activities of *Artemisia vulgaris* L. leaves and flowers against on test microorganisms

A. Antimicrobial activities of leaves against on *Pseudomanas aeruginosa*

B. Control against on *Pseudomanas aeruginosa*

C. Antimicrobial activities of leaves against on *Staphylococcus aureus*

D. Control against on *Staphylococcus aureus*

E. Antimicrobial activities of leaves against on *Candida albicans*

F. Control against on *Candida albicans*

G. Antimicrobial activities of flowers against on *Bacillus pumalis*

H. Control against on *Bacillus pumalis*

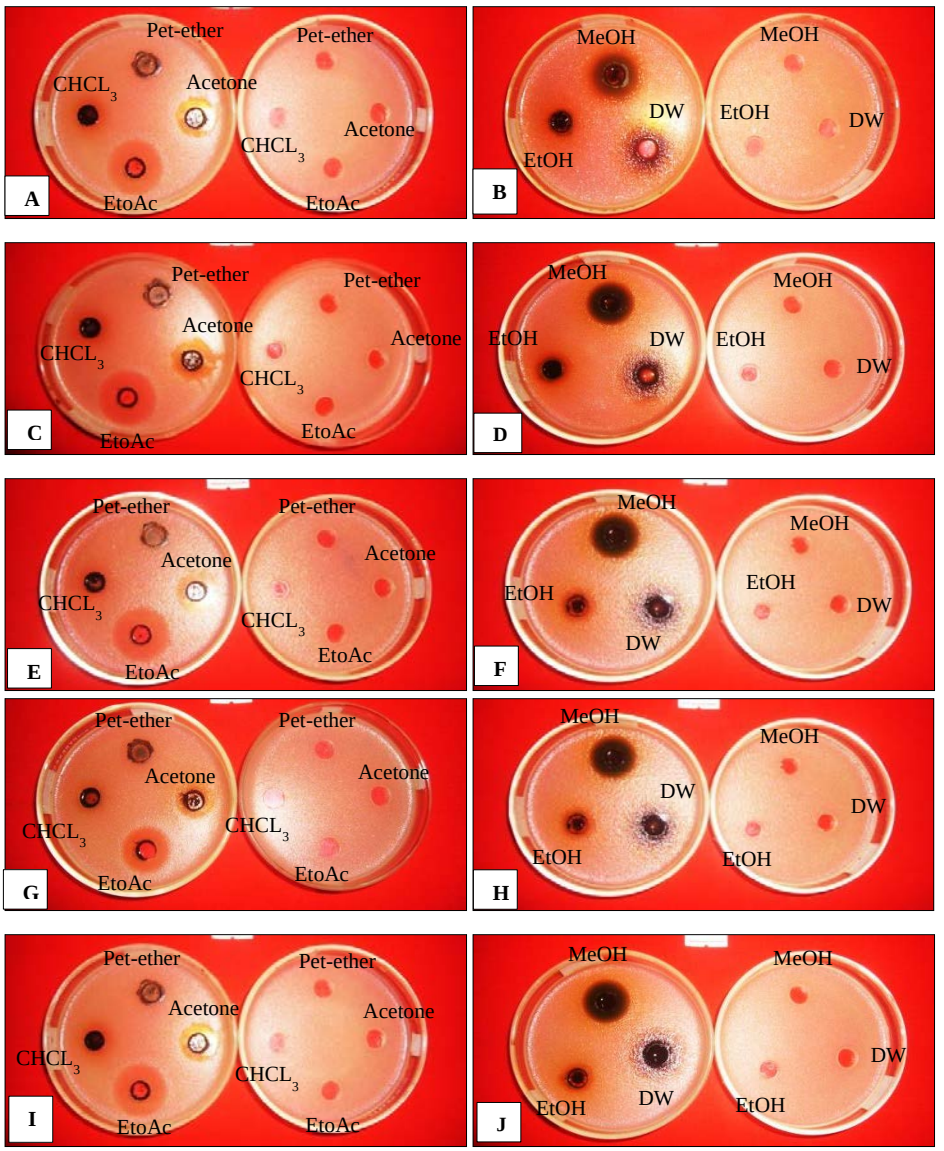


Figure 4. Antimicrobial activities of *Artemisia vulgaris* L. flowers against on test microorganisms

A-B. Antimicrobial activities and control against on *Bacillus subtilis*
C-D. Antimicrobial activities and control against on *Escherichia coli*
E-F. Antimicrobial activities and control against on *Pseudomonas aeruginosa*
G-H. Antimicrobial activities and control against on *Staphylococcus aureus*
I-J. Antimicrobial activities and control against on *Candida albicans*

Table 6. Results of antimicrobial activities

Solvent Samples		***Bacillus pumalis***	***Bacillus subtilis***	***Escher-ichia coli***	***Pseudomonas aeruginosa***	***Staphyloco-ccus aureus***	***Candida albicans***
Pet-ether	Leaves	12 mm	-	-	12 mm	12 mm	12 mm
	Flowers	15 mm	14 mm	15 mm	-	-	14 mm
Chloro-form	Leaves	14 mm	12 mm	15 mm	14 mm	13 mm	13mm
	Flowers	22 mm	25 mm	25 mm	15 mm	22 mm	14 mm
Ethyl acetate	Leaves	26 mm	15 mm	27 mm	28 mm	24 mm	25mm
	Flowers	35 mm	34 mm	38 mm	40 mm	25 mm	20 mm
Acetone	Leaves	18 mm	12 mm	19 mm	18 mm	17 mm	19mm
	Flowers	18 mm	23 mm	22 mm	20 mm	15 mm	18mm
Methanol	Leaves	22 mm	22 mm	23 mm	23 mm	22 mm	23 mm
	Flowers	20 mm	15 mm	20 mm	21 mm	20 mm	-
Ethanol	Leaves	15 mm	-	18 mm	27 mm	15 mm	15 mm
	Flowers	20 mm	16 mm	21 mm	15 mm	20 mm	-
D/W	Leaves	13 mm	-	14 mm	-	13 mm	-
	Flowers	-	12 mm	-	-	-	-

Agar well = 10 mm, **-** = no inhibition

11 – 13 mm = weak activity, 14 – 18 mm = high activity, >18 mm = very high activity

Discussion and Conclusion

In this study, qualitative and quantitative analysis and antimicrobial screening of leaves and flowers of *A. vulgaris* L. were performed.

Kumar and Kumud (2010) reported that the aerial parts of *A. vulgaris* L. contains saponins, phytosterols, carbohydrates, proteins and amino acid, and flavonoid. In this study, alkaloids, α-amino acids, carbohydrates, starch, reducing sugars, glycosides, phenolic compounds, saponins, tannins, steroids/terpenoids and flavonoids except the cyanogenic glycosides were detected from the leaves and flowers of *A. vulgaris* L.. The findings of metabolites in this study agreed with those mentioned by Kumar and Kumud (2010). According to the results, this plant is rich in metabolites.

Kumar and Kumud (2010) also stated that the moisture content of aerial parts of *A. vulgaris* L. was 12.54-16.15%. Schuman and Howard (1977) stated that average ash content of *A. vulgaris* L. at various growth stages was 13.7%. In this study, the moisture content of leaves was 13.35% and the flowers moisture content was 9.75%. The total ash content in leaves was 10.21 % and 6.56 % in flowers. The results of moisture content and total ash content of leaves in this study are almost similar with the above reports who stated by Schuman and Howard (1978) and Kumar and Kumud (2010).

In this study, toxic metals, arsenic, mercury and lead were not found in both samples of *A. vulgaris* L. except cadmium. Cadmium was found in leaves (0.03 ppm) and flowers (0.006 ppm) respectively. WHO (2011) stated the estimated lethal oral dose for humans is 350 - 3500 mg of cadmium. According to this literature, *A. vulgaris* L. can be used safely as an edible or a medicinal plant for human.

Baykanerela *et al*. (2012) stated that the methanolic extracts of *Artemisia vulgaris* L. showed inhibition on *P. aeruginosa* as 23 mm diameter zone. In this study, the methanolic leaves extract showed inhibition as 23 mm and flowers extracts showed 21 mm on *P. aeruginosa.* Therefore, the findings of antimicrobial activity on *P. aeruginosa* agree with the results mentioned by Baykanerela *et al*. (2012).

Mensah *et al*. (2015) described that the acetone extracts of *Artemisia vulgaris* L. have a greater activity against *Bacillus subtilis* than ethanol extract. In this study, acetone extracts of leaves showed weak activity (12 mm) and acetone extracts of flowers showed very high activity (23 mm) against *Bacillus subtilis.* No inhibition of ethanolic leaves extracts showed on *Bacillus subtilis*. The ethanolic flower extract showed high activity (16 mm) against *Bacillus subtilis.* The acetone extracts of leaves and flowers showed greater activities than ethanolic leaves and flower extracts against on *Bacillus subtilis*. These findings agreed with Mensah *et al*. (2015). The locality of the plant material and the extraction procedure cause differences in the antimicrobial activity and the presence of metabolites of this plant.

In conclusion, this study can support for future researches concerning the production of plant based antimicrobial products for local needs.

Acknowledgement

I would like to express my special thanks to Dr Moe Moe Lwin, Professor and Head, Department of Botany, Kyaing Tong University for her supervision, invaluable guidance and advices throughout my research.

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Taxonomic Study on Five Species of Families Gesneriaceae and Linderniaceae in Indaw and Banmauk Townships of Sagaing Region

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Abstract

Taxonomic studies on some species of families Gesneriaceae and Linderniaceae in Indaw Township and Banmauk Township of Sagaing Region were conducted. In this research, *Paraboea sinensis* (Oliver) B. L. Burtt and *Rhynchoglossum gardneri* Theobald & Grupe are included in the family Gesneriaceae. The three species, *Lindernia antipoda* (L.) Alston, *Lindernia dubia* (L.) Pennell and *Torenia fournieri* Lind., are belonging to the family Linderniaceae. The collected species were systematically arranged according to families stated in APG IV classification system of Byng *et al*. (2016). Most species were shrubs and some were herbs and trees. The detailed description of the species was presented with relevant photographs. An artificial key to the species was also included.

Keywords: Gesneriaceae, Linderniaceae, Indaw Township, Banmauk Township, Taxonomy

Introduction

The present study deals with some species of families Gesneriaceae and Linderniaceae growing in Indaw Township and Banmauk Township of Sagaing Region. The Indaw Township lies between North Latitude 24°13′ and East Longitude 96°08′. The elevation of Indaw is 118-132 m above sea level. The Banmauk Township stands between North Latitude 24°24′ and East Longitude 95°51′. The elevation of this area is 266-820 m above sea level.

The Gesneriaceae family shows many adaptations to specialized habitats in both vegetative and floral characters. Many epiphytic or epilithic species have the ability to dry out for short periods and revive themselves in wetter conditions. In the Old World, the cotyledons develop unequally, and in some genera the only adult vegetative structure is one much-enlarged cotyledon that lies flat against a tree-trunk or rock. Floral adaptations are

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associated with pollinating agents, involving often brightly colored corolla and sometimes calyx. In some species, the pollinator is attracted by extra-floral structures, such as coloured hairs or leaves, Many New World genera have developed brightly colored fleshy fruits, which are adapted to seed dispersal by birds, bats, monkeys or ants. The colourful flowers and comparative case of cultivation have made many genera important in horticulture, but few are hardy in the temperate regions. The family also provides many medicinal plants (Heywood 1978).

The recently described Linderniaceae are a monophyletic group that emerged in the course of the disintegration of the Scrophulariaceae in the last years. First molecular studies sampled only a small fraction of the genera assigned to the Linderniaceae, but later *Lindernia* was shown to be non-monophyletic (Fischer *et al*. 2013).

Although many researchers had done on taxonomic research works on several families, the species of the families Gesneriaceae and Linderniaceae are still remained to be studied.

The aim and objectives of this research were to classify and identify the plants of families Gesneriaceae and Linderniaceae, to describe the detail characteristics of the collected specimens, to contribute the floristic information for natural scientific researches.

Materials and Methods

The field observation and collection of the fresh flowering plant specimens were undertaken from Indaw Township and Banmauk Township of Sagaing Region. All the collected specimens were recorded as photographs while flowering and labeled by collection numbers. Field notes were stated with detailed plant description and habitat and precise location by using Global Positioning System (GPS). According to the plant collection and preservation techniques, the collected specimens had been air dried and pressed. The longitudinal and transverse sections of the ovary were also dissected for the classification and identification of the collected species.

For morphological characteristics, the collected specimens were identified with the help of various flora and monographs. Identification of collected specimens was carried out by referring to Hooker (1881 to 1887), Brandis (1906), Backer (1965) and Dassanayake (1980 to 2001). The final

verification was confirmed according to the recent online information of international plant name index and plant information of Tropicos website. The collected species were systematically arranged according to the classification system of Byng *et al*. (2016). An artificial key to the species was constructed and presented. All the collected species were prepared to herbarium specimens and the voucher specimens were deposited at the herbarium of Botany Department, University of Mandalay.

Results

The collected 5 species belonging to 4 genera of 2 families were systematically arranged into families according to APG IV System (2016). Then the genera and species were also arranged by alphabetically as shown in Table 1.

Table 1. List of Collected Species

Group	Order	Family	No.	Scientific Name
Asterids	Lamiales	Gesneriaceae	1.	*Paraboea sinensis* (Oliver) B. L. Burtt
			2.	*Rhynchoglossum gardneri* Theobald & Grupe
		Linderniaceae	3.	*Lindernia antipoda* (L.) Alston
			4.	*Lindernia dubia* (L.) Pennell
			5.	*Torenia fournieri* Lind.

1. ***Paraboea sinensis*** (Oliver) B. L. Burtt, Notes Roy. Bot. Gard. Edinburgh. 38: 471. 1980. (Figure 1.)

Phylloboea sinensis Oliver, Hooker's Icon. Pl. 18: pl. 1721.

Myanmar name : Unknown

English name : Unknown

Flowering period : June to October

Perennial, shrubs, about up to 1.0 m high; stems and branches terete, brown pannose, glabrescent. Leaves simple, opposite and decussate, exstipulate, petiolate; petioles canaliculate above, about 0.5-8.0 cm long; blades elliptic, 2.0-13.0 cm by 1.0-6.0 cm, cuneate at the base serrate along the margin, acute at the apex, puberulent on both surfaces. Inflorescences axillary, solitary. Flowers whitish purple, about 1.5 cm in diameter, bisexual, zygomorphic, pentamerous, hypogynous; bracts orbicular, about 8.0 mm long; pedicel about 1.0 cm long. Calyx 5-lobed, oblong, about 7.0 mm long, puberulent without, glabrous within. Corolla 5-lobed, whitish purple, glabrous without, hairy within; tubes about 1.0 cm long; lobes 5.0 mm long. Stamens 2, free, epipetalous, inserted; filaments filiform, short, about 3.0 mm long, pubescent; anthers dithecous, dorsifixed, brown. Disc inconspicuous. Carpels 2, syncarpous; ovary oblong, bilocular, many ovule in each locule on the 2 projecting inward parietal placenta; style filiform, about 2.0 cm long; stigma simple. Capsule spirally twisted, about 2.5-5.6 cm long, glabrous. Seeds unappendaged.

Specimens Examined : Sagaing Region, Ban Mauk Township, Za Lone Moutain Area, N Latt 24° 31.399' and E Long 095° 49.107', Elevation 820 m; 22th October 2018; May Phyoe Thynn; Collection No. 32.

Figure 1. *Paraboea sinensis* (Oliver) B. L. Burtt
A. Inflorescence B. L.S of Flower C. Stamens
D. Pistil E. L.S of Ovary F. T.S of Ovary

2. *Rhynchoglossum gardneri* Theobald & Grupe, Ceylon J. Sci., Biol. Sci. 10: 70. 1972. (Figure 2.)

Klugia zeylanica Gardn., Calcutta J. Nat. Hist. 6: 498. 1846; Trimen, Handb. Fl. Ceylon 3: 278. 1895.

Myanmar name : Pan-pyar

English name : Unknown

Flowering period : May to October

Annual, erect or prostrate herbs, about 20-30 cm high; stems and branches quadrangular, succulent, with a sparsely villous line down one side of stem. Leaves simple, alternate, exstipulate, petiolate; petioles 0.5-3.0 cm long, glabrous or villous above; blades ovate to ovate-oblong, 4.5-15.0 cm by 1.5-6.5 cm, cordate or rounded at one side of the unequal base, distinctly incurved, entire along the margin, acuminate at the apex, membranous, bright green above, paler beneath, glabrous to scabrous above, glabrous beneath. Inflorescence pseudo-raceme, terminal, appearing lateral and opposite leaf, one-sided, many-flowered; peduncles 1.0-2.5 cm long; glabrous to villous along one side. Flower bright blue, 5.0 mm in diameter, bisexual, zygomorphic, pentamerous, hypogynous; bracts linear, 1.0-2.0 mm long, glabrous; pedicels 5.0 mm long. Calyx campanulate or tubular-campanulate; tube 5-angled, equal and slightly winged, 5.0 mm long; lobes lanceolate-acuminate, 4.0 mm long, greenish-white. Corolla

bilabiate, personate, 5-lobed; tube white, 8.0 mm long, glabrous without, pubescent within; upper lip white, about 3.0 mm by 3.0 mm, glabrous; lower lip bright rich blue with a yellow spot at base, 6.0 mm by 7.0 mm, glabrous. Stamens 4, free, epipetalous, included; filaments unequal, filiform, glabrous, about 5.0 mm long; anthers dithecous, dorsifixed, glabrous. Disk cupular. Carpels 1; ovary ovoid, glabrous, 1.0 mm long, unilocular, with many ovules on the parietal placentae; style slender, 4.0 mm long, glabrous; stigma oblique, sub-capitate. Capsules ovoid, 4.0-5.0 mm by 3.0-4.0 mm, membranous.

Specimens Examined : Sagaing Region, Ban Mauk Township, Za Lone Moutain Area, N Latt 24° 31.399' and E Long 095° 49.107', Elevation 820 m; 22th October 2018; May Phyoe Thynn; Collection No. 41.

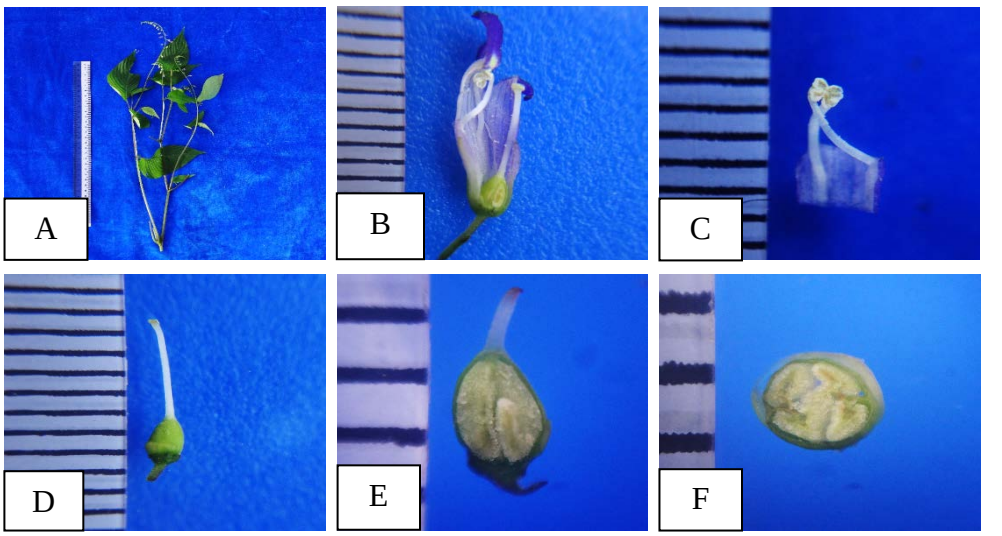


Figure 2. *Rhynchoglossum gardneri* Theobald & Grupe

A. Inflorescence B. L.S of Flower C. Stamens
D. Pistil E. L.S of Ovary F. T.S of Ovary

3. *Lindernia antipoda* (L.) Alston in Trimen, Hand. Fl. Ceylon. 6 (suppl.): 24. 1931. (Figure 3.)

Ruellia antipoda L., Sp. Pl. 2: 635. 1753.

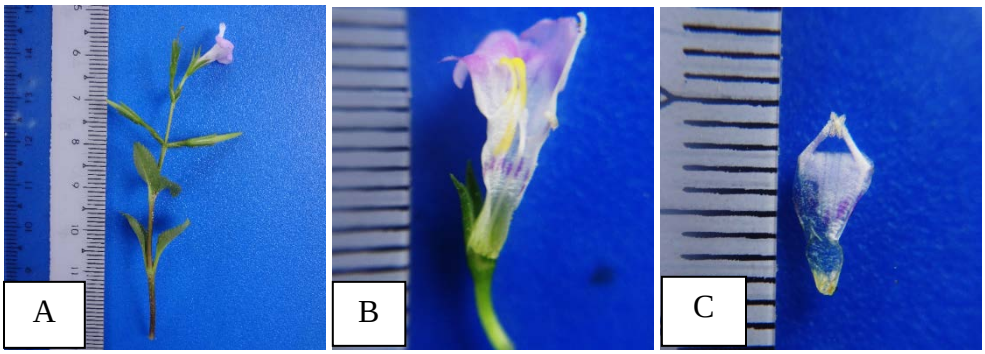
Myanmar name : Unknown

English name : Yellow seed false pimpernel

Flowering period : June to October

Annual, herbs, up to 0.5 m high; stems and branches quadrangular, suberect or prostrate basally and rooting from lower nodes then ascending, many branched, channeled, glabrous. Leaves simple, opposite and decussate, exstipulate, petiolate; petioles about 0.3-1.0 cm long; blades elliptic, 1.5-3.0 cm by 1.0-1.5 cm, cuneate at the base, serrate along the margin, obtuse at the apex, glabrous on both surfaces. Inflorescences terminal or axillary, racemes. Flowers pale purple, about 7.0 mm in diameter, bisexual, zygomorphic, pentamerous, hypogynous; bracts linear or linear-lanceolate to oblong-lanceolate, serrulate at the margins; pedicels about 3.0 mm long, deflexed in fruit. Calyx 5-lobed, cleft to base; tubes 1.0 mm long; lobes linear-lanceolate, about 2.0 mm long, subequal, hispidulous along midrib and edges. Corolla 5-lobed, bilabiate, pale purple; tube about 4.0 mm long, whitish or pale yellow towards base; the lower lip 3-lobed, orbicular, midlobe slightly larger than lateral ones with usually purplish spots at the base; the upper lip 2-lobed, linear-oblong, concave. Stamens 2, unequal, free, epipetalous, inserted; filaments filiform, about 4.0 mm long; anthers dithecous, dorsifixed. Carpels 2, syncarpous; ovary oblong, bilocular, with many ovules in each locule on the axile placentae; style slender, 1.0 cm long; stigma bifid. Capsule linear-cylindric or subulate-cylindric, two and a half times as long as calyx, glabrous, often dull purple. Seeds oblong to ellipsoid, reticulate, tawny.

Specimens Examined : Sagaing Region, Indaw Township, Nam Khar Village, N Latt 24° 12.354' and E Long 096° 08.657', Elevation 132 m; 23th October 2018; May Phyoe Thynn; Collection No. 44.



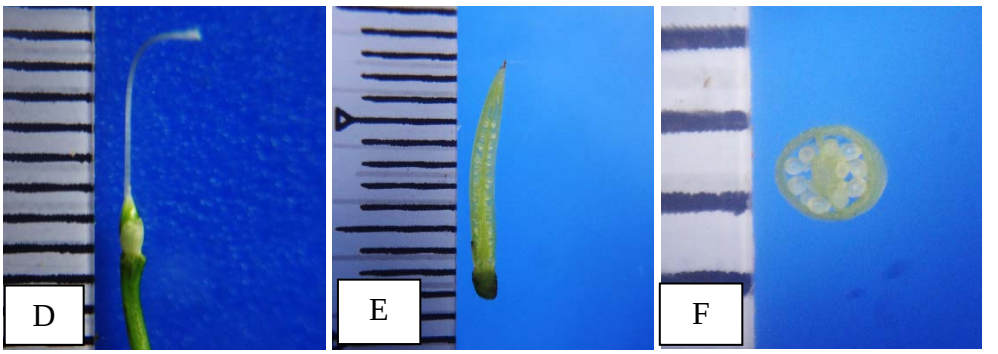


Figure 3. *Lindernia antipoda* (L.) Alston

A. Inflorescence	B. L.S of Flower	C. Stamens
D. Pistil	E. L.S of Ovary	F. T.S of Ovary

4. *Lindernia dubia* (L.) Pennell, Acad. Nat. Sci. Philadelphia Monogr. 1: 141. 1935. (Figure 4.)

Gratiola dubia L., Sp. Pl. 1: 17. 1753.

Myanmar name : Unknown

English name : Unknown

Flowering period : June to October

Annual, herbs, up to 0.5 m high; stems and branches quadrangular, rooting at lower nodes, much branched, puberulous. Leaves simple, opposite and decussate, exstipulate, sessile; blades elliptic, 0.6-0.9 cm by 0.5-0.1 cm, rounded or slightly cuneate at the base, entire along the margin, acute at the apex, glabrous on both surfaces. Inflorescences axillary, solitary. Flowers pale blue, about 0.7 cm in diameter, bisexual, zygomorphic, pentamerous, hypogynous, showy; pedicels 1.0-2.0 cm long. Calyx 5-lobed, broadly cupular, green, glabrous; tubes 0.1 cm long; lobes about 0.2 cm long, hispidulous above. Corolla 5-lobed, bilabiate, pale blue; the lower lip 3-lobed; the upper lip 2-lobed, galeate; lobes sharply pointed. Stamens 4, free, epipetalous, inserted; filaments filiform, about 0.4 cm long; anthers dithecous, sagittate, basifixed; staminode 2. Disc short, annular, greenish. Carpel 2, syncarpous; ovary ovate, bilocular, with many ovules in each locule on the axile placentae; style slender, 0.5 cm long; stigma

capitate, flattened. Fruit a capsule, oblong, about 0.3 cm long, rounded at both ends. Seeds ellipsoid.

Specimens Examined: Sagaing Region, Indaw Township, Nam Khar Village, N Latt 24° 12.459' and E Long 096° 08.354', Elevation 120 m; 21th October 2018; May Phyoe Thynn; Collection No. 40.

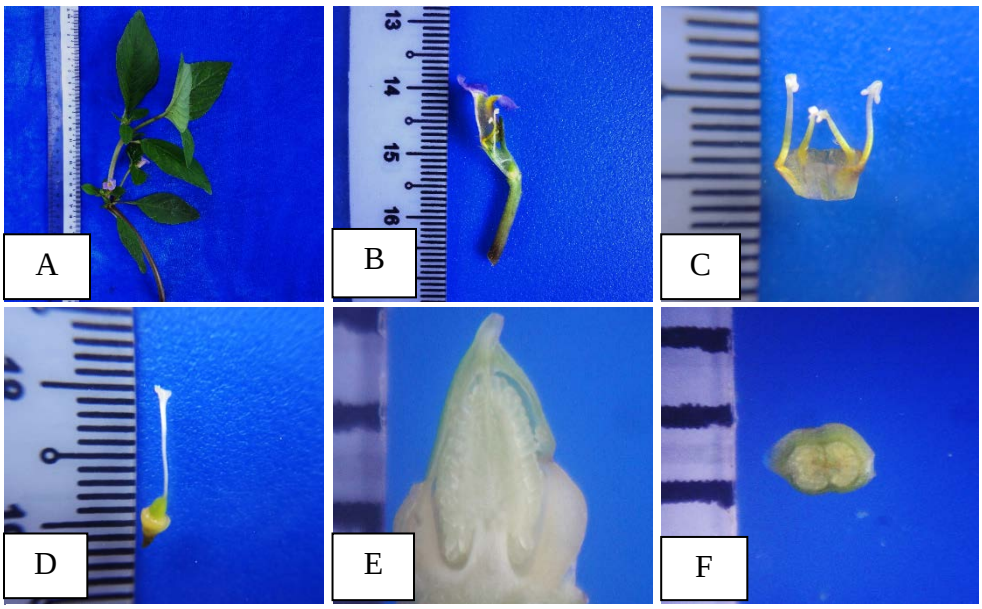


Figure 4. *Lindernia dubia* (L.) Pennell

A. Inflorescence B. L.S of Flower C. Stamens
D. Pistil E. L.S of Ovary F. T.S of Ovary

5. *Torenia fournieri* Lind. ex Fourn., Illustr. 1. Hortic. 23: 129. 1876. (Figure 5.)

Myanmar name : Kya pazat gale

English name : Torenia

Flowering period : August to January

Annual erect herbs, up to 5.0 cm high; stems and branches quadrangular, no rooting at the lower node, spreading branch, pubescent. Leaves simple, opposite and decussate, exstipulate, petiolate; petioles slender, 3.0-4.0 mm long; blades ovate-oblong, 1.7-3.0 cm by 1.0-1.5 cm, cuneate at the base, serrate along the margin, acute at the apex, glabrous on both surfaces. Inflorescences axillary and solitary. Flowers purple, about 2.2

cm in diameter, bisexual, zygomorphic, pentamerous, hypogynous; bracts lanceolate, about 0.8 cm long; pedicel about 1.5 cm long, sparsely pubescent. Calyx tubular, 5-lobed; tubes winged strigose. Corolla bilabiate, tubular, 5-lobed; tube expand above, about 1.5 cm long, upper lip-2-lobed; orbicular, about 1.0 cm long; lower lip 3-lobed, about 0.7 cm long. Stamens 4, didynamous, epipetalous, inserted; filaments filiform, appendage at base, about 5.0 mm long; anthers dithecous, basifixed, longitudinal dehiscing. Carpels 2, syncarpous; ovary superior, oblong, about 2.0 mm long, bilocular with many ovules in the locule on axile placenta; style terminal, about 1.0 cm long; stigma bilobed. Fruits capsular, oblongoid, many-seeded, 5.0-7.0 mm long. Seeds orbicular-oblong, 0.5-1.0 in diameter, yellow.

Specimens Examined: Sagaing Region, Ban Mauk Township, Za Lone Moutain Area, N Latt 24° 31.587' and E Long 095° 49.571', Elevation 774 m; 22th October 2018; May Phyoe Thynn; Collection No. 42.

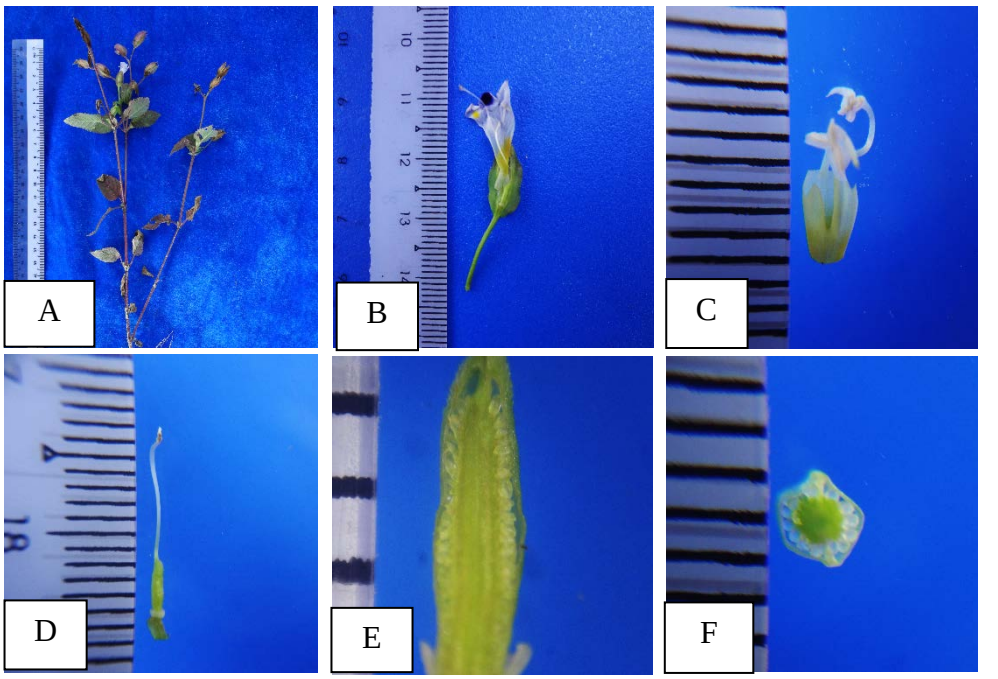


Figure 5. *Torenia fournieri* Lind.

A. Inflorescence B. L.S of Flower C. Stamens
D. Pistil E. L.S of Ovary F. T.S of Ovary

An Artificial Key to the Species

1. Plants shrubs; stems and branches terete ---------- **1. *Paraboea sinensis***

1. Plants herbs; stems and branches quadrangular ---------- 2

2. Leaves alternate; carpels 1; ovary unilocular ----------
---------- **2. *Rhynchoglossum gardneri***

2. Leaves opposite and decussate; carpels 2; ovary bilocular ------- 3

3. Stems and branches no rooting at the lower node; leaves ovate-oblong; stamens 4 ---------- **5. *Torenia fournieri***

3. Stems and branches rooting at the lower node; leaves elliptic; stamens 2 or 4 ---------- 4

4. Leaves petiolate; inflorescences racemes; flowers pale purple; stigma bifid ---------- **3. *Lindernia antipoda***

4. Leaves sessile; inflorescences solitary; flowers pale blue; stigma capitate ---------- **4. *Lindernia dubia***

Discussion and Conclusion

The present research work deal with the taxonomic some species of the families Gesneriaceae and Linderniaceae. In this research, the flowering specimens were collected from Banmauk Township and Indaw Township of Sagaing Region.

In Gesneriaceae, two species, were collected from the study area. *Paraboea sinensis* (Oliver) B. L. Burtt and *Rhynchoglossum gardneri* Theobald & Grupe were collected from the Za Lone Mountain area of Ban Mauk Township of Sagaing Region. Shrubs, axillary solitary type of inflorescences, ovary with 2 projecting inward parietal placentation and spirally twisted capsules are the distinct characters of *Paraboea sinensis* (Oliver) B. L. Burtt. *Rhynchoglossum gardneri* Theobald & Grupe possess the characters of annual, prostrate herbs, arrangement of leaves alternate, terminal pseudo-raceme type of inflorescences with many-flowered, corolla bilabiate, carpel one, unilocular ovary with the parietal placentation, capitate stigma. The characters of the above species were agreed with Wentsai *et al*. (1998) and Dasssanyake (1981).

In this research work, the two species, *Lindernia antipoda* (L.) Alston and *Lindernia dubia* (L.) Pennell, were collected from Nam Khar Village of Indaw Township of Sagaing Region. The one species of this family, *Torenia fournieri* Lind., was found in Za Lone Mountain area of Ban Mauk Township of Sagaing Region.

The two species, *Lindernia antipoda* (L.) Alston and *Lindernia dubia* (L.) Pennell were annual herbs. The stems and branches of these species is suberect or prostrate basally and rooting from lower nodes. The petioles were absent in the leaves of *Lindernia dubia* (L.) Pennell. These two species posses bilabiate corolla. The anthers of *Lindernia dubia* (L.) Pennell were sagittate and the stigma were capitate. The characteristics of the above species were similar to Dassanayake (1981) and Kottaimuthu (2016). The species *Torenia fournieri* Lind. possess axillary and solitary type of the inflorescences, calyx with winged strigose, bilabiate corolla and bilobed stigma. The fruit type of this species was a capsule with many-seeded. These distinct characters were agreed with Dassanyake (1981).

In this research work, the specimens including both medicinal plants and threatened species were collected. *Lindernia antipoda* (L.) Alston was mentioned as a threatened species in the IUCN Red List (2011).

The present study will contribute towards a deep, better understanding and knowlege on morphological characters of the families Gesneriaceae and Linderniaceae. These two study areas are very intersting for its richness of natural plant resources and various diversity of plants. Therefore, it is hoped that the research work of floristic study will provide the valuable taxonomic information and distribution of plant species for further scientific research.

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Phytochemical, Elemental, Physicochemical and Antioxidant Activities from Fruit Pulp of *Persea americana* Miller

Naw Al Shar Phaw[1] & Soe Myint Aye[2]

Abstract

In this study, phytochemical, elemental, physicochemical and antioxidant activities from the fruit pulp of *Persea americana* Miller (Avocado) were carried out. The fruits of *Persea americana* Miller were collected from Pyin Oo Lwin Township during the year of 2017-2018. Preliminary phytochemical examinations, elemental analysis, physicochemical characterization, and the antioxidant activity were laid out on the fruit pulp of *Persea americana* Miller. According to the phytochemical examinations, alkaloid, glycoside, steroid, terpene, reducing sugar, carbohydrate, phenolic compound and tannin were detected. In elemental analysis, sodium, potassium, calcium, iron, zinc and iridium were present. Physicochemical characterization showed the content of moisture (3%), soluble matter of hexane (32%), ethanol (32%), pet-ether (32%) and water soluble matter of ash (72.6%). To test the antioxidant activity, four solvents, ethanol, n-hexane, ethyl acetate and water were used for the crude extracts. Among the four extracts, ethanol fractions exhibited the highest values of antioxidant activies (17.36 ± 1.256 IC_{50}).

Keywords: *Persea americana* Miller, phytochemical, elemental analysis, physicochemical, antioxidant activity

Introduction

Avocados are an important food crop in Central America. Elsewhere in the tropics they are grown for home consumption and the local markets. Commercial production is confined to California, Florida, Cuba, Argentina, Brazil, South Africa, Australia and Hawaii. In temperate countries the avocado is still regarded as a luxury fruit, but it is likely to gain in popularity as more people become acquainted with it. Production in the United States increased from 130 tons in 1924 to approximately 21,000 tons in 1948 and 40,000 tons in 1958 (Purseglove 1968).

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[2] Rector, Sagaing University

The chemical composition of the avocado shows that the percentage of water, about 60%, is relatively low compared to the average of other fruits, consequently, there is a higher percentage of other components, increasing the nutritional values of the fruit-lipids are present in large amounts, 30%. The digestible carbohydrates are only 3 to 10%. As well as proteins and mineral salts, there are many vitamins, from A to K, including B_1, essential for normal metabolism, and B_2, which stimulates growth, also vitamin C (antiscorbutic) and pantothenic acid. The avocado is therefore highly nutritious, supplying about 230 calories per 3½ ounces (100 grams) of pulp. It is eaten fresh and, because of the low percentage of sugars, can be used in antipasto and salads (Bianchini & Corbetta 1976).

Plant natural products are involved in many aspects of human existence. These natural products may be used as purified compounds or as components of complex mixtures which serve as medicines, pesticides, flavorings, herbicides, etc. (Atta *et al.* 2013).

Phytochemicals are the individual chemicals from which the plants are made. Phytochemical is simply a word that means plant chemicals. Plants have the ability to synthesize mixtures of structurally diverse bioactive compounds with multiple and mutually potential therapeutic effects. The plants have the capacity of manufacturing the secondary products. Hundreds of phytochemicals are currently being studied. Many are believed to have a major positive impact on human health (Duke & Sternberg 2005 as cited in Raaman 2006). Important plant secondary metabolites have been isolated over a period of time from natural sources (Raaman 2006).

