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Editorial Board

Contents

Study of Pathogenic Fungi Infected on Stem of *Piper betle* L.

Kyaw Kyaw Lwin*

Abstract

In this study, the pathogenic fungi were isolated from the stem of *Piper betle* L.. It was collected from Kalay Township during 2018-2019. In the present study, two kinds of fungi were isolated, namely *Fusarium* sp. and *Alternaria* sp.. The isolation of pathogenic fungi were carried out by the methods of Suto (1999). The macroscopical and microscopical characters were also observed by the methods of Barnett (1956). The local people are used the leaves of *Piper betle* L. as medicine to cure the eye disease.

Keywords: Pathogenic fungi, stem of *Piper betle* L.

Introduction

Angiosperms or flowering plants form the largest group of plant kingdom, including about 300 families (411 families, Hutchinson), 8000 genera and 300000 species. They are considered to be highest evolved plants on the surface of the earth. (Pandey, 1999).

Plant pathology is a science that deals with two subjects disease in plants and diseases of plants. Disease in plants is continuous malfunction diseases of plants are specific examples of different kinds of malfunctions. 2 nt diseases reduce both the quantity and the quality of plant products (Whetzel, 1929).

A variety of relationships exist between fungal endophytes and their host plants, ranging from mutualistic or symbiotic to antagonistic or slightly pathogenic. The term "pathogen" means the organism that incites diseases on living being. It may be fungus, bacteria, virus, etc. The fungal disease are importance because they sometimes cause much damage and destruction. Affected cells and tissues of diseased plants are usually weakened or destroyed by disease causing agents. The ability of such cells

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and tissues to perform their normal physiological functions is reduced or the plant dies (Amold, 2007).

The *Piper betle* L. were collected from Indaing-gyi village, Kalay Township. Kalay District is situated in the Western part of Sagaing Region. It consists of three Township, Kalay Township, Kalaywa Township and Mingin Township. Latitudinally, it lies between 23° 11' 15" N and 23° 11' 33" N. Longitudinally it lies between 94° 04' 21" E (Google, 2019). It is nearly 3337 square miles in extent, from the North to South is 84 miles and 58 miles from East to West. It is surrounded by Tamu District and Mawleik District in North, by Shwebo District in East, by Monywa District and Gangaw District in South, and by Chin State in West. Kalay Township is the smallest in Kalay District. The altitude of Kalay is 182.8 meter above sea level. Kalay is surrounding by hills.

In Myanmar, the *Piper betle* L. are economically importance pla[illegible]3
The local people are used the leaves of *Piper betle* L. as medicine to cu[illegible]
the eye disease.

Therefore, pathogenic fungi was investigated from the stem of *Piper betle* L.. In this study two pathogenic fungi were isolated, namely *Fusarium* sp. and *Alternaria* sp.. The aims and objectives of this paper are to know the variety of pathogenic microorganisms from the diseases infected stem of *Piper betle* L. and to study their macroscopical and microscopical characters of pathogenic microorganisms.

Literature Review

In plants, disease can be defined as the malfunctioning of host cells and tissues that results from their continuous irritation by a pathogenic agent or environmental factor and lead to the development of symptoms. Disease is a condition involving abnormal changes in the partial impairment or death of the plant or its parts. As a science, plant pathology attempts to increase our knowledge of the causes and the development of plant diseases. It is also a science with a more practical goal: develop controls for all plant diseases in order to save the produce that today is destroyed by plant diseases and to make it available to the hungry and ill-clothed millions of our increasingly overpopulated world (Wilson, 1995).

The family Piperaceae are erect or scandent herbs, shrubs, leaves alternate, rarely opposite, petiolate, palmately or penninerved, often fleshy;

flowers very minute, bracteate, bisexual or unisexual, in dense fleshy spikes or the spikes umbellate; perianth absent; stamens 1-10, hypogynous, anthers each of 2 distinct or confluent cells, ovary superior, 1-loculed, the carpels 2-5, the ovule solitary, the style 0-1, the stigma 1-5, fruit a small drupe; seed small (Lawrence, 1951).

Whenever plants are disturbed by pathogens or by certain environmental conditions and one or more of these functions is interfered with beyond a certain deviation from the normal, then the plants become diseased. The primary causes of disease are either pathogenic living organisms (pathogens) or factors in the physical environment. Pathogens may cause disease in plants by (1) weakening the host by continually absorbing food from the host cells for their own use; (2) killing or disturbing substances they secrete; (3) blocking the transportation of food, mineral nutrients, and water through the conductive tissues; and (4) consuming the contents of the host cells upon contact (Gimenez *et al.*, 2007).

Plant diseases are sometimes classified according to the symptoms they cause (root rots, cankers, wilts, leaf spots, scabs, blights, anthracnose, rusts, smuts, mosaics, yellows), according to the plant organ they affect (root diseases, stem diseases, foliage diseases, fruit diseases), or according to the types of plants affected (field crop diseases, vegetables), or accord to the types of plants affected (field crop diseases of ornamental pla (Agrios, 1988).

Sinha (2008) stated that the genus *Fusarium* is belonging to the family Tuberculariaceae. The family includes about 152 genera. *Fusarium* and *Tubercularia* are the two commonly known among these. The genus *Fusarium* includes a large number of species and many forms within species. Many of these are saprobic or saprophytic. Some are only mild facultative parasites. Others are parasitic. All however, have a saprophytic stage. Some of the parasitic species are soil inhabitants and can live indefinitely as soil saprohytes but some are soil invaders. The latter soon die if they are not able to infect a suitable host plant. The parasitic form-species cause rot of stores fruits, vegetables and other commodities and are responsible for dry rot of potato tubers of colocasia corms or soft rot of rhizomes of ginger during storage. Some are mild root parasities and some primarily cortical invaders which cause stem cankers, food rots and pre-emergence damping off diseases.

According to Barnett (1969), the characters of genus *Fusarium* are mycelium extensive and cottony in culture, often with some tinge of pink, purple or yellow, in the mycelium or medium; conidiophores variable, slender and simple or stout, short, branched irregularly or bearing a whorl of phlalides, single or grouped into sporodochia; conidia hyaline, variable, principally of two kinds often held in a mass of gelatinous material; macroconidia several-celled slightly curved or bent at the pointed ends, typically canoe-shaped: microconidia 1-celled, ovoid or oblong, born singly or in chains; some conidia intermediate, 2- or 3- celled, oblong, oblong, borne slightly curved; parasitic on higher plants or saprophytic on decaying plant material. A large and variable genus, sometimes placed in the Tuberculariaceae because some species produce sporodochia.

The genus *Alternaria* belonging to the family Dematiaceae. It includes imperfect fungi in which both the mycelium and the conidia are dark-coloured. Most of the species are saprophytes. Some are plant pathogens and a few parasitize men and animals. The mycelium is not large and extensive but is short, septate, branched, light brown but becoming darker with age. The colonies of *Alternaria* are woolly but more compact with the underside very dark-coloured. In the parasitic species, the hyphae are intercellular at first, but later penetrate cells of the invaded tissues and thus become intracellular. The cells are usually multinucleate. The conidia are produced at the tips of ordinary hyphae which are comparatively short and dark coloured. Special hyphae termed conidiophores are not recognizable. The conidia are large, dark coloured, several celled and beaked. The number of cells varies from 8-14 or even more. The component cells occur packed in muriform, conical masses. The septa dividing the spore into cells are both transverse and vertical and their number is not fixed (Sinha 2008).

The general characteristics of the genus *Altermaria* are: Conidiophore dark, simple, rather short or elongate, typically bearing a simple or branched chain of conidia: conidia dark typically with both cross and longitudinal septa: variously shaped, obclavate to elliptical or ovoid, frequently borne acropetally in long chains, less often borne singly and having an apical simple or branched appendage. Parasitic or saprophytic on plant material (Barnett 1969)

Materials and Methods

Collection of plant samples

The diseases infected stem of *Piper betle* L. (Figure 1. A and B) was collected from Indaing-gyi village, Kalay Township during 2018 to 2019. The plant samples were placed in sterile plastic bags and brought to the laboratory for isolation of pathogenic fungi. The collected plant was identified according to the morphological characters shown as the literature of Hooker (1885).