The primary metabolites have an essential role in photosynthesis, respiratory, growth and development. Secondary metabolites are not directly involved into normal growth, development. These secondary metabolites play a major role in defense mechanism against herbivores. Various effective contents are present in the medicinal plants. Alkaloids, flavonoids, glycosides, tannins, saponins etc., are the most effective contents against various diseases. These contents are also used for the development of new medicines by which people can develop their immune system (Shashank *et al.* 2015).

The aim of the present study was concerned with the phytochemical constituents of *Persea americana* Miller. The objectives of this research were

to study the phytochemical constituents, to examine the physicochemical characterization, to determine the elemental analysis and to observe the antioxidant activity of the fruit pulp of *Persea americana* Miller growing in Pyin Oo Lwin Township.

Materials and Methods

Preliminary Phytochemical Studies

The preliminary phytochemical study was carried out on the fruit pulp of *Persea americana* Miller in order to determine the presence of alkaloid, glycoside, steroid, terpene, reducing sugar, carbohydrate, cyanogenic glycoside, phenolic compound, tannin and acid/ base/ neutral. The methods were used according to Trease and Evans (2002) and Raaman (2006). The qualitative analysis was undertaken at the Department of Chemistry, University of Mandalay and Pharmaceutical Laboratory, Biotechnology and Material Science Research Department, Ministry of Science and Technology, Kyaukse, Myanmar.

Elemental Analysis by Using WDXRF

The elemental analysis of the fruit pulp of *Persea americana* Miller was examined by using the Wave Length Dispersive X-ray Fluorescence (WDXRF, Super Mini 200, Rigaku, Japan) at the Department of Geology, Universities' Research Center, University of Mandalay.

Physicochemical Characterization of *Persea americana* Miller

Physicochemical characterization consisting of moisture, total ash; acid insoluble ash, water soluble ash, and solubility of ethanol, n-hexane, methanol, petroleum ether, ethylacetate and water soluble matter of fruits was carried out by the methods of British Pharmacopoeia (1973). All the quantitative analysis was carried out at the Department of Botany, University of Mandalay.

Antioxidant Activity of Crude Extracts from the Fruit Pulp of *Persea americana* Miller by Using DPPH Free Radical Scavenging Method

The antioxidant activity of the fruit pulp of *Persea americana* Miller was determined using four different fractions extracted with ethanol, n-hexane, ethyl acetate and water. This study was tested at Biotechnology and Material Science Research Department, Ministry of Science and Technology, Kyaukse, Myanmar.

Procedure of Antioxidant activity

The antioxidant activity of the different fractions of crude extracts was determined by 1, 1-diphenyl- 2- picrylhydrazyl (DPPH) free radical scavenging assay according to Lee *et al*. (2004). The reaction mixture containing 5 µL of test sample (16, 8, 4, 2, 1, 0.5, 0.25 mg/ml) and 95 µL of DPPH (200 µmol) dissolved in ethanol, was taken in a 96-well micro-titer plate and kept standing at 37°C for 30 minutes. The absorbance was measured at 518 nm by using 96-well microplate reader (TC 96-ELISA Microplate reader). Ascorbic acid was used as a standard. Percent Radical Scavenging Activity (% RSA) was calculated by using the following formula:

$$\% \text{ RSA} = [1 - (\text{OD test compound} / \text{OD control})] \times 100$$

RSA = Radical Scavenging Activity

OD = Optical Density

Results were expressed as Mean ± Standard Error Mean (SEM) of three independent experiments for each test samples. The IC_{50} of the samples was determined by using the statistical programs, Graph Pad Prism Version 5.01.

Results

Phytochemical Activities

According to the preliminary phytochemical tests, alkaloid, glycoside, steroid, terpene, reducing sugar, carbohydrate, phenolic compound, tannin and organic base were present. However, cyanogenic glycoside was not detected. The results of the preliminary phytochemical activities were shown in Table 1.

Elemental Analysis

The elemental analysis of the fruit pulp of *Persea americana* Miller was shown in Table 2 and Figure 1. According to the elemental analysis, sodium 16.23%, potassium 70.31%, calcium 9.06%, iron 2.48%, zinc 0.76% and iridium 0.44% respectively.

Physicochemical Characterization

The physicochemical characterization, such as moisture, ethanol, hexane, methanol, petroleum ether, ethylacetate, water, total ash, acid insoluble ash and water soluble ash were shown in Table 3. According to the physicochemical activities, moisture contents in the fruit pulp was 3%. The ethanol, n-hexane and petroleum ether soluble matters were found to be 32% respectively. The ash contents in water extract was 72.6%.

Antioxidant Activities

In this experiment, seven different concentrations (16, 8, 4, 2, 1, 0.5, 0.25 mg/ml) were used for four different crude extracts namely ethanol, n-hexane, ethyl acetate and water. Ascorbic acid was used as standard.

Comparison on Inhibition % and IC_{50} Values of Four Different Crude Extracts from *Persea americana* Miller Fruits Pulp

The comparative inhibition % and IC_{50} values of each crude extract from the fruits pulp of *Persea americana* Miller and ascorbic acid at the concentration 4 mg/ml were shown in Table .4, Figure 2 and 3.

The inhibition % and IC_{50} values of ethanol extract were 21% and 17.36 ± 1.256 mg; n-hexane, 1% and 92.8 ± 1.052 mg; ethyl acetate, 70% and 24.67 ± 1.197 mg; water, 17% and 98.13 ± 1.067mg; ascorbic acid, 81% and 0.00064 ± 2.286 mg, respectively.

Table 1. Preliminary phytochemical tests on the fruit pulp of *Persea americana* Miller

No.	Constituent	Extract	Reagent used	Observation	Results
1	Alkaloid	HCl	- Dragendroff's reagent - Wagner's reagent	- Pale orange - Reddish brown ppt.	+ +
2	Glycoside	H_2O	10% lead acetate solution	Yellow ppt.	+
3	Steroid	EtOH	$CHCl_3$, Conc: H_2SO_4, aceticanhydride	Deep green	+
4	Terpene	EtOH	Conc: H_2SO_4	Yellowish brown	+
5	Reducing sugar	Distilled water	Benedict solution	Reddish brown ppt.	+
6	Carbohydrate	Distilled water	10% α-naphthol, Conc: H_2SO_4	Purple ring	+
7	Cyanogenic glycoside	Distilled water	Conc: H_2SO_4, Sodium picrate paper	Pink colour	-
8	Phenolic compound	Distilled water	Ferric chloride solution	Green colour	+
9	Tannin	Distilled water	$FeCl_3$, conc: H_2SO_4	Yellow ppt.	+
10	Acid / Base/ Neutral	Distilled water	Bromocresol green	Blue	Base

Table 2. Percentages of elements consisting in fruit pulp of *Persea americana* Miller

No.	Elements	Mass Percentage (%)
1.	Sodium	16.23
2.	Potassium	70.31
3.	Calcium	9.06
4.	Iron	2.48
5.	Zinc	0.76
6.	Iridium	0.44

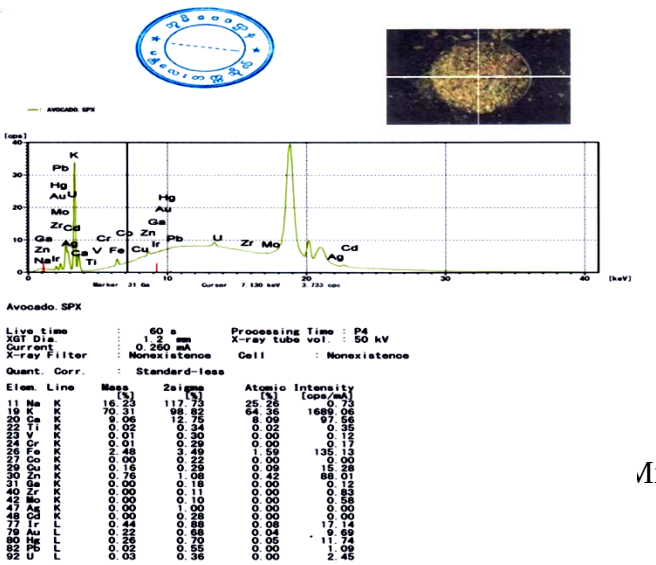

Figure 1. E Miller

Table 3. Physicochemical characterization of powdered fruit pulp of *Persea americana* Miller

No.	Physicochemical Characters	Quantity Determination (%)
1.	Moisture	3
2.	Ethanol soluble matter	32

No.	Physicochemical Characters	Quantity Determination (%)
3.	Hexane soluble matter	32
4.	Methanol soluble matter	28
5.	Petroleum ether soluble matter	32
6.	Ethylacetate soluble matter	6
7.	Water soluble matter	6
8.	Total ash	3.56
9.	Acid insoluble ash	21
10.	Water soluble ash	72.6

Table 4. *In vitro* antioxidant activity of the different fractions of avocado extracts (IC_{50}) against DPPH radicals

No.	**Samples**	**Conc. (mg/ ml)**	**% RSA**	**IC_{50} (mg) ± SEM**
1	Ethanol Fraction	4	21.51162791	17.36 ± 1.256
2	n-Hexane Fraction	4	1.162790698	92.8 ± 1.052
3	Ethyl acetate Fraction	4	70.34883721	24.67 ± 1.197
4	Water Fraction	4	17.44186047	98.13 ± 1.067
5	Ascorbic acid	4	81.39534884	0.00064 ± 2.286

RAS = Radical Scavenging Activity
SEM = Standard Error Mean
Conc. = Concentration
IC_{50} = The half maximal inhibitory concentration

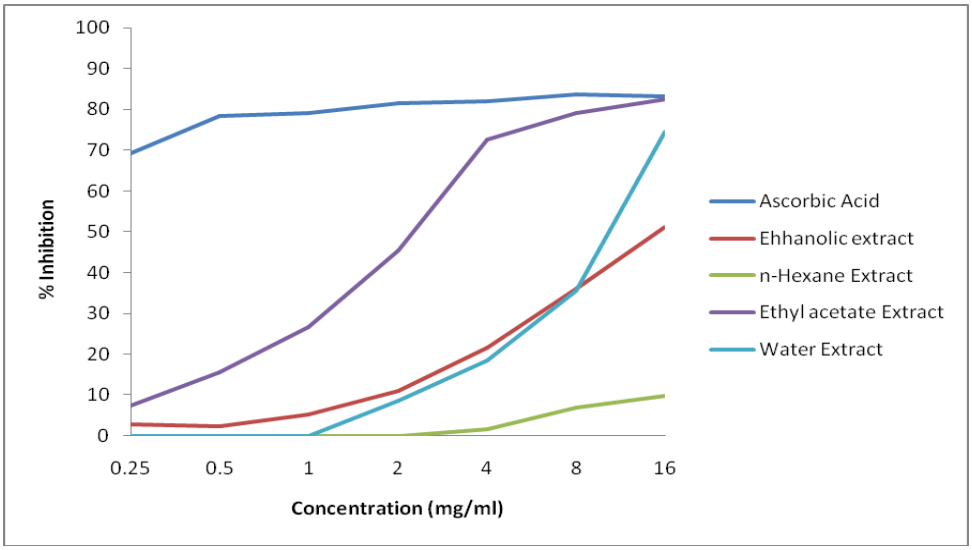

Figure 2. Inhibition % in different concentration of four crude extracts from fruit pulp of *Persea americana* Miller

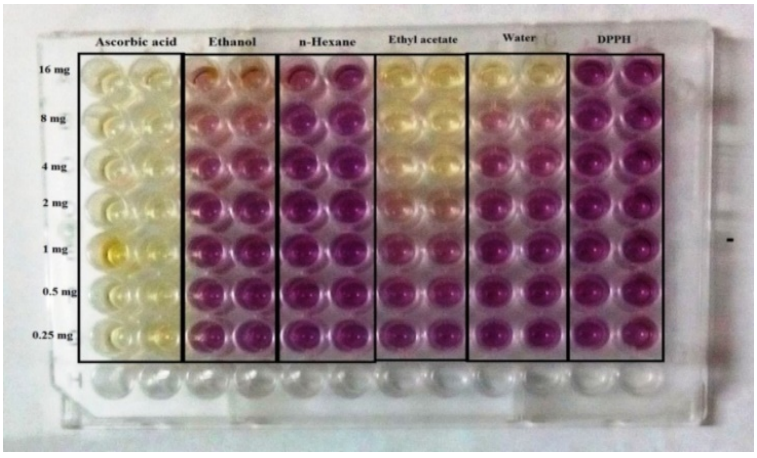

Figure 3. DPPH free radical scavenging assay with 96-well micro-titer plate

Discussion and Conclusion

In the present study, the phytochemical investigation, elemental analysis, examination of physicochemical characterization, and antioxidant activity were performed on the fruit pulp of *Persea americana* Miller.

The phytochemical study showed that alkaloids, glycosides, steroids, terpenes, reducing sugars, carbohydrates, phenolic compounds, tannins and organic bases were present. Alkaloids are vegetable bases containing nitrogen. Consequently they are of the utmost importance in medicine and constitute some of the most valuable drugs (Hill 1952). They are important therapeutically significantly plant secondary metabolites (Arukwe *et al*. 2012). Glucosides are similar to alkaloids in their properties, but they are derived from carbohydrates rather than proteins. They probably have a protective function. They may serve to regulate the acidity and alkalinity of plant cells. Glucosides are useful to man as drugs (Hill 1952). Arukwe *et al*. (2012) stated that steroids are antioxidants *in vitro*. Their values in the fruit pulp samples are appreciable and could add to their medicinal properties. Raaman (2006) stated that biological activity of various substances has been related with terpenoids. Many sesquiterpenes and diterpenes are used as antibiotics. Several sesquiterpenes have been found to be active against experimental tumours and the plant growth hormones like gibberellins and diterpenoids. As some terpenoids exhibit biological activity, namely, insecticidal anthelmintic or antiseptic, those are used in pharmacy. In the present study, alkaloid, glycoside, steroid and terpene are present as secondary metabolites in avocado fruits. Therefore, apart from its use as edible food, the avocado can be utilized for the valuable medicinal purposes such as antioxidant, antibiotic and antimicrobial properties.

Mineral elements in plants become important when their health benefits are considered in the body of organism. Most of these minerals occur as chemical compounds in solution form hence, they are able to diffuse to different part of plants. High sodium content in the body has been associated with high blood pressure in the body but this may not be possible in a situation of higher potassium content. Potassium was the highest to other minerals investigated in the present study. Potassium is necessary for electrolyte balance, controls high pressure, etc. Zinc plays role in wound healing, iron is known for haem formation, manganese and copper aid iron absorption in the body. The absence of lead, cadmium, and chromium could be an indication that the investigated samples are free of toxic metals (Arukwe *et al.* 2012).

Sodium is vital component of nerves as it stimulates muscle contraction. Sodium also helps to keep calcium and other minerals soluble

in the blood, as well as stimulating the adrenal glands. Sodium acids in preventing heat prostration or sunstroke (Obikoya 2015).

Iridium in the human body is a powerful antioxidant that stabilises the body's metabolism and destroys free radicals. Iridium is involved with processes that are associated with the spinal cord and pituitary gland (Anonymous 2015).

Iron is an important trace mineral which enters into the vital activity of the blood and glands. It is the master mineral which creates warmth, vitality and stamina. It is required for the health complexion and for building up resistance in the body. Iron also improves physical performances, can help preventing cancer, prevents and cures anaemia, helps preventing learning problems for children, increases immunity, raises energy levels, holds the energy level stable and promotes a calm sleep (Anonymous 2015).

According to the elemental analysis of the present study, avocado fruit pulp contains the important mineral elements, such as, sodium, potassium, calcium, iron, zinc and iridium. Those elements are beneficial for the human body to be healthy.

According to the physicochemical characterization, moisture content was found to be 3%. Vinha *et al.* (2013) stated that the moisture content is one of the most important indices evaluated in foods, especially fruits. It is a good indicator of their economic value because it reflects solid contents and serves to assess its perishability. Sanmugarajah *et al.* (2013) mentioned that the less value of moisture content of drugs could prevent content bacterial, fungal or yeast growth through storage. The ash values total ash; water soluble ash and acid insoluble ash from the present study were found to be 3.56%, 72.67% and 21% respectively. Total ash of 6.40% was low implying that the crude plant has low inorganic components. The aim of ashing was to remove all traces of organic matter. The total ash values can be used to detect foreign organic matter and adulteration with sand and earth, therefore, reflecting the kind of care that must be taken in preparing the plant for drug (Ibrahim *et al.* 2012). Sanmugarajah *et al.* (2013) stated that ash values used to find out quality, authenticity and purity of unsophisticated drug and also these values are important quantitative standards. The solubility percentages of *Persea americana* Miller fruit pulp in the ethanolic, hexane and pet-ether extracts were 32% respectively. Sanmugarajah *et al.* (2013) described that the extractive values are valuable

to estimate the chemical constituents present in the crude drug and furthermore assist in evaluation of definite constituents soluble in a particular solvent.

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegeneerative diseases (Parkinson's and Alzheimer's) (Buhler & Miranda 2000).

In this research work, according to the observation on different crude extracts from the fruit pulp of avocado fruits by using the ascorbic acid as a standard it was found that the IC_{50} values of ethanolic extract were 17.36 ± 1.256. From this study, fruits of *Persea americana* Miller contained the antioxidant activities which is helpful in preventing the various oxidative stresses.

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Morphological and Anatomical Characters of *Oryza sativa* L. var. Aye Yar Min and *Oryza sativa* L. var. PR 23

Thet Thet Zin[1], Naw Al Shar Phaw[2] & Nu Nu Yee[3]

Abstract

In the present study, morphological and anatomical investigation on two rice varieties of *Oryza sativa* L. var. Aye yar min and *Oryza sativa* L. var. PR 23 belonging to family Poaceae were studied. The seeds of rice were obtained from Department of Agriculture, Patheingyi Township, Mandalay Region and School of Agriculture, Yunnan University, China in the year of 2019 - 2020. In the morphological studies, the plant height of *O. sativa* L. var. Aye yar min greater than in *O. sativa* L. var. PR 23. In addition, *O. sativa* L. var. Aye yar min possessed the greater number in leaf length and width, panicle length, filament length and caryopsis length than *O. sativa* L. var. PR 23. In the anatomical studies, two varieties were found basically similar but slightly different in sizes and shapes of cells and thickness of tissue layers. The greater thickness of lamina, midribs, culm and root diameters were observed in *O. sativa* L. var. PR 23.

Keywords: *Oryza sativa* L. var. Aye yar min .var. PR 23, morphology, anatomy

Introduction

The family Poaceae is worldwide in distribution, estimated to be the principal component of some 20% of the earth's cover of vegetation. The family contains about 9000 species distributed among 651 genera (Dassanayake 1994).

The a great wide geographical distribution of the rice plant *Oryza sativa* L. and its long history of cultivation in Asian countries have led to the development of a great diversity of varietal types. The cultivated rice plant *Oryza sativa* L. belonging to the tribe Oryzeae under the sub-family Oryzoideae in the grass family Poaceae. Biosystematics recently divided into genus *Oryza* into several sections and placed *O. sativa* L. under series Sativa in section Sativae. *O. sativa* is indigenous to Asia. The rice plant may be characterized as an annual grasses with round, hollow,

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jointed culms, rather flat, sessile leaf blades, and a terminal panicle (Chang *et al*. 1965).

The palea, lemma, sterile lemmas, and rachilla constitute the hull of indica rice. In japonica rice, however, because of the non-shattering characteristic, the hull usually includes rudimentary glumes and perhaps apportion of the pedicel. The lemma is larger than the palea and covers about two-thirds of the surface area of matured rice. The edges of the palea fit inside those of the lemma, the hull closes tightly. The indica rice are widely grown in tropical regions such as Southeast Asia; japonica rice, which are adapted to cooler areas, are largely grown in temperate countries such as central and northern China, Korea and Japan. Both indica and japonica rice can also be grown in subtropical regions such as Taiwan (Yoshida 1981).

Rice is the staple food of about half the world's population, of which more than 90 percent of the rice consumers live in Asia. Therefore, rice plays an important role in proving food security, and alleviating malnutrition and poverty in Asia and the world. The great success of hybrid rice in China has encouraged other countries like India, Vietnam, Philippines, Indonesia and Bangladesh to follow the adoption of hybrid rice technology since the 1900s, and more recently, Myanmar strongly advocating hybrid rice production (FAO 2014). Rice is a critically important staple food and the demand for production is expected to continue to increase, especially in developing countries. Deployment of perennial rice could meet important needs, such as increased production per growing season, reduced risks for farmers, lower labour requirements, less water needed, and protecting soil from erosion (Sacks 2013).

The aim and objectives of this research was to know the morphological and anatomical variations between the same species of *Oryza sativa* L. var. Aye yar min and *Oryza sativa* L. var. PR 23.

Materials and Methods

In the present study, *Oryza sativa* L. var. Aye yar min was obtained from Department of Agriculture, Patheingyi Township Mandalay Region and *Oryza sativa* L. var. PR 23 from School of Agriculture, Yunnan University, China in the year of 2019 - 2020. The vegetative and floral parts of fresh specimens were studied. Classification and identification were

made by referring Hooker (1897), Johanson (1940), Backer and Brink (1968), Lawrence (1968) and Dassanayake (1994). Microtome sections were made by the method of Johansen's (1940), at the Department of Research, University of Traditional Medicine, Mandalay.

Results

Morphological study

Oryza sativa L. var. Aye yar min (Figure 1)

Myanmar name : Aye yar min

English name : Paddy

Flowering period : September to October

Annual, erect, rhizomatous tufted grasses, 135 - 146 cm high. Culms, terete, unbranched, glabrous; nodes glabrous; internodes long, glabrous. Leaf-sheath, glabrous, hairy at the mouth; ligule membranous, leaf-blades linear-lanceolate, 22 - 110 cm long, 1 - 2.5 cm wide, narrowed at the base, scabrous along the margin, acuminate at the apex, scabrous on both surfaces. Panicle raceme, 16 - 34 cm in length. Glumes 2, reduced into upper and lower flattened minute lips, obconical, chartaceous. Florets solitary, oblong-ovate, about 8 mm long, bisexual, awn present. Sterile lemmas subulate, about 3 mm long, 1-nerved, entire along the margin, acute at the apex, coriaceous, scarbrous, pale green; sterile palealancolate, about 3 mm long, entire along the margin, acute at the apex, coriaceous, pale green, glabrous. Fertile lemma boat-shaped, 7 - 8 mm long, nerved, acute at the apex, cariaceous, scabrescent; fertile palea elliptic-oblong or boat-shaped, 7 - 8 mm long, nerved, acute at the apex, coriaceous, scabrescent. Stament 6; filaments filiform, about 7 mm long, white; anther dithecous, about 2 mm long, white or creamy; ovaries ovoid, 0.5 mm long, green; styles, about 1 mm long, white; stigmas 2, plumose, 0.5mm long, laterally exserted, pale yellow. Caryopsis ovoid, 7 - 9 mm in length, 2.0 - 2.5 mm in width, greenish yellow.

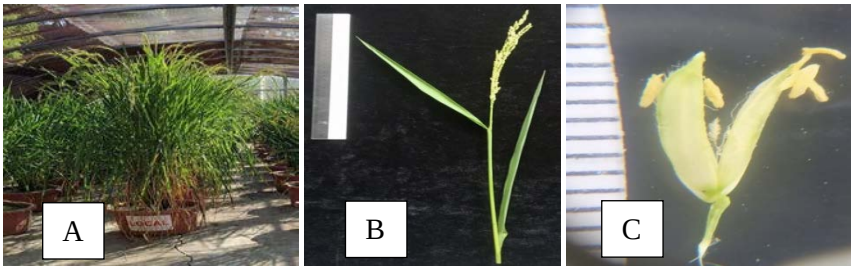


Figure 1. Morphological characteristics of *Oryza sativa* L. var. Aye yar min
A. Habit B. Inflorescence C. Floret

***Oryza sativa* L.var. PR 23 (Figure 2)**

Myanmar name : Saba

English name : Paddy

Flowering period : September to October

Perennial, erect, rhizomatous tufted grasses, 110 - 118 cm high. Culms, terete, unbranched, glabrous; nodes glabrous; internodes long, glabrous. Leaf-sheath, glabrous, hairy at the mouth; ligule membranous, leaf-blades linear-lanceolate, 29 - 79 cm long, 0.8 - 1.9 cm wide, narrowed at the base, scabrous along the margin, acuminate at the apex, scabrous on both surfaces. Panicle raceme, 9 - 30.5 cm in length. Glumes 2, reduced into upper and lower flattened minute lips, obconical, chartaceous. Florets solitary, oblong-ovate, about 7.5 mm long, bisexual, awn present. Sterile lemmas subulate, about 3 mm long, 1-nerved, entire along the margin, acute at the apex, coriaceous, scarbrous, pale green; sterile palealancolate, about 3 mm long, entire along the margin, acute at the apex, coriaceous, pale green, glabrous. Fertile lemma boat-shaped, about 7 mm long, nerved, acute at the apex, cariaceous, scabrescent; fertile palea elliptic-oblong or boat-shaped, about 7 mm long, nerved, acute at the apex, coriaceous, scabrescent. Stament 6; filaments filiform, about 3 - 4 mm long, white; anther dithecous, about 2 mm long, white or creamy; ovaries ovoid, 0.5 mm long, green; styles, about 1 mm long, white; stigmas 2, plumose, 0.5 mm long, laterally exserted, pale yellow. Caryopsis ovoid, 7 - 8 mm in length, 3 - 4mm in width, greenish yellow.

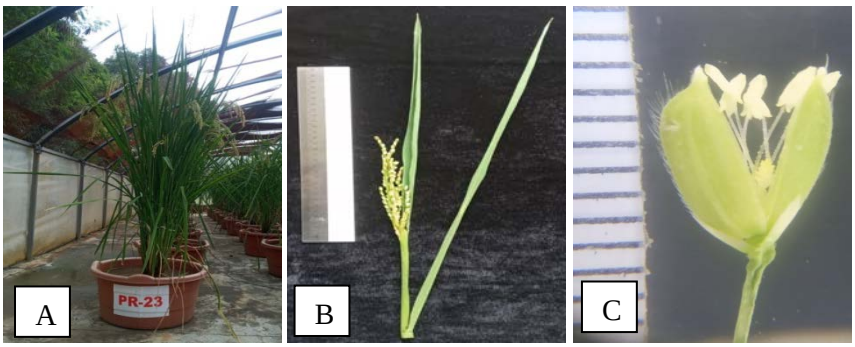


Figure 2. Morphological characteristics of *Oryza sativa* L. var. PR 23
A. Habit B. Inflorescence C. Floret

Anatomical Study

Internal structure of the leaf of *Oryza sativa* L. Aye yar min (Figure 3)

Lamina

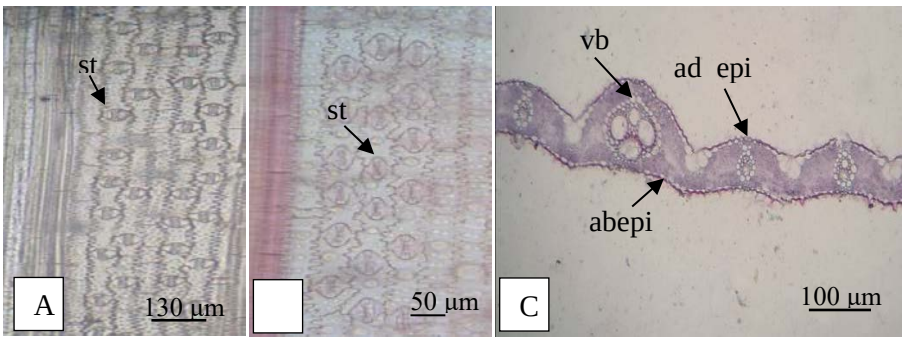
In transverse section, the lamina of *O. sativa* L. var. Aye yar min was dorsiventral with parallel venation, 62.4 - 91.2 µm thick, distinguishable into dermal, ground and vascular tissue system.

Dermal Tissue System: In surface view, epidermal cells of both surfaces were parenchymatous, elongated in shape, cell wall wavy; adaxial epidermal cells 50.0 - 362.5 µm in length, 7.5 - 17.5 µm in width; abaxial epidermal cells 55 - 250 µm in length, 12.5 - 17.5 µm in width. Stomata present on both surfaces, graminaceous type, 25.0 - 27.5 µm in length, 15 - 20 µm in width, subsidiary cells triangular or dome shaped. Silica and papillae present. In transverse section, both adaxial and abaxial epidermis composed of oval or barrel shaped cells, the cells 4.8 - 7.2 µm in length, 4.8 - 7.2 µm in width, bulliform cells found at the furrows of the adaxial epidermis, oval or circular in shape and distinct. Cuticle present. **Ground Tissue System**: Mesophyll cells were composed of chlorenchymatous cells, 48.0 - 81.6 µm thick. **Vascular Tissue System:** Vascular bundles were embedded in the mesophyll tissue, more or less circular or elliptical in outline, the vascular bundle 28.8 - 105.6 µm in length, 19.2 - 110.4 µm in width, leaf interveinal distance 24 - 144 µm in length, collateral type, phloem at the adaxial side and xylem at the abaxial side. Phloem polygonal, 6.0 - 19.2 µm in length, 4.8 - 12.0 µm in width, phloem composed of sieve tube elements, companion cells, and phloem parenchyma; xylem polygonal

in shape,1 - 4 cells, 8.4 - 43.2 µm in length, 6.0 - 43.2 µm in width, composed of vessel elements, tracheids, xylem fibers and xylem parenchyma.

Midrib

In transverse section, the midribs of *O. sativa* L. var. Aye yar min studied were adaxially flat and abaxially hemispherical, 1344 - 1440 µm in length, 1020 - 1080 µm in width, distinguishable into dermal, ground and vascular tissue system. **Dermal Tissue System:** In transverse section, the adaxial and abaxial epidermis oval or barrel in shape, one-layered, the cells 6.0 - 9.6 µm in length, 4.8 µm - 7.2 µm in width. Cuticle thick and trichome present. **Ground Tissue System:** It was composed of chlorenchymatous and parenchymatous tissues and lacunae. The chlorenchyma 30 - 50 µm thick. The parenchyma radiating like a strand, the cells polygonal in shape, the cells 19.2 - 62.4 µm in length, 19.2 - 52.8 µm in width. Large lacunae were present. **Vascular Tissue System**: Vascular bundle were scattered in the ground tissue, rounded or elliptic in shape, the vascular bundles 67.2 - 134.4 µm in length, 62.4 - 115.2 µm in width, closed collateral type, phloem lying adaxial side and xylem lying abaxial side. Phloem composed of sieve tube elements, companion cells, and phloem parenchyma; phloem 10 - 17.5 µm in length, 7.5 - 12.5 µm in width; xylem composed of vessel elements, tracheids, xylem fibers and xylem parenchyma, vessel elements polygonal in shape, 2 - 3 cells, 20 - 55 µm in length, 15 - 40 µm in width.



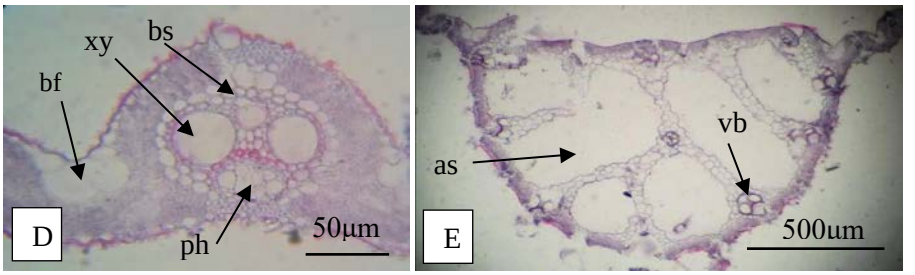


Figure 3. Internal structure of the leaf of *Oryza sativa* L. var. Aye yar min A. Adaxial surface view of lamina B. Abaxial surface view of lamina C. T.S of lamina D. Close up view of lamina showing vascular bundle E. T.S of midrib(st-stomata, vb-vascular bundle, ad epi-adaxial epidermis, abepi-abaxial epidermis, bf-bulliform cell, bs-bundle sheath, xy-xylem, ph-phloem, as-air space)

Internal structure of the culm of *Oryza sativa* L. var. Aye yar min (Figure 4)

In transverse section, the culms of *O. sativa* L .var. Aye yar min studied were circular in outline, hollow in the center, the culm wall 528 - 672 µm thick, distinguishable into dermal, ground and vascular tissue systems. **Dermal Tissue System**: In transverse section, the epidermal cells parenchymatous, barrel or rectangular-shaped, one-layered, 3.6 - 7.2 µm in length, 1.2 - 2.4 µm in width. Cuticle thick. **Ground Tissue System:** Differentiated into sclerenchymatous and parenchymatous tissue, sclerenchymatous cells oval or polygonal in shape, 2.4 - 14.4 µm in length, 2.4 - 8.4 µm in width; parenchymatous cells circular or polygonal in shape, 19.2 - 115.2 µm in length, 19.2 - 86.4 µm in width. Intercellular spaces present. **Vascular Tissue System:** Vascular bundles were embedded in the ground tissue, closed collateral type, arranged in two rows, scattered in the periphery and in the center of the ground tissue. The peripheral vascular bundles were oval in shape, 57.6 - 96.0 µm in length, 33.6 - 43.2 µm in width, culm interveinal distance 307.2 - 480.0 µm. The central ones oval in shape, 168 - 192 µm in length, 86.4 - 134.4 µm in width, culm interveinal distance 264.0 - 417.6 µm. In individual vascular bundles, phloem lying adaxial side and xylem lying abaxial side. Phloem composed of sieve tube elements, companion cells, and phloem parenchyma, the cells 10 - 20 µm in length, 10 - 15 µm in width; xylem composed of vessel elements, tracheids,

xylem fibers and xylem parenchyma, vessels 2 - 3 cells, 10 - 60 µm in length, 10 - 30 µm in width.

Internal structure of the roots of *Oryza sativa* L. var. Aye yar min (Figure 4)

In transverse section, the roots of *O. sativa* L. var. Aye yar min studied were rounded or circular in shape, 1100 - 1125 µm in diameter, distinguishable into dermal, ground and vascular tissue systems. **Dermal Tissue System:** In transverse section, the piliferous layer detached. **Ground Tissue System:** Composed of hypodermis, sclerenchymatous fiber, parenchyma, endodermis and pericycle. Hypodermis one-layered, oval in shape, the cells 15.0 - 22.5 µm in length, 12.5 - 15 µm in width. Sclerenchymatous fiber one-layered, oval in shape, the cells 3.75 - 10.0 µm in length, 3.75 - 12.5 µm in width. Parenchyma cells radiating in cortical zone, 380 - 490 µm thick. Endodermis and pericycle one-layered, parenchymatous. Intercellular cavity present. **Vascular Tissue System:** The vascular bundles circular in shape, about 180 µm in diameter; phloem composed of sieve tube elements, companion cells, and phloem parenchyma; the cells 10 - 20 µm in diameter; xylem composed of vessel elements, xylem fibers and xylem parenchyma, vessel elements polygonal in shape, 3 - 4 cells, 45 - 50 µm in diameter.

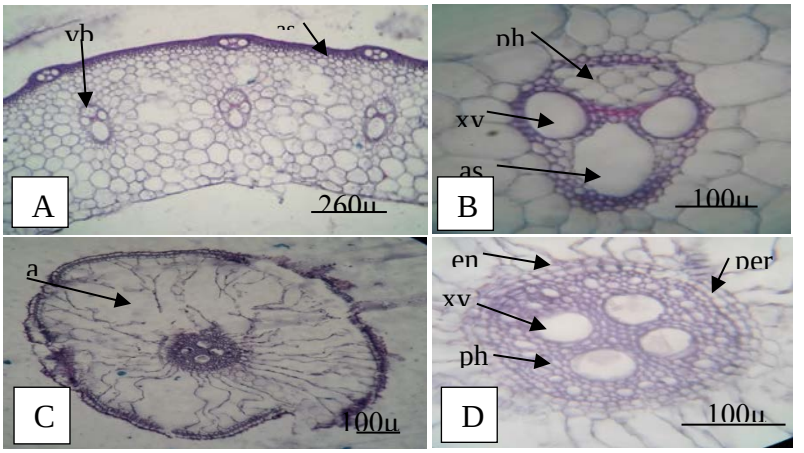


Figure 4. Internal structure of the culm and roots of *Oryza sativa* L. var. Aye yar min A. T.S of culm B. Close up view of culm showing vascularbundle C. T.S of root D. T.S of root showing vascular bundle(vb- vascular bundle, sc-sclerenchyma, xy-xylem, ph-phloem, as-air space, en-endodermis, per-pericycle)

Internal structure of the leaf of *Oryza sativa* L. var. PR 23 (Figure 5)

Lamina

In transverse section, the lamina of *O. sativa* L. var. PR 23studied was dorsiventral with parallel venation, 90 - 130 µm thick, distinguishable into dermal, ground and vascular tissue system. **Dermal Tissue System**: In surface view, epidermal cells of both surfaces were parenchymatous, elongated in shape, cell wall wavy; adaxial epidermal cells 30 - 75 µm in length, 12.5 - 22.5 µm in width; abaxial epidermal cells 37.5 - 95 µm in length, 12.5 - 17.5 µm in width. Stomata present on both surfaces, graminaceous type, 22.5 - 27.5 µm in length, 10 - 20 µm in width, subsidiary cells triangular or dome shaped. Silica and papillae present. In transverse section, both adaxial and abaxial epidermis composed of oval or barrel shaped cells, the cells 10 - 20 µm in length, about 10 µm in width, bulliform cells found at the furrows of the adaxial epidermis, oval or circular in shape and distinct. Cuticle present. **Ground Tissue System**: Mesophyll cells were composed of chlorenchymatous cells, 50 - 110 µm thick. **Vascular Tissue System:** Vascular bundles were embedded in the mesophyll tissue, more or less circular or elliptical in outline, the vascular bundle 30 - 140 µm in length, 30 - 130 µm in width, leaf interveinal distance 40 - 190 µm in length, collateral type, phloem at the adaxial side and xylem at the abaxial side. Phloem polygonalin shape, 10 - 20 µm in length, 10 - 15 µm in width, phloem composed of sieve tube elements, companion cells and phloem parenchyma; xylem polygonal in shape,2 - 4cells, 10 - 60 µm in diameter, composed of vessel elements, tracheids, xylem fibers and xylem parenchyma.