Isolation of pathogenic Microorganisms

Isolation of pathogenic fungi was done according to the methods described by Suto (1999). Firstly, the diseases infected stem segment was washed in running water for 15 min. The stem was cut into about 1 cm pieces. Then it soaked in 95% alcohol for 15 sec and washed with distilled water. They were cut into smaller pieces. Then the smaller pieces were dried on the sterilized paper for 30 min. and placed on agar plate.

Preparation of culture medium

In isolation of pathogenic fungi, the two media (WGA and PGA) were used for the isolation of pathogenic fungi. PGA medium is used to study for the macroscopical characters and WGA medium for the microscopical characters of pathogenic fungi.

Preparation of culture medium (Suto, 1999)

Macroscopical characters

In this screening, PGA medium was employed for the macroscopical characters. The isolated strains were incubated on to the PGA medium for 3-5 days.

PGA medium (Potato Glucose Agar Medium)

Potato powder	0.5 g
Glucose	1.6 - 1.8 g
Agar	1.8 - 2.0 g
D.W	100 ml
pH	7

(For fungi, Penicillin (0.8 g) was added to the medium after autoclaving)

Microscopical characters

The WGA medium was utilized for the microscopical characters. The isolated fungi were inoculated onto the WGA medium and incubated or 5-7 days.

WGA medium (Water Glucose Agar Medium)

Glucose	1.6 - 1.8 g
Agar	1.8 - 2.0 g
D.W	100 ml
pH	7

(For fungi, Penicillin (0.8 g) was added to the medium after autoclaving)

Results

Morphological study of the collected plant specimens

Scientific Name - *Piper betle* L. **(Figure-1- A and B)**

Family - Piperaceae

English Name - Betel pepper, Betel vine

Myanmar Name - Kun

Outstanding features

Perennial, woody, smooth climber, dioecious; stem is swollen at the nodes. Leaves simple, alternately, petiolate, exstipulate, coriaceous; blade ovate to ovate oblong, cordate at the base, entire at the margin, acuminate at the apex, with 2-3 pairs of curved veins from the base and one pair from the midrib, shiny bright green. Inflorescence cylindrical; spike pendulous. Male spike, crowded with small flowers with 2 stamens. Female spike, crowded with 3-5 stigma of female flowers. Fruit fleshy drupe.

Part used - Stem

Uses -The leaves are astringent, aromatic, antiseptic, carminative, aphrodisiac, stimulant, expectorant and sialagogue. The leave juice with honey is given to children in colic, indigestion,

diarrheas and laryngitis. The leaves are chewed to remove foul odour from the mouth. The leaf juice is used as eye drops in ophthalmic and other painful eye diseases and night blindness (Pandey, 1978).

Macroscopical and Microscopical Characters of microorganisms

Isolation of Pathogenic Microorganisms

Universities Research Journal 2020, Vol. 13 namely *Fusarium* sp. and *Alternaria* sp. were ısolated from the dısease ınfected stem of *Piper betle* L.

Macroscopical characters of KKL-1 (*Fusarium* sp.)

After 3-5 days cultivation, it was observed that KKL-1 was white purple colour in colonies at 25ºC on PGA medium (Figure 1. C).

Microscopical characters of KKL-1 (*Fusarium* sp.)

After 5-7 days of incubation, the fungus KKL-1 was observed that the mycelium was extensive and cottony in culture, often with some tinge of pink, purple or yellow in medium and become dark-coloured at maturity; the conidiophores was variable, slender and simple, short, branched irregularly, bearing a whorl of phlalides, the conidia was hyaline, variable, the macroconidia are several-celled, slightly curved or bent at the pointed ends (Figure 1. D).

Macroscopical characters of KKL -2 (*Alternaria* sp.)

After 3-5 days cultivation, it was observed that KKL-2 was light brown colour in colonies at 25ºC on PGA medium (Figure 1. E).

Microscopical characters of KKL-2 (*Alternaria* sp.)

After 5-7 days of incubation, the fungus KKL-2 was observed that not large and extensive but it is short and septate, branched, light brown but becoming darker with age. Conidiophores dark and simple, rather short and typically bearing a simple or branched chain of conidia; Conidia dark, typically with both cross and longitudinal septa; variously shaped and obclavate to elliptical or ovoid (Figure 1 . F).

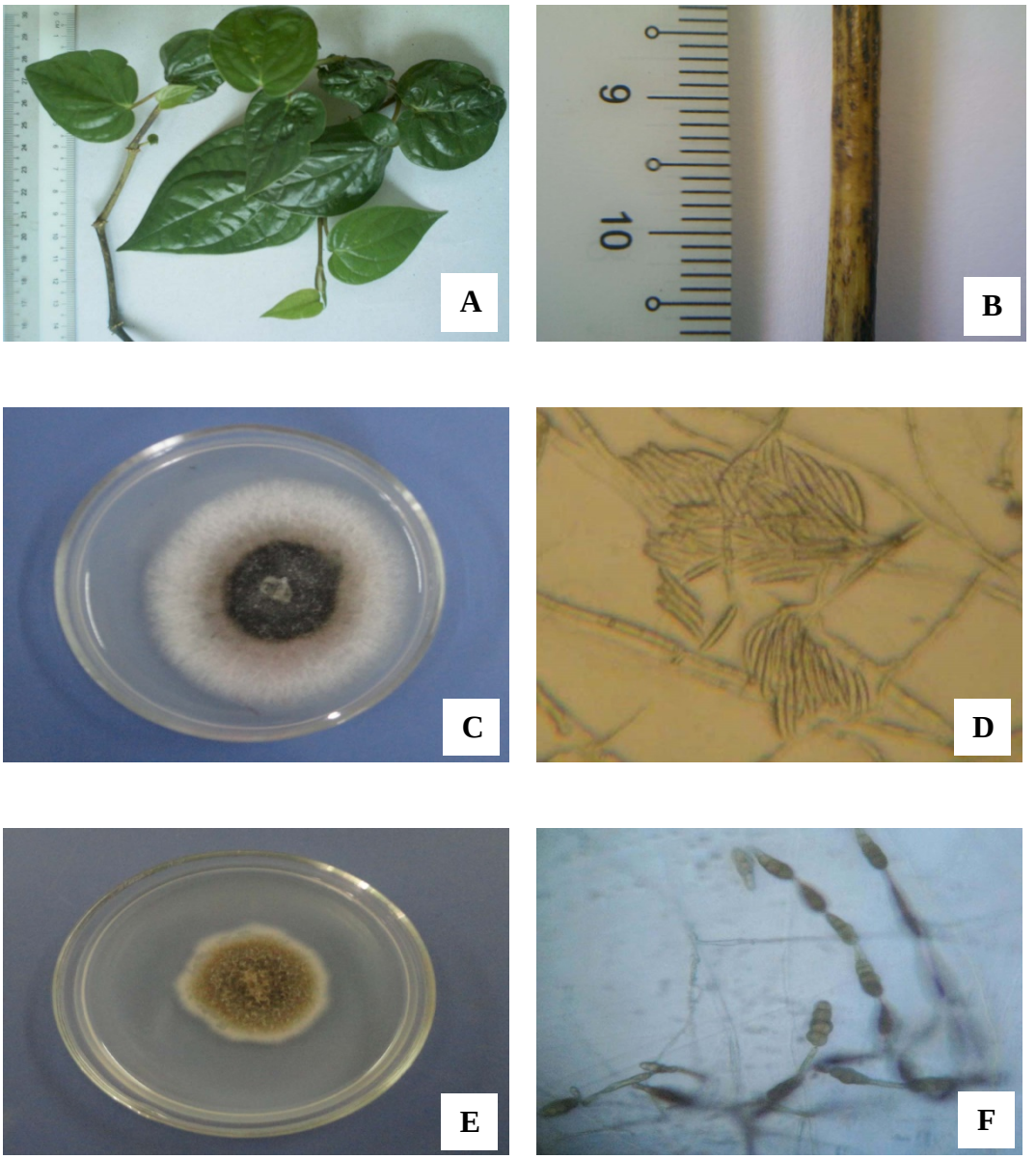

Figure 1. Macroscopical and Microscopical characters of KKL-1 (*Fusarium* sp.) and KKL-2 (*Alternaria* sp.)

A. Habit of *Piper betle* L.

B. Diseases infected stem of *Piper betle* L.

C. Macroscopical character of pathogenic fungi on PGA medium

D. Microscopical character of KKL-1 (*Fusarium* sp.)

E. Macroscopical character of pathogenic fungi on PGA medium

F. Microscopical character of KKL-2 (*Alternaria* sp.)

Discussion and Conclusion

In the present study, two pathogenic fungi were isolated from the stem of *Piper betle* L. These macroscopical and microscopical characters are agreed with the Barnett (1969) and Sinha (2008). Therefore, the KKL-1 fungus is the *Fusarium* sp..

These macroscopical and microscopical characters are agreed with the Barnett (1969) and Sinha (2008). Therefore, the fungus KKL-2 is the *Alternaria* sp..

According to Sinha (2008), the genus *Fusarium* sp. is belonging to the family Tuberculariaceae and the family includes about 152 genera. The genus *Fusarium* sp. includes a large number of species and saprophytic or parasitic. The dark mycelium produces thick bands which plug the vascular tissues and produce toxic secretions and the plant wilts and dies.

Barnett (1969) stated that the *Fusarium* sp. is parasitic on higher plants or saprophytic on decaying plant material. These characters are agreeable with the present study.

Sinha (2008) showed that *Alternaria* sp. is belonging to the family Dematiaceae. Most of the species are saprophytes. Some are plant pathogens and a few parasitize men and animals.