Midrib

In transverse section, the midribs of *O. sativa* L. var. PR 23 were adaxially flat and abaxially hemispherical, 1500 - 1800 µm in length, 1250 - 1625 µm in width, distinguishable into dermal, ground and vascular tissue system. **Dermal Tissue System:** In transverse section, the adaxial and abaxial epidermis oval or barrel in shape, the cells 7.5 - 12.5 µm in length, 5- 10 µm in width. Cuticle thick and trichome present. **Ground Tissue System:** It was composed of chlorenchymatous and parenchymatous tissues and lacunae. The chlorenchyma 40 - 80 µm thick. The parenchyma radiating like a strand, the cells polygonal in shape, the cells 40 - 100 µm in length, 40 - 60 µm in width. Large lacunae were present. **Vascular Tissue System**: Vascular bundle were scattered in the ground tissue, rounded or

elliptic in shape, the vascular bundles 50 - 200 µm in length, 50 - 190 µm in width. Closed collateral type, phloem lying adaxial side and xylem lying abaxial side. Phloem composed of sieve tube elements, companion cells, and phloem parenchyma; phloem 7.5 - 15.0 µm in length, 7.5 - 12.5 µm in width; xylem composed of vessel elements, tracheids, xylem fibers and xylem parenchyma, vessel elements polygonal in shape, 2 - 3 cells, 10 - 65 µm in length, 15 - 40 µm in width.

Internal structure of the culm of *Oryza sativa* L. var. PR 23 (Figure 6)

In transverse section, the culms of *O. sativa* L. var. PR 23 were circular in outline, hollow in the center, the culm wall 400 - 600 µm in thick, distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System: In transverse section, the epidermal cells parenchymatous, barrel or rectangular-shaped, about 10 µm in length, about 10 µm in width. Cuticle thick. **Ground Tissue System:** Differentiated into sclerenchymatous and parenchymatous tissue, sclerenchymatous cells oval or polygonal in shape, 5.0 - 12.5 µm in length, 5.0 - 12.5 µm in width; parenchymatous cells circular or polygonal in shape, 20 - 80 µm in length, 20 - 60 µm in width. Intercellular spaces present. **Vascular Tissue System:** Vascular bundles were embedded in the ground tissue, closed collateral type, arranged in two rows, scattered in the periphery and in the center of the ground tissue. The peripheral vascular bundles were oval in shape, 50 - 80 µm in length, 60 - 100 µm in width, culm interveinal distance 50 - 170 µm. The central ones oval in shape, 170 - 200 µm in length, 110 - 150 µm in width, culm interveinal distance 230 - 500 µm. In individual vascular bundles, phloem lying adaxial side and xylem lying abaxial side. Phloem composed of sieve tube elements, companion cells, and phloem parenchyma, the cells 10 - 20 µm in length, 10 - 20 µm in width; xylem composed of vessel elements, tracheids, xylem fibers and xylem parenchyma, vessels 2 - 4 cells, 20 - 50 µm in length, 15 - 40 µm in width.

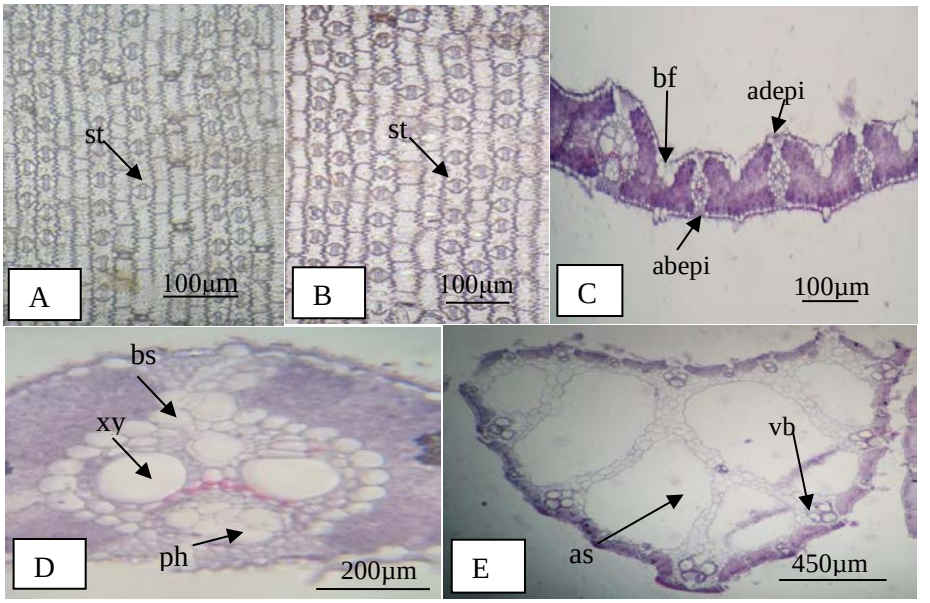


Figure 5. Internal structure of the leaf of *Oryza sativa* L. var. PR 23
A. Adaxial surface view of lamina B. Abaxial surface view of lamina C. T.S of lamina D. Close up view of lamina showing vascular bundle E.T.S of midrib (st-stomata, vb-vascular bundle, ad epi-adaxial epidermis, abepi- abaxial epidermis, bf-bulliform cell, bs-bundle sheath, xy-xylem, ph-phloem, as-air space)

Internal structure of the root of *Oryza sativa* L. PR 23 (Figure 6)

In transverse section, the roots of *O. sativa* L. PR 23 were rounded or circular in shape,1875 - 2125 µm in diameter, distinguishable into dermal, ground and vascular tissue systems. **Dermal Tissue System:** In transverse section, the piliferous layer detached. **Ground Tissue System:** Composed of hypodermis, sclerenchymatous fiber, parenchyma, endodermis and pericycle. Hypodermis one-layered, rounded or oval in shape, the cells 10 - 20 µm in length, 12.5 - 17.5 µm in width. Sclerenchymatous fiber one-layered, barrel in shape, the cells 7.5- 12.5 µm in length, 7.5 - 10.0 µm in width. Parenchyma cells radiating in cortical zone, 180 - 320 µm thick. Endodermis and pericycle one-layered, parenchymatous. Intercellular cavity present. **Vascular Tissue System:** The vascular bundles circular in shape, about 150 - 200 µm in diameter; phloem

composed of sieve tube elements, companion cells, and phloem parenchyma; the cells 5 - 15 µm in length and 5 - 10 in width; xylem composed of vessel elements, tracheids, xylem fibers and xylem parenchyma, vessel elements polygonal in shape, 3 - 4 cells, 30 - 40 µm in diameter.

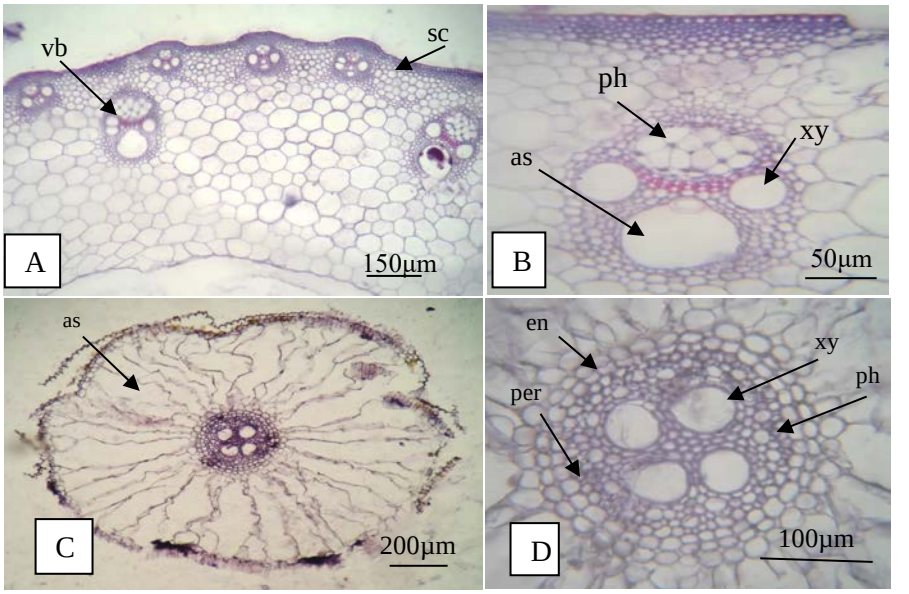


Figure 6. Internal structure of the culm and roots of *Oryza sativa* L. var. PR 23 A. T.S of culm B. Close up view of culm showing vascular bundle C. T.S of root D. T.S of root showing vascular bundle (vb-vascular bundle, sc-sclerenchyma, xy-xylem, ph-phloem, as-air space, en-endodermis, per-pericycle)

Discussion and Conclusion

In the present study, the two varieties of *Oryza sativa* L. belonging to family Poaceae. The seeds of rice were obtained from Department of Agriculture, Patheingyi Township, Mandalay Region and School of Agriculture, Yunnan University, China in the year of 2019 - 2020. The morphological and anatomical characters were studied, described, compared and discussed.

Plant is herb, erect and slender. Leaf blades flat, linear-lanceolate narrow at the base, scabrous along the margin, acuminate at the apex; panicle raceme; glume 2; floret includes the lemma, palea and the enclosed

flower. The flower consists of six stamens and a pistle. The pistle contains one ovule. The short style bears the bifurcate, plumose stigma. The upper surface of the blades has many ridges formed by the parallel veins. The most prominent ridge on the lower surface is the midrib. All morphological characters were agreed with Hooker (1897), Chang (1965), Backer and Brink (1986), Dassanayake (1994). The morphological investigation of *O. sativa* L. Aye yar min possessed higher number of plant height, leaf length and width, panicle length, filament length and caryopsis length than the *O. sativa* L. var. PR 23.

The epidermal cells of the surface view of lamina were parallel venation. The cells were two types, long and short, walls sinuous. Bulliform cells are found in furrows of adaxial epidermis of lamina in transverse section. Epidermis was one layered thick. Close collateral type of vascular bundles were found in all parts of leaf. In cross section, the blade contains many large and small vascular bundles; stomates are both sides of the surface, and large air spaces are found between the vascular bundles this character was agreed with Metcalfe (1960) and Esau (1956) Yoshida (1981).

In anatomical investigation, dermal tissue system was composed of one layer of epidermal cells on both surfaces. Stomata are present on both sides, graminaceous types, these characters were coincided with Metcalfe (1960) and Esau (1956). The greater thickness of lamina was found in *O. sativa* L. var. PR-23.

Midrib conspicuous, owing to a prominent, rounded, abaxial, and a less pronounced, flattened, adaxial projection; containing a number of vascular bundles, but none present in the vertical septum between the 2 large air-canals occupying a large part of the midrib characters was agreed with Metcalfe (1960).

The epidermis of the culm is single layered in two rice varieties. Hypodermis which lies beneath the epidermis, consists of sclerenchymatous cells. The numbers of hypodermal layers varies among the two cultivars. The vascular bundles were found two rows the outer and inner. The outer vascular bundles were smaller and inner bundles are larger. In two cultivars, the outer vascular bundles were embedded in the hypodermis. All the inner vascular bundles were embedded in the ground tissue, these characters were agreed with Metcalfe (1960) and Sarwar & Prodhan (2000).

In transverse section of roots, were circular in outline. The parenchymatous epiblema cells are one layered and rectangular to quadrangular in shaped. The ground tissue is thin-walled, parenchymatous, composed of cortex, endodermis and pericyclean pith. Vascular bundles are triarch and tetraarch, radial type. Roots is the present of large air spaces, this characters agreement with Yoshida(1981). The thickness of cortex was found in *O. sativa* L. var. PR 23.

Morphology and anatomy of rice roots are quite similar to other monocots including cereal crops (Esau 1965). One characteristic of rice roots is the presence of large air spaces in mature roots. These air spaces are connected with those in the culms and leaves, providing an efficient air passage system from shoot to root (Yoshida 1981).

In conclusion, the morphological and anatomical characters of two rice varieties (Aye yar min and PR 23) can be recorded. This characters will be partially fulfilled the classification of rice varieties in Myanmar.

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We would like to express our special thanks to every individual member of Perennial Rice (PR member) in Department of Botany, University of Mandalay.

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Taxonomy and Phylogenetic Relationships of Some Orchid Species on Pondaung-Ponnya Ranges between Pauk and Htilin Townships

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Abstract

Taxonomy and phylogenetic relationships of some orchid species on Pondaung Ponnya Ranges between Pauk and Htilin Townships within Magway Region were studied from 2018 to 2020. Totally 10 species belonging to 8 genera under 4 tribe of Orchidaceae were verified. They are one species from the genus *Aerides*, *Ascocentrum*, *Bulbophyllum*, *Coelogyne*, *Cymbidium*, *Pholidota*, *Vanda* and 3 species from *Dendrobium*. All species are epiphytes. The numbers of pollinia are 2 in *Aerides*, *Ascocentrum*, *Cymbidium* and *Vanda* whereas the numbers of pollinia are 4 in other species. The similarities among genera were 42.2%. *Bulbophyllum*, *Coelogyne*, *Dendrobium* and *Pholidota* were more closely related species and more primitive. *Ascocentrum* and *Vanda* were more closely related species and more advanced. The phenogram for the phylogenetic relationships among genera was shown in figure 4. The present results can be applied as information of plant resources.

Keywords: phylogenetic relationships, Orchidaceae, similarities among genera, related species, phenogram

Introduction

The flowering plants are dominant and successful plants in the world. Among them, the Orchidaceae is one of the largest families. Singh (2010) stated that it is second largest family and consist of 788 genera, 8500 species. It is most common in moist tropical forests and also well distributed in subtropics and temperate regions.

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It is a family of considerable economic importance in horticulture and floristry but also in the pharmaceutical industries. They also repay the fungi by producing some nutrients during photosynthesis that help the fungi to survive in a given ecosystem.

Simpson (2006) stated that Taxonomy includes description, identification, nomenclature and classification. Singh (2010) indicated that identification is recognizing an unknown specimen and nomenclature deals with the determination of a correct name. He described classification as an arrangement of organisms into groups on the basis of similarities.

Phylogenetic methods aim at developing a classification based on analysis of phylogenetic data and developing a cladogram. Sneath and Sokal (1973) define numerical taxonomy as grouping by numerical methods of taxonomic units into taxa on character states (Singh 2010).

Grant reported that Myanmar orchids include a total of 86 genera and 581 species in 1895. In 1961, Hundley and Chit Ko Ko stated that 128 genera and 739 species of orchids were distributed in Myanmar. Kress *et al.* (2003) listed 738 species of 132 genera belonging to Orchidaceae in the checklist of Myanmar.

In Myanmar, many workers had studied not only taxonomy but also pollen morphology on Orchidaceae. But it is still lacking to study on Pondaung-Ponnya ranges, therefore focus on this area to study. It lies between Pauk and Htilin Townships, which situated in the western part of Magway Region. The ranges lie as north-south direction. The elevation is over 1000 m above sea level. The plant collection area is between 21° 38' and 21° 40' North Latitude and between 94° 15' and 94° 17' East Longitude.

The aim and objectives of the present research are to collect some orchid species from Pondaung-Ponnya ranges, to verify the name based on taxonomic descriptions, to construct the phenogram of phylogenetic relationships among genera and to contribute the knowledge and information of plant resources to other researchers.

Materials and Methods

The orchid species on Pondaung-Ponnya ranges between Pauk and Htilin Townships were collected and verified from 2018 to 2020. All the specimens were recorded by taking photographs. Identification of specimens was carried out by referring to the Flora stated by Hooker

(1894), Seidenfaden (1992) and Dassanayake (1981). The scientific names were confirmed by the websites of IPNI and online Botanical Database of Tropical Plant (TROPICO). The outstanding characteristics were presented with the photographs of inflorescences and pollinium. Myanmar names were referred to Hundley and Chit Ko Ko (1987) and Kress *et al*. (2003). The phenogram of the phylogenetic relationships among similarities of genera was constructed. An artificial key based on morphological characters was also created.

Results

Altogether 10 species belonging to 8 genera under 4 tribe of the family Orchidaceae were verified. The list was presented in Table 1. The comparable characteristics of all genera were presented in Table 2. Numerical data matrix of genera and selected characters were presented in Table 2.1 and the t x t matrix presenting similarities between taxa was stated in Table 2.2. The phenogram among the genera based on overall selected characters was shown in Figure 4.

Table 1. List of the studied species

Kingdom	-	Plantae
Clade	-	Tracheophytes
Clade	-	Angiosperms
Clade	-	Monocots
Order	-	Asparagales
Family	-	Orchidaceae
Subfamily	-	Epidendroideae

Tribe	No.	Scientific name
Cymbidieae	1	*Aerides multiflora* Roxb.
Vandeae	2	*Ascocentrum ampullaceum* (Roxb.) Schlechter
Dendrobieae	3	*Bulbophyllum suavissimum* Rolfe.
Arethuseae	4	*Coelogyne rigida* Parish & Rchb.f.
Cymbidieae	5	*Cymbidium aloifolium* (L.) Sw.
Dendrobieae	6	*Dendrobium chrysotoxum* Lindl.
	7	*Dendrobium crepidatum* Lindl. &Paxton
	8	*Dendrobium infundibulum* Lindl.
Arethuseae	9	*Pholidota articulata* Lindl.
Vandeae	10	*Vanda bensonii* Veitch & Rchb.

Orchidaceae (A. L. de Jussieu, 1789)

Perennial herbs. Stems base often thickened to form pseudobulb, aerial roots present. Leaves alternate, simple, sheathing base. Flowers bisexual, zygomorphic. Sepals 3, lateral more or less adnate to the ovary. Middle petal forming labellum. Androecium adnate to style and stigma forming a column. Pollen grains agglutinated into pollinia, each pollinium with a caudicle, 2 to 8 pollinia. Carpels 3, ovary inferior, unilocular, parietal placentation; stigma 3, one often transformed into a sterile rostellum, latter often having a sticky pad called viscidium (Singh 2010).

1. *Aerides multiflora* Roxb., Pl. Corom. 3. 68.1820. (Figure 1 A. B)

Myanmar name : Sarkalay thit khwa

English name : Unknown

Flowering period : January to March

Monopodial epiphytes. Roots cylindrical, drooping and clinging. Pseudobulb absent. Leaf-blades linear, 15.0-25.0 cm by 1.5-2.0 cm, base sheathing, apex emarginate, fleshy, coriaceous. Inflorescences axillary racemes, pendulous, 40- to 50-flowered; peduncular bracts 4, ovate-lanceolate. Flowers pinkish white with pale violet blotch, fragrant; floral bracts triangular. Dorsal sepals suborbicular; two lateral sepals ovate. Two

lateral petals ovate-lanceolate, coriaceous. Labellum 3-lobed, obovate-oblong, lateral lobes rounded; mid-lobes ovate, coriaceous; spur short. Column white; column foot short; anthercaps orbicular, white. Pollinia 2, globoid, yellow. Caudicles absent. Visidium small. Ovary triangular.

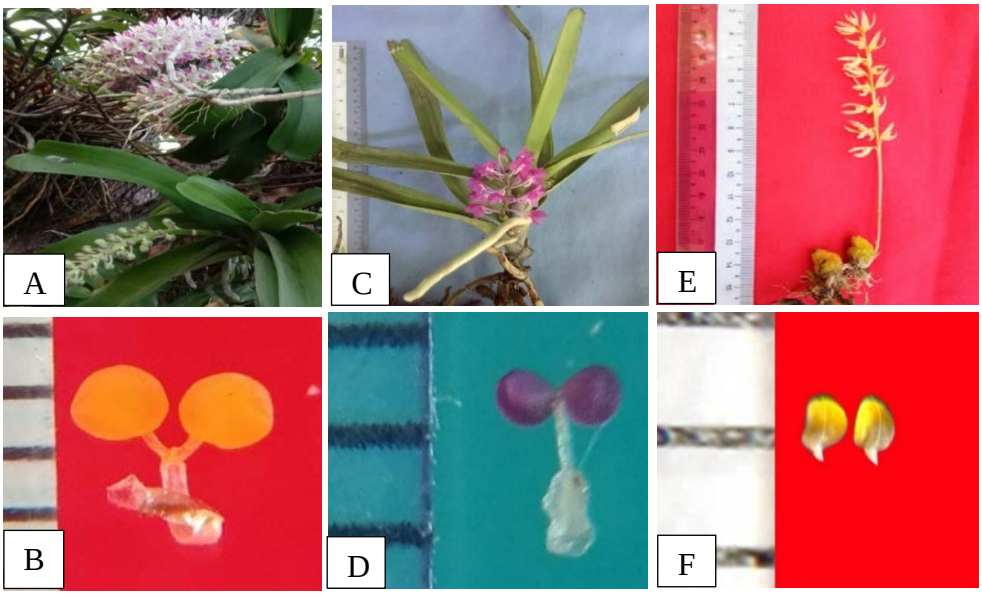

Figure. 1 A. Habit of *Aerides multiflora* Roxb.
B. Pollinarium of *Aerides multiflora* Roxb.
C. Habit of *Ascocentrum ampullaceum* (Roxb.) Schlechter
D. Pollinarium of *Ascocentrum ampullaceum* (Roxb.) Schlechter
E. Habit of *Bulbophyllum suavissimum* Rolfe.
F. Pollinarium of *Bulbophyllum suavissimum* Rolfe.

2. *Ascocentrum ampullaceum* (Roxb.) Schlechter, Repert. Spec. Nov. Region. Veg. Beih. 1(13): 975. 1914. (Figure 1 C. D)

Myanmar name : Thandar lay pyar

English name : Unknown

Flowering period : April to May

Monopodial epiphytes. Roots cylindrical, cinereous. Pseudobulbs absent. Leaves 6-8 in number; blades linear, 15-28 cm by 1-2 cm, base

sheathing, apex mucronate. Inflorescences axillary racemes, 10- to 18-flowered; peduncles arisen from lower node; peduncular bracts 3. Flowers purple, 0.8-1.2 cm in diameter, dropping; pedicels ribbed; bracts deltoid. Dorsal sepals ovate; 2-lateral sepals ovate. Two lateral petals obovate. Labellum 3-lobed, mid-lobes emarginate; spurs curved forward, swollen above. Column purplish white; column foot white. Anthercaps oval, convex. Pollinia 2, globoid, purple. Caudicle white; viscidium oval. Stigmatic surface white; rostellum projection pale brown. Ovary oblongoid.

3. *Bulbophyllum suavissimum* Rolfe, Gard. Chron. Ser. 35: 297. 1889.

(Figure 1 E. F)

Myanmar name : Thazin

English name : Unknown

Flowering period : December to March

Sympodial epiphytes. Roots clinging, cylindrical. Stem pseudobulbs, one-jointed, ovoid, 1.8-2.5 cm long. Leaves 2 per pseudobulb; blades elliptic-oblanceolate, 10-15 cm by 2-3 cm, sheathing base, apex acute, leafless at anthesis. Inflorescences basal racemes, many-flowered; peduncles 1 on each pseudobulb; peduncular bracts 3-4, sheathing, persistent. Flowers yellow, 0.6-0.8 cm in diameter, fragrant. Dorsal sepals linear-oblong, 3-nerved; two lateral sepals ovate-lanceolate. Two lateral petals ovate. Labellum liguliform, golden yellow. Spur indistinct. Column creamy. Anthercaps ovoid, muricate without. Pollinia 4, ovoid, compressed in pairs, bony, yellow. Caudicles and viscidium absent. Ovary oblongoid.

4.*Coelogyne rigida* Parish & Rchb.f., Trans. Linn.Soc. London. 30. 146.

1874. (Figure 2 A. B)

Myanmar name : Unknown

English name : Unknown

Flowering period : June to July

Sympodial epiphytes. Roots clinging, cylindrical. Pseudobulbs, one-jointed, subovoid, 2 cm long. Leaves 2 per pseudobulb, blades suboblong oblanceolate, 19-25 cm by 3-5 cm, base cuneate, apex acuminate, leathery.

Inflorescences terminal racemes, 5- to 8-flowered; peduncles 1 on each pseudobulb; peduncular bracts 10, sheathing, forming a tube, persistent. Flowers yellow, 0.7 cm in diameter; bracts not deciduous at anthesis. Dorsal sepals 3-veined; two lateral sepals suboblong, 3-veined. Lateral petals linear, 1-veined. Labellum subovate, 3-lobed; mid-lobe margin undulate, apex emarginate, callus with 3 longitudinal lamellae; lamellae distinctly crisped. Column winged. Anthercaps ovoid. Pollinia 4, subclavate, cohering in pair, yellow. Caudicles and viscidium absent. Ovary trigonous.

5. ***Cymbidium aloifolium*** (L.) Sw., Nova.Acta Reg. Soc. Sci. Upsal., ser. 26: 73. 1799. (Figure 2 C. D)

Myanmar name : Thit tet lin nay

English name : Unknown

Flowering period : April to May

Sympodial epiphytes. Roots fibrous and clinging, cylindrical, white. Pseudobulbs 3- to 4-jointed, ovoid, covered with permanent leaf-sheaths. Leaves linear-oblong, 20.0-30.0 cm by 2.0-3.0 cm, coriaceous, tip bilobed. Inflorescences axillary racemes, up to 45 cm long, dropping, 20- to 30-flowered; peduncular bracts 4. Flowers dull yellow with purplish-brown, 2.5-3.0 cm in diameter; bracts triangular. Dorsal sepals ligulate-elliptic; lateral sepals oblong, pubescent within. Lateral petals narrowly oblong to elliptic, pubescent within. Labellum distinctly 3-lobed; midlobe elliptic, two-parallel ridged of callus. Column foot yellow with purplish brown base. Anthercaps sub-globoid. Pollinia 2, flabellate, pale yellow, waxy. Caudicles absent. Viscidium short, white. Ovary oblongoid ridged.

6. ***Dendrobium chrysotoxum*** Lindl., Edwards's Bot. Region. 33, ad pl. 19. 1847. (Figure 2 E. F)

Myanmar name : Mout khan wa

English name : Unknown

Flowering period : December to February

Sympodial epiphytes. Roots fibrous and adventitious. Pseudobulbs one-jointed, fusiform, 9-13 cm long. Leaves oblong-lanceolate, 10.5-13.5

cm by 2.5-3.5 cm; base sheathing, apex bifid. Inflorescences terminal raceme, many-flowered; peduncles 5.0-15.0 cm long; peduncular bracts 3,

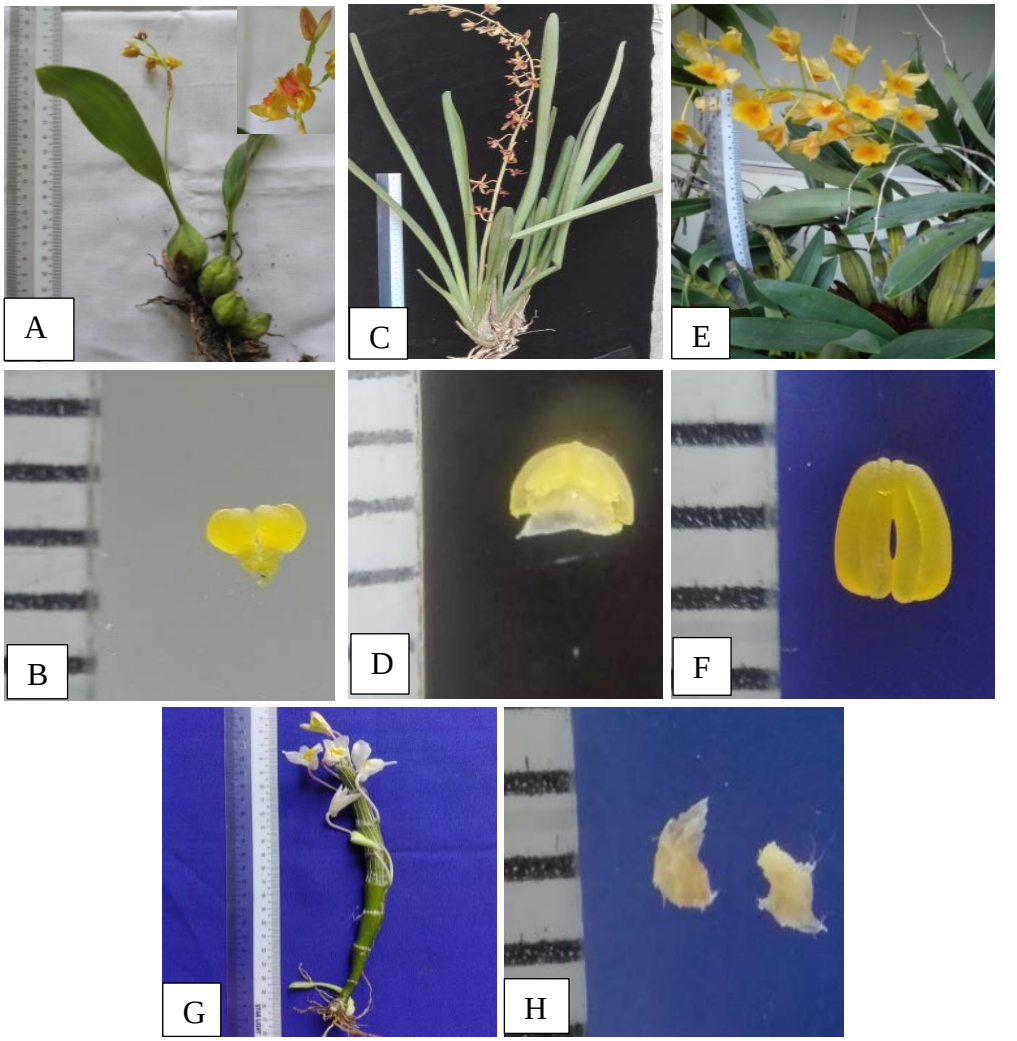

Figure. 2 A. Habit of *Coelogyne rigida* Parish & Rchb.f.
B. Pollinarium of *Coelogyne rigida* Parish & Rchb.f.
C. Habit of *Cymbidium aloifolium* (L.) Sw.
D. Pollinarium of *Cymbidium aloifolium* (L.) Sw.
E. Habit of *Dendrobium chrysotoxum* Lindl.
F. Pollinarium of *Dendrobium chrysotoxum* Lindl.
G. Habit of *Dendrobium crepidatum* Lindl. &Paxton
H. Pollinarium of *Dendrobium crepidatum* Lindl. &Paxton

ovate. Flowers yellow, fragrant, 2.5-3.2 cm in diameter; floral bracts triangular. Dorsal sepal ovate-oblong, tip bifid, brightly yellow; lateral sepals oblong-obtuse, tip bifid, brightly yellow. Lateral petals bright yellow, tip bifid. Labellum orbicular, margin fimbriae, tip emarginate, dark yellow blotched. Column pale yellow. Anthercap oblong. Pollinia 4, yellow, clavate, waxy. Caudicles and viscidium absent. Ovary ellipsoid, longitudinal grooved.

7. *Dendrobium crepidatum* Lindl. &Paxton, Paxt.Fl.Gard.1: 63, f. 45. 1853.

(Figure 2 G. H)

Myanmar name : Ganaing na bay pauk

English name : Unknown

Flowering period : March to April

Sympodial epiphytes; stems longitudinal striated. Roots cylining, cylindrical, cinereous. Pseudobulbs oblong. Leaves 4-6 in number; blades linear-lanceolate, 4-10 cm by 2-3 cm, base sheathing, apex aristate. Inflorescences axillary and terminal racemes, 10- to 13-flowered; peduncles short, arisen from upper node, resupinate. Flowers white, 1.0-1.5 cm in diameter; bracts triangular. Dorsal sepals elliptic; 2-lateral sepals fused, oblong, white with tinged purple at the apex. Two lateral petals elliptic, apex truncate. Labellum 3-lobed, mid-lobes orbicular, apex obcordate, margin sinuate, yellow in the middle and base; basal-foot very short. Column curved; column foot short. Anthercaps subovoid, white, convex. Pollinia 4, oblong, 0.1 cm long, yellow, waxy. Stigmatic surfaces concave; rostellum projection white. Ovary oblong, curved.

8. *Dendrobium infundibulum* Lindl., J. Proc. Linn. Soc., Bot. 3: 16. 1859.

(Figure 3 A. B)

Myanmar names : Ngwe pale; Taung ngwe tu

English name : The small-funneled lip Dendrobium

Flowering period : February to March

Sympodial epiphytes; roots clinging, cylindrical, white. Stems pseudobulb, many-jointed, terete, stout. Pseudobulbs linear-oblongoid. Leaf

blades oblong-lanceolate, 5.0-9.0 cm by 1.5-2.3 cm, sheathing base, apex obliquely emarginate. Inflorescences terminal and axillary racemes, 1- to 3-flowered; peduncular bracts 3. Flowers white, 5.5-6.0 cm in diameter; bracts ovate. Dorsal sepals oblong; 2-lateral sepals oblong-ovate. Two lateral petals obovate, margin undulate. Labellum infundibuliform, attached to the base of column, distinctly 3-lobed; side-lobe obovate, pubescent within, midlobes obcordate, margin crispate, tip emarginate, orange ridged on lower part; spurs funnel-shaped. Column straight; column foot present. Anthercaps oblongoid. Pollinia 4, oblongoid, yellow, waxy. Caudicles and viscidium absent. Stigmatic surfaces oblong. Ovary trigonous.

9. *Pholidota articulata* Lindl., Gen. Sp. Orchid. Pl. 38. 1830. (Figure 3 C.D)

Myanmar name : Myauk let hnyo; Kwyet mee pan myokywe

English name : Jointed pholidota

Flowering period : May to June

Sympodial epiphytes; stems erect, many-jointed. Roots cinereous, adventitious. Pseudobulb tetragonal, in pair at the node of pseudobulb. Leaves 2-4 in number; blades lanceolate, 5.0-12.0 cm by 2.0-4.0 cm, base attenuate, apex acuminate. Inflorescences terminal racemes 3- to 5-flowered; peduncles arisen from upper node, dropping; peduncular bracts 4. Flowers creamy, 0.8-1.2 cm in diameter, fragrant; bracts rhomboid. Sepals convex; dorsal sepals ovate; 2-lateral sepals free, equal, ovate. Lateral petals lanceolate. Labellum 3-lobed; mid-lobes boat-shaped, concave, 4-ridded, apex obcordate; spurs absent. Column creamy. Anthercaps convex. Pollinia 4, obovate-oblong, yellow. Caudicles absent. Viscidium short. Stigmatic surfaces 3-lobed; rostellum small. Ovary 3-ridged.

10. *Vanda bensonii* Veitch & Rchb., F.Gard. Chron.1867:180.1867.

(Figure 3.E.F)

Myanmar name : Moe thuzar

English name : Unknown

Flowering period : December to May

Monopodial epiphytes; stems 10-13 cm long, leafy. Pseudobulb absent. Leaf blades linear, narrow, recurved, thick, coriaceous, 20.0-24.5

cm by 1.5-2.0 cm, base sheathing, margin entire, apex mutinous. Inflorescences axillary and terminal raceme, 6- to 10-flowered; peduncular

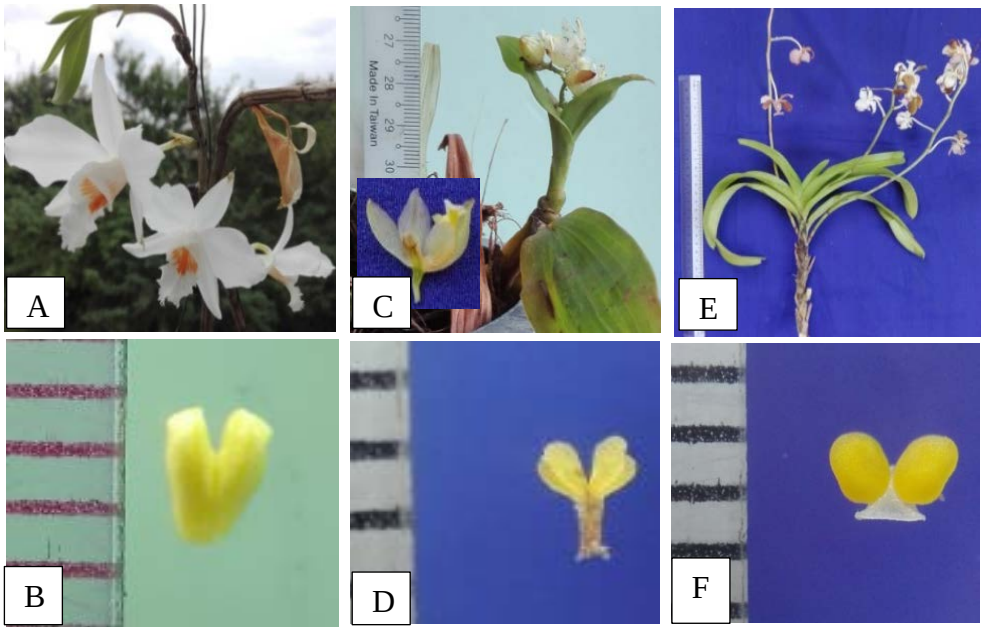

Figure. 3 A. Habit of *Dendrobium infundibulum* Lindl.
B. Pollinarium of *Dendrobium infundibulum* Lindl.
C. Habit of *Pholidota articulata* Lindl.
D. Pollinarium of *Pholidota articulata* Lindl.
E. Habit of *Vanda bensonii* Veitch & Rchb.
F. Pollinarium of *Vanda bensonii* Veitch & Rchb.

bracts ovate; peduncle 20-25 cm long. Flowers yellowish brown, 5-6 cm in diameter; bracts triangular, clasped the rachis. Dorsal sepal obovate, 5-veined; two lateral sepal broadly obovate, 7-veined, coriaceous. Two lateral petals subobovate, with brown spots, coriaceous. Labellum bluish-purple; lobes triangular, recurved; spur conical. Column short; anthercaps ovoid, white. Pollinia 2, yellow, waxy; stipes round, white. Ovary triangular.

Phylogeny among the genera

The phylogenetic relationships were studied based on the morphological characters of 8 genera of all species. The ten selected characters used in scores are (1) habit (2) pseudobulb (3) number of pollinium (4) shape of pollinium (5) colour of flowers (6) colour of pollinium (7) caudicle (8) stipe (9) viscidium and (10) attachment of pollinium. The individual characters were designated as primitive (0) and advanced (1) characters. Total score of advanced characters for the genera *Aerides, Ascocentrum, Bulbophyllum, Coelogyne, Cymbidium, Dendrobium, Pholidota* and *Vanda* are 9, 10, 2, 2, 7, 2, 4, 10 (Table 2.1). The similarities among genera were shown in Figure 4. According to the selected characters, all genera are similar in 42.2%. *Bulbophyllum Coelogyne*, *Dendrobium* and *Pholidota* have 100% similarities. *Ascocentrum* and *Vanda* have 100% similarities. *Aerides* similars with *Ascocentrum* and *Vanda* in 90%. *Cymbidium* similars with *Aerides*, *Ascocentrum* and *Vanda* in 80%.