In the present study, the genus *Alternaria* sp. is found as the parasitic pathogenic fungus. Therefore, these characters are agreeable with the Barnett (1969) and Sinha (2008).

In this paper, two pathogenic fungi *Fusarium* sp. and *Alternaria* sp. were isolated from the infected stem of *Piper betle* L. Their macroscopical and microscopical characters were studied.

Pandey (1978) showed that the leaves juice of *Piper betle* L. is used as eye drops in ophthalmic and other painful eye diseases. In study area, the

Piper betle L. are economically importance plant and the leaves are used to cure the eye disease.

Therefore, these pathogenic fungi should be control. It is necessary to further study for the properties and structure of the compound extracted from the leaves of *Piper betle* L..

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Pollen Morphology of Genus *Strobilanthes*

Khin Su Su Kyaw*

Abstract

The pollen morphology of ten species of genus *Strobilanthes* from family Acanthaceae was presented. In the present paper, all the collected species of *Strobilanthes* are wild. The tricolporate aperture type, prolate or perprolate grain shape, distinctly reticulate or wavy flanges exine ornamentation were studied in this genus. In *Strobilanthes*, colpi were mostly longicolpate and pori were mostly circular and lalongate. An artificial key was constructed based on aperture type, grains shape, size, pseudocolpi and exine ornamentation. The pollen description and colour photographs were also presented.

Keywords: Pollen morphology, *Strobilanthes*

Introduction

Family Acanthaceae is one of the widely distributed families in tropical to subtropical and temperate regions. It is a large family of about 240 genera and 2200 species in the world (Laurence 1951). Kress *et. al*. (2003) stated that 43 genera and 280 species were attributed to Myanmar.

Erdtman (1969) described that Acanthaceae pollen are isopolar, bilateral, di-polyaperturate, 2- to 4-porate; perprolate, prolate; colporate grains are sometimes provided with pseudocolpi. Some of the pollen in Acanthaceae are similar to those of the Bignoniaceae and the Pedaliaceae.

The genus *Strobilanthes* is palynologically diverse and its pollen morphology has been used in previous classifications. The pollen are ellipsoidal and spheroidal and tricolporate and triporate with pseudocolpus. The pseudocolpi divide the exine into 12- 21 longitudinal ribs; which are usually bireticulate, with a coarse ladder-like reticulum and a depressed regular microreticulum in the lumina.(Bennett and Scotland 2003).

Carine (1998) proposed that, in some species of *Strobilanthes* have long and narrow ectoapertures, circular and lalongate endoaperture. These pollen grains has longitudinal ribs, each rib with a ladder-like reticulum. Moore *et al* (1991) stated that morphological characteristics of pollen grains

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also can be useful phylogenetic marker in studies of plant taxonomy because many pollen traits are influenced by the strong selective forces involved in various reproductive processes, including pollination, dispersal and germination.

The present paper focuses on type, shape, size, sculpture of pollen of ten species of genus *Strobilanthes* from family Acanthaceae. Thus, this research is carried out to the information of only genus of pollen morphology that will be useful in plant identification and to evaluate the morphological difference of pollen grains among the flowering plants.

Materials and Methods

The specimens were collected from Pyin Oo Lwin Township (Mandalay Region) and Ywa ngyn Township (Southern Shan State). The specimens were identified by referring to Hooker (1878), Backer (1965), Dassanayake (1998) and Qi-ming (2008). Myanmar names of the collected species were referred to Kress *et. al.* (2003). Pollen samples were freshly collected from the anthers of blooming flowers and stored in glass vial with glacial acetic acid.

Pollen samples were acetolysed by the method of Erdtman (1952). The acetolysis solution was mixed with 9 parts of glacial acetic acid and 1 part of concentrated sulphuric acid was added. Acetolysis mixture 1cc was poured into the test-tube containing the pollen samples. The sample bottle was stirred with a glass rod. The test-tube was heated in a water-bath at 80°C for 15 minutes. The test-tube was allowed to cool, and the sample diluted with distilled water and centrifuged for 30 minutes at 3000 rpm. After centrifuging, the distilled water was removed and then the specimen was transferred to the storage bottles. The mounted slides were observed under light microscope to study the pollen morphology. For each species, more than 10 pollen grains were measured and recorded. The terminology used in the identification of pollen is according to Erdtman (1952), Hoen (1999) and Hesse *et al.* (2009).

Results

Ten species of genus *Strobilanthes* from family Acanthaceae had been identified and studied the morphological characteristics of pollen grains.

Pollen Morphology of Study Species

1. *Strobilanthes asper* Wight (Fig. 1. A, B)

Tricolporate, perprolate, 100-115 × 39-50 μ in length and breadth; pori lalongate, about 15×20 μ in length and breadth ; colpi longicolpate, 90-110× 2.5μ in length and breadth, distinctly pseudocolpi present, the number of pseudocolpi 12, the ribs ladder like reticulum ; exine about 2.5μ thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 2.5-6.0 μ in width, the muri simplibaculate, about 1μ wide .

Location : Southern Shan State, Ywa ngyn Township

2. *Strobilanthes auriculatus* (Wall.) Nees (Fig. 2. A, B)

Tricolporate, perprolate, 80-90 × 40-45 μ in length and breadth; pori lalongate, about 12.5 × 15 μ in length and breadth ; colpi longicolpate, 70-80 × 4μ in length and breadth, distinctly pseudocolpi present, the number of pseudocolpi 15, the ribs ladder like reticulum; exine about 2.5μ thick, sexine as thick as nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 2.5-5.0 μ in width, the muri simplibaculate, about 1μ wide .