Table 2. Comparable characteristics of genera of all species

SN	Taxa	Characters									
		Habit	Pseudobulb	Number of Pollinium	Shape of pollinium	Colour of flower	Colour of Pollinium	Caudicle	Stipe	Viscidium	Attachment of pollinium
1	*Aerides*	Monopodial	Absent	2	Orbicular	Pinkish-white	Yellow	Absent	Present	Present	Present
2	*Ascocentrum*	Monopodial	Absent	2	Orbicular	Purple	Purple	Present	Present	Present	Present
3	*Bulbophyllum*	Sympodial	Present	4	Oblong	Yellow	Yellow	Absent	Absent	Absent	Absent
4	*Coelogyne*	Sympodial	Present	4	Subcla-vate	Yellow	Yellow	Absent	Absent	Absent	Absent
5	*Cymbidium*	Sympodial	Present	2	Flabellate	Dull Yellow	Pale Yellow	Absent	Present	Present	Present
6	*Dendrobium*	Sympodial	Present	4	Oblong	Yellow or white	Yellow	Absent	Absent	Absent	Absent
7	*Pholidota*	Sympodial	Present	4	Obovate-oblong	Creamy	Yellow	Absent	Absent	Present	Present
8	*Vanda*	Monopodial	Absent	2	Ovate	Yellowish brown	Yellow	Present	Present	Present	Present

Table 3. Numerical data matrix of genera and selected characters scored as 0 (plesiomorphic) and 1 (apomorphic)

SN	Taxa	Characters										
		Habit	Pseudobulb	Number of Pollinium	Shape of pollinium	Colour of flower	Colour of Pollinium	Caudicle	Stipe	Viscidium	Attachment of pollinium	
1	*Aerides*	1	1	1	1	1	1	0	1	1	1	9
2	*Ascocentrum*	1	1	1	1	1	1	1	1	1	1	10
3	*Bulbophyllum*	0	0	0	0	1	1	0	0	0	0	2
4	*Coelogyne*	0	0	0	0	1	1	0	0	0	0	2
5	*Cymbidium*	0	0	1	1	1	1	0	1	1	1	7
6	*Dendrobium*	0	0	0	0	1	1	0	0	0	0	2
7	*Pholidota*	0	0	0	0	1	1	0	0	1	1	4
8	*Vanda*	1	1	1	1	1	1	1	1	1	1	10

Table 4. t x t matrix presenting similarities between genera

No.	Taxa	*Aeri-des*	*Ascocen-trum*	*Bulbo-phyllum*	*Coelo-gyne*	*Cymbi-dium*	*Dendro-bium*	*Pholi-dota*	*Vanda*
1	*Aerides*	-							
2	*Ascocentrum*	9	-						
3	*Bulbophyllum*	3	2	-					
4	*Coelogyne*	3	2	10	-				
5	*Cymbidium*	8	7	5	5	-			
6	*Dendrobium*	3	2	10	10	5	-		
7	*Pholidota*	5	4	8	8	5	10	-	
8	*Vanda*	9	10	2	2	7	2	4	-

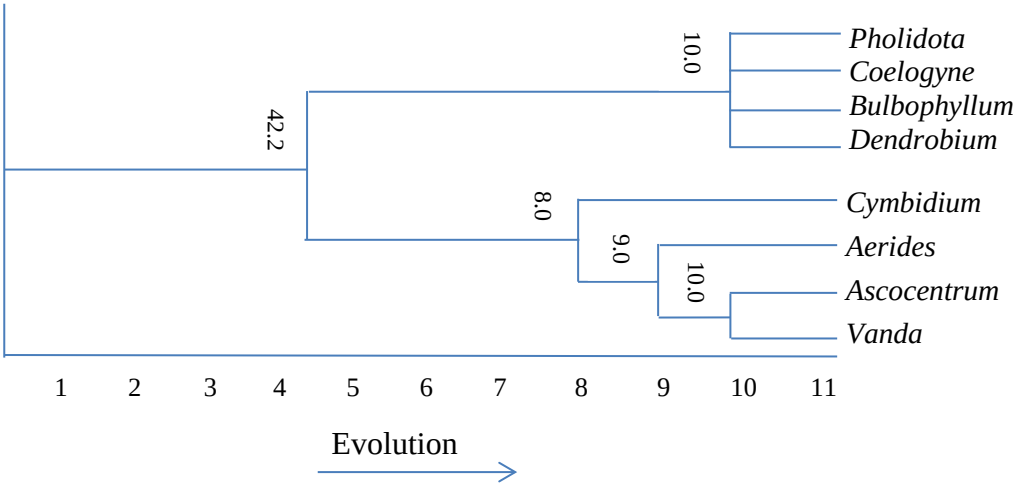

Figure 4. The phenogram among the genera based on overall selected characters

An Artificial key to the studied species:

1. Stems monopodial ---------- 2
1. Stems sympodial ---------- 4

2. Inflorescences more than 38-flowered; caudicles absent ----------
---------- 1. *Aerides multiflora*

2. Inflorescences less than 18- flowered; caudicles present ---------- 3

3. Flowers purple, less than 2 cm in diameter; dorsal sepal ovate ----------
---------- 2. *Ascocentrum ampullaceum*

3. Flowers yellowish brown, more than 5 cm in diameter; dorsal sepal obovate ---------- 10. *Vanda bensonii*

4. Pollinia 2, stipe present ---------- 5. *Cymbidium aloifolium*

4. Pollinia 4, stipe absent ---------- 5

5. Racemes basal of pseudobulbs ---------- 3. *Bulbophyllum suavissimum*

5. Racemes axillary or terminal of pseudobulbs ---------- 6

6. Peduncular bracts 10; pollinium subclavate in shape ---------- 4. *Coelogyne rigida*

6. Peduncular bracts 4 or less; pollinium oblong in shape ---------- 7

7. Pseudobulbs one-jointed ---------- 6. *Dendrobium chrysotoxum*

7. Pseudobulbs many-jointed ---------- 8

8. Pseudobulbs tetragonal ---------- 9. *Pholidota articulate*

8. Pseudobulbs cylindrical ---------- 9

9. Inflorescences more than 12 flowers; peduncular bracts absent; spur absent ---------- 7. *Dendrobium crepidatum*

9. Inflorescences less than 4 flowers; peduncular bracts present; spur present ---------- 8. *Dendrobium infundibulum*

Discussion and Conclusion

The present research work deals with the study on taxonomy and phylogenetic relationship of some orchid species. Altogether 10 species belonging to 8 genera under 4 tribe of the family Orchidaceae were studied.

All of the studied species are under the subfamily Epidendroideae. They were found as wild epiphytes. Among them, *Coelogyne rigida* Parish & Rchb.f. and *Pholidota articulata* Lindl. were rarely found. *Dendrobium* species were abundantly distributing in the study area.

According to the morphological characteristics of the studied species, *Aerides*, *Ascocentrum* and *Vanda* are monopodial epiphytes whereas the other 7 species are sympodial epiphytes. *Aerides*, *Ascocentrum* and *Vanda* have pseudobulb stems. The inflorescences are axillary or terminal racemes except in *Bulbophyllum suavissimum* Rolfe., in which the inflorescences was basal racemes. These characters are agreed with those of Dassanayake (1981) and Chen (2009).

The number of pollinia was 2 in *Aerides*, *Ascocentrum*, *Cymbidium* and *Vanda* while in the other genera, the pollinia are 4 in number. These characters are agreed with those of Dassanayake (1981).

The shapes and size of pollinium are variable. The pollinium of *Ascocentrum ampullaceum* (Roxb.) Schlechter was found as purple colour

but the other species were yellow. The caudicles occur in the *Ascocentrum ampullaceum* (Roxb.) Schlechter and *Vanda bensonii* Veitch & Rchb whereas the other species have no caudicles. These characters are agreed with those of Dassanayake (1981) and Chen (2009).

In *Aerides*, *Ascocentrum*, *Cymbidium* and *Vanda*, the stipes, viscidium and the attachment of pollinarium were found but these characters were not found in other species. These characters are agreed with those of Dassanayake (1981) and Chen (2009).

The total score of the genera *Aerides*, *Ascocentrum*, *Bulbophyllum*, *Coelogyne*, *Cymbidium*, *Dendrobium*, *Pholidota* and *Vanda* were 9, 10, 2, 2, 7, 2, 4 and 10 respectively. The most primitive total score 2 was found in *Bulbophyllum, Coelogyne* and *Dendrobium*. The most advanced total score 10 was observed in *Ascocentrum* and *Vanda*.

According to the phylogenetic point of view, all genera have the similarities in 42.2%. *Bulbophyllum*, *Coelogyne*, *Dendrobium* and *Pholidota* have 100% similarities. *Ascocentrum* and *Vanda* have 100% similarities. *Aerides* similar with *Ascocentrum* and *Vanda* in 90%. *Cymbidium* similar with *Aerides*, *Ascocentrum* and *Vanda* in 80%.

It is hoped that the present research work will know the morphology and the phylogenetic relationship of all study species and will give the valuable information to the students and other researchers.

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Morphological, Microscopical Characteristics and Phytochemical Constituents from Leaves of *Aloe vera* L. Burm. f. (Shazaungletpat)

Yin Yin Aye*

Abstract

Aloe vera (L). Burm. f., is the medicinal plant which belonging to the family Asphodelaceae. It is locally known as Shazaungletpat in Myanmar. The specimens were collected from Amarapura Township, Mandalay Region. The plant is a perennial succulent herb. Leaves are densely rosulate and sharp with pointed apex. The flowers are bisexual, zygomorphic, yellow or orange, crowded into a rosette and in panicles. In the microscopical study, paracyctic type of stomata on both surfaces and more abundant on the lower surfaces. The epidermal cells were rectangular or polygonal to barrel shaped. Mucilage parenchymatous cells are found at the centre. The vascular bundles were collateral types and embedded in the mesophyll tissue. The leaves powder were showed parenchymatous cells, stomata, vessel, fibre, starch grains and crystals. The sensory characters were found in yellowish brown in color and granular. Odour was strongly aromatic and bitter taste. In phytochemical study, presence of alkaloid, glycoside, phenol, tannin, saponin, carbohydrate, flavonoid and polyphenol, lipophilic and absent in reducing sugar of leaves were investigated.

Keywords: *Aloe vera* L. Burm. f., morphological, microscopical, phytochemical

Introduction

The Asphodelaceae is consisted of 5 genera and 700 species (Cronquist 1981). Two species of the genus *Aloe* were found in Myanmar (Kress *et al.* 2003). *Aloe vera* (L). Burm. f., is native to southern Africa and introduced into northern Africa, China, Mediterranean countries and west India (WHO 1999). The plant was rosettes of large succulent; subulate leaves, flat or concave on the upper surface, strongly spines at the apex and red or yellow flower spikes with fruit a capsule (Wallis 1967). Transverse section of *Aloe vera* (L). Burm. f., leaves showed a strongly cuticularized epidermis with numerous stomata on both surfaces. The parenchymatous

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cells contained chlorophyll, starch and crystal. A central region had large mucilage parenchymatous cells. The vascular bundles lied at the junction of two previous layers and had well-marked pericycle and endodermis (Kapoor 2001). The vascular bundles in the leaves were isolated and formed a line parallel with a short distance within the mesophyll (Wallis 1967). The powder of *Aloe* leaves was yellowish brown to reddish brown which contained amorphous minute crystals (WHO 1999).

The *Aloe vera* (L). Burm. f. leaves contained anthraquinones (aloin), tannins and polysaccharides (Chevallier 1996). *Aloe* gel consisted of a major sugar component, lipid (WHO 1999). *Aloe* contained c-glycosides and resins. *Aloe* gel was very rich water, saponin, reducing sugar and polysaccharides and sterol (Burneton 1995). Purgative properties of aloes are due to the presence of three pentoside (Kapoor 2001). The leaves juice of *Aloe vera* (L). Burm. f. were used for intestinal worm in children, haemophilia, skin and uterine disorders, liver and spleen enlargement, burns painful, fever, purgative, carminative, cough, asthma and dysuria (Duke 2002). Externally, it is used for burns and pains. Internally, for jaundice, habitual constipation, loss of appetite, gas formation in the stomach and leucorrhoea (WHO 1999). Aloe leaves are used as indigestion, menstrual disorders, eye and ear disease, constipation, skin diseases and tonic agent (Ministry of Health 2000).

The aim and objectives of this research are to study the morphological, microscopical characters and phytochemical constituents of *Aloe vera* L. Burm. f. from Amarapura Township, Mandalay Region.

Materials and Methods

The species of *Aloe vera* L. Burm. f. was collected from Amarapura Township, Mandalay Region. The collected plants were identified with the help literatures Hooker (1885) and Dassanayake (2000). After the collection, the fresh specimens of leaves were preserved in 50% ethyl alcohol for further study. For microscopical study, the specimens were examined by preparing microtome sections according to Johnson (1940) method. Macerations of leaves were made by Jeffery (1917) method. Then, the diagnostic characters of powder leaves were used to examine the samples according to Wallis (1967) and Trease and Evans (1978). The photographs were taken with the help of a camera.

For phytochemical test, the fresh specimens were collected, air dried and grinded to get powder and stored in air tight containers. After that ethanol, 1% HCL and water extracts were tested for the presence or absence of phytochemical constituents according to Harbone (1984) method. The phytochemical tests for alkaloid, glycoside, phenol, tannin, saponin, reducing sugars, carbohydrate, lipophilic, flavonoid and polyphenols were carried out by using the various reagents. The morphology, microscopy and phytochemistry of *Aloe vera* L. Burm. f. were studied at the Department of Botany, University of Mandalay and University of Distance Education, Mandalay.

Phytochemical Tests

Test for Alkaloids

Two grams of dried leaves samples were boiled with 1% diluted HCL for 20 minutes and filtered off. The filtrate was divided into three portions and tested with 2 - 3 drops of Dragendroff's reagent, Mayer's reagent and Wagner's reagent.

Test for Glycosides

Two grams of dried leaves samples were boiled with 10 ml of distilled water for 20 minutes, allowed to cool and filtered. The filtrate was tested with 3 drops of 10% lead acetate solution ($Pb(OCO)^3$.

Test for Phenols

Two grams of dried leaves samples were boiled with 10 ml of distilled water for 20 minutes and filtered. The filtrates were treated with 3 drops of fleshy prepared (1:1) mixture containing 1% potassium ferric cyanide ($K_3Fe(CN)_6$) and 1% ferric chloride solution ($FeCl_3$).

Test for Tannins

Two grams of dried leaves samples were boiled with 10 ml of distilled water for 20 minutes and filtered. The filtrates were treated with 2 drops of gelatin 1% solution and ferric chloride solution ($FeCl_3$) 1%.

Test for Polyphenols

Two grams of dried leaves samples were boiled with 10 ml of 95% ethanol for 20 minutes and filtered. The filtrates were treated with 2 - 3 drops of 10% ferric chloride solution ($FeCl_3$).

Test for Saponins

Two grams of dried leaves samples were boiled with 10 ml of distilled water for 20 minutes and filtered. The filtrate was introduced into a test tube and 3 drops of sodium bicarbonate ($NaHCO_3$) or distilled water and the mixture was vigorously shaken for few minutes. The appearance of frothing was showed the presence of saponin.

Test for Reducing Sugars

Two grams of dried leaves samples were placed into test tube and was heated with 10 ml of distilled water on a water bath for 20 minutes. Then, it was filtered and the filtrate was tested with 2 - 3 drops of Benedict's solution.

Test for Carbohydrates

Two grams of dried leaves samples were boiled with 10 ml of distilled water for 20 minutes and filtered. The filtrate was placed in a test tube and added 2 - 3 drops of Benedict's solution and concentrated sulphuric acid (Conc: H_2SO_4).

Test for Flavonoids

Two grams of dried leaves samples were boiled with 10 ml of 95% ethanol for 20 minutes and filtered. The filtrate was placed in a test tube. Then 5 - 10 drops of concentrated hydrochloric acid (Conc: HCL) and a small piece of Magnesium (Mg) were added and the mixture was boiled for a few minutes.

Test for Lipophilic compounds

Two grams of dried leaves samples were boiled with 10 ml of distilled water for 20 minutes and filtered. The filtrates were placed in a test tube and added 1 - 2 drops of Potassium hydroxide (KOH) solution.

Results

Morphological characters

Aloe vera L. Burm. f.

Family - Asphodelaceae

Myanmar name - Shazaungletpat

English name - Bitter Aloe

Part used - Leaves

Flowering and Fruiting Period - January to April

The species is found in wild or cultivated in medicinal garden.

Perennial, rhizomatous, succulent herb with a short stem. Leaves simple, alternate or densely rosulate, blades linear-lanceolate with soft spines along the margin, glabrous. Inflorescences terminal scapose racemes with numerous flowers. Flowers bisexual, zygomorphic, yellow or orange. Perianth 6-lobed, tubular-campanulate with two series of 3 - segments each. Stamens 6 in two series of 3 each, adnate to the base of the perianth tube; filaments of outer whorl flat, inner whorl slender; anthers oblongoid, dithecous, dorsifixed. Ovary superior, ellipsoid, trilocular with many ovules in each locule on the axile placentae; style filiform; stigma simple. Fruits capsule, ovate, glabrous. Seeds numerous and black (Figure 1A).

Microscopical Characters

Microscopical Characters of *Aloe vera* L. Burm. f.

The macroscopical characters of the leaves of *Aloe vera* (L). Burm. f., its microscopical characters and its diagnostic characters of powder were described.

Macroscopical Characters of Leaves

The leaves were simple, densely alternate or spiral, sessile, succulent, long and gradually tapering at the tip, tips acicular-acuminate, cresent- shaped at the base, spiny at the margin, the blades linear - lanceolate, slightly concave above, slightly convex beneath, green, becoming pale brownish green in the dry season (Figure 1B).

Microscopical Characters of Leaves

In surface view, the epidermal cells of both surfaces were smooth-walled and somewhat polygonal in shape, various in size and parenchymatous cells. The stomata were present on both surfaces and more abundant on the lower surface. Upper epidermis of stomata number were 2 per field in high power, stomata index 7. Lower epidermis of stomata number were 5 per field in high power, stomata index 13. The type of

stomata was paracytic and lanceolate shaped in outline, with two guard cells, chloroplast present reniform in the guard cells.

In transverse section, the cuticle was present on both surfaces and slightly wavy, 5.5 µm thick. The epidermis was made - up of parenchymatous cells with straight anticlinal walls and one - layered thick. The upper and lower epidermal cells were thin - walled, rectangular, polygonal, barrel, oval or rounded in shapes and compactly arranged. The outer and inner walls were slightly convex. The outer spongy parenchyma cells were larger than the epidermal cells and 2 to 3 layers thick with elongated cells. The inner spongy parenchyma cells were oval or rounded in shape and 5 to 8 layers thick. They were thin-walled and parenchymatous cells with abundant chloroplast. Large mucilage parenchymatous cells are found at the centre. The vascular bundles were collateral type and embedded in the mesophyll tissue. They are 6 to 8 in number and arranged in a ring, along the transverse plane. Each bundle is surrounded by a layer of parenchymatous bundle sheath. A group of sclerenchymatous cells is covered the bundle at the outer side and forming a bundle cap. The bundle consisted of xylem in the inner and phloem in the outer side. Phloem is composed of sieve tube and companion cells. Xylem is composed of vessel elements, fibers and xylem parenchyma (Figure 2 A-F).

Diagnostic Characters of Powder Leaves

The powder showed fragments of parenchyma, stomata, vessel with annual or spiral thickening and single fibers. The starch grains were found simple or compound, oval or rounded with eccentric hilum and single or bundles of calcium oxalate crystals (Figure 3 A-F).

Sensory Characters of Leaves (Figure 1C).

Colour - yellowish brown

Texture - granular

Odour - strongly aromatic

Taste - bitter

Table 1. Microscopical Measurement of cells; starch grains and crystals on leaves of *Aloe vera* L. Burm. f.

Characters	Length (µm)	Width (µm)
Upper epidermal cells	10.0 - 15.0 (mean 12.0)	6.5- 12.5 (mean 10.0)
Lower epidermal cells	7.5 - 18.5 (mean 15.5)	7.5 - 12.5 (mean 9.5)
Outer spongy mesophyll cells	10.0 - 25.0 (mean 16.5)	7.5 - 15.0 (mean 12.5)
Inner spongy mesophyll cells	7.5 - 20.0 (mean15.5)	10.5 -17.5(mean 15.0)
Vessel elements	150.0 - 300.0 (mean 280.0)	10.0 - 20.0 (mean18.5)
Fiber	200.0 - 250.0 (mean 250.5)	10.0 - 20.0 (mean16.5)
Parenchyma cells	48.0 - 80.0 (mean 65.0)	8.0 - 15.0 (mean12.5)
Stomata	25.0 - 30.0 (mean 28.0)	5.0 - 15.0 (mean12.5)
Starch grain	10.0 - 15.0 (mean 12.0)	5.0 - 10.0 (mean 7.5)
Crystal	100.0 - 250.0 (mean 200.0)	30.0 - 50.0 (mean 40.0)

Table 2. Phytochemical constituents of *Aloe vera* L. Burm. f.

No.	Phytochemical Constituents	Extract	Reagent used	Observation	Result
1.	Al kaloids	1% HCl	Dragendroff's reagent	Orange ppt.	+
			Mayer's reagent	Cream ppt.	+
			Wagner's reagent	Reddish brown ppt.	+
2.	Glycoside	Water extract	10%lead acetate	White ppt.	+
3.	Phenol	Water extract	1% $K_3Fe(CN)_6$ + 1% $FeCl_3$	Dark blue ppt.	+
4.	Tannin	Water extract	1% Gelatin + 1% $FeCl_3$	Pale brown ppt	+
5.	Saponin	Water extract	$NaHCO_3$	Frothing	+
6.	Reducing sugar	Water extract	Benedict's solution	Not ppt.	-
7.	Carbohydrate	Water extract	Benedict's solution + H_2SO_4	Pale yellow ppt	+
8.	Lipophilic	Water extract	KOH solution	Deep colour change	+
9.	Flavonoid	EtOH extract	Conc: HCl+ Mg	Orange	+
10.	Polyphenol	EtOH extract	10% $FeCl_3$	Dark green	+

(+) = present, (–) = absent, ppt = precipitation

Phytochemical tests indicated that the presence of alkaloids, glycosides, phenol, tannin, saponin, carbohydrate, lipophilic, flavonoid, polyphenol and absence of reducing sugars were shown in Table 2.

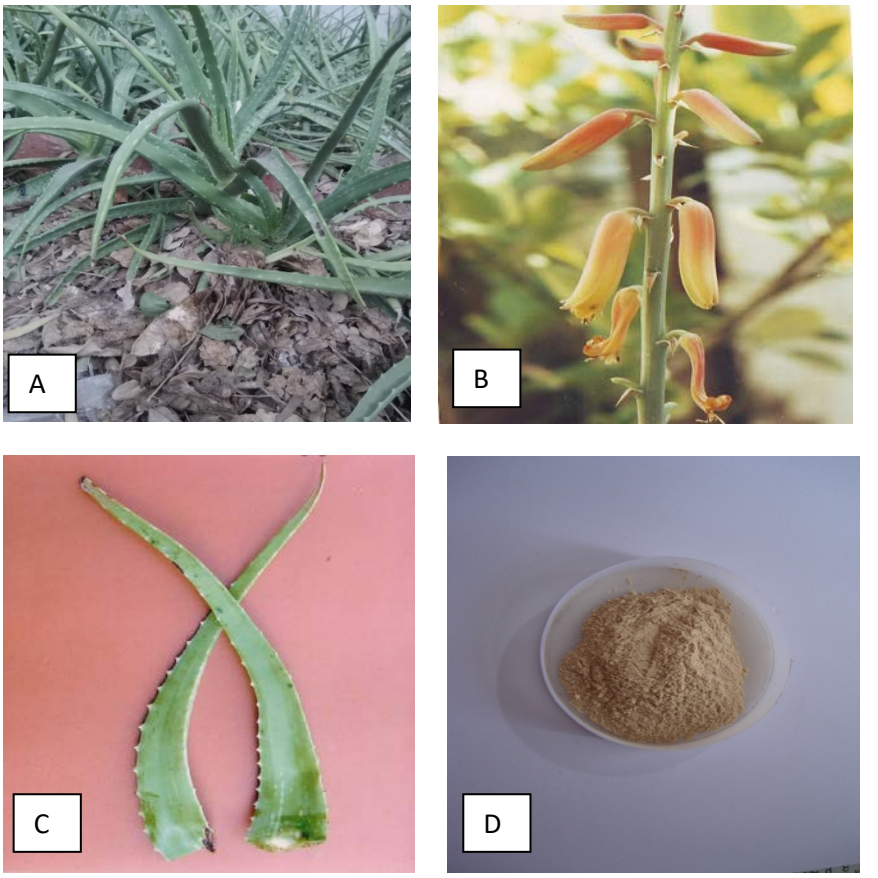

Figure 1. Morphological Characters of *Aloe vera* L. Burm. f.

A. Plant in natural habit

B. Inflorescence

C. Fleshy leaves

D. Dried Leaf powder

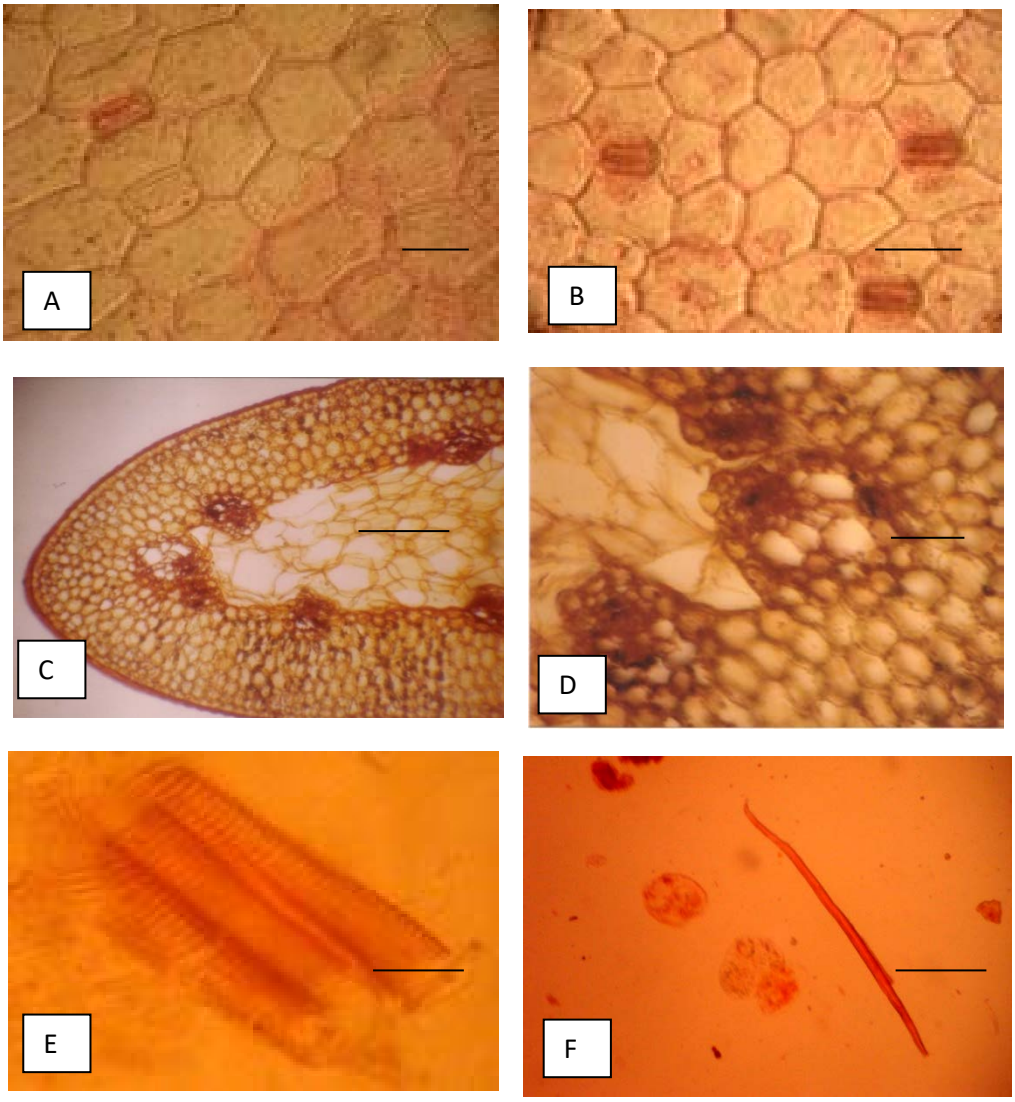

Figure 2. Microscopical characters on leaves of *Aloe vera* L. Burm. f.
A. Adaxial surface view of lamina (15 µm)
B. Abaxial surface view of lamina (18 µm)
C. T.S of lamina (90 µm)
D. Close-up view of vascular bundle (50 µm)
E. Vessel elements (50 µm)
F. Fiber (50 µm)

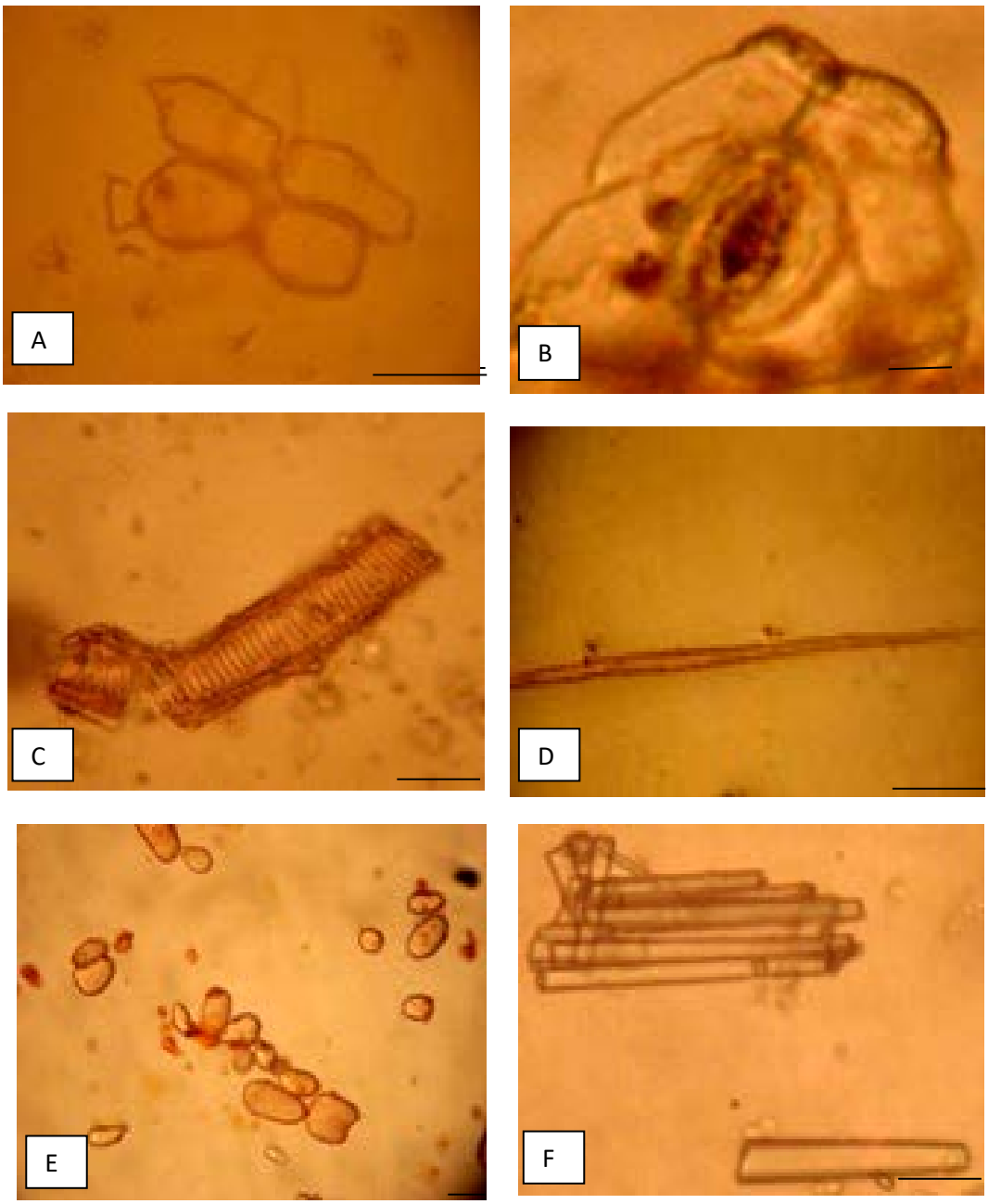

Figure 3. Powder microscopical characters on leaves of *Aloe vera* L. Burm. f.
A. Parenchyma cells (50 µm) B. Stomata (15 µm) C. Vessel element (50 µm) D. Fiber (80 µm) E. Starch grains (10 µm) F. Crystals (50 µm)

Discussion and Conclusion

In this research, the medicinal plant, *Aloe vera* L. Burm. f., belongs to the family Asphodelaceae was studied. The morphology of vegetative and reproductive parts, microscopical characters and phytochemical constituents were described and discussed. The plants of *Aloe vera* L. Burm. f. was succulent perennial herb with densely rosulate leaves and glabrous. Inflorescences are terminal scapose racemes spike and many flowers. Flowers are bisexual, zygomorphic and they have stamens 6 in two series of 3 each and ovary superior. These characters were agreed with Hooker (1975) and Dassanayake (2000). The microscopical characters of the lamina of *Aloe vera* (L). Burm. f., the cuticle was present on both surfaces and slightly wavy and stomata are found to be paracytic and present on both surface. The upper mesophyll cells were 2 to 3 layers thick on the adaxial side and lower mesophyll cells 5 to 8 layers thick on the abaxial side. Mucilage parenchymatous cells are found at the centre. The vascular bundles were 5 to 8 in number and embedded in the mesophyll tissue and arranged in a ring along the trasverse plane. These characters were agreed with Wallis (1967) and Kapoor (2001). The dried powder of leaves were showed that yellowish-brown colour; epidermis with paracytic stomata; and fragments of parenchymatous cells, stomata, vessel elements with annular or spiral thickening, fibers, single or 2 - 3 groups starch grains; single or bundles of calcium oxalate crystals. These characters were agreed with Wallis (1967) and WHO (1999). In the present study, phytochemical analysis of *Aloe vera* L. Burm. f., showed that the presence of alkaloids, glycosides, phenol, tannin, saponin, carbohydrate, lipophilic, flavonoid, polyphenol and absence of reducing sugars. Among them alkaloids, tannin, saponin, phenol and giycosides were found in this species. These characters were agreed with Chevallier (1996) and Burneton (1995).

It can be concluded that the morphological characters and microscopical characters of the plant and powdered drugs are useful for identification and standardization of the medicinal drugs. Moreover, the substitution and adulteration of the drugs can be detected by the use of its known characters of the true medicinal plants. Therefore, the bioactive chemical constituent of this plant is effectively on preparing of medicine. This research will be provided the basic information in Myanmar traditional medicine.

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Extraction and Identification of Antibacterial Metabolite Producing Soil Fungi

Tin Moe Aye[1] & Moe Moe Aye[2]

Abstract

Furarium lunatum was isolated from the soil sample collected of Ma Minbu Pagoda, Minbu Township, Magway Region. *Pseudomonas* sp. was isolated from the diseased leaf of *Sesamum indicum* L. (Hnann). Fermentation fort the production of antibacterial metabolite was established as fermentation medium. According to the R_f values solvent No. 4 ethyl acetate is suitable for the extraction of antibacterial metabolite from the fermented broth. Ethyl acetate extract was showed the antibacterial activity against *Pseudomonas* sp. (41.93 mm). Chloroform-methanol mixture (9:1), fractions No. 43-44 exhibited the antibacterial activity on *Pseudomonas* sp. Based on the macroscopical and microscopical characters, MF- 10 was confirmed as *Fusarium* sp.

Keywords: antibacterial metabolite, antibacterial activity, *Fusarium* sp.

Introduction

Fungi are sensitive to nutritional and environmental factor and either growth and sporulation are therefore greatly influenced by the composition of the nutrient media pH and temperature. The temperature is a variable the directly affects the growth rate of the microorganisms (Hajiji *et al*. 2013). Chromatography is a laboratory technique that separate components within a mixture by using the differential affinities of the components for a mobile medium and for a stationary adsorbing medium through which they pass.

In paper chromatography, substances are distributed between stationary phase and a mobile phase. Chromatograph partially characterized by the medium on which the separation occurs. This medium is commonly identified as the "stationary phase".

TLC is a method for identifying substance and testing the purity of compounds. TLC is a useful technique because. It is relatively quick and requires small quantities of materials. Separation in TLC involve

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distributing a mixture of two or more substances between a stationary phase and a mobile phase. The aim of the research is to study the growth of fungi by using carbon and nitrogen utilization of *Furarium lunatum* for the growth.

Materials and Methods

The materials and methods used for the present research are described in the following figure 1.

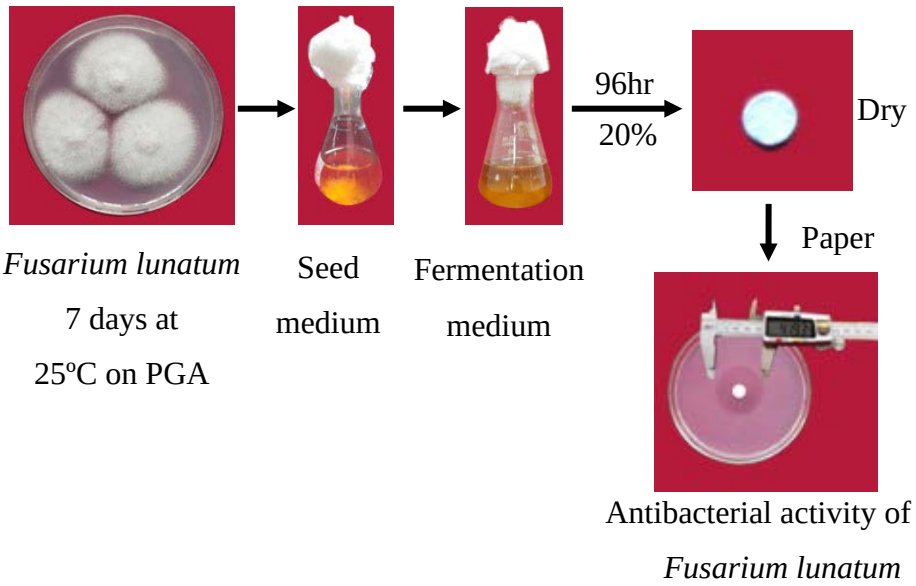


Figure 1. Procedure of fermentation

Antibacterial activity methods

Seed Medium		**Fermentation medium**	
Glucose	1.0 g	Glucose	2.0g
Yeast extract	0.4g	Molasses	1.0ml
NZ amine type A	0.3g	Yeast extract	0.3 g
K_2HPO_4	0.001g	NZ amine type A	0.3g
DW	100 ml	K_2HPO_4	0.001 g

pH	7.0	$CaCO_3$	0.1 g
		DW	100 ml

Assay Medium

Glucose	1.0g
Yeast extract	0.2g
Agar	1.8 g
DW	100ml

Effects of pH on the fermentation

The effects of pH on the fermentation for antibacterial metabolite production was done by carrying out the fermentation at six different pH 3.0,4.0, 5.0, 6.0, 7.0, and 8.0.