Location : Mandalay Region, Pyin Oo Lwin Township

3. *Strobilanthes divaricatus* T.Anders. (Fig. 3. A, B)

Tricolporate, prolate, 75-80 × 60-70 μ in length and breadth; pori circular, about 15 μ in diameter ; colpi longicolpate, 70-80 × 2.5μ in length and breadth, distinctly pseudocolpi present, the number of pseudocolpi 12, the ribs ladder like reticulum; exine about 4μ thick, sexine as thick as nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 3.0-4.5 μ in width, the muri simplibaculate, about 1.5μ wide .

Location : Mandalay Region, Pyin Oo Lwin Township

4. *Strobilanthes dupeni* Bedd. (Fig. 4. A, B)

Tricolporate, perprolate, 100-110 × 50-55 μ in length and breadth; pori lalongate, about 17.0 × 20 μ in length and breadth ; colpi

longicolpate,90-100 × 5μ in length and breadth, distinctly pseudocolpi present, the number of pseudocolpi 12, the ribs ladder like reticulum; exine about 3.5μ thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 1.5-2.5 μ in width, the muri simplibaculate, about 1.0μ wide .

Location : Mandalay Region, Pyin Oo Lwin Township

5. *Strobilanthes glabratus* Nees (Fig. 5. A, B)

Tricolparate, perprolate, 80-90 × 40-45 μ in length and breadth; pori circular, about 11.5 μ in diameter ; colpi longicolpate, 70-80 × 6.0μ in length and breadth, distinctly pseudocolpi present, the number of pseudocolpi 15, the ribs ladder like reticulum; exine about 3μ thick, sexine as thick as nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 2.5-3.5 μ in width, the muri simplibaculate, about 1.25μ wide.

Location : Mandalay Region, Pyin oo lwin Township

6. *Strobilanthes helicoides* (Nees) T.Anders. (Fig. 6. A, B)

Tricolporate, perprolate, 110-120 × 65-80 μ in length and breadth; pori lalongate, about 12.0 × 20 μ in length and breadth ; colpi longicolpate, 105-110 × 5.0μ in length and breadth, distinctly pseudocolpi present, the number of pseudocolpi 12, the ribs ladder like reticulum; exine about 4μ thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 3.5-5.0 μ in width, the muri simplibaculate, about 1.25μ wide.

Location : Mandalay Region, Pyin Oo Lwin Township

7. *Strobilanthes helictus* T.Anders. (Fig. 7. A, B)

Tricolporate, prolate, 70-75 × 60-65 μ in length and breadth; amb sub-rounded; pori lalongate, about 13.0 × 15 μ in length and breadth ; colpi longicolpate, 65-70 × 5.0μ in length and breadth, distinctly pseudocolpi present, the number of pseudocolpi 9; exine about 2.5μ thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 3.0-3.5 μ in width, the muri simplibaculate, about 1.25μ wide.

Location : Mandalay Region, Pyin Oo Lwin Township

8. *Strobilanthes papillosus* T.Anders. (Fig. 8. A, B)

Tricolporate, prolate, 70-75 × 60-65 μ in length and breadth; pori circular, about 11.5 μ in diameter ; colpi longicolpate, 68-72 × 5.0μ in length and breadth, distinctly pseudocolpi present, the number of pseudocolpi 18; exine about 3μ thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 1.5-2.5 μ in width, the muri simplibaculate, about 1μ wide.

Location : Southern Shan State , Ywa ngyn Township

9. *Strobilanthes phyllostachyus* Kurz (Fig. 9. A, B)

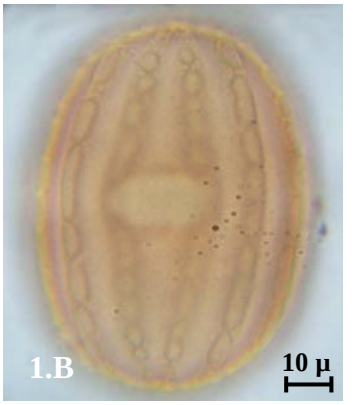
Tricolporate, prolate, 70-75 × 55-60 μ in length and breadth; pori lalongate, about 18 × 23 μ in length and breadth ; colpi longicolpate, 63-70 × 5μ in length and breadth, distinctly pseudocolpi present, the number of pseudocolpi 9, the colpi alternate with two pseudocolpi ; exine about 3μ thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 0.25-1. 5 μ in width, the muri simplibaculate, about 0.5μ wide.