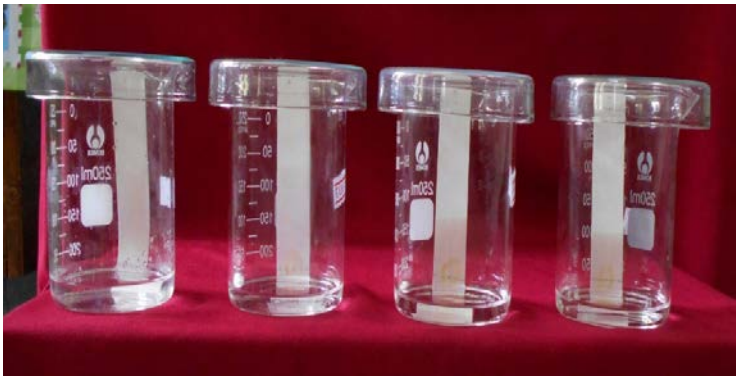
Paper chromatography

Solvents employed in Paper chromatography (Tomita, 1988)

1. 20% NH_4Cl
2. *n*-butanol saturated with water
3. *n*- BuOH - acetic acid - water (3:1:1)
4. ethyl acetate saturated with water

A preliminary study of paper chromatography involves; selection of fermentation type filters. Preparation of sample, spotting of sample-fermented broth was to be spotted at paper position on the paper using suitable a capillary tube. After allowed to dry, this paper was immersed into the solvent. The solvent moves over the sample on the paper. Drying of the paper using an air drier. Each paper was put on assay agar plate. After one hour, the paper was moved out. These assay agar plates were incubated for one hour. In this case, the inhibitory zone was measured R_f value of the metabolite.

Fermented broth samples were applied on the paper and dry



1. 20% NH_4Cl 3. *n*- BuOH - acetic acid - water (3:1:1)

2. *n*-butanol saturated with water 4. ethyl acetate saturated with water

Figure 2. Procedure for the paper chromatography

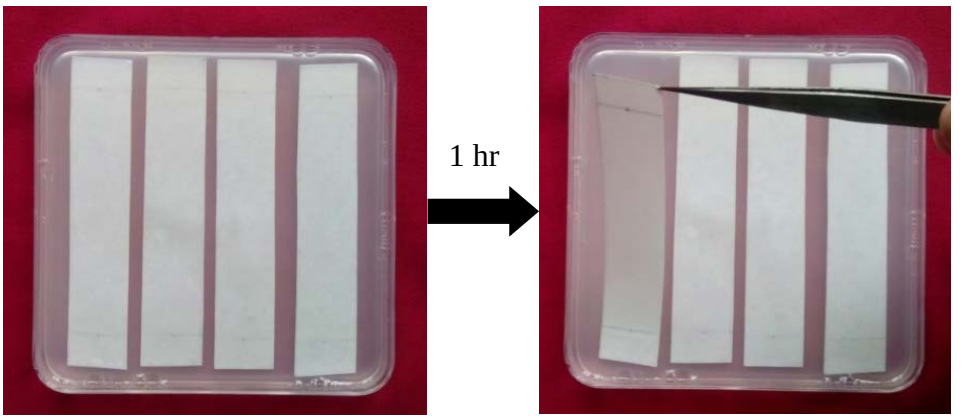


Figure 3. Procedure for the paper chromatography

Figure 4. Extraction of antibacterial metabolite

Thin Layer Chromatography and Bioautographic Overlay Assay (Simon and Gray, 1998, Cannel, 1998)

The obtained EtOAc extracted samples (20 µL) were applied on the TLC plate and allowed to dry. The TLC plate were developed in the solvent of chloroform and chloroform - methanol mixture (9:1), Toluene and Toluene - methanol mixture (9:1).

Each TLC plate was placed on assay agar plates. After one hour, they were peel off and the plate was incubated for 24 hours. Then bioautography was done to check the antibacterial activity.

$$R_f \text{ value} = \frac{\text{Distance of compound from origin}}{\text{Solvent front from origin}}$$

Silica Gel Column Chromatography

The silica gel was dissolved in chloroform and silica gel column was packed. EtOAc extract was then passed through silica gel column, and eluted with chloroform-methanol solvent (9:1); and then tested the activity against *Pseudomonas* sp.

Two mL of each fraction was collected and examined the activity.

Identification of the fungus *Fusarium lunatum*

The macroscopical and microscopical were observed by the methods of:

- Barnett, H.L. 1956: L Fungi Imperfecti.
- Domsch, 1993, Pendro *et al*, 2009.
- Ando and Inaba 2004: Taxonomy of fungi

Results

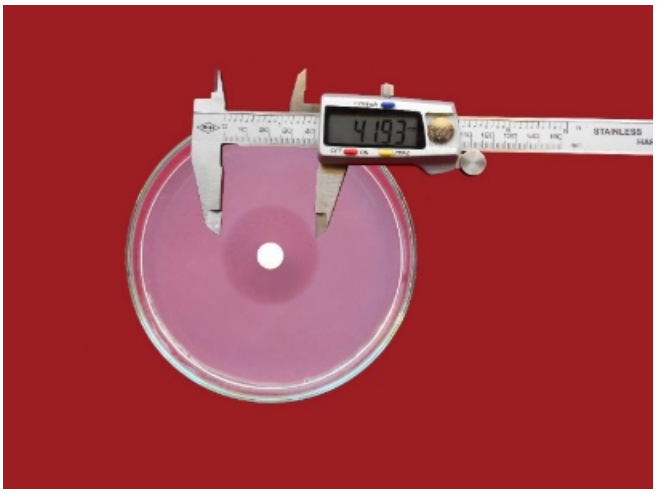
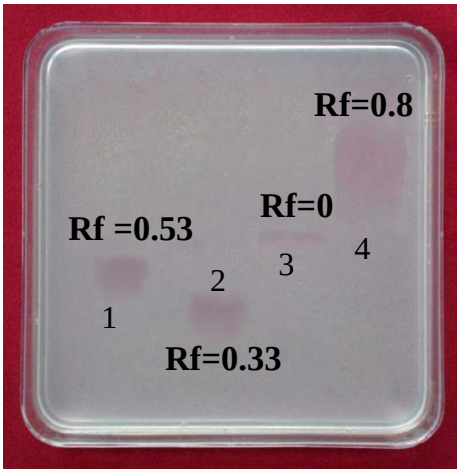


Figure 5. Antibacterial activity of *Fusarium lunatum* on the fermentation medium (7 days fermentation)



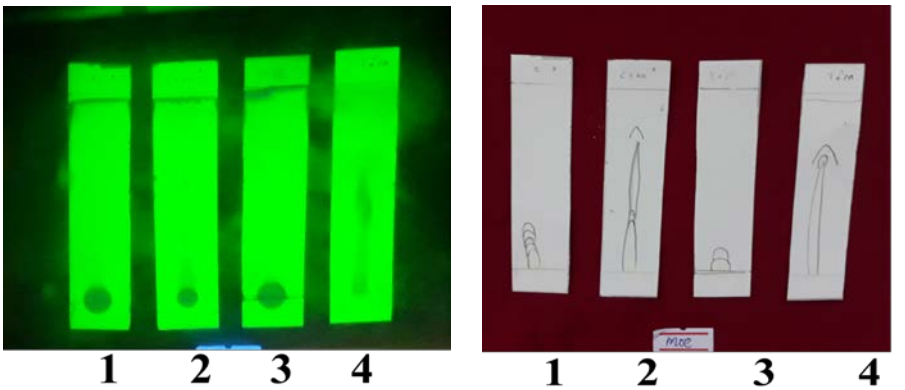
1. **20% NH_4Cl**
2. **Water saturated n-BuOH**
3. **n-BuOH-acetic acid-water (3:1:1)**
4. **Water saturated ethyl acetate**

Figure 6. Paper Chromatography Bioautographic Assay

pH 3 pH 4 pH 5

pH 6 pH 7 pH 8

Figure 7. Effects of pH on the fermentation

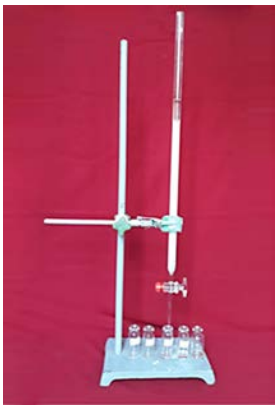


1. Chloroform
2. $CHCl_3$-MeOH (9:1)
3. Tolurene
4. Tolurene -MeOH (9:1)

Figure 8. Bioautography (Thin Layer Chromatography)

1. Chloroform
2. CHCl3-MeOH (9:1)
3. Toluene
4. Toluene - MeOH

Figure 9. Bioautography (Thin Layer Chromatography)



Column Size …….. 55cmx1.3cm
Flow rate 1.0ml/min
Solvents ……. $CHCl_3$ (80mL),
$CHCl_3$- MeOH (9:1) 60mL
$CHCl_3$- MeOH (8:2) 70mL

Figure 10. Silica gel column chromatography

Table 1. The effects of fractions on *Pseudomonas* sp

Fraction No.	**Activity**	**Eluting solvent**
1- 36	No activity	Chloroform only
37-42	No activity	Chloroform-methanol (9:1)
43-44	Activity	Chloroform-methanol (9:1)
45-64	No activity	Chloroform-methanol (9:1)
65-100	No activity	Chloroform-methanol (8:2)

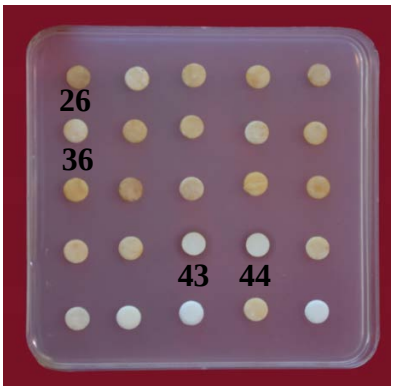
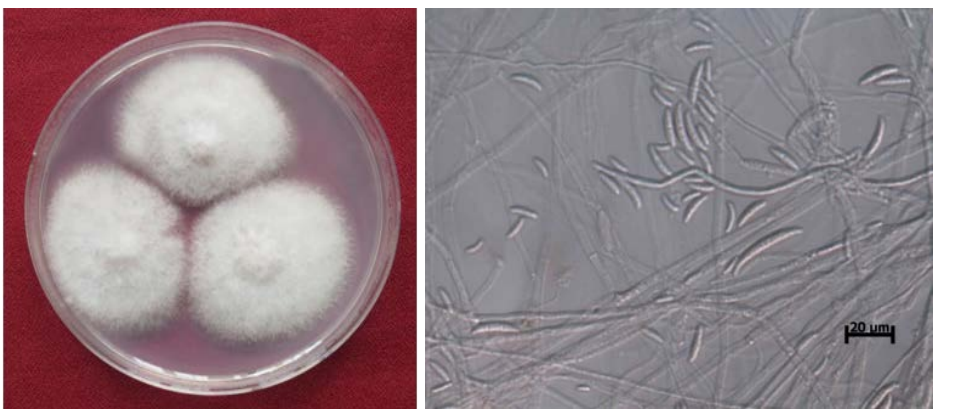


Figure 11. The effect of Chloroform and Chloroform-methanol (9:1) fractions on *Pseudomonas* sp. (Silica gel column chromatography)

Isolation and identification of fungal strain MF-10



A. Front view of fungal strain B. Photomicrograph of fungal strain

Figure 12. Morphology and photomicrograph of fungal strain MF-10 (7 days old culture on PGA medium**)**

Morphological characters of MF-10

Morphological characteristic of this fungus includes extensive cotton like mycelium in culture, often with some tinge of pink, purple or yellow. *Fusarium* spp. grow rapidly and produces wooly to cottony, fat, spreading colonies. Colonies are usually fast growing, pale or bright-

coloured (depending on the species) with or without cottony aerial mycelium. The colour of the thallus varies from whitish to yellow, pink, red, or purple shades.

Microscopical characters of MF-10

Conidia microconidia ellipsoid, slightly curved, moderately truncate at the base, mostly 5-17×2.8-5 µm; macroconidia fusiform with a rather blunt apical and slightly pedicellate basal cell, rather thin-walled, mostly 3-4 (-5) septate, 27-55×4.2-6µm. Conidiophores long, scarcely branched conidiophores bearing microconidia in watery droplets; more compact and sporodochia conidiophores bearing the macroconidia in slimy masses. Chlamydospores commonly produced, terminal, lateral or sometimes intercalary, hyaline, roughened.

Identification Key for *Fusarium*

1. Synnemata not form ……2
2. Conidium axis not curved through more than 180……..…..3
3. Conidia shape not above……..…..…4
4. Length/width ratio of conidium body less than 15:1 …….5
5. Conidium with 1 or more septa ……..…..…6
6. Conidium with more than 1 septa……..…7
7. Conidium with only transverse septa……Phragmoconidium

   Phragmoconidium

   1. Conidiophores not produced or not clear
   2. Conidia enteroblastic
   3. Conidia phialidic ……*Fusarium*

In the literature references, the characters of fungal strain MF-10 was identified was *Fusarium.*

Kingdom : Fungi

Phylum : Ascomycota

Class : Sordariomycetes

Order : Hypocreales

Family : Nectriaceae

Genus : *Fusarium*

Discussion and Conclusion

In the study of pH effect on fermented broth, 84hrs age of inoculum and 20% size of inoculum were shown the highest antibacterial activity of 36.26 mm at 7 days of fermentation (pH 6). Other pH value of 4 showed the distinct inhibitory zones of 16.66 mm, pH 5 showed 19.25mm, pH 7 showed 20.55mm, pH 3 and 8 did not showed inhibitory zones. Therefore, the fermented broth with pH 6 showed the best of inhibitory zone against *Pseudomonas* sp.

In this study, four kinds of different solvents were used to observe the optimum extraction ability of secondary metabolites. According to R_f value, ethyl acetate showed the excellent extraction than other. Therefore, solvent No.4 ethyl acetate is suitable for the extraction of antibacterial metabolite from the fermented broth.

In thin-layer chromatography, chloroform-methanol (9:1), toluene-methanol (9:1) were used. According to bioautography, Chloroform, Chloroform-methanol (9:1) was selected.

In the investigation of silica gel column chromatography with chloroform- methanol mixture (9:1), fractions No. 43-44 exhibited the antibacterial activity on *Pseudomonas* sp. In the study of identification, fungus MF-10 possessing antibacterial activity was identified as *Fusarium sp.* on the basis of morphological-microscopical characters and reference keys.

Acknowledgements

We would like to express thanks Dr. Khin Maung Oo, Rector, University of Magway, Our special thanks are Dr. Aye Aye Kyi, Professor and Head, Department of Botany, and Dr. Thandar, Professor, Department of Botany, and University of Magway for their thoughtful suggestion and many precious advices at this paper. Finally, many thanks to express my gratitude to me for their parental love support my study.

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Morphological identification of *Aspergillus novofumigatus* from the soil of Beikthano Ancient City

Khin Ni Lar Oo*

Abstract

The present study was conducted to identify *Aspergillus novofumigatus* isolated from Beikthano Ancient City, Taungdwingyi Township, Magway Region. In this study six differential media such as, Czapek Dox Agar (CZA), Malt Extract Agar (MEA), Potato Glucose Agar (PGA), Czapek Yeast Agar (CYA), Yeast Extract Agar (YEA) and Oat Agar (OA) were used for the identification of *Aspergillus novofumigatus* using macroscopic features such as colony growth, conidal color, colony reverse, and microscopic characteristic including conidiophores, vesicle, metulae, phialides and conidia. In the study of carbon and nitrogen utilization *Aspergillus novofumigatus* for the growth, it was observed that yellow diffusible pigment on corn agar.

Keywords: *Aspergillus novofumigatus*, identification, macroscopic feature, microscopic characteristics

Introduction

Aspergillus is a very large genus containing about 250 species, which are currently classified in to seven sub genera that are in turn subdivided into several sections comprised of related species (Klich, 2002).

As with fungi in genera, *Aspergillus* taxonomy is complex and ever evolving. The genus is easily identified by its characteristic conidiophores, but species identification is complex, for it is traditionally based on a range of morphological features. Macro morphological features which are considered include conidial and mycelia color, colony diameter, colony reverse color, production of exudates ad soluble pigments. Micro morphology characterization is mainly dependent seriation, shape and size of vesicle, conidia ad stipe morphology and ascospores (Klich, 2002).

In this study we emphasize on morphological methods including macroscopic features of colonies and microscopic characteristics for identification of *Aspergillus* species from soil.

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Materials and Methods

Identification of selected fungus *Aspergillus novofumigatus*

The selected fungus was cultured on six differential media such as Czapek Dox Agar (CZA), Malt Extract Agar (MEA), Potato Glucose Agar (PGA), Czapek Yeast Agar (CYA), Yeast Extract Agar (YEA), Oat Agar (OA). After seven days of incubations, macroscopic characteristics such as colony diameter, colony color and microscopic characteristics including conidiophore, vesicles, phialides and conidia were observed.

Microscopic examination method

The morphological and microscopical characters of fungus ***Aspergillus novofumigatus*** were examined by the methods of Domsch *et al.*, 1993, 2007; Samson *et al.*, 2004, 2007 and Nyongesa *et al*, 2015. Microscopical characters were studied by Olympus Sato Shouji Camera attached microscope.

Medium used for the isolation of fungi (Ando, 2004)

Czapek - Dox Agar (CZA)
Components per liter

Sucrose	30g
$NaNO_3$	2g
K_2HPO_4	1g
$MgSO_4.7H_2O$	5g
$FeSO_4$	0.5g
KCl	0.01g
Agar	18g
pH	6.5

Czapek -Yeast Agar (CYA)
Components per liter

Sucrose	30g
$NaNO_3$	2g

Malt Extract Agar (MEA)
Components per liter

Malt Extract	20g
Agar	20g
pH	6.5

Potato Glucose Agar (PGA)

Potato	20g
Glucose	20g
Agar	18g
pH	6.5

Yeast Extract Agar (YEA)
Components per liter

Yeast Extract	20g
Agar	20g

K_2HPO_4	1g	pH	6.5
$MgSO_4.7H_2O$	5g	**Oat Agar (OA)**	
$FeSO_4$	0.5g	**Components per liter**	
KCL	0.01g	Oat	20g
Yeast	10g	Agar	20g
Agar	18g	pH	6.5
pH	6.5		

Carbon and nitrogen utilization for the growth of NLF-12

Carbon and nitrogen source for the growth of NLF-12 was carried out by the methods of Omura, 1985 and Cruger and Cruger, 1989. In this study, lactose, sucrose, potato, tapioca, corn and glucose were utilized for the carbon sources. Potassium nitrate, malt extract, yeast extract, meat and peptone were utilized for nitrogen sources.

Results

Identification of selected fungus

After seven days of incubating, plates were observed for macroscopic characteristics such as colony diameter, surface and reverse color. Colony diameter after incubation for 7 days, on CZA 3.6-3.8 cm; on MEA 5.3-5.5 cm; on PGA 5.0-5.3 cm; on CYA 4.0- 4.3 cm; on YEA 5.7-6.0 cm; on OA 4.0- 4.3 cm respectively. On CZA, the surface color was dull green and reverse color was white. On MEA, YEA and OA, both surface color were grey and reverse color were white and pale green. On PGA and CYA, both surface colors were greenish grey and reverse color were white and pale green respectively. On corn agar, the colonies 3.0- 3.2 cm, surface color was greenish blue with yellow reverse color.

Table 1. Colony morphology of NLF-12on different media

No.	Media	Colony surface color	Colony reverse color	Size of Colonial growth(cm)
1	CZA	Dull green	White	3.6-3.8
2	MEA	Grey	White	5.3-5.5
3	PGA	Greenish grey	White	5.0-53
4	CYA	Greenish grey	Pale green	4.0-4.3
5	YEA	Grey	White	5.7-6.0
6	OA	Grey	Pale green	4.0-4.3

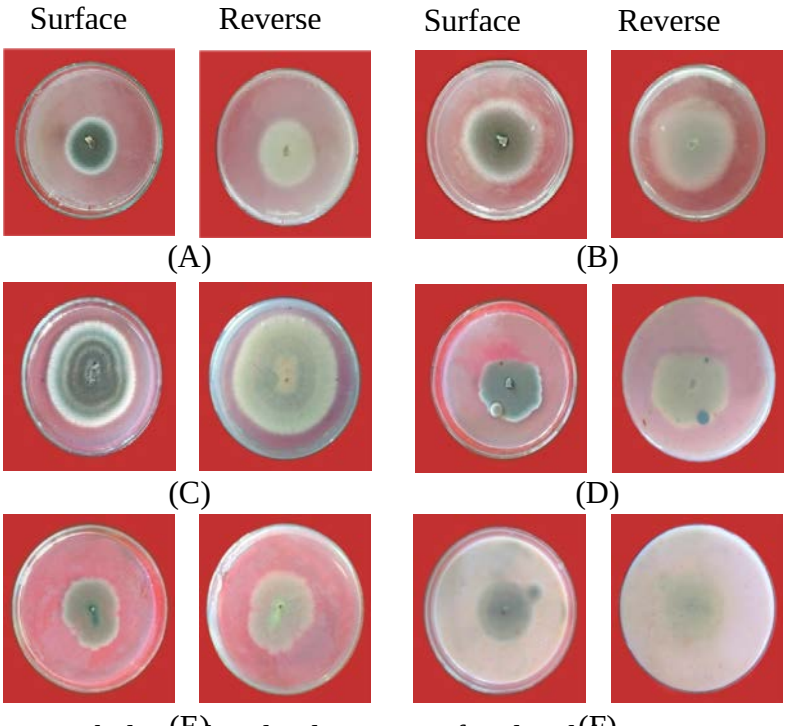

Figure 1. Morphological and colony size of isolated fungus NLF-12on (A) CZA, (B) MEA and (C) PGA media, (D) CYA, (E) YEA and (F) OA media (7 days old culture)

Microscopic examination of selected fungus NLF-12

In the microscopic characters, the isolated fungus NLF-12 was uniseriate with long columnar conidia head. Majority was pyriform to radiate 11-21µm in diameter vesicle. Conidiophores were long 240- 440 (600) × 4.8-12.9 µm and septate. The conidia ranged between 3.5µm globose. The colonies on corn agar were greenish blue mycelia and yellow diffusible pigment. Those macroscopial and microscopical charactes were similar to the investigation on the *Aspergillus novofumigatus* of Domsch *et al.*, 1993, 2007; Samon *et al*., 2004, 2007 and Nyongesa *et al.*, 2015.

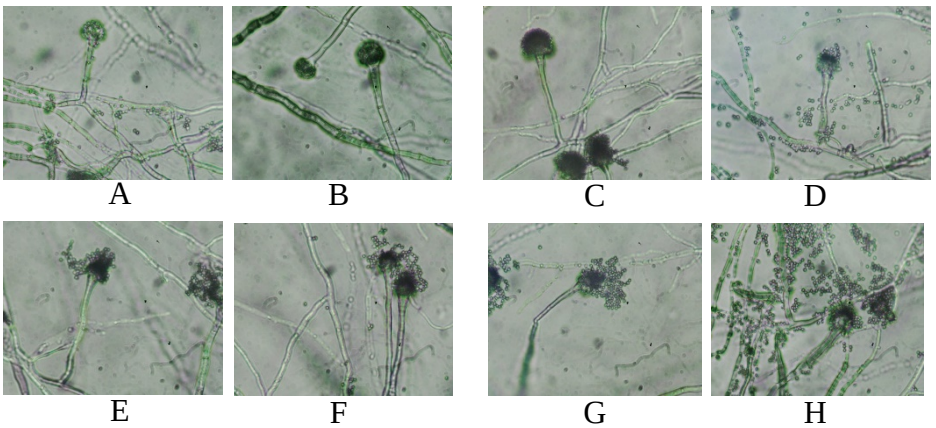

Figure 2. Photomicrograph (200 X) of fungus NLF-12on MEA medium for (A) 3 days, (B) 4 days (C) 5 days (D) 6 days (E) 7 days, (F) 8 days (G) 9 days and (H) 10 days at 25ºC

Table 2. Morphology of NLF-12 on different carbon sources for 7 days at 25ºC

Carbon source	Colony size (cm)	Front View	Reverse view
Lactose	3.2 - 3.4	Whitish grey	Pale white
Sucrose	3.0 - 3.3	Grey	Pale green

Carbon source	Colony size (cm)	Front View	Reverse view
Potato	4.5 - 4.7	Dark brown	Greenish grey
Tapioca	4.4 - 4.6	Grey	Greenish grey
Corn	**3.0 - 3.2**	**Greenish blue**	**Yellow**
Glucose	3.2 – 3.4	Grey	Pale greenish grey

It was found that yellow diffusible pigment on corn agar.

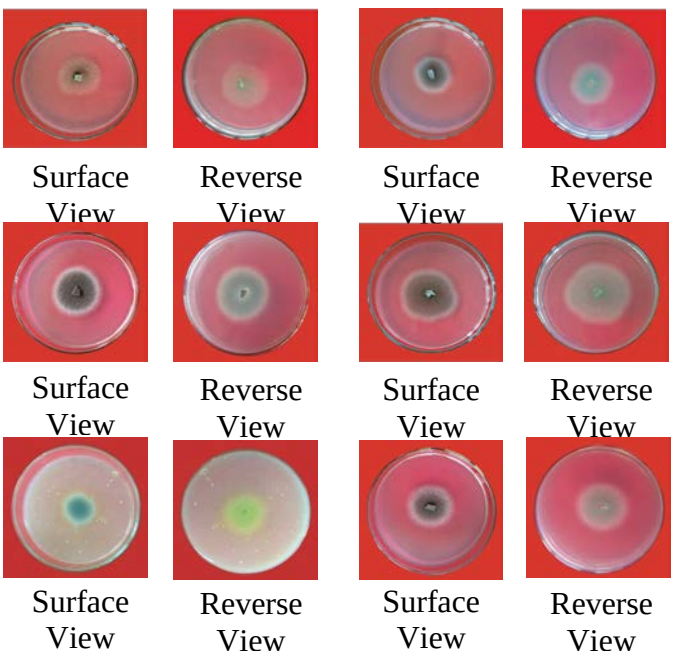

Figure 3. Morphology of NLF-12on different carbon sources for 7 days at 25ºC

Table 3. Morphology of NLF-12 on different nitrogen sources for 7 days at 25ºC

Nitrogen source	Colony size (cm)	Surface View	Reverse view
KNO_3	3.5 - 3.7	Pale	Pale
Malt extract	6.0 - 6.2	Greenish grey	Pale grey
Yeast extract	6.1 - 6.4	Dark green	Pale grey
Meat	2.5 – 2.7	Pale grey	White
Peptone	3.5- 3.7	Greenish blue	White

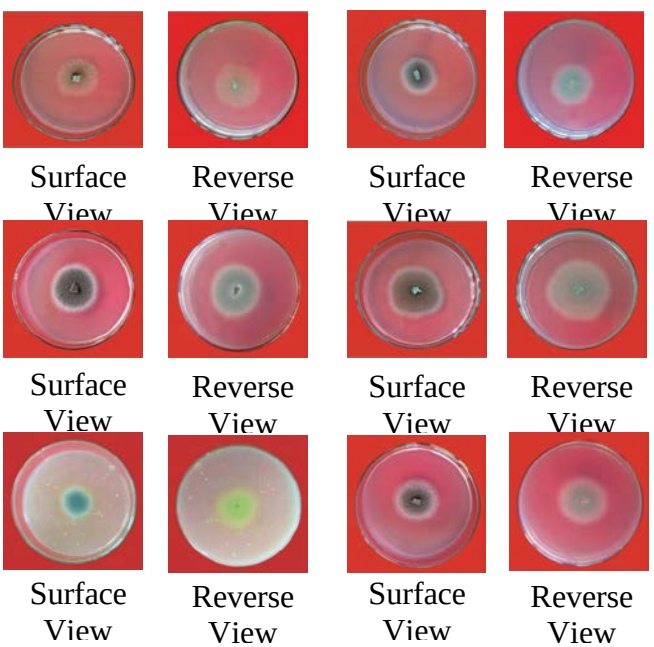

Figure 4. Morphology of NLF-12 on different nitrogen sources for 7 days

Identification key for *Aspergillus*

KEY TO GENUS

(Domsch *et al*, 1993, 2007; Samson, 2007 and Nyongesa *et al*, 2015)

KEY TO GROUP

1. **Conidium laking septum……. Ameroconidium**
2. Conidium with 1 septum ….. ……Didymoconidium
3. Conidium with more than 1 septum and only transverse septa.. ……Phragmoconidium
4. Conidium body subdivided by intersecting septa in more than one plane ….. …….. Dictyoconidium

Identification Key of Fungus NLF-12

(Domsch *et al*, 1993, 2007; Samson, 2007 and Nyongesa *et al*, 2015)

I. Ameroconidium

A. Conidiophore not produced, conidiogenous cells integrate in mycelial cells

B. Conidiophore not produced or not clear

C. Conidiophores with or without septate developed single, not branched

1. Conidia holoblastic

2. Conidia enteroblastic

i. Conidia phialidic

a. Mono- phialidic

b. Multi – phialidic...*Penicillium, Aspergillus,*

Purpureocillium, Paecilomyces, **NLF-12**

According to these key steps, the fungus **NLF-12** may be the genus ***Penicillium, Aspergillus, Purpureocillium* and *Paecilomyces.***

Table 4. Comparison of Microscopical characters of *Penicillium, Aspergillus, Paecilomyces, Purpureocillium* and fungus NLF-12

Genus of Fungi	Distinct Characters
Penicillium *	Phialides have thicker apices
***Aspergillus* ***	**Vesicle present**
Paecilomyces *	Phialides are basically swollen, taper towards their apices are slightly apart from each other
Purpureocillium *	Phialides are basically swollen, taper towards their apices are slightly apart from each other
Fungus NLF-12	**Vesicle Present**

***(Domsch *et al*, 1993, 2007; Samson, 2007 and Nyongesa *et al*, 2015)**

Key to the species

(Domsch *et al*, 1993, 2007; Samson, 2007 and Nyongesa *et al*, 2015)

1. Isolates were predominantly Uniseriate----------2

1. Isolates were predominantly Uni or Biseriate----------10

10.Condia head were clavate----------11

10.Conidia head globose, radiate or columnar----------13

13. The colonies were coffee brown on MEA and cocoa brown on CYA, they formed distinct radial furrows, had globose and ellipsoidal conidial heads with vesicle diameter ranging between 29 -45 µm----------A. japonicus

13. The colonies were in shades of black and green----------14

14. The colonies were black and date brown on MEA and showed they first growth on all the media with the colony diameter rangin between 38 - 60 mm, they had very wide vesicle with diameters of 45 -74 µm and short conidiophores----------15

14. The colonies were yellow green to dark green with abundant conidation on sporulation on all the media ----------16

16. Colonies appeared in shades of yellow green as they formed sporulation rings with white mycelia on MEA colonies on CYA and CZA were yellow with smooth to finely rough conidia (3.5- 5 µm)---------- ---------- A. *flavus*

16. Colony growth was restricted on CZA, they produced sclerotia on CYA and CZA, produced with rough and green conidia--------17

17. They had radiate conidia heads----------18

17. They had columnar conidia heads----------19

19. They produced soluble pigments on CYA, had pyriform to sub clavate to clavate vesicle short conidia heads,thick walled stipe ----------20

19. Lacked soluble pigments on CYA----------21

21. They have varied shapes of the vesicles and the attachment of the phialides on the vesicle----------22

21. Had large conidia 4- 7 µm with varied neck on stipe, some stipe expanded towards the vesicle others retained the same width, large vesicle 21-29 µm phialides loosely attached onto the vesicle ----------A. *novofumigatus* (A)

22. Had small conidia 3 - 5 µm, small vesicle 11- 21 µm, phialides compactly packed and covered nearly half of the vesicle---------- ----------A. *novofumigatus* (B)

Distinguished characters of fungus NLF-12

After incubation for 7 days at 25°C;

On PGA, the colonies 5.0-5.3cm in size, greenish grey with white mycelia. On MEA, the colonies 5.3-5.5 cm in size, grey with white mycelia. On CYA, the colonies 4.0-4.3cm in size, greenish grey with white mycelia. **On Corn agar the colonies 3.0-3.2 cm in size, greenish blue with yellow reverse colours.**

Conidiophore 240 -440 (600) × 4.8 - 12.9 µm and septate. The vesicle were pyriform to radiate,11 - 21 µm in diameter. Conidia heads were long

columnar, uniseriate with some having phialides compactly packed on the vesicle. Conidia ranged between 3- 5 μm, globose.

Table 5. Macro and micro morphological characteristics of representatives of the *Aspergillus* species

Species	Colony sizes (cm) on MEA	Vesicle shape	Vesicle diameter (μm)	Conidia Sizes (mm)
A. parasiticus	1.5-2.0	Pyriform	24-30	4-5.8
A. duricaulis	3.0-6.0	Pyriform	10-26	2-3.5
A. novofumigatus	5.3-5.5	Pyriform	11-21	3-5
A. nidulans	4.5-5.5	Pyriform	9-16	3-4
Fungus NLF-12	**5.3-5.5**	**Pyriform**	**11-21**	**3-5**

According to morphological and microscopical characters and references keys (Domsch *et al*, 1993 & 2007; Samson, *et al*, 2007 & Nyongesa *et al*, 2015, selected fungus NLF-12 was identified as *Aspergillus novofumigatus* S.B. Hong, Frisvad & Samson, 2006.

Kingdom - Fungi
Division - Ascomycota
Class - Eurotiomycetes
Order - Eurotiales
Family - Trichocomaceae
Genus - *Aspergillus*
Species - *A. novofumigatus*

Discussion and Conclusion

In the present study, the identification of selected fungus NLF-12, morphology and microscopical morphological characteristic have been used on six differential media. The genus has been classified in to section based on seriation either uniseriate, biseriate or both, the shape of conidia head; globose, radiate, columnar or clavate. Colony diameter after incubation for 7days, on CZA 3.6-3.8 cm; on MEA 5.3-5.5 cm; on PGA 5.0-5.3 cm; on CYA 4.0-4.3 cm; on YEA 5.7 to 6.0 cm; on OA 4.0-4.3 cm respectively. On CZA, the surface color was dull green and reverse color was white. On MEA, YEA and OA, both surface color were grey and reverse color were white and were pale grey. On PGA and CYA, both surface colors were greenish grey and reverse color were white and pale green respectively. On corn agar, the colonies 3.0-3.2 cm, surface color was greenish blue with yellow reverse color. The conidiophores measured 240-440 (600) × 4.8-12.9 μm and septate. The vesicles were pyriform to radiate, 11-2 μm in diameter. Conidia heads were long columnar, uniseriate with some having phialides, conidia arranged between 3-5 μm, globose.

In the present study, six kinds of different carbon and nitrogen source were used to observe that yellow diffusible pigment on corn agar.

According to the results, the selected fungus NLF-12 was identified as *Aspergillus novofumigatus*. These results were in agreement with Domsch *et al.*, 1993, 2007; Samon *et al*., 2004, 2007 and Nyongesa *et al.*, 2015.

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I profound gratitude goes first to Dr. Khin Maung Oo, Rector of Magway University, for this encouragement and administrative assistance. I would like to record my deep thank to Professor Dr. Aye Aye Kyi, Head of Botany Department, University of Magway and Professor Dr. Thandar, Department of Botany, University of Magway 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 Bago, for her valuable instructions, encouragement and overall supervision for the successful completion of this research paper.

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Taxonomic Characters of some Monocotyledonous Species in Lashio University Campus

Mar Mar Wai*

Abstract

The present paper, were study on taxonomic characters of 10 monocotyledonous species in Lashio University Campus. The recorded species are belonging to family Amaryllidaceae, Agavaceae, Cannaceae and Zingiberaceae. Totally 7 genera belong to 4 family were collected and studied. All the collected specimens have been classified, identified and described their morphological characters with scientific names, synonyms, Myanmar names, English names, family names, flowering period, specimens examined. In addition, color photographs for habits and characters of inflorescences in respective species were reported. An artificial key to the identified species was constructed base on the vegetative and reproductive characters.

Keywords: Taxonomy, angiosperms, monocotyledonous, species, Lashio University.

Introduction

The present paper, species belong to family Amaryllidaceae, Agavaceae, Cannaceae and Zingiberaceae were collected in Lashio University Campus. The study area is located in Lashio, Northern Shan State of Myanmar. It is situated between the North latitude 22°57′42″ and East longitude 97°44′23". The total area is 122,215.172 m square and elevation is 825.83 m. (Department of Geography, Lashio University).

The occurrence of vegetation found in this area is mixing of trees, shrubs, herbs and climbers. In the present paper, 4 families have been described and families have been arranged according to classify for order and family of flowering plants, Angiosperm Phylogeny Group, APG IV (2016).

Taxonomy is a science that includes identification, nomenclature, and classification of objects, and is usually restricted to objects of biological origin; when limited to plants, it is often referred to as systematic botany. Plants taxonomy as a science is treated in its orthodox sense, that is, a

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science based fundamentally on morphology with support of all interrelated sciences. Identification is the determination of a taxon as being identical with or similar to another and already known elements; the determination may or may not be arrived at by the aid of literature or by comparison with plants of known identity. Nomenclature is concerned with the determination of the corrected name of a known plant according to a nomenclatural system. Every species is classified as a member of a particular genus, every genus belong to a particular family (Lawrence 1964).

Therefore, the family, genus and scientific name of individual species have been identified by habit, morphology of rhizome, bulb, stems, leaves, inflorescences and their floral parts. They are generally found as ornamental plants in University Garden and through the Campus, except the *Globba racemosa* Smith, as found as wild plant.

The aims and objectives of this study are to be identify and classify of selected monocotyledonous species in Lashio University Campus, to make a confirm of family names, scientific names, Myanmar names, English names and contribute the morphology characters of respective species to be easily identification. Moreover, to give the knowledge of taxonomic information for botany students and in part useful references for further study.

Materials and Methods

The fresh specimens of study species were collected in Lashio University Campus during June to August in 2019. All the collected species were recorded by photographs to study their habit, leaves arrangements, inflorescences portion, flowers and fruits characters. The specimens were kept within the plastic bags to identify and classify. Data of all species had been investigated with the help of dissecting microscope. The first step of identification was solving the family names of the specimens followed by scientific names according to their morphological characters. The floristic literatures of Lawrence (1964), Hooker (1897), Cronquist (1981) and Dassanayake (1983 and 2003) have been used to classified and identified. Myanmar names and English names (common names) were presented by using references of Hundley and Chit Ko Ko (1961) and Kress *et al*. (2003). The index for nomenclatural data referred is index kewensis by which the names and synonyms of plants up to rank of species being confirmed. The family names of studied species were arranged in according to Plant

Systematics (APG IV). Genera and species names are arranged in alphabetically.

Results

1. *Crinum asiaticum* L. Sp. Pl. 292.1753.

Myanmar name : Koyan Gyi

English name : Lily Poison Bulb, Spider Lily

Family : Amaryllidaceae

The morphological characters of this species are:

Perennial aromatic herbs, about 1.0 m high; bulbs spherical, about 3.0 by 3.0 cm, red, glabrous. Leaves simple, distichous, exstipulate, sessile; blades narrowly lanceolate, parallel venation, channeled along the middle of the upper surface, attenuate at the base, undulate along the margin, acuminate at the apex, coriaceous, smooth, bright green, glabrous. Inflorescences terminal umbellate cymes, flowers many (about 10-13); peduncles very long, purplish green, glabrous. Flowers bisexual, actinomorphic, epigynous, trimerous, bracteate, pedicellate, bracteolate, salver-form, white, fragrant, showy; perianth lobed-6, linear lanceolate, revolute and recurved, obtuse at the apex, white, glabrous; perianth tubes straight, cylindrical, pale green, lighter distally, glabrous. Stamens 6, inserted, equal in length; filaments filiform, adnate to the throat of the perianth tube; spreading, deflexed, purple in the distal half, glabrous; anthers dithecous, introse, curved, midfixed, longitudinal dehiscence, yellow, linear, turning purple, glabrous. Ovary inferior, syncarpous, trilocular with two ovules in each locule on the axile placentae, oblong, green, glabrous; style terminal, simple, filiform, decline, white, purple in the distal half, glabrous; stigma trifid, decline, white, purple in the distal half, glabrous. Fruits and seeds unavailable.

Flowering period : June to July

Specimen examined : Found in the Lashio University Garden.

2. *Zephyranthes carinata* Herb. App. (to Bot. Reg .7)36. 1821.

Z. grandiflora Lindl., Bot. Reg .t .902. 1825.

Myanmar name : Hnin Pan

English name : Rosepink Rain Lily, Rosepink Magic Lily,

Family : Amaryllidaceae

The morphological characters of this species are:

Perennial herbs about 0.5 m high; bulb tunicated, globular, 1.5 by 1.0cm, wine-red, glabrous. Leaves simple, distichous, exstipulate, sessile; blades narrowly linear, parallel venation, attenuate at the base, entire along the margin, actue at the apex, dark green, glabrous. Inflorescences terminal, solitary, pedunculate cymes, peduncles very long, dark green, whitish at the base, glabrous. Flowers bisexual, actinomorphic, epigynous, trimerous, bracteate, pedicellate, bracteolate, funnel-form, rose red, showy; perianth lobed-6, oblong lanceolate, rose red, glabrous; perienth tubes cylindrical, straight, light green, glabrous. Stamens-6, inserted, equal in length; filaments filiform, adnate to the mouth of perianth tube, white, glabrous; anthers dithecous, introse, versatile, longitudinal dehiscence, oblong, yellow, glabrous. Overy inferior, syncarpous, trilocular with many ovules in each locule on the axile placentae, oblong, green, glabrous; style terminal, simple, stout, erect, white, greenish in the distal half; stigma trifid, recurved, white, glabrous. Capsules subglobose, 3- lobed, brown, glabrous. Seeds compress, black, glabrous.

Flowering period : June to August

Specimen examined : Found on the west side of L.H 10 Building, Lashio University.

3. *Yucca aloifolia* L. SP. PL. 319. 1753.

Local name : Thagya Hlan Thwa

English name : Aloe Yucca, Spanish Bayonet

Family : Agavaceae

The morphological characters of this species are:

Perennial evergreen, large, semi-woody, shrubs; stems short, thick, unbrached, about 1.6 m high. Leaves simple, alternate, stipulate, rosette, crowded, sessile; blades lanceolate, parallel venation, fleshy, large, fibrous, attenuate at the base, entire along the margin, spine-tipped at the apex, dark green, glabrous. Inflorescences large, terminal paniculate racemes;

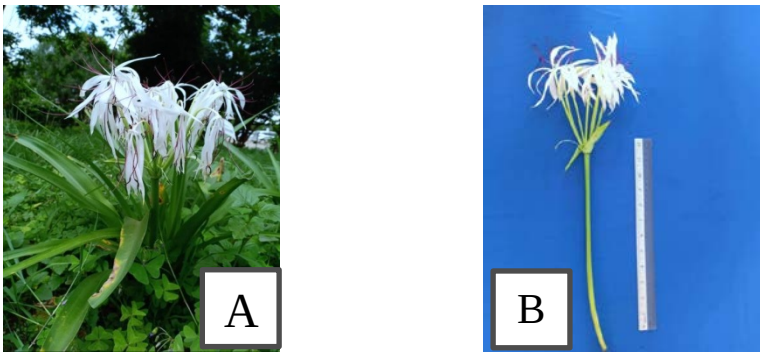

Figure 1. Morphological characters of *Crinums asiaticum* L.
A. Habit B. Flowering branch

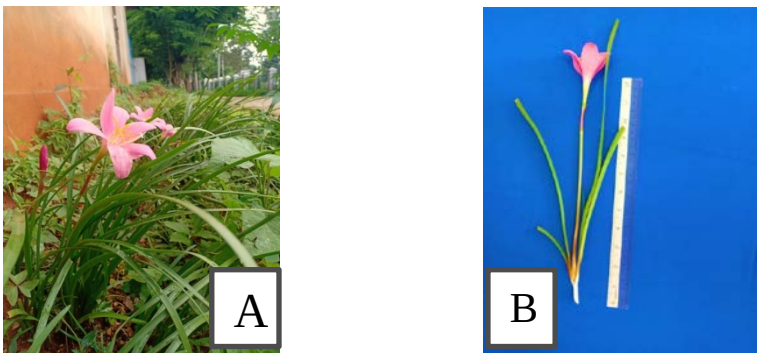

Figure 2. Morphological character of *Zephyranthes carinata* Herb.
A. Habit B. Flowering branch

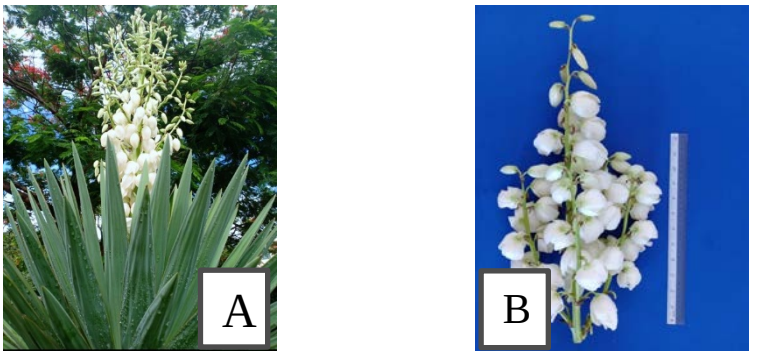

Figure 3. Morphological characters of *Yucca aloifolia* L.
A. Habit B. Flowering branch

peduncles very long, with many flowers, stout, green, glabrous. Flowers bisexual, zygomorphic, epigynous, trimerous, bracteate, pedicellate, bracteolate, egg-shaped, creamy white, showy. Perianth lobed-6, ovate oblong, creamy white, glabrous. Stamens 6, inserted, equal in length; filaments free, flattened, reflexed, white, glabrous; anthers dithecous, extrose, basifixed, longitudinal dehiscence, oblong, yellowish brown, glabrous. Ovary inferior, syncarpous, trilocular with two ovule in each locule on the axile placentae, oblong, green, glabrous; style terminal, simple, stout, erect, white, glabrous; stigma 3-lobed, bifid at the tips, white, glabrous. Fruits and seeds unavailable.

Flowering period : June to August

Specimen examined : Found in the Lashio University Garden.

4. *Canna indica* L. Sp. Pl. 1. 1753.

Myanmar name : Budatharana

English name : Canna, Red Canna Lily

Family : Cannaceae

The morphological characters of this species are:

Perennial aromatic rhizomatous herbs, large, about 2.0 m hight; rhizomes cylindrical, fleshy with tuberous roots, about 5.0 by 5.0 cm, brown. Leaves simple, distinchous, exstipulate, petiolate, large, cauline; blades oblong, midrib distinct, pinnately parallel venation, attenuate at the base, slightly undulate along the margin, acuminate at the apex, dark green,

shiny, glabrescent. Inflorescences terminal, pedunculate racemes, peduncles very long with many flower, dark red, glabrescent. Flowers bisexual, zygomorphic, epigynous, trimerous, bracteate, pedicellate, bracteolate, infundibuliform, large, red, showy. Outer tepals 3, persistent, lanceolate, red, glabrescent; inner tepals 3, red, oblanceolate, striation, unequal in size, red glabrous. Stamen 6, inserted, unequal in length, fertile stamen 1+ sterile stamens 2+2, filaments flatten, free, wing, yellowish brown, glabrous; anthers dithecous, introse, dorsifixed, longitudinal dehiscence, oblong creamy white, glabrous, labellum lanceolate, red, glabrous. Ovary inferior, syncarpous, trilocular with many ovules in each locule on the axile placentae, ellipsoid, red, tuberculate. Style terminal, simple, flat, erect, showy, dark red, glabrescent; stigma 3-lobed, red, glabrescent. Capsules, subglobose, hard, red, bristles; seeds spherical, small, black, stiff hairy.

Flowering period : June to August

Specimen examined : Found in the Sabal Saung (3), Lashio University.

5. *Globba racemosa* Smith, Exot. Bot. 2: 115. 1808.

Myanmar name : Pa Dein Ngo

English name : Dancing Girl

Family : Zingiberaceae

The morphological characters of this species are:

Perennial aromatic rhizomatous herbs, about 0.8 m high; rhizomes cylindrical, fleshy with tuberous roots, about 4.0 cm by 1.0 cm, brown, glabrous. Leaves simple, alternate, exstipulate, petiolate; blades lanceolate; parallel with pinnate lateral veins venation, mid-rib distinct, attenuate at the base, entire along the margin, caudate at the apex, dark green above, pale green beneath, glabrous. Inflorescences terminal pedunculate racemes, decurved, many flowers. Flowers bisexual, zygomorphic, epigynous, trimerous, bracteate, pedicellate, ebracteolate, funnel-shaped, orange; pedicels slender, long, green, glabrous; bracts persistent, oblanceolate, green, glabrous. Calyx lobed-3, campanulate, orange, glabrous; lobes lanceolate, orange, glabrous. Corolla lobed-3, orange, glabrous; lobes ovate, orange, glabrous; corolla tube long, orange, glabrous. Stamens 6, exerted, unequal in length, 1fertile + 4 sterile and 1 rudiment, fertile stamen at

medium, subtanted by 4 appendages; orange, glabrous; sterile, labellum showy, orange with red spots on the middle, glabrous; staminal tubes very long, cylindrical, orange, glabrous; filaments very short, free, yellow, glabrous; anthers dithecous, parallel, dorsifixed, longitudinal dehiscence, oblong, orange, glabrous. Ovary inferior, syncarpous, papillae, unilocular with one ovule in each locule on the parietal placentae, ovoid, green, glabrous. Style terminal, filiform, light green, glabrous; stigma simple, capitate, green, glabrous. Capsules glabrous. small, orbicular, appendages on each side, brown, glabrous. Seeds one, aril, black,

Flowering period	:	June to Augus.
Specimen examined	:	Found behind the 12 Hostel Building, Lashio University

6. *Kaempferia pulchra* L. Sp. PL. 1:2:1753

Myanmar name	:	Unknown
English name	:	Peacock Ginger, Silverspot
Family	:	Zingiberaceae

The morphological characters of this species are:

Perennial aromatic, rhizomatous herbs, about 0.13 m high; rhizomes cylindrical, fleshy with tuberous roots, about 5.5 by 1.5 cm, brown, glabrous. Leaves simple, distichous, exstipulate, petiolate; petioles long, pale purple, glabrous; blades obovate, rounded at the base, entire along the margin, attenuate at the apex, dark green and motted above, a series of black eyelash marks above, gray beneath, glabrous. Inflorescences terminal solitary cymes, contemporaneous with the rhizome. Flowers bisexual, zygomorphic, epigynous, trimerous, bracteates, pedicelate, bracteolate, infundibuliform, fragrant, purple, showy. Calyx lobed-3, oblanceolate, adnate to the corolla lobed, white, glabrous; calyx tubes cylindrical, white, glabrous. Corolla lobed-3, orbicular, rotate, purple, glabrous; corolla tubes short, glabrous; Stamens 12, inserted, unequal in length, fertile stamens 3, sterile stamens 7, labellum purple, glabrous; filaments filiform, free, white, sparsely hairy; anthers lanceolate, dithecious, erect, basifixed, longitudinal dehiscence, purple, sparsely white hairy. Ovary inferior, syncarpous trilocular with two ovules in each locule on the axile placentae, oblongoid,

white, glabrous; style terminal, simple, short, white, glabrous; stigma simple, white, glabrous. Fruits and seeds unavailable.

Flowering period : June to August

Specimen examined : Found in the Botanical garden, Lashio University.

Discussion and Conclusion

Cronquist (1981) stated that the Division Angiosperms consist of two classes, dicotyledonous and monocotyledonous. In this study, all of the species were studied from the class monocotyledonous in Angiosperms. Among the present species, except *Globba racemosa* Smith, is wild and rest of the species were found as cultivated for ornamental purpose.

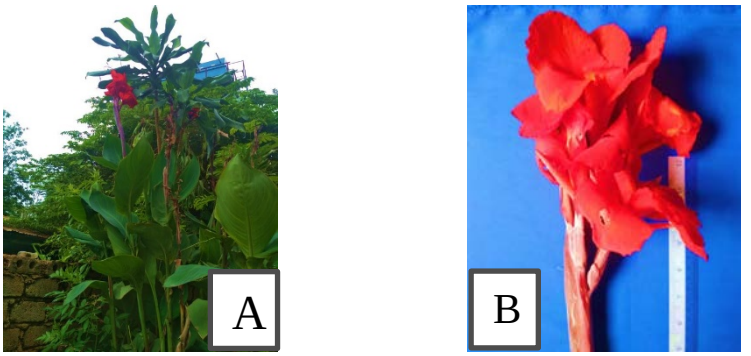

Figure 4. Morphological characters of *Canna indica* L.

A. Habit B. Flowering branch

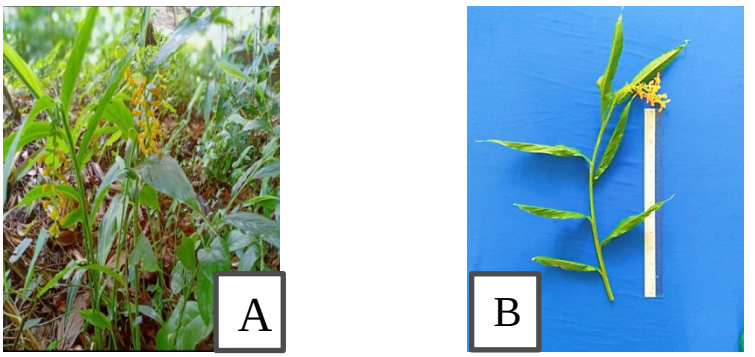

Figure 5. Morphological characters of *Globba racemosa* Smith

A. Habit B. Flowering branch

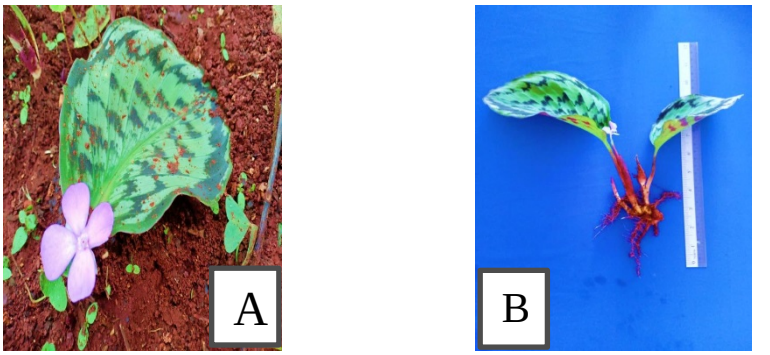

Figure 6. Morphological characters of *Kaempferia pulchra* L.

A. Habit B. Flowering branch

In the present paper, 6 species had been reported. They are 2 species belong to 2 genera of family Amaryllidaceae, 1 species of family Agavaceae, 1 species of family Cannaceae and 2 species belong to 2 genera of family Zingiberaceae.

The *Crinum asiaticum* L. and *Zephyranthes carinata*, Herb. are members of family Amaryllidaceae. *Crinum asiaticum* L. is distinguished from other species by its terminal umbellate cymose inflorescences and very long peduncles with many fragrant flowers. White color petals with purple in the half length and distal. Equal in length stamens are spreading and decline. Simple filiform style and trifid stigma are decline, *Crinum asiaticum* L. plant or plant part is used for gastrointestinal disorders, skin diseases, fever, earache, tonsillitis, mumps and antidote to poison.

The diagnostic features of *Zephyranthes carinata* Herb., is perennial truncate bulbous herbs, simple sessile, exstipulate and distichous Leaves. Terminal and solitary pedunculate cymose inflorences. Equal in length stamens are adnate to the mouth (throat) of the perianth tube. Many ovules contain in each locule. Capsules contain oblong, compress, black seeds. The membranes of *Zephyranthes* plants contain various toxic alkaloids including lycorine and haemanthamine. They can cause vomiting, convulsions and death to humans, livestock and poultry.

Yucca alifolia L. is one of the species of family Agavaceae. The distinct characters of *Yucca aloifolia* L. are perennial ever green, large, semi- woody shrubs. Short thick unbranched stems with simple alternate stipulate, rosette and crowed sessile leaves. Lanceolate flesh and large blades with fibrous, entire margins and spine- tipped at the apex. Terminal

paniculate large racemes with egg- shaped creamy white, showy flowers. The leaves of *Yucca aloifolia* L. contain spinous and possess anti-inflammatory activity. A hard fiber obtained from the leaves is used for making ropes, baskets and mats.

Canna india L. is one of the members of family Cannaceae. The distinct features of *Canna india* L. are perennial rhizomatous herbs, simple distichous exstipulate leaves. Terminal pedunculate racemes inflorescences with large and red zygomorphic flowers. Fertile stamens 1 and flattened with wing- like filaments. Hard and red color capsules with bristles hairs. The leaves of *Canna india* L. are suitable for wrapping and as plates. Both the leaves and the rhizomes can be used as cattle feed. *Canna* is also well known as a garden ornamental because of its beautiful flowers and foliage of various colors.

Among the present species, 2 species of family Zingiberaceae would be described. They are *Globba racemosea* Smith, and *Kaempferia pulchara* L. They are distinguished from the other species by perennial aromatic rhizomatous herbs. Rhizomes are cylindrical in shaped and fleshy with tuberous roots. Zygomorphic flowers with sterile stamens and showy labellums are present. *Globba racemosa* Smith, (Yunna China) is used as traditional medicine to cancer cure. In Myanmar, Isopimarane diterpenoids compounds were collected from rhizomes of *Kaempferia pulchra* L. to their Vpr inhibitory activity (Viral protein, an accessory gene of HIV).

As a result, point out *Yucca aloifolia* L. belong to family Agavaceae is perennial shrubs and unbranched stem with crowded leaves. Rest of the members of the family Amaryllidaceae, Cannaceae and Zingiberaceae are perennial herbs and distinchous leaves. All of the stated species of flowers are epigynous, trimerous, bracteates, bracteolate. Anthers are dithecous and longitudinal dehiscence. Ovary is inferior, syncarpous and trilocular. Placentation is axile and one to many ovules each locule. Moreover, medicinal and other uses of respective species had been reported by literatures.

In this paper, according to the collected specimens, 6 species belong to 6 genera from 4 families could be identified. This is hoped that, gives the knowledge of the morphological characters of presented species and to provide the basic of taxonomic information for other subjects of further scientific research and botany students.

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Isolation of Soil Fungi and their Antifungal Activity Against Plant Pathogenic Fungi

Thet Thet Khaing[1], Moe Moe Aye[2] & Nyunt Phay[3]

Abstract

In the course of the isolation of fungi, fungi were isolated from the soil samples collected at Yenanchaung Township, Magway Region by the method of soil dilution method. In the investigation of the isolation of pathogenic fungi were isolated from the diseased leaf of *Arachis hypogaeo* L. (Myay pe) by direct inoculation method. In the screening of antifungal metabolite possessing fungi, soil fungi were exhibited highly antifungal activity against plant pathogenic fungi. Plant pathogenic fungi were identified as *Aspergillus niger*.

Keywords; Soil fungi, Pathogenic fungi, Antifungal activity and Identification

Introduction

Soil is the upper layer of most of the earth's surface and varies in depth from inches to over twenty feet. It s a production of weather rock but quite distinct in its characteristic. Soils are excellent cultural media for the growth of many types of organisms (Angelov, 2008). Microorganisms have significant functions in ecosystem and are found in all kinds of habitats, there will be an increasing demand for microorganisms with unusual properties (Kurtzman, 1992 and Subramanian, 1982).

Plants are constantly exposed and threatened by a variety of pathogenic microorganisms present in their environment. Diseases caused by plant pathogenic fungi significantly contribute to the overall loss in crop yield worldwide (Savary, *et al*., 2006; Montersinos, 2007). Plant pathogenic fungi differentiate morphologically distinct hyphal structure at various times during infection pathogenic fungi are major plant pathogens, which cause significant losses in economically important crops (Shepherd, *et al.*, 2003). Pathogenic fungi are the main infectious agent in plants, causing alternations during developmental stages including post harvest. In fruit and vegetables, there is a wide variety of fungal genera causing quality

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problems related to aspect, nutritional value, organoleptic characteristics and limited shelf life (Agrios, 2004). Antifungal substances which are obtained from plant have no side effect against environment thus giving significant advantage. Due to the present antifungal substances in this plant, it can use as biospesticide.

Most of identification systems for microorganism which are based on morphological, physiological and molecular data have been developed to a species level identification (Boehout, 2002). Identification of microorganisms that produce bioactive compounds was of great interest in the development of new molecules to fight against many pathogens. The fungal kingdom includes many species with unique and unusual biochemical pathways (Keller, *et al.*, 2005).

Materials and Methods

Isolation of Fungi from soil samples

Soil samples were collected from different places in Yenanchaung Township. Soil samples were carried out from 11 to 13, May, 2020. All samples were collected at depth of 2-6 inches. Soil sample were transferred directly to sterile plastic bags. These were transported to the laboratory and processed immediately for the isolation and cultivation of fungi.

Isolation of soil fungi was undertaken by Soil Dilution Method (NITE, 2013) using Low Carbon Agar (LCA) medium and Patato Glucose Agar (PGA) medium.

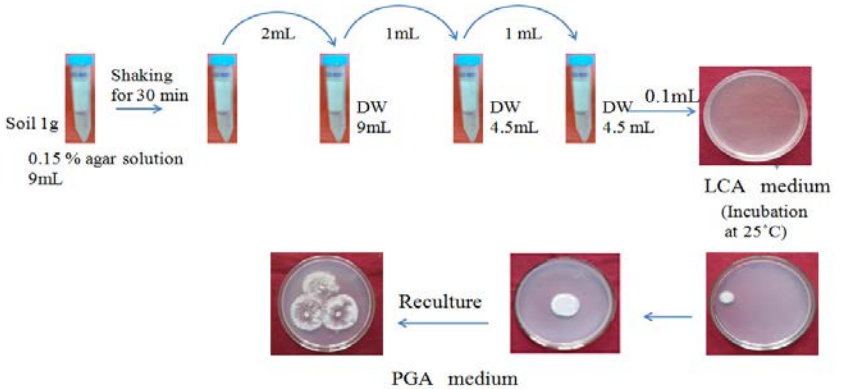


Figure 1. Soil dilution method

Medium Used for Isolation of Fungi

LCA medium (Low Carbon Agar medium) Ando, 2004

Component per liter	
Glucose	2.0 g
Sucrose	2.0 g
K_2HPO_4	1.0 g
$MgSO_47H_2O$	0.5 g
KNO_3	1 g
KCL	0.5 g
Agar	18 g
pH	6.5 ±

(After autoclaving chloramphenicol was added to the medium.)

Potato Glucose Agar (PGA) medium

Component per liter	
PGA	19 g
pH	6.5 ±

(After autoclaving chloramphenicol was added to the medium.)

Isolation of Plant Pathogenic fungi

Pathogenic fungi were isolated from the diseased leaf of *Arachis hypogaea* L. (Myay pe).

Figure 3. Habit of *Arachis hypogaea* L. (Myay-pe)

Scientific Name	-	*Arachis hypogaea* L.
Family	-	Fabaceae
Myanmar Name	-	Myay-pe
English Name	-	Groundnut

Annual prostrate or erect; stem subterete. Leaves simple 4-foliolate, paripinnately compound alternate, stipulate; leaftets 4. Inflorescence axillary short raceme with 2-7 flowers. Flowers bisexual, zygomorphic, hypogynous, yellow with striped with red. Calyx campanulate with 5 lobes. Corolla papilionaceous; standard suborbicular; wings free; keels keel beaked. Stamens 10, moadelphous, anther dithecous basifixed. Carpels monocarpellary; ovary unilocular, 1-7 ovuled, marginal placentation; style long, stigma minute. Pods idehiscent, oblongoid, slightly constricted. Seeds 1-3, cotyledons rich in oil, red.

Medium Used for the Isolation of Pathogenic Fungi
Isolation medium
Component per liter

Glucose	2 g
K_2HPO_4	1 g
$MgSO_4 7H_2O$	0.5g
Yeast extract	1 g
KCL	0.5 g
Agar	18 g
pH	6.5 ±

(After autoclaving chloramphenicol was added to the medium.)

PGA Medium
(Component per Liter)

PGA	39 g
pH	6.5 ±

(After autoclaving chloramphenicol was added to the medium.)

Screening of Antifungal Metabolite Producing Soil Fungi

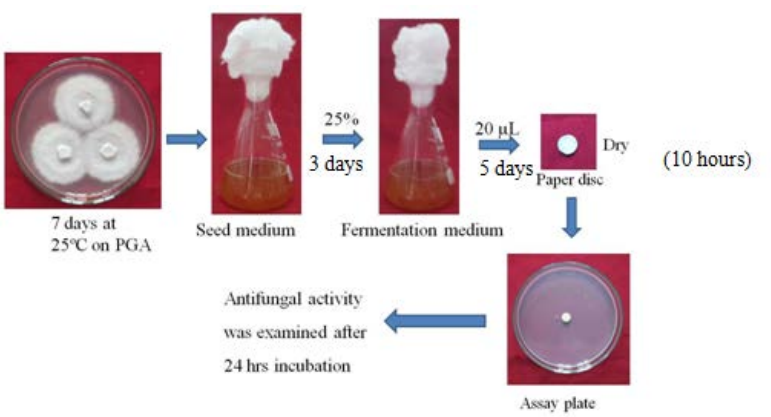


Figure 2. Study on the antifungal activities NITE (2004)

Seed Medium

Component per liter	
Glucose	15 g
Yeast extract	7 g
Polypeptone	5 g
K_2HPO_4	0.01g
$MgSO_4.7H_2O$	0.01g
pH	6.5 ±

Fermentation Medium

Component per liter	
Glucose	15 g
Glycerol	1 g
Yeast Extrast	15 g
Polypeptone	06 g
$MgSO_4.7H_2O$	0.01 g
K_2HPO_4	0.01 g
$CaCO_3$	1 g
pH	6.5 ±

Assay Medium

Component per liter	
Glucose	1 g
Yeast extract	3 g
pH	6.5±

Identification Plant Pathogenic Fungi

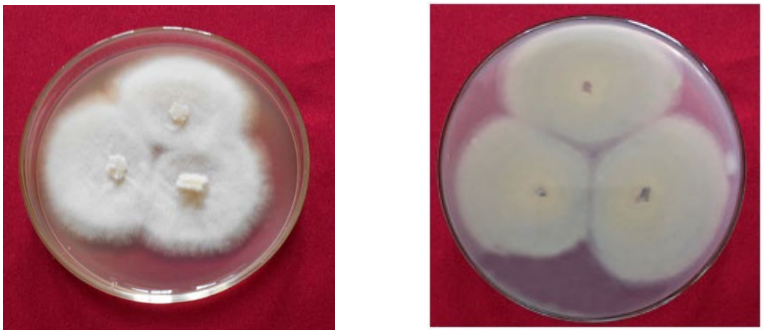
Pathogenic fungi was carried out by observing both macroscopic and microscopic features of fungal colonies. For microscopic screening, slides were prepared. Mycelia from each isolate were taken from PGA plate and spread onto a clean slide mount with a drop of water, covered with cover slip and then observed under a light microscope using 400 X magnification. The branching patterns of conidiophores and the shapes and sizes of conidia were examined. The macroscopic and microscopic features were compared to the characteristics described by Ando and Inaba, 2004.

The macroscopical and microscopical were observed by the methods of Barnett, 1956. Domsch, 1993, Pendro, *et al.*, 2009, Abarca, *et al.*, 2004, Taxonomy and significance of black Aspergilla. The morphological and microscopical characters were observed by the methods of Ando and Inaba, 2004. For the study of macroscopical (Morphological) characters, PGA, CzA and MEA agar medium were employed. Each plate was inoculated at three points, equidistant from the centre and incubated for 7 days at 25°C. Microscopical characters were studied by microscope. Comparison of these characters with reference keys was undertaken to identify.

Results

Isolation of Fungi from Soil Samples

In the isolation of soil fungi, soil sample were collected from of Yenanchaung Township. Soil Fungi were isolated from soil sample. Fungi were collected by Soil Dilution Method.



Front view Reverse view

Figure 4. Morphology of soil fungi (7 days old culture on PGA medium)

Isolation of Plant Pathogenic Fungi

In this study, plant pathogens were isolated from diseases plants leaf of *Arachis hypogaea* L. (Myay pe)

Figure 5. Habit of *Arachis hypogaea* L. (Myay-pe)

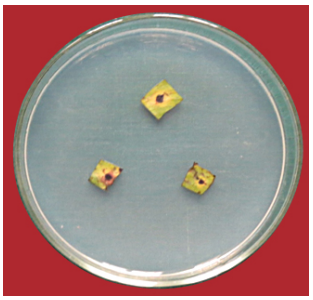


Figure 6. Pathogenic fungi isolated from *Arachis hypogaea* L.

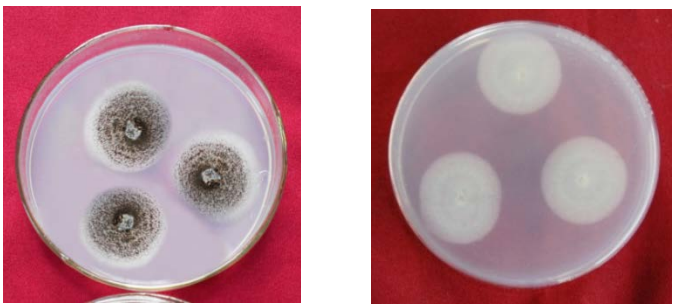
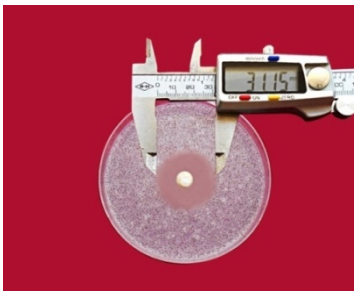


Figure 7. Morphology of pathogenic fungi isolated from *Arachis hypogaea* L.

Preliminary Study for Antifungal Activities by Paper Disc Diffusion Assay

In this investigation, soil fungi showed the antifungal activities against pathogenic fungi.



FM-3

Figure 8. Antifungal activity of isolated soil fungi against pathogenic fungi (*Arachis hypogaea* L.) (Myay pe)

Identification of Plant Pathogenic fungi

Figure 9. Morphology and photomicrograph of fungi (7 days old culture on PGA medium)

Morphological Characters of Pathogenic Fungi

Colonies are usually fast growing, white, yellow, yellow brown, brown to black or shades of green, mostly consisting of a dense flat of erect conidiophores.

Microscopical Characters of Pathogenic Fungi

Conidiophores are short, smooth walled and have conical shaped terminal vesicles, which support a single row of phialides on the upper two thirds of the vesicle. Conidial heads are typically columnar up to 400×50 µm but often much shorter and smaller and uniseriate. Hyphae are septate; an unbranched conidiophores arise from a specialized foot cell. The conidiophore is enlarged at the tip, forming a swollen vesicle are completely or partially covered with flask shaped phialides which may develop directly on the vesicle (uniseriate form) or be supported by a cell known as a metula (biseriate form). The phialides produce chains of mostly round, sometimes rough, conidia. Vesicle are spherical, conidia arise circumferentially from vesicle uniseriate conidia subglobose, echinate.

Identification of Plant Pathogenic Fungi

Identification Key for *Aspergillus* (Nyongesa, *et al.*, 2015, Waternabe, 2002)

1. Synnemata not form ……2

2. Conidium axis not curved through more than 180……3

3. Conidium only one axis; any protuberances, other than setulate, if present, not more than ¼ the length of the spore bod……..…..4

4. Length/width ratio of conidium body less than 15:1 ….…….5

5. Conidium lacking septa……Ameroconidium

Ameroconidium

A. Conidiophore not produced, condiogenous cells integrate in mycelial cells

B. Conidiophores not produced or not clear

C. Conidiophore with or without septa develop single, not branch

1. Conidia holoblastic

2. Conidia enteroblastic

i. Phialoconidium

a. Single philida

b. Multi-phialide with parallel arrangement ….. *Aspergillus*

KEY STEP SPECIES for *Aspergillus niger* (Nyongesa, *et al.*, 2015, Waternabe, 2002)

1. Isolates were predominantly Biseriate ……..2

2. Colonies appeared in shades of black to brown…… 3

3. Colonies with smooth edges on MEA with large vesicle between 32 - 60 µm……..4

4. Diameter of the colonies on CYA 25˚C was between 45 - 55 mm and conidia range of 3 - 6 µm……5

5. Colonies formed radial furrows on PGA, had small white edge of the colony on MEA and a large black area of conidiation……*Aspergillus niger*

In the literature references, the characters of pathogenic fungi was identified was *Aspergillus niger* Van Tieghem 1867.

Discussion and Conclusion

Present study carried out for an effort to understand the soil fungi diversity. The soil moisture has a direct effect on the population of fungi positively. Hence, at higher moisture the tolerance and colonization is badly affected (Adams, 1999). Several reviews exist of recent, successful techniques for isolating fungi from nature. (Labeda, 1992) includes basic techniques for isolation specific habitat. In the isolation of soil fungi were isolated from the soil samples collected at Yenanchaung Region soil dilution method using LCA medium.

In the course of the isolation of pathogenic fungi was isolated from diseased plants of *Arachis hypogaea* L. (Myay pe) by direct inoculation method. In this investigation, soil fungi showed the antifungal activities against plant pathogenic fungi.

Plant pathogens cause great losses to agricultural crops and thus threaten food resources all over the world (Baniasadi, *et a.l*, 2009). Due to the plant pathogens such as fungi, bacteria and virus, the yields of crops are lost every year and result in serious economic losses to crops (Pitt and Hocking, 1997). Peanut (*Arachis hypogaea* L.) is an important food and oil seed crop worldwide. Diseases pose a major threat to the production of peanuts each year and prevention of disease in peanut is a major concern for producers (Porter, 1997). Fungi can be rendered as the most harmful microorganism and so far, 46 fungal diseases were recorded on groundnut and 67 fungi were associated with various symptoms type (Wikipedia 2012).

In our country, plant pathogens such as fungi, bacteria and virus are challenges to agricultural farmers. So it is necessary to control the plant pathogens, especially in crops in our country that this research was undertaken.

In the identification of pathogenic fungi, on the basis of literature reviews, reference keys and morphological and microscopical characters, pathogenic fungus was determined to be identified as *Aspergillus niger* Van Tirghten 1867.

Acknowledgements

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Morphological Study and Germination of *Vigna radiata* (L.) Wilczek (Pedi-Shwe-Wa)

Su Hlaing Winn [*]

Abstract

The morphological and germination of *Vigna radiata* (L.) Wilczek (mungbean) was conducted in the garden of Botany Department, University of Yangon in 2015. In this work, the verification of *Vigna radiata* (L.) Wilczek was carried out using standard literatures. The germination percentage of *Vigna radiata* (L.) Wilczek was also tested and the result showed that the germination rate of mungbean was 96.8%.

Keywords: *Vigna radiata*, standard literatures, germination percentage

Introduction

Vigna radiata (L.) Wilczek (mungbean) is one of the members of family Fabaceae. It is synonymous with *Phaseolus aureus* Roxb. The more common vernacular names are mungbean, green gram, golden gram (Aykroyd and Doughty, 1964).

Mungbean is an annual legume (Skerman *et al*., 1988). It is an annual, semi-erect to erect, deep-rooted herb (Gentry, 2010). It is an annual herb, erect, twining, or creeping (Purseglove, 1969). Stems are slightly hairy. Junctions of branches and stems are stipuled (Skerman *et al*., 1988). The stems branch from the base are covered with short fine brownish hairs (Gentry, 2010). Stems have hispid with brown spreading hairs (Purseglove, 1969). The leaves are alternate and trifoliate; leaflets are medium to dark green, broadly ovate, sometime lobed, rounded at the base and pointed at the apex (Gentry, 2010). Stipules peltate, ovate; petiole present; leaflets ovate, sometimes 3-veined from base, base broadly cuneate or rounded, apex acuminate or acute (Purseglove, 1969). The first flowers appear seven to eight weeks after planting and the crop reaches maturity in 12 to 14 weeks (Skerman *et al*., 1988). Racemes axillary, four to several flowered. Calyx tube, glabrous; lobes narrowly deltoid. Standard yellowish green outside, sometimes pink inside, suboblate; wings yellow, ovate; keel falcate and incurved through 180° (Purseglove, 1969). Pods clothed in long,

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spreading, deciuous silky hairs (Skerman *et al*., 1988). Seeds 8-14, greenish or yellow-brown, shortly cylindric; hilum white. The seeds are edible and are used medicinally (Purseglove, 1969).

The optimum temperature for germination should be 25-28°C. Germination will take place within 3 to 7 days. Seeds soak in water before sowing for better germination. Improper depth of seeding leads to poor germination (FAO, 2004). The base soil temperature for emergence is 10.5°C, and plant growth is maximised at ambient temperatures of 28-30°C. Optimum seed germination and plant growth occur at temperatures of 28-33°C. Seed appearance and quality are of paramount importance (Website, 1).

Seeds are broadcast or dibbled in the hills or in rows. Mungbean is grown mainly on smallholdings, often as mixed crops or inter-crop. Associated crops are usually of longer duration than the mungbean (sugar-cane, cotton, sorghum) but sometimes of shorter duration (Madura maize, Indonesia). To make use of short cropping period, short-duration mungbean is often relay-cropped (Maesen and Somaatmadja, 1992). Increasing food production, the planting of high-quality seed is as vital to increasing yields as irrigation, fertilizers, pest control, and improved cultural conditions (Noggle and Fritz, 1983).

It is a very important source of human food. Seeds may sprout in the pod under very humid conditions (Maesen and Somaatmadja, 1992). Green pods are eaten as a vegetable. It is an important pulse crop providing vegetable protein for people throughout Asia. Mungbean is widely grown in monoculture in dry and semi-dry regions, as well as being used as an intercrop throughout much of the country because of its drought tolerance and nitrogen-fixing soil fertilization (Duke, 1981).

The study aimed to confirm the morphological characters of mungbean and to record the germination percentage of mungbean.

Materials and Methods

Time and Place of the Study

The experiment was conducted in the garden of Botany Department, University of Yangon in 2015.

Planting Materials

The planting material, mungbean seeds of cultivar Yezin-1 (Pedi-Shwe-Wa), was collected from Vegetable and Fruit Research Development Center (VFRDC), Yemon, Hlegu Township, Yangon Region.

Morphology of *Vigna radiata* (L.) Wilczek

The confirmation of the morphological characters of *Vigna radiata* (L.) Wilczek was carried out using the references of Purseglove (1969), Hooker (1879), Duke (1981), Summerfield and Roberts (1985) and Dassanayake (1991).

Germination of *Vigna radiata* (L.) Wilczek

The seed germination medium was the mixture of soil, burnt rice husks and sand. One part of soil, one part of burnt rice husks and one part of sand were thoroughly mixed, then wet by the water and then it was filled in the trays containing small pots. In germination, full cheek and almost the same size of mungbean seeds were selected, then soaked in water for 10 hours before sowing in the medium. One centimeter depth holes were made in the medium of the pots for seed germination. One seed was sown in a hole. Fifty seeds of mungbean were germinated in a tray and these were replicated by five. During germination, 70% soil moisture level was maintained. The rate of seed germination, days for seed germination were daily recorded. The rate of germination was calculated using the following formula developed by Soupe (2009).

$$\text{Germination (\%)} = \frac{\text{Total number of germinated seedlings}}{\text{Total number of cultivated seed}} \times 100$$

Results

Morphology of *Vigna radiata* (L.) Wilczek

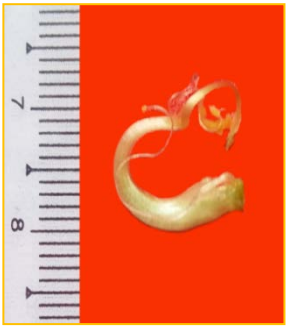
In morphological studies, *Vigna radiata* (L.) Wilczek (mungbean) is annual herb. The stems are erect or suberect, terete, green, ribbed, fulvous. Leaves are alternate, trifoliolate compound, petiolate, stipulate; leaflets ovate-subobicular, deltoid to rounded at the base, entire at the margin, obtuse-acuminate at the apex, dark green above and pale green beneath, pilose on both surfaces. Inflorescences are axillary and terminal, subumbellate or pseudoracemes, few to many flowered. Flowers are greenish yellow to yellow, bracteate, bracteolate, pedicellate, complete, bisexual, irregular, zygomorphic, pentamerous, cyclic, hypogynous. Calyx five lobed, campanulate, greenish yellow; glabrescence; tube short; lobes triangular (upper two and lower three), ciliate. Corolla five; standard suborbicular, emarginated at the tip, reflexed, striated, glabrous; wings obovate-oblong, short clawed; keels prolonged into spirally twisted, beak at the tip, glabrous. Stamens ten, diadelphous, included creamy white; free filament filiform, the adelphous filaments filiform, unequal; anthers oblong, uniform, brownish yellow, dithecous, introrse, dorsifixed, longitudinal dehiscence. Ovary linear to oblong, monocarpellary, unilocular with many ovules in the locule on marginal placenta, green, pilose; style filiform, twisted, creamy white; stigma globose, superior. Pods oblong, swollen, slightly curved, green in young and dark brown in age, ten to twenty seeded, more or less distinctly septate. The seeds are ovate, light to dark green in unripe and grey, greyish green, brown or blackish in age, hard, glabrous (Figure 1).

A. Habit

B. Leaf

C. Inflorescences

D. Flower



E. Androecium

F. Gynoecium

G. Fruits

H. Seeds

Figure 1. Morphological characters of *Vigna radiata* (L.) Wilczek

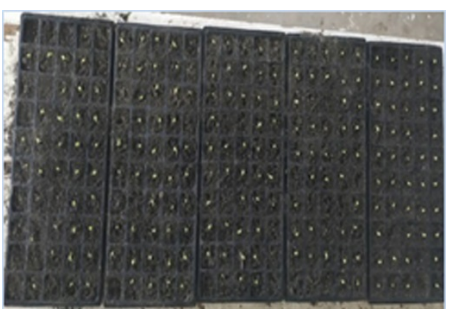
Germination of *Vigna radiata* (L.) Wilczek

The total of 250 seeds were germinated in this experiment. In day two, none of seeds were germinated. At day three, 213 seeds were germinated and day four, 29 seeds were again germinated. There were no more seeds germinated until 7 days. Therefore, the germination percentage of mungbean was 96.8% in this experiment (Table 1), and the seeds used in this experiment were certified and expected for better growth and yield.

Table 1. Germination of *Vigna radiata* (L.) Wilczek

Tray	Number of sown seeds	Germinated seedlings		Germination (%)
		Day 3	Day 4	
1	50	39	47	94
2	50	43	49	98
3	50	46	49	98
4	50	39	48	96
5	50	46	49	98
Total	250	213	242	96.8

First Day



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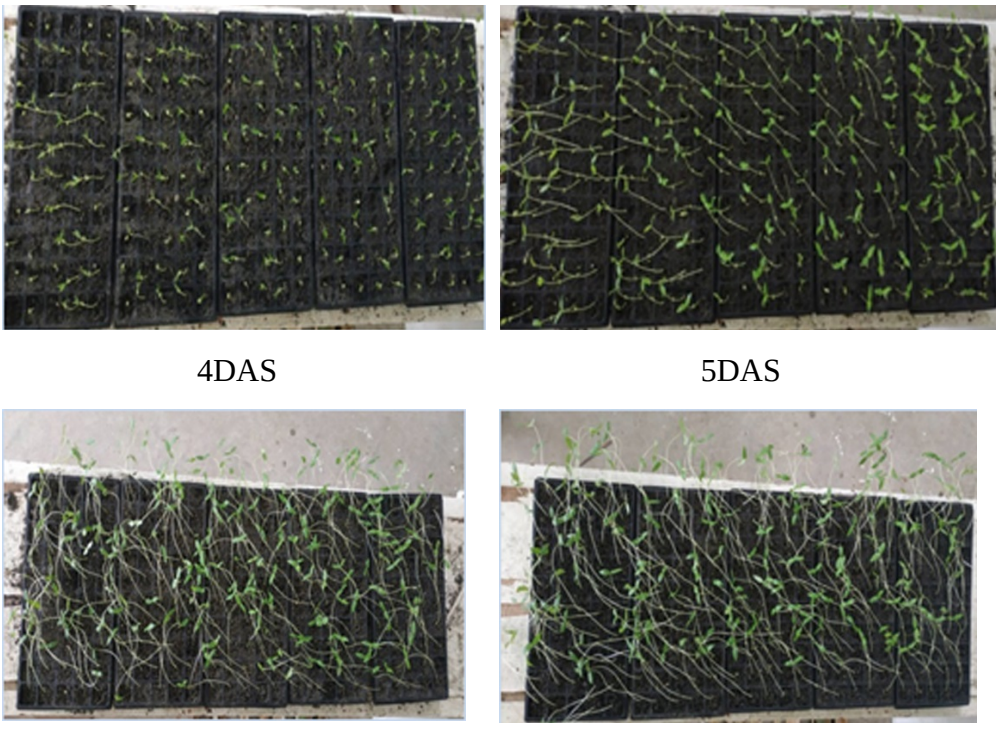

4DAS 5DAS

6DAS 7DAS

Figure 2. Seeds germination of *Vigna radiata* (L.) Wilczek

Discussion

In morphological studies, *Vigna radiata* (L.) Wilczek (mungbean) are erect or suberect herbs. Leaves are alternate, trifoliolate compound. Inflorescences are axillary and terminal. Above these characters were in agreement with the literatures of Hooker (1879), Purseglove (1969), Duke (1981), Summerfield and Roberts (1985), Tun Than (1988) and Maesen and Somaatmadja (1992).

In this experiment, flowers were greenish yellow to yellow, bisexual, zygomorphic, 5-merous, cyclic, hypogynous. Pedicels were very short. Calyx were 5-lobed, campanulate, greenish yellow, glabrescent. Corolla were 5, papilionaceous. Stamens were 10, diadelphous, longitudinal dehiscence. Ovary was superior. The flower characters were in accordance with Hooker (1879), Purseglove (1969), Tun Than (1988), Dassanayake (1991), Maesen and Somaatmadja (1992) and Ismaiel (2004).

Pods were oblong, slightly curved, ten to twenty seeded, more or less distinctly septate in this experiment. This finding was confirmed with Summerfield and Roberts (1985), Tun Than (1988) and Ismaiel (2004).

The results of seed germination showed that seeds of mungbean germinated in three days after sowing which was in agreement with Hick (1978). He stated that soybean will sprout in three days. Duke (1981) mentioned that mungbean seeds germinated in three to five days. Noggle and Fritz (1983) reported that during germination and emergence the food reserves were mobilized and transported to the embryonic plant, where they were first converted into root material (days 3 to 6). However, one of the studies mentioned that mungbean seeds germinated within four to five days (Website, 2). In this experiment, germination percentage of *Vigna radiata* (L.) Wilczek was 96.8 percent which was in agreement with Gentry (2010) who reported the germination rate of mungbean was 80 to 90 percent. Seed germination is important to the growers owing to its concerns to the yield. Nisar *et al*. (2011) reported that efficient crop yield is generally associated with efficient seed germination.

Acknowledgements

I would like to express my sincere indebtedness to Rector, Dr. Thet Lwin; Pro-rector, Dr. Aye Lwin, Pyay University for giving permission to carry out this research work. I would like gratitude to Dr. Hla Hla Win, Professor and Head, and Dr. Aye Aye Mar, Professor, Department of Botany, Pyay University, for their valuable suggestions and guidance. My deepest gratitude goes to my supervisor Dr. Thanda Aye, Professor, Department of Botany, Shwebo University, for her invaluable advice, overall supervision, criticism, suggestions and guidance on the experiments.

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Screening on the Antimicrobial Activity of Soil Fungi Against Eight Test Organisms

Zar Zar Yin[1], Myat Myat Phyo[2] & Khin Min Min Kyaw[3]

Abstract

This research work was focused on the antimicrobial activity of soil fungi against eight test organisms. Soil samples were collected from three villages of Mudon Township during 2019. Soil fungi were isolated by serial dilution method from these samples and cultured on Blaskeslee's Malt Extract Agar (BMEA Medium), Czapek- Dox Agar (CZA Medium), Malt Extract Agar (MEA Medium), Dichloram Rose Bengal – Chloramphenicol Agar (DRBC Medium), Glucose Ammonium Nitrate Agar (GAN Medium), Potato Glucose Agar (PGA Medium) and incubated for 3-7 days at room temperature. A total of eleven fungi were isolated and the surface colors of all strains were different color and size. In the colony morphology, the margins of isolated fungi were entire, and their elevation were convex, flat, raised, and the form were circular and irregular. Moreover, physicochemical properties of soil samples were analyzed. Antimicrobial activity of all fungi was observed by agar well method on eight test organisms. Among them, ZM- 8 exhibited the highest antifungal activity (28.65 mm) against *Malassezia furfur* at 7 days. These results suggested that the selected fungus may be utilized for screening the antimicrobial activity and to treat the diseases caused by pathogenic microorganisms.

Keywords: soil fungi, colony morphology and antimicrobial activity

Introduction

Soil is an excellent culture media for the growth and development of various microorganisms. Numerous antibiotics have been isolated from a variety of microorganism; however, studies are still being conducted to identify novel antibiotics effective against pathogenic fungi and bacteria (Cavalcanti, *et. al.*, 2006).

Soil are the foundation of all terrestrial ecosystems and are home to a vast diversity of bacteria, archaea, fungi, insects, annelids and other invertebrates as well as plants and algae. These soil dwellers provide food

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or nutrients that support organisms that live above and below ground. Soils also play critical roles in buffering and filtering freshwater ecosystems. Consequently, soils are extremely important to human societies (Dominati, 2010). The number and species of microbes in soil vary directly in response to environmental conditions such as nutrient availability, soil texture and type of vegetation cover (Atlas, *et. al*. 1998). 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).

Fungi are an important component of the soil microbiota typically constitution more of the soil biomass than bacteria depending on soil depth and nutrient conditions (Ainsworth & Bisby, 1995). Fungi represent a very important biological resource with an estimated 1.5 million species in the world. The tropics are generally recognized as embracing the greatest variation on earth and in the case of plants about two-thirds (180,000 species) are believed to occur there (Raven, 1988).

Therefore, soil sample is the most effective and popular materials for especially isolating a number of fungi (Ando, 2004). Wide spread efforts have been carried out by many scientists in order to screen for novel antibiotic production microbes (Oskey, 2004).

Soil is a naturally occurring loose mixture of mineral and organic particles, still remains the most important target for most researchers in their efforts to discover novel antibiotics which have pharmaceutical values (Nejad, 2013).

Therefore, the aim and objectives of this paper was to isolate soil fungi, to study the cultural characteristics of isolated soil fungi on six different media, to investigate the colony morphology of isolated fungi and to determine the antimicrobial activity of isolated fungi.

Materials and Methods

Collection of soil samples

The soil samples were collected from three different places – Tarpaton (N 16˚ 20.643′ E 97˚ 40.965′), Tharyar gone (N 16˚ 20.819′ E 97˚ 40.741′), Kyaukta lone (N 16˚ 19.732′ E 97˚ 42.082′) villages of Mudon Township, 2019. The soil samples were collected from different places (up

to 15 cm depth) into sterilized polythene bags after removing the surface soil for the isolation of fungi and brought to the laboratory of Botany department at Mawlamyine University.

Physicochemical analysis of Soil Samples

The collected soil samples were characterized for its physicochemical properties. Physiochemical parameters include organic nitrogen, phosphorous, potassium oxide, pH, temperature, moisture and texture. Temperature and color of the soil samples was recorded on the spot. The other physicochemical parameters of the soil samples were analyzed at Land Use, Perennial Crops Research & Development center (Mawlamyine).

Serial Dilution Method (Dubey, 2002)

One gram of soil sample was introduced into a conical flask containing 99 ml of distilled water. The flask was than shaken for about 30 minutes in order to make the soil particles free from each other. This solution was then serial diluted from 10^{-3} to 10^{-7} dilution in separate test tubes and 0.5 ml each of the above dilution was separately transferred into sterile petridishes under aseptic condition. The sterilized medium in conical flask was cooled down to about 45^{o} C and separately poured into each of the petridish containing the respective soil dilutions. The inoculated plates were shaken clock-wise and anti-wise direction for about 5 minutes so as to make uniform distribution of the fungi inoculums. When the agar was solidified, the inoculated plate were inverted and incubated at 27^{o}C- 30^{o}C for 3-7 days. Isolated pure fungi were preserved into slant culture containing BMEA Medium for further experimentations.

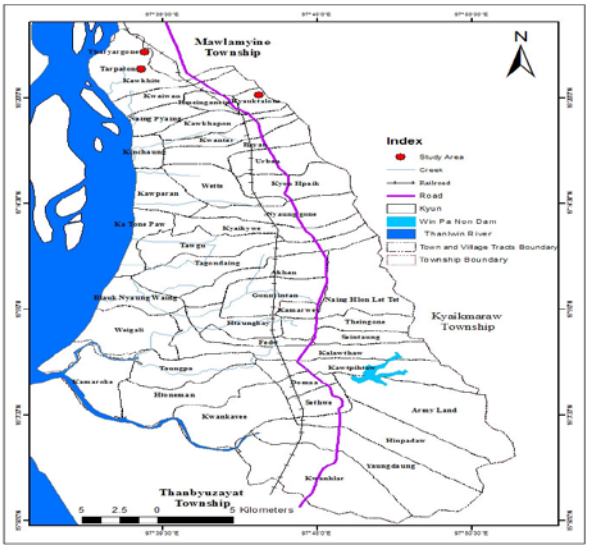


Figure 1. Map of collected soil sample area (Mudon Township)
Source: Department of Geography, Mawlamyine University

Agar Well Method (Collins, 1964)

Isolated strains were tested by agar well method for the preliminary antimicrobial activities. Cork borer was used to make the wells (8 mm in diameter) in the autoclaved basal antimicrobial test-medium. Wells impregnated with 3- 6 days old culture fermented broths (20 µL) were incubated at room temperature for 24- 28 hours. After 24- 48 hours of incubation, the clear zones were measured. Therefore, the diameter of clear zones had seen observed as potent activity as shown by respective strain. Clear zones surrounding the wells indicated the presence of antimicrobial activities which inhibit the growth of the test organisms selectively.

Test Organisms

The test organisms used for the experiment were *Escherichia coli* AHU5436, *Bacillus subtilis* IFO 90571, *Bacillus pumilus* IFO 90571, *Candida albicans* NITE 09542, *Pseudomonas fluorescens* IFO94307, *Staphlylococcus aureus* AHU8465, *Agrobacterium tumefaciens* NITE 09678 and *Malassezia furfur* AUV 0255. The organisms were obtained from National Institute of technology and Evaluation (NITE, Japan) and Pharmaceutical Research Department, Yangon, Myanmar.

Results

In present research work, 11 fungal strains were isolated and the results of the physicochemical properties of soil samples showed that soil environments of Tarpaton and Tharyargone village were sandy loam and Kyauktalone village were sandy clay loam.

In the pH values, Tarpaton, Tharyargone and Kyauktalone villages had moderately acidic pH - 6.42, 6.16 and 6.52 respectively. The temperature of soil environments had between 20.75ºC and 20.85ºC with variation in present moisture content (1.18, 1.85 and 2.99), organic nitrogen (0.09 and 0.15), phosphorus (2.40, 5.88 and 5.94), potassium dioxide (5.09, 6.19 and 6.61). The color of soil samples were red and brown. These results were shown in Table 1.

Table 1. Physico-chemical Properties of Soil Samples collected from three different places of Mudon Township

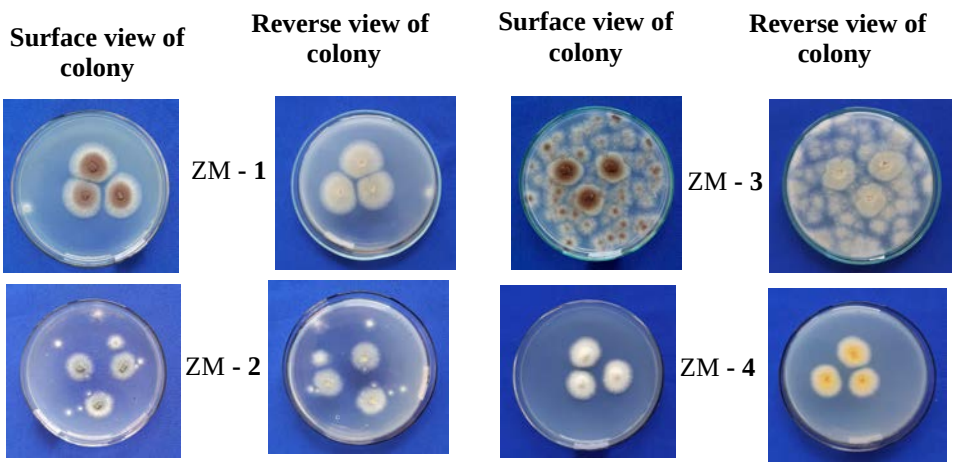
**SL- Sandy Loam, SCL- sandy clay loam, N- Nitrogen, P- Phosphorous, K_2O- Potassium oxide

No	Place	Soil color	Texture	pH	T(Cº)	Moisture (%)	Nitrogen (%)	Nutrients	
								P (ppm)	K_2O (mg)
1	Tarpaton	Brown	SL	6.42	20.75	1.85	0.15	5.94	6.61
2	Tharyar gone	Brown	SL	6.16	20.85	1.18	0.09	5.88	5.09
3	Kyaukta lone	Red	SCL	6.52	20.75	2.99	0.09	2.40	6.19

In the present research, out of 11 fungal isolates were obtained and seven strains from Kyauktalone, two strains from Tharyargone and each two strains from Tarpaton. In the culture media, 8 strains were isolated from BMEA Medium, 2 strains from DRBC Medium and 1 strain from PDA Medium. These results were shown in Table 2. The isolated fungi were designated as ZM- 1 to ZM- 11.

Table 2. Isolation of fungi on six different media

No	Places	BMEA	DRBC	PDA	MEA	CZA	GAN	Total
1	Tarpaton	ZM - 1	ZM - 2	-	-	-	-	2
2	Tharyar gone	ZM - 3	-	ZM - 4	-	-	-	2
3	Kyaukta lone	ZM - 5, 6, 7, 8, 10, 11	ZM - 9	-	-	-	-	7
		8	2	1	-	-	-	11



Photomicrograph ($\times$ 40)

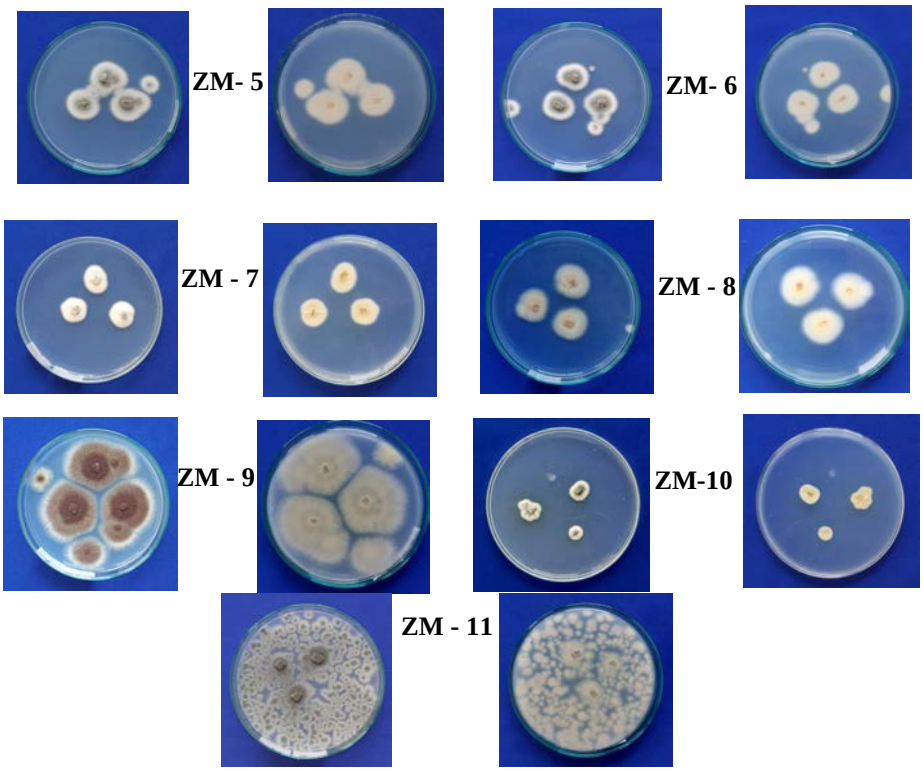


Figure 2. Morphological characters of isolated fungi (ZM – 1 to 11)

All strains were tested by eight test organisms for preliminary study of antimicrobial activity. Among them, 11 strains showed different level of antimicrobial activity. In 6 days old culture, ZM- 8 showed the antibacterial activity (22.62 mm) against *Escherichia coli*. And then, ZM- 8 showed (21.92 mm) on *Bacillus subtilis* at 6 days. Again, ZM-4 showed the antibacterial activity (18.29 mm) against *Bacillus pumilus* at 6 days. Similarly, ZM- 8 showed the highest antifungal activity (20.12 mm) against *Candida albicans*.

ZM-4 and ZM-3 showed the antibacterial activity (18.78 mm) against *Pseudomonas fluorescens* and (17.69 mm) against *Staphylococcus aureus* at 6 days of fermentation periods. And then, ZM- 1 exhibited the highest antibacterial activity (20.35 mm) against *Agrobacterium tumefaciens* and ZM- 8 showed the antifungal activity (28.65 mm) on *Malassezia furfur* at 7 days of fermentation periods.

Table 3. Antibacterial activity of 11 fungal strains against *Escherichia coli*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)				
		3 days	4 days	5 days	6 days	7 Days
1	ZM- 1	13.81	13.79	13.76	14.11	12.46
2	ZM - 2	+	12.73	12.61	13.09	11.70
3	ZM - 3	17.20	13.78	11.90	14.90	13.28
4	ZM - 4	+	15.89	13.03	16.51	17.60
5	ZM - 5	-	15.24	12.78	13.49	12.30
6	ZM - 6	+	-	-	-	-
7	ZM - 7	-	-	-	-	-
8	ZM - 8	19.81	17.40	13.66	**22.62**	20.21
9	ZM - 9	-	+	-	15.28	12.29
10	ZM - 10	13.65	12.25	-	16.51	+
11	ZM - 11	12.14	+	14.11	18.70	18.52

(+) Activity present, (-) No activity, Well size = 8mm

Figure 3. Antibacterial activity of isolated fungal strains against *Escherichia coli*

Table 4. Antibacterial activity of 11 fungal strains against *Bacillus subtilis*

No.	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)				
		3 days	4 days	5 days	6 days	7 Days
1	ZM - 1	+	+	16.31	13.71	+
2	ZM - 2	+	+	15.96	+	+
3	ZM - 3	+	+	14.62	+	16.83
4	ZM - 4	-	-	-	+	-
5	ZM - 5	+	+	18.60	+	+
6	ZM - 6	+	-	19.39	-	-
7	ZM - 7	-	-	-	-	-
8	ZM - 8	-	-	12.10	**21.92**	15.78
9	ZM - 9	-	-	-	-	+
10	ZM - 10	-	-	-	-	+
11	ZM - 11	+	-	-	14.38	+

(+) Activity present, (-) No activity, Well size = 8mm

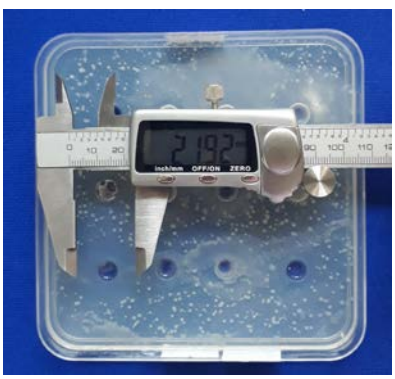


Figure 4. Antibacterial activity of isolated fungal strains against *Bacillus subtilis*

Table 5. Antibacterial activity of 11 fungal strains Against *Bacillus pumilus*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)				
		3 days	4 days	5 days	6 days	7 days
1	ZM - 1	+	13.30	13.18	15.47	13.12
2	ZM - 2	+	13.14	13.17	13.89	12.71
3	ZM - 3	+	13.78	12.16	15.38	16.83
4	ZM - 4	+	15.97	11.86	**18.29**	17.58
5	ZM - 5	+	+	14.22	13.74	12.60
6	ZM - 6	+	13.33	-	11.44	+
7	ZM - 7	-	-	-	-	-
8	ZM - 8	18.29	18.68	12.26	12.39	15.67
9	ZM - 9	-	-	-	13.49	15.52
10	ZM - 10	+	+	13.18	15.94	+
11	ZM - 11	+	11.93	12.57	16.34	16.68

Figure 5. Antibacterial activity of isolated fungal strains against *Bacillus pumilus*

Table 6. Antifungal activity of 11 fungal strains against *Candida albicans*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)				
		3 days	4 days	5 days	6 days	7 days
1	ZM - 1	+	12.94	12.45	15.56	12.86
2	ZM - 2	+	12.79	11.83	13.32	-
3	ZM - 3	+	14.84	12.73	15.41	13.30
4	ZM - 4	14.88	15.86	-	14.47	19.78
5	ZM - 5	+	15.22	13.65	13.39	13.88
6	ZM - 6	+	12.49	-	+	-
7	ZM - 7	-	-	-	+	-
8	ZM - 8	15.52	18.46	18.24	**20.12**	25.98
9	ZM - 9	-	-	-	15.61	12.70
10	ZM - 10	-	16.22	-	16.24	+
11	ZM - 11	12.70	-	-	14.09	17.90

(+) Activity present, (-) No activity, Well size = 8mm

Figure 6. Antifungal activity of isolated fungal strains against *Candida albicans*

Table 7. Antibacterial activity of 11 fungal strains against *Pseudomonas fluorescens*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)				
		3 days	4 days	5 days	6 days	7 days
1	ZM - 1	-	-	-	-	-
2	ZM - 2	-	-	-	-	-
3	ZM - 3	-	-	-	+	-
4	ZM - 4	-	-	-	**18.78**	+
5	ZM - 5	-	-	-	11.44	+
6	ZM - 6	-	-	-	-	-
7	ZM - 7	-	-	-	-	-
8	ZM - 8	-	-	-	12.58	12.03
9	ZM - 9	-	-	-	-	-
10	ZM - 10	-	-	-	-	-
11	ZM - 11	-	-	-	11.37	12.66

(+) Activity present, (-) No activity, Well size = 8mm

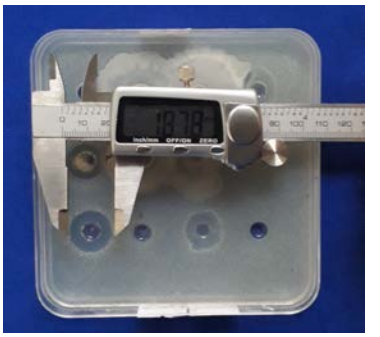


Figure 7. Antibacterial activity of isolated fungal strains against *Pseudomonas fluorescens*

Table 8. Antibacterial activity of 11 fungal strains against *Staphylococcus aureus*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)				
		3 days	4 days	5 days	6 days	7 days
1	ZM - 1	-	-	-	+	+
2	ZM - 2	-	-	-	+	+
3	ZM - 3	+	+	-	**17.69**	+
4	ZM - 4	-	-	-	-	-
5	ZM - 5	-	-	-	-	-
6	ZM - 6	-	-	-	-	-
7	ZM - 7	-	-	-	-	-
8	ZM - 8	-	-	-	12.58	12.03
9	ZM - 9	-	-	-	-	-
10	ZM - 10	-	-	-	-	-
11	ZM - 11	-	+	-	-	-

(+) Activity present, (-) No activity, Well size = 8mm

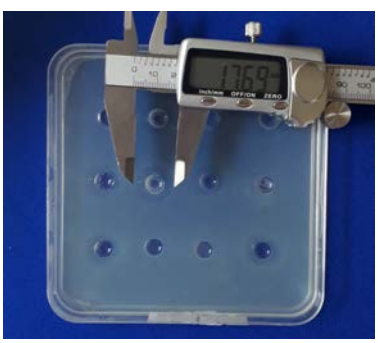


Figure 8. Antibacterial activity of isolated fungal strains against *Staphylococcus aureus*

Table 9. Antibacterial activity of 15 fungal strains against *Staphylococcus aureus*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)				
		3 days	4 days	5 days	6 days	7 days
1	ZM - 1	+	15.78	11.48	16.33	**20.35**
2	ZM - 2	+	12.57	+	+	20.30
3	ZM - 3	+	+	+	+	+
4	ZM - 4	-	-	-	-	-
5	ZM - 5	+	14.16	11.80	17.69	23.64
6	ZM - 6	+	11.54	+	+	+
7	ZM - 7	-	-	-	+	+
8	ZM - 8	-	-	-	11.93	+
9	ZM - 9	-	-	-	-	-
10	ZM - 10	-	+	-	-	+
11	ZM - 11	+	+	+	12.88	16.17

(+) Activity present, (-) No activity, Well size = 8mm

Figure 9. Antibacterial activity of isolated fungal strains against *Agrobacterium tumefaciens*

Table10. Antifungal activity of 11 fungal strains against *Malassezia furfur*

No	Isolated Fungi	Fermentation Period (days) and Inhibitory zone (mm)				
		3 days	4 days	5 days	6 days	7 days
1	ZM - 1	+	+	13.81	12.14	+
2	ZM - 2	+	13.67	13.06	12.38	+
3	ZM - 3	+	14.87	13.38	19.13	+
4	ZM - 4	15.52	18.70	12.78	-	+
5	ZM - 5	+	14.34	12.78	12.24	13.26
6	ZM - 6	+	+	+	+	+
7	ZM - 7	-	-	-	-	-
8	ZM - 8	18.78	15.19	18.96	24.15	**28.65**
9	ZM - 9	-	-	-	17.40	+
10	ZM - 10	+	13.50	14.78	17.40	+
11	ZM - 11	+	+	14.51	16.75	18.27

(+) Activity present, (-) No activity, Well size = 8mm

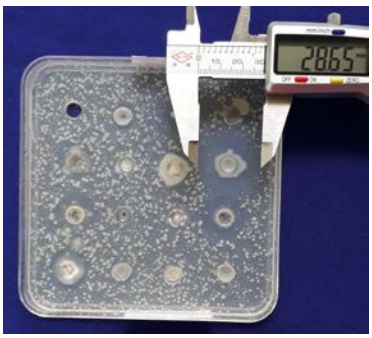


Figure 10. Antifungal activity of isolated fungal strains against *Malassezia furfur*

Discussion and Conclusion

Scientists are continuously searching for novel antibiotic producing microbes because drug resistant strains of pathogen emerge more quickly than the rate of discovery of new drugs and antibiotics (Kumar *et al*., 2010).

In the present work, physicochemical analysis of soil samples showed that pH of the soil is acidic and is rich with both macro and micro nutrients which is favorable for the growth of fungi. Fungal diversity of any soil depends on a large number of factors of the soil such as pH, organic content and moisture (Rangaswami, 1998).

Ramann *et al*., 1899 also reported that due to the accumulation of more litter in scrub and deciduous forest more percentage of fungi are present in the soil for the purpose of recycling of dead organic matter. It is known that the bacteria thrive well in neutral and alkaline soils, whereas fungi show the best activity under acidic conditions.

Isolation of eleven fungi was cultured on six culture media. For the human health and nutrition fungi are well known to produce both beneficial and deleterious natural agents and continue to be explored as useful sources of natural antimicrobial agents. In comparison to plants, microorganisms are highly diverse but narrowly explored (Chioma *et al*., 2016).

All strains were tested by eight test organisms for preliminary study of antimicrobial activity. Among them, 11 strains showed different level of antimicrobial activity. In this research, ZM-8 showed the strong antimicrobial activity against *Escherichia coli, Bacillus subtilis, Bacillus pumilus, Candida albicans and Malassezia furfur*.

It was concluded that the present research was to isolate the fungi from different soil samples and to study the antimicrobial activity of isolated fungi on eight test organisms. Further study should be performed on the fermentation conditions of selected fungus and extraction of antimicrobial compounds.

Acknowledgements

I am indebted to Dr Aye Aye Tun, Rector of Bago University and Dr Yin Yin Than, Pro-Rector of Bago University, for their encouragement and supports in preparing this work. I would like to acknowledge to the following persons who have supported for this research work: Dr. San Wai Aung, Professor and Head of Department of Botany,

Bago University for her encouragement, Dr. Tin Moe Aye, Professor, Department of Botany, Bago University for her suggestions. Especially, I'm grateful thanks to Daw Myat Myat Phyo and Daw Khin Min Min Kyaw for their helps in this paper.

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Preliminary Study of Some Myanmar Subtropical Wild Orchids of Bon-Taung Reserved Forest in Taungoo Area

Moe Sandar Shein[1], Thant Zaw Win[2], & Tin Moe Aye[3]

Abstract

The present study is concerned with the subtropical wild orchids of natural habitat in Taungoo Area; mainly study sides are Bon Taung reserved, Taungoo vicinity, Taungoo, Oat Twin, Phyu high way road side and Phyu cities in the subtropical region. In this recent study has chosen the subtropical area where native orchids have been grown well on the old trees ***Albizia lebbeca*** (L.) Benth (Kuk-ko) and ***Tamarindus indica***) L. (Ma-Gyi). In this paper included two genera and nine species which have been recorded from study sites, two genera are ***Dendrobium, pathoglotis.*** Most of them are epiphytes and terrestrials. The recorded specimens were identified, classified and described with color photographs of their natural habitats and their inflorescence. The distinguished characters of each species has been emphasized with their own artificial keys. In addition, they are recorded with GPS location system.

Keyword : Bon Taung reserved forest, Taungoo Area, subtropical, epiphytes and terrestrials, artificial keys

Introduction

In this recent study the wild orchids have been conducted in Bon-Taung reserved forest in Oat- Twin Township and Taungoo urban area of Taungoo District. Which places are subtropical region. Some wild orchids grown well in these places. Family Orchidaceae stretch out of the world exceptional of ice capped and desert region. Some orchidologist predict about 35,000 species around the world **Seidenfadan, (1992**). The family orchidaccea are probably largest family among Angiospermae, Monocotyledonae. The study gold area are Bon- Taung reserved forest in Oat- Twin township and Taungoo urban area of Taungoo district. Oat-Twin Township is located on the east by Htan-ta-pin Township, on the west by Pauk-khaung Township, on the south by Phyu Township and on the north by Toungoo Township. It lie between North latitude18˚15′ and18˚54′

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East longitude 98˚13′and 98˚51′.There are four reserve forest and one protective public forest in Oat-Twin Township. They are Bon Taung reserve forest, Mya-yar-pin reserve forest, Phyu kun reserved forest and Kha-Baung reserved forest and Kha-Baung protected public forest under controlled by forest department. Bon-Taung reserve forest is tropical rain forest type, saturated in Southern Westpart between Oat-Twin Township. The area of this forest is about 23886 acres and lie between North Lattitude 18˚ 42′ -18˚ 45′ and East longitude 98˚ 13′ -98˚ 20′ and average temperature 34˚C.

In this recent study, (1) Subfamilies belong to (1) Tribes (2) Subtribes, (2) genera and (9) species have been collected from this study area including epiphyte, and terrestrial. They are genus S***pathoglottis and Dendrobium.*** In this recent study ***Spathoglottis plicata ,Dendrobium palpebrae, D.aggregatum, D. Chrysotosum, D.parashiim D.moschatum, D.pulchellum, perulatum*** and ***D.dixanthum*** are recorded from study area. The classification and taxonomic description of collected specimens are provided with coloured photographic and keys of subtribe, genera and species are also constructed.

Methodology

The specimens were collected from Oattwin Township. All these specimens were colourful photographed to record their actual habitat and the nature of inflorescence. The collected specimens were classified according to Dresseler's classification Dresseler(1927) and identified by Seidenfaden (1992), Grant (1966), Nantiya Vaddhanaputi (2006), Hooker (1954), Seidenfaden and Smitch (1965) and Dassanayake (1981) method and GPS location system. Herbarium specimen well prepared and submitted to Botany Department Yangon University.

Arrangement of the Subfamily, Tribe, Subtgribe and Genera in the Present Study

Class	:	Liliopsida (Monocotyledoneae)
Subclass	:	Orchidales
Family	:	**Orchidaceae**
Subfamily	:	(I)Epidendroideae
I. Subfamily	:	Epidendroideae

Tribe : Arethuseae

Subtribe : Blettinae

Genera : (1) ***Spathoglottis***

Tribe : Epidendrae

Subtribe : Dendrobiinae

Genera : (2) ***Dendrobium***

The classification of Subfamilies in the study is in accordance with **Dresseler (1927)** and the key below is cited from **Seidenfaden** and **Wood. (1992)** described in "The Orchids of Indochina".

Results

Subfamily	**Tribe**	**Subtribe**	**Genus**	**Species**	**Myanmar Name**
Epidendroideae	Arethuseae	Bletinae	Spathoglottis	S.plicata	Ohn Thitkwa
	Epodendreae	Dendrobinae	*Dendrobium*	*D.palpebrae*	None
				D.aggregatum	Yadana Shwe Ket
				D.chrysotoxum	Shwetu Mouk Kham Wa
				D.Parashii	Kayan Yaung Lwin Pyin
				D.moschatum	Waso Pan
				D.pullchullum	Sinma Myak Kwin
				D.perrulatum	None
				D. dixanthum	Shwe Wah Kalay

In this paper one subfamily, two tribes, 2 subtribes and two genera and 9 species have been collected from study area.

Key to the tribe of subfamily *Epidendoideae.*

1. Terrestrial. Plants usually plicate leaves, pollinia 8 ---- **Aruthuseae**

1 Epiphyte. Plants usually conduplicate leaves. Pollinia 8 or 4 ---------- ---------- **Epidendeae**

In this recent study only one genus ***Spathoglottis*** of subtribe Blettinae under Tribe Aruthuseae was recorded from study area.

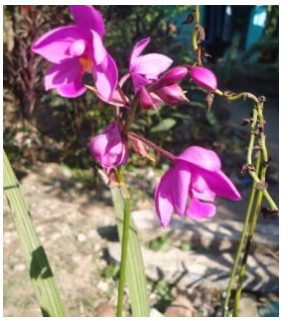
Subfamily Epidendoideae

Spathoglottis

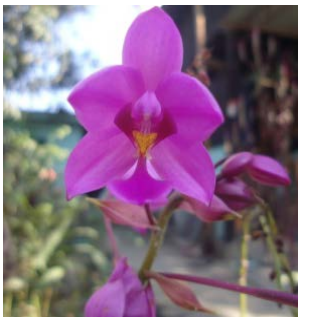
Terrestrials, the mostly subterraneous tubers ovoid, sometimes depressed, each bearing a few plicate leaves. Inflorescence from a basal leaf axile, scape tall and slender, bearing a succession of many flowers. Sepals and petals about equal. Lip strongly 3-lobed, side lobes narrow (lacking in one species), oblong, curved upwards, midlobe, with a very narrow claw at the base of which are two small ovoid calli and two small laterally spreading teeth, the end of the blade more or less widening and sometimes cleft.Column slender, curved, without foot. Pollinia 8, slender in two groups of 4.

***Spathoglottis plicata* Bl.**

Habit



Inflorescence



Flower

Spathoglottis plicata Bl. Bijdr, 401.1825.J.J.S.,FL. Buit.6:219, f.162. Ridl., Flora 4:117.

Terrestrial, pseudobulb small, leaves 2-4 feet long and 3-4 cm wide, acuminate with long petiole. Inflorescence long and slender, raceme. Flower purple, often medium. Petals broder than the sepals. Lip trilobed, sidelobes equal, erect, oblong obtuse, middle narrow spathulate, rounded end, two yellow calli at the base of the midlobe. Anther 2-celled. Pollinia 8.

Myanmar Name - Ohn-pan

Flowering Period - June - December

Occurrence - Taungoo urban area (Taungoo Township)
(N 18 ˚ 56′- E 96˚ 25′)

Distribution - Sumatra, (Native), Malaya, Borneo Java and Philipippine Newguinea **Holttum, (1964)**

In this paper only one genus ***Dendrobium*** of Subtribe Dendrobinae under Tribe Epidendrae was collected from study area.

Dendrobium.

This genus have more or less elongated cylindrical leafy pseudobulbs at stems, the leaves being generally bifarious, alternate and flat , they differ as in habit, so in size. The flowers are lateral and either solitary, in fasicles or in raceme. The sepals and petals all the segment of the flowers except the lip are nearly uniform in shape the general difference being that of the outer segment or sepal , two lateral sepals are larger than the other and adhere commonly to the side of the column, or usually prolonged into a blunt spur. The lip is always sessile. Pollinia 4 in pairs side by side, quite free, anther 2-celled.

Key to the species of genus *Dendrobium*

1. Pseudobulb stout and short---------- 2
1. Pseudobulb slender and long---------- 3

2. Stem quadarangular. Inflorescence with loose flower. Flower white with yellow in the centre.---------- 1. ***D. palpebrae***

2. Stem with furrow. Inflorescence with many flowers. Flower other

coloured---------- 4

3. Inflorescence pendulous with many flowers ---------- 5
3. Inflorescence suberect with afew folwers ---------- 6

4. Stem short and tuff. Inflorescence pendulous. Flower thin texture ----------2. ***D. aggregatum***

4. Stem stout fusiform narrow at the base. Inflorescence sub erect. Flower thick and thick texture.---------- 3. ***D. chrysotoxum***

5. Leaves brownish purple. Flowers large and orange yellow. Lip cup shaped ---------- 5. ***D. moschatum***
5. Leaves green . Flower creamy white. Lip rounded in curved with purple crimson blotch on each side of hypochile ---------- ---------- 6. ***D. pulchellum***

6. Pseudobulb suberect, flower last one weak. Flower golden yellow. Lip rounded with deep yellow at the base ---------- ---------- 8. ***D. dixanthum***

6. Leaves green. Flower medium size. Lip rounded ---------- 7

7. Pseudobulb pendulous. Flower last two month. Flower white. Lip spathulate, white with yellow at the base. ---------- 7**.** ***D. perulatum***
7. Pseudobulb not too long, covered with sheath. Flower deep purple. Inflorescence two flowers per node ---------- 4. ***D. parashii***

***Dendrobium palpebrae* Lindl.**

Habit

Inflorescence

Flower

***Dendrobium palpebrae* Lindl.**

Epiphyte. Stem clavate four angled. Leaves close, 3-5 oblong-lanceolate acute. Flower in lax inflorescence, white, lasting a few days, lateral raceme. Sepals oblong, petals broder clawerd ciliorate. Lip oblong softly downy, base shortly clawed convolute and fringed, short median callus at the base of the lip, orange with a white brown along edge. Floral bracts small.

Myanmar Name - None

Flowering Period - March

Occurrence - Bon Taung reserved forest, Phyu Township. Taungoo District (N 18 ˚ 14′- E 96˚ 13′)

Distribution - NE India, Myanmar, Thailand (**Seidenfaden, 1992)**. Myanmar (**Grant,1966)**

***Dendrobium aggregatum* Roxb.**

Habit

Inflorescence

Flower

***Dendrobium aggregatum* Roxb FL iii 477.**

***Dandrobium lindleyi* Steud**

***D. jenkinsii* Wall.**

Epiphyte dwarf, evergreen species, clustered. Stems fusiform with furrow pseudobulbs about 5-6 cm long and 3.00 cm wide. Leaves solitary, oblong ovate, tip notched. Inflorescence loop drooping lateral raceme. Flower are deep golden yellow with an orage yellow stain at the hypochile, about 3.00cm across. Sepal ovate obtuse spreading. Petals much broader ovate, base cuneate. Mantum subglobose. Lip shortly clawed transversely oblong from a short convolute at the base, pubescent, entire ciliolate. Column yellow. Pollen masses 4 in pairs.

Myanmar Name - Yadana Shwe Khat

Flower period - February- March

Occurrence - Bon-Toung reserved forest. Oat twin Township.
(N 18 ˚ 55′- E 96˚ 25′)

Distribution - Myanmar, Deccar, Sikkim, Bhutan, NE India, Thailand and China **(Seidenfaden ,1992)**
Arrancan, Martabow, Tenassenim Hook. f. (**Grant, 1966**)

***D. chrysotoxum* Lindl.**

Habit

Inflorescence

Flower

***D. chrysotoxum* Lindl. In Bot. Reg. 1847. Under t-19 and t. 36**

***D. suavissimum* Rchb.f. in Gard. Chrsn 1874. 406.**

Epiphyte, evergreen species. Stem stout, short, club-shaped with deeply furrowed, clustered from a slender base, yellowish when old, about 26-30 cm long. Leaves oblong acute, leathery. Inflorescence obliquely erect or slightly drooping with many flowers, well spread about 30cm long. Flowers golden yellow about 2.5cm across. Sepal oblong elliptic, petals broadly obovate. Mentum insignificant. Lip suborbicular with finely imbricate at margin. Very deep orange at the base, concave. Column short. Pollan 4 in pairs, waxy.

Myanmar Name - Shwetu Mouk Kham Wah

Flower period - February- March

Occurrence - Bon-Toung reserved forest. Oat twin Township.

(N 18 ˚ 46′- E 96˚ 17′)

Distribution - Native in Myanmar (**Holttum,1964**), WE India, Myanmar, Thailand and China (**Seidafaden,1992**). Arracan and Burma Myanmar (**Grant,1966).**

***Dendrobium parashii* Rchb.f.**

Habit

Inflorescence

Flower

***Dendrobium parashii* Rchb. f.**

Epiphyte, Stem stout their entire length and decurved. Leaves oblong, lanceolate. Flower 1-3 with short peduncle, rose purple; 3.0 cm across. Sepal oblong acuminate, petal broadly elliptic. Lip shortly clawed convolute with a small ovate obtuse tomentose and ciliate recurved limb. Column white anther purple.

Myanmar Name - Ka-yan-young-lwin-pyin

Flowering period - April – May.

Occurrence - Myanmar Bon-taung reserved forest of Oat Twin Township. Tangoo District. (N 18 ˚ 56′- E 96˚ 25′)

Distribution - Myanmar, NE India, Thailand, China, (**Seidenfaden, 1992).**

***Dendrobium maschatum* (Buch. Ham.) Sw.**


Habit


Inflorescence


Flower

***Dendrobium moschatum* (Buch. Ham.) Sw.**

Epiphyte. Stem brown slender. Leaves linear oblong, reddish green. Raceme 5-6 lax flowers arising on the top of the stem. Flower yellowish orange, large showy, about 7.00 cm long and wide. Sepals ovate-obtuse. Petals broadly rounded, larger than the sepals, glabrous. Edge of lip incurved forming a pouch, yellow with two maroom blotch at epichile and long ciliate veings, outside pubescent on incurved edge. Column long with red spot. Anther 2-celled. 4 pollinia.

Myanmar Name - Wah-so -Pan

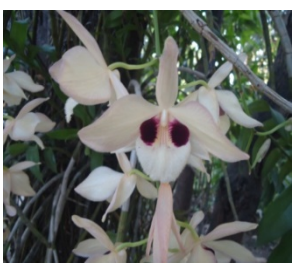
Flowering Period - May to June

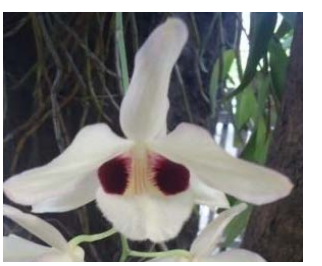
Occurrence - Bon-Taung reserved forest Oat Twin Township, Taungoo Township, Phyu Township, Bago District.(N 18 ˚ 56′- E 96˚ 25′)

Distribution - Himalaya, Myanmar, Thailand and China (**Seidenfaden, 1992).**

***D.endrobium pulchellum* Roxb.**


Habit


Inflorescence


Flower

***Dendrobium pulchellum* Roxb ex. Linl.Gen, and SP,Orchid,82:FL .ind, 486**

***D. dalhousiearum*, Wall.**

***Dendrobium pulchellum* Roxb ex. Linl**

Epiphyte, evergreen species. Stem stout, terete subfusiform about 20 50 cm high, markly red purple line. Leave linear oblong, base cordate. Raceme drooping lateral 5-10 frowers. Frower, very large rosy, cream cold, about 6-8 cm long and wide .Sepals oblong acute, petals much broader than the sepal, mentum rounded. Lip shortly clawed orbicular oblong tip and side densely glandular villous on epichile, two large dark crimson blotches at the base of the lip. Colum short and stout, colum and anther dark purple. Pollen masses 4 in pairs.

Myanmar Name - Sin –ma myat -kwin

Flowering period - May-June

Occurrence - Bon-Taung- reserved forest, Taungoo District , Taungoo and Phyu Township. (N 18 ˚ 56′- E 96˚ 22′)

Distribution - Myanmar, Nepal, NE India, Thailand, China and Malaysia. **(Seidenfaden,1992)** Native to Assam and Tenasser Singapore Island, Malaya **(Holttum,1964).**

Dendrobium perulatum Gagnep. Bull.

Habit

Inflorescence

Flower

Dendrobium perulatum Gagnep. Bull. Mus. Paris 2.22(3): 396, 1950Seidenfaden 1979: 49. Averyanov 1988 f: 149. 1990:79

Epiphyte. Stem pendulous, about 15-20cm long, moderately slender, terete. Leaves lanceolate, acuminate. Flowers 2-3 on a short peduncle from the leafless stem. Sepals and petals oblong acute. Petals not twisted. Flower white except the centre on lip which is yellow. The upper surface of lip sparsely papillose. The upper bract lanceolate, thin, hyphae. The older stem glossy, bamboo yellow. The flower bare stem still surrounded by disintegration sheath. Flowering last time two month from bud to flower.

Myanmar Name - Not known

Flowering period - May-June

Occurrence - Bon-Taung- reserved forest, Taungoo District , Taungoo and Phyu Township. (N 18 ˚ 49′- E 96˚ 16′)

Distribution - Endemic **(Seidenfaden,1992)**

Dendrobim dixarthum **Rchb.f**

Habit

Inflorescence

Flower

Dendrobim dixarthum **Rchb.f**

Epiphyte, stem long slender, yellow, erect. Leaves lanceolate. Raceme on the leafless stem. Flower golden greenish yellow 2-4 flowers, 3-5 cm wide. Sepal oblong subacute, petals broder obtuse ciliolate. Lip orbicular from short convolute base, deeper yellow blotch in the centre, mentum subglobose.

Myanmar Name - Shwe Wah kalay

Flowering period - March to April

Occurrence - Bon-Taung reserved forest Oat twin Township, Taungoo district. (N 18 ˚ 47′- E 96˚ 14′)

Distribution - Myanmar, Thailand (**Seidenfaden, 1992**.)

Discussion

The Orchidaceae is the largest family of the flowering plants kingdom, comprising a large number of species .Some author suggest 12,000 to15,000 species and other as many as 35,000 species (**Dressler, 1927**).Some reveal that more or less 800genera ,35,000 species(**Margaret Hodgson,1968**),72 genera and 554 species (**Grant.B.,1966**), **Handeley** and **Chit Ko Ko** reported that 113 genera and 850 species in 1961 and128 genera and 739 species in 1986 as Myanmar native orchid. In this paper included one subfamily, Epidendroideae and two subtripe Blettinae and Dendrobiinae two genera *Spathoglottis* and *Dendrobium* and nine species. This classification cited by Dressler classification and all of these species are epiphyte and only terrestrial which is genus *Spathoglottis*. The present work listed two genera of orchid as follow; 1 species *Spathoglotis* and ***Dendrobium*** with nine species, In this paper sub family Epidendroideae, genus *Spathoglottis* has collected only one species *Spathoglottis plicata* BL. which possesses narrow spathulate midlobe with distinct two yellow calli in the base of the midlobe. Eight species of Genus *Dendrobium* has found in recent study. They are *D. palpebrae* Lindl. which possesses quadarangular stem and white flower. *D. aggregatum* Roxb. contains shortly tuft stem and transversely oblong golden yellow lip. *D. chrysotoxum* Lindl.has club-shaped stem with deep furrow and finely imbricate margin lip. *D. parashii* Rchb. contains two deeply purple blotch at the base and ciliate and recurved limb. *D. moschatum*(Buch.Ham.)Sw possesses cup shaped lip. *D. pulchellum*Roxb has strongly erect stem and purple crimson blotch on each side of rounded lip. *D. perulatum* Gagnep.Bull contains spathulate white lip with yellow at the base. *D. dixanthum*Rchb.f has rounded lip with deep yellow at the base. Among them ***Drodrobium perulatum*** Gagnep.Bull is endermis **(Seidenfaden, 1992) ,** Some Myanmar orchids has been revealed as native which is ***Dendrobium aggregatum*** Steud**,** ***D.aphyllum*** (Roxb.) C.E.C.Fisher**,** ***D.Chrysotoxum*** Lin**.,** ***D.dixanthum*** Rchb**.**f.and ***D.pulchellum*** Roxb ex Lindl**. (Holttum,1964)**

Conclusion

Today wild orchids migrate from the jungle to the urban area by the human activity so some wild orchid have to seen and maintain in vicinity and downtown but some of them are died according to habited and some disappear completely by human activity. Therefore all nationality must be find out and maintain the living jewels for natural resource of Myanmar. In fact the orchidologist have to find out and report to government for protection of natural heritage and contribute the knowledge about value of orchids to native sellers, orchid hunters and local dwellers how to protect and evaluate the wild orchids for natural conservation. The present study explored and thoroughly described the natural resource of Myanmar.

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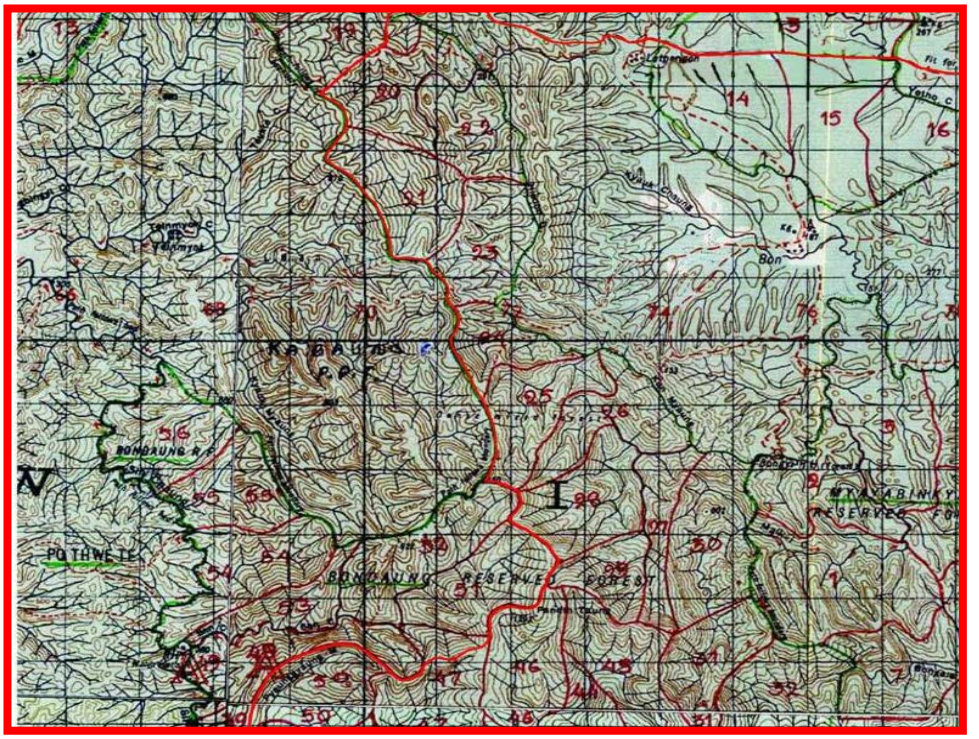
Topographic map of the study area

96˚ 15′
96˚ 20′



Topomap No. 94B/5
Scale – 1″ = 1 mile

Biochemical Characterization and Identification of Phosphate Solubilizing Bacterium *Bacillus* sp.

Myint Myint Than[1] & Zar Zar Yin[2]

Abstract

The present research was focused on the isolation and identification of phosphate solubilizing bacteria from the different soil. Soil samples were collected from ten different soil at Taung-Yar-Kone village and Ywar-Thit-Kone village of Pathein Township, Ayeyawady Region. These samples are cultured on two different culture media and a total of 47 phosphate solubilizing bacterial colonies were isolated and designated as MMT 1-47. Cell morphology of isolated strains was studied by gram staining and these strains were rod, short rod and cocci. Seventeen strains were gram positive and thirty strains were gram negative. Moreover, the antimicrobial activity of all strains were tested by agar well diffusion method on ten test organisms. Among them, MMT-3 showed the highest antibacterial activity (26.11 mm) against *Agrobacterium tumefaciens.* Therefore, MMT-3 was selected and identified by morphological, microscopical and biochemical characteristics as well as KB 013 identification kit and classified as belonging to the genus *Bacillus* sp. This research will provide the knowledge of isolation and identification of *Bacillus* sp.

Keywords: Biochemical characterization, identification of *Bacillus* sp.

Introduction

Microorganisms grow in unique and extreme habitats, they may have the capability to produce unique and unusual metabolites. Generally, the reason why they produce such metabolites is not known, but it is believed that many of these metabolites may act as chemical defense of microbes competing for substrates (Gallo *et al.,* 2004). Manipulating nutritional or environmental factors can promote the biosynthesis of secondary metabolites and thus facilitate the discovery of new natural products (Wang *et al.,* 2011).

Phosphate solubilizing microorganisms include different groups of microorganisms, which not only assimilate phosphorus from insoluble

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forms of phosphates, but they also cause a large portion of soluble phosphates to be released in quantities in excess of their requirements. Among the bacterial genera with this capability are *Pseudomonas, Azospirillum, Bacillus, Rhizobium, Burkholderia, Arthrobacter, Alcaligenes, Serratia, Enterobacter, Acinetobacter, Flavobacterium* and *Erwinia* (Rodriguez *et al.,* 1996).

The ability to produce endospores allows *Bacillus* to survive extreme environmental conditions as those occurring in food processing. Many of the *Bacillus* spp. are reported to occur in a number of fermented or non-fermented food products, such as dry cured sausages, cheeses, traditional fermented milks, sourdough, etc., as a result of the relationships between processing and natural contamination of raw ingredients. The *Bacillus* spp. in food cooperates with other microorganisms during fermentation, releasing metabolites including antimicrobial substances, amylases, lipases and proteases (Graumann, 2007).

Search for new natural biologically active compounds and their characterization is one of the urgent tasks in modern biotechnology. Microorganisms are an important source of such compounds (Esikova *et al.,* 2001).

Therefore, the present study was carried out the isolation and identification of phosphate solubilizing bacteria from soil samples. The aim and objectives of this study were to observe the biochemical properties of phosphate solubility bacteria and to identify the selected phosphate solubility bacteria.

Materials and methods

Ten different soil samples were collected from each five different places of Taung-Yar-Kone village and Ywar-Thit-Kone village in Pathein Township, Ayeyawady Region from June to July, 2016. This experiment was carried out at 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. In order to isolate bacteria from soil samples, these soil samples were dried in air and ground, and finally kept in a clear flask.

Serial dilution of pour plating and streaking technique were used to isolate the microorganisms from soil according to Collins 1965.

The identification of selected bacterium was carried out using colony morphology, gram staining methods (Dubey and Maheshwari, 2002), spore staining method (Prescott, 2002) and biochemical tests which include the catalase test (Salle, 1948), oxidase test (Dickey and Kelman, 1988), motility test (Prescott, 2002).

Oxygen requirement (aerobic/anaerobic) (Precott, 2002), methyl red test (Bisen and Verman, 1998), acetyl-methylcarbinol production test (Voges-Proskauer-VP Test) (Cruickshank *et al.*,1963), citrate utilization test (Atlas, 1993) urea hydrolysis test (Christenson, 1946), hydrogen sulphide test (Cowan, 1975), phenylpyruvic acid test (PPA) (Atlas, 1993) nitrate reduction (Harrigan and Mc Cane, 1996), salt tolerance (Atlas, 1993), sugar fermentation test (Cowan, 1975), starch hydrolysis test (Pelezar and Chan, 1972), gelatin hydrolysis test (Cruickshank, 1968), casein hydrolysis test (Pelezar and Chan, 1972), potato plug test (Atlas, 1993) and esterase activity test (Prescott, 2002).

Identification Kit

KB 013 is a combination of 12 tests for identification of *Bacillus* species. Kit contains sterile media for Malonate, Voges Proskauer's, Citrate, ONPG, Nitrate reduction, Catalase, Arginine and 5 different carbohydrate utilization tests (Sucrose, Mannitol, Glucose, Arabinose and Trehalose). These results were shown in Figure 1.

Result Interpretation chart

No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reactioin	Negative reaction
1	Malonate	-	Malonate utilization	Bluish green	Dark Blue	Bluish green
2	Voges Proskauer's	1-2 drops of Baritt reagent A and 1-2 drops of Baritt reagent B	Detects acetoin production	Colourless/ Light yellow	Pinkish red	Colourless/ slight copper

No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reactioin	Negative reaction	
3	Citrate	-	Citrate utilization	Light Green	Dark Blue	Light Green	
4	ONPG	-	Detects Beta galactosidase	Colourless	Yellow	Colourless	
5	Nitrate Reduction	1-2 drops of sulphanilic acid and 1-2 drops of N, N-Dimethyl-1-Naphylamine	Detects Nitrate reduction	Colourless/ Light yellow	Pinkish Red	Colourless	
6	Catalase	3% H_2O_2 solution	Detects Catalase activity	Colourless	Effervescence coming out from the loop	No Effervescence seen	
7	Arginine	-	Arginine utilization	Olive Green to Light Purple	Purple/ Dark Purple	No Change in	color or yellow
8	Sucrose	-	Carbohydrate utilization	Pinkish Red/ Red	Yellow	Red/ Pink	
9	Mannitol	-	Carbohydrate utilization	Pinkish Red/ Red	Yellow	Red/ Pink	
10	Glucose	-	Carbohydrate utilization	Pinkish Red/ Red	Yellow	Red/ Pink	
11	Arabinose	-	Carbohydrate utilization	Pinkish Red/ Red	Yellow	Red/ Pink	
12	Trehalose	-	Carbohydrate utilization	Pinkish Red/ Red	Yellow	Red/ Pink	

Figure 1. Result Interpretation chart for KB 013 HiBacillus™ identification Kit

Results

The pure isolate was cultured on Pikovskaya's (PVK) medium. After 48 hour, morphological characters of colonies were cream colour, round, small size, entire margin and raise form. Then, slow growing yellow, mucoid, round, smooth and domed shape with entire margin were found on the medium. According to gram stain, selected bacterium MMT-3 was rod

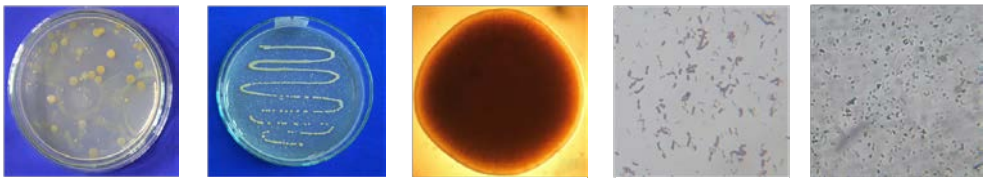
shape, gram positive and endospore present. The results of some biochemical test are presented in Table 1 to 5 and Figure 2 to 10.

Table 1. Cell morphology and biochemical characteristics of selected bacterium MMT-3

Sr. No.	Test	Results
1	Cell morphology	rod
2	Gram staining	Gram-positive
3	Spore staining	(+)
4	Catalase test	(+)
5	Oxidase test	(-)
6	Motility	(+)
7	Aerobic/Anaerobic	Facultative anaerobic
8	Citrate utilization	(+)
9	Methyl red (MR) test	(-)
10	Voges-Proskauer (VP)	(-)
11	Urea hydrolysis	(+)
12	Hydrogen sulphide (H_2S) production	(+)
13	Phenylpyruvic acid (PPA)	(-)
14	Nitrate reduction test	(+)
15	Salt Tolerance (1.5%-6.5%)	(+)

(+) positive reaction (-) negative reaction

Colony character and cell morphlogy



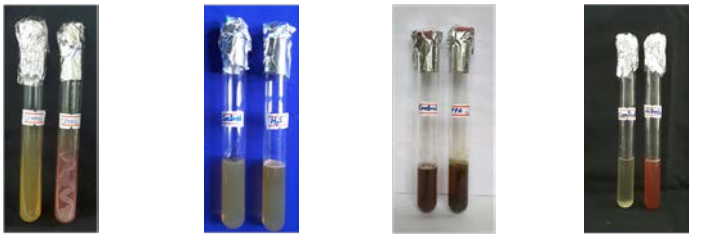
Starter culture from soil sample | **Colony character** | **Colony morphology** | **X 100 Gram staining (+)** | **X 100 Spore staining (+)**

Figure 2. Starter culture, colony character and cell morphology of selected bacterium MMT-3

Catalase test **Oxidase test**

Figure 3. Biochemical test for catalase and oxidase

Aerobic/Anaerobic test | **Motility test** | **Citrate utilization** | **Methyl red (MR) test** | **Voges-Proskauer (VP)**



Urea hydrolysis | **Hydrogen sulphide** | **Phenylpyruvic acid (PPA)** | **Nitrate reduction test**

Figure 4. Biochemical test for aerobic/anaerobic, motility, citrate utilization, methyl red (MR), voges-proskauer (VP), urea hydrolysis,hydrogen sulphide, phenylpyruvic acid (PPA) and nitrate reduction

Figure 5. NaCl salt tolerance of selected bacterium MMT-3

Table 2. Fermentation of carbohydrate on selected bacterium MMT-3

Sr. No.	Source	Carbohydrate fermentation	
		Acid production	Gas production
1	Dextrose	(++)	(+)
2	Sucrose	(+)	(-)
3	Maltose	(+)	(-)
4	Lactose	(+)	(-)
5	Xylose	(+)	(-)
6	Galactose	(+)	(-)
7	Fructose	(+)	(-)
8	Arabinose	(+)	(-)

(++) Maximum activity (+) minimum activity

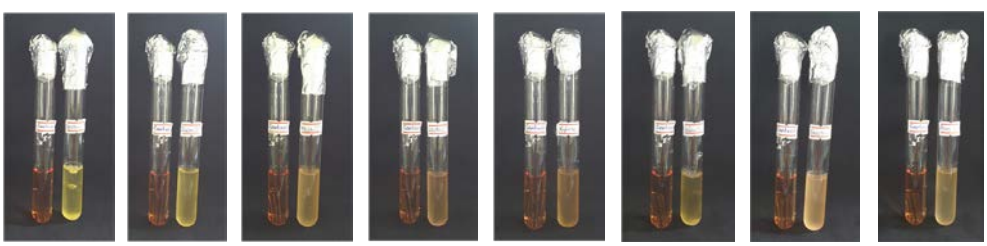


Figure 6. Carbohydrate fermentation test for dextrose, sucrose, maltose, lactose, xylose, galactose, fructose and arabinose

Table 3. Starch hydrolysis tests of selected bacterium MMT-3

Sr. No.	Source	Results
1	Rice	(-)
2	Sticky rice	(-)
3	Glue	(-)
4	Tapioca	(-)
5	Soluble starch	(-)
6	Corn	(-)
7	Potato	(-)
8	Wheat	(+)

(+) Little hydrolysis (-) non-hydrolysis

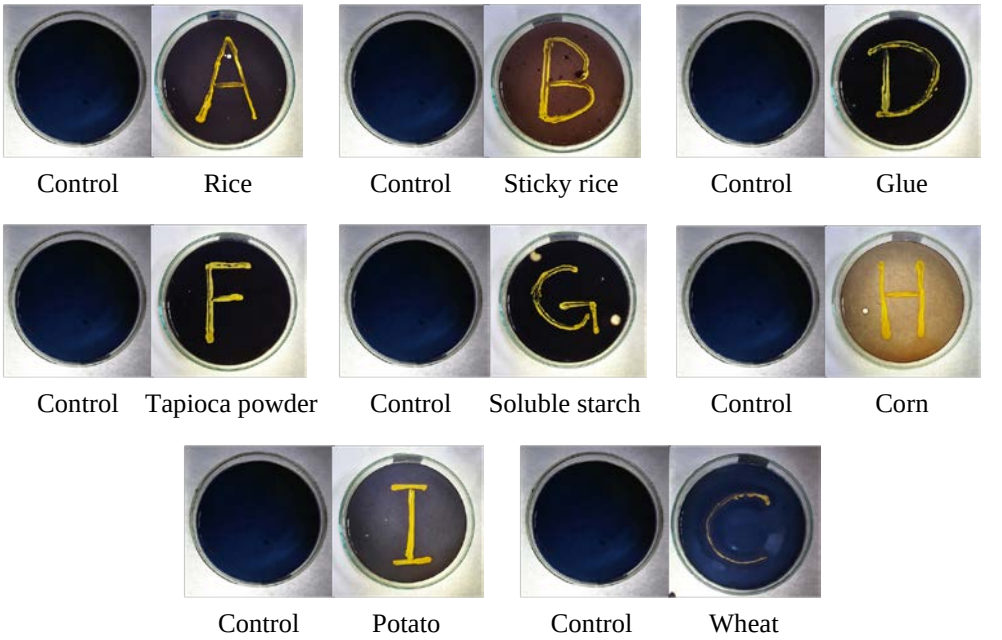


Figure 7. Rice, sticky rice, glue, tapioca powder, soluble starch, corn, potato and wheat of MMT-3

Table 4. Biochemical tests of selected bacterium MMT-3

Sr. No.	Test	Results
1	Gelatin hydrolysis	(-)
2	Casein hydrolysis	(+)
3	Potato plug	(-)
4	Esterase hydrolysis	(+)

(-) non-hydrolysis (+) hydrolysis

Figure 8. Gelatin and casein hydrolysis test of selected bacterium MMT-3

Figure 9. Potato plug test and asterase activity of selected bacterium MMT-3

Biochemical test recorded using KB013 HiBacillus^{TM} identification Kit (India)

Each KB 013 is a standardized, colorimetric test system based on carbohydrate utilization and other biochemical tests specific for the identification of *Bacillus* species. The tests are based on the principle of pH change and suitable utilization. *Bacillus* species on incubation exhibit metabolic changes which are indicated by a colour change in the media that

can be either interpreted visually or after addition of reagent wherever required. These results are shown in Table 5 and Figure 10.

Table 5. Biochemical test results of selected bacterium MMT-3

Sr. No.	Biochemical tests	Results
1	Malonate	(+)
2	Voges Proskauer's	(-)
3	Citrate	(+)
4	ONPG	(+)
5	Nitrate Reduction	(+)
6	Catalase	(+)
7	Arginine	(-)
8	Sucrose	(+)
9	Mannitol	(-)
10	Glucose	(+)
11	Arabinose	(+)
12	Trehalose	(+)

(+) = positive reaction (-) = negative reaction

Figure 10. Biochemical tests of selected bacterium MMT-3

Identification of selected phosphate solubilizing bacterium MMT-3

The selected bacterium, MMT-3 was characterized by morphological characters. Morphological analysis indicated that, the strains was facultative anaerobic, non-pigmented and non-sporulating bacteria. By Gram's staining procedure, gram-positive, rod-shaped bacteria and spore-staining was found to be positive. Catalase and nitrate reduction tests were positive. These results were presented in Figure 11.

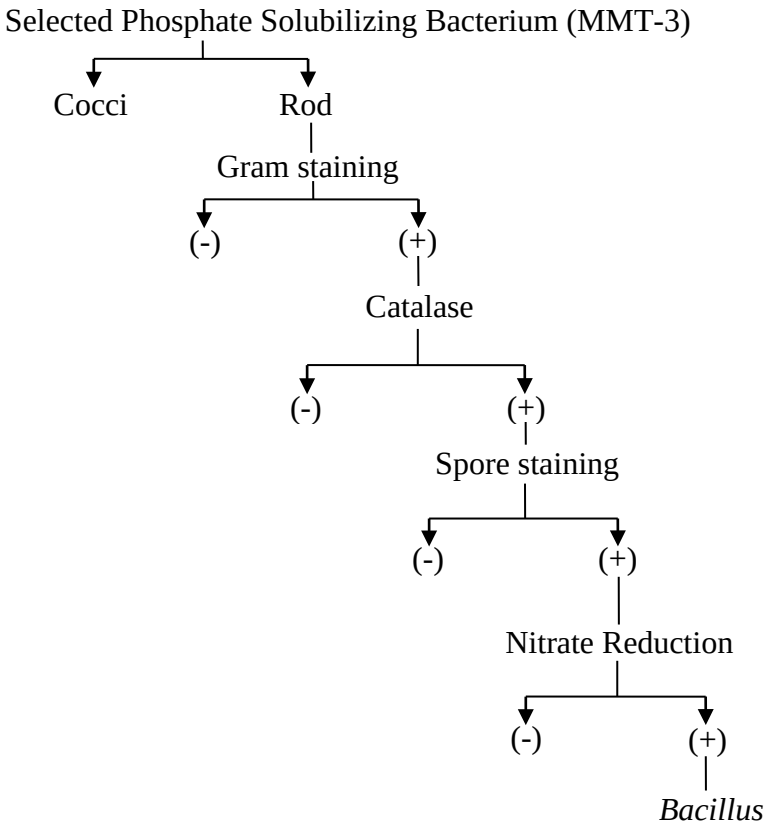


Figure 11. Schematic diagram of selected phosphate solubilizing bacterium MMT-3 for possible genus *Bacillus* (Bradshaw, 1992)

Discussion and Conclusion

The morphological and biochemical method of identification of bacteria is the classical method of characterization of bacteria (Holt *et al.*, 1994).

In the biochemical characterization, MMT-3 was gram-positive and rod-shape, spore present, catalase positive, oxidase test negative, motility present, facultative anaerobic, methyl red (MR) and Voges-Proskauer (VP) test negative; H_2S was produced, citrate utilization positive, urea hydrolysis positive, phenylpyruvic acid (PPA) test and nitrate reduction test negative; MMT-3 can grow in NaCl salt (1.5% to 6.5%).

In the carbohydrate fermentation, acid was produced in the sugar dextrose, sucrose, maltose, lactose, xylose, galactose, fructose and arabinose and gas was not produced except dextrose; MMT-3 can not hydrolyse rice, sticky rice, glue, tapioca, soluble starch corn and potato except wheat; gelatin hydrolysis negative, casein hydrolysis positive and esterase activity present, no-growth on potato slice.

These characters were similar to the investigation on Genus *Bacillus* by Buchanan, 1974. Therefore, MMT-3 was classified as the Genus *Bacillus.*

Graumann, 2007 described that the *Bacillus* genus is a heterogeneous group of Gram-positve, facultative anaerobic, endospore-forming bacteria and ubiquitous in nature.

It can manage to get the results by using manual and identification kit. However, manual test did not differe in getting results from identification kit. Moreover, *Bacillus* was also identified by using KB 013 HiBacillus^{TM} (Identification kit). Therefore, these results were the same and confirmed as the genus *Bacillus.*

It was concluded that the present work was to study some biochemical characterization. Further work will be study on the purification and identification of isolated compounds of MMT-3 and minimum inhibitory concentration (MIC).

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