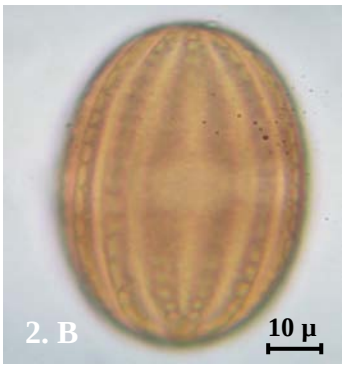
Location : Mandalay Region, Pyin Oo Lwin Township

10. *Strobilanthes walkeri* Arn. (Fig. 10. A, B)

Tricolporate, oblate, 65-75 × 80-85 μ in length and breadth; pori circular, about 13 μ in diameter ; colpi longicolpate, 60-65 × 4.0μ in length and breadth, distinctly pseudocolpi present, the number of pseudocolpi 15; exine about 4μ thick, sexine thicker than nexine; sculpturing wavy flanges, which run longitudinally and anastomose at various points, the flanges around each aperture in the shape of a short colpus .

Location : Southern Shan State , Ywa ngyn Township





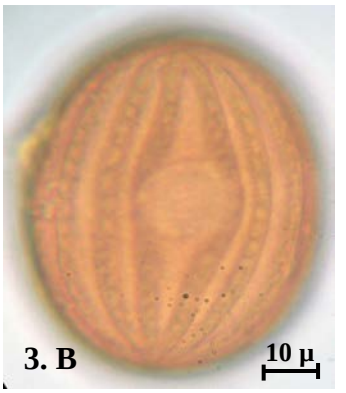
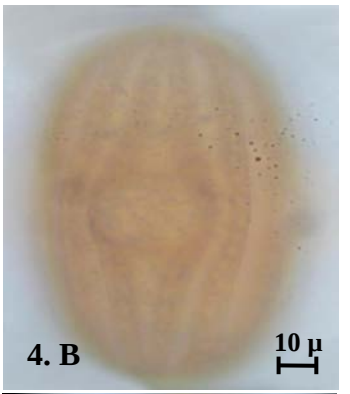


Figure 1. *Strobilanthes asper* Wight
A. Habit B. Equatorial view
Figure 2. *Strobilanthes auriculatus* (Wall.) Nees
A. Habit B. Equatorial view
Figure 3. *Strobilanthes divaricatus* T.Anders.
A. Habit B. Equatorial view



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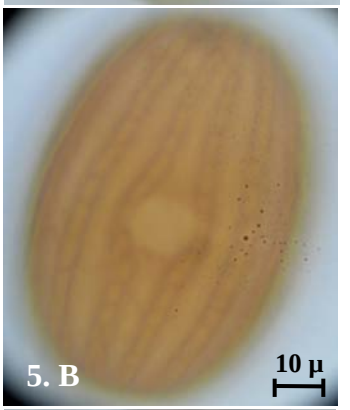

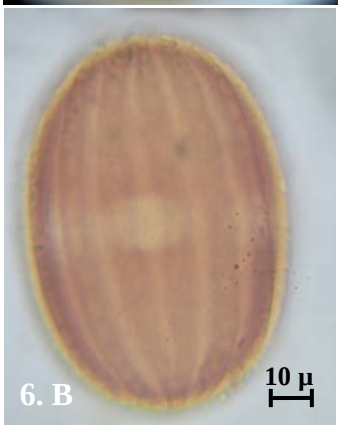

Figure 4. *Strobilanthes dupeni* Bedd.
A. Habit B. Equatorial view
Figure 5. *Strobilanthes glabratus* Nees
A. Habit B. Equatorial view
Figure 6. *Strobilanthes helicoides* (Nees) T.Anders.
A. Habit B. Equatorial view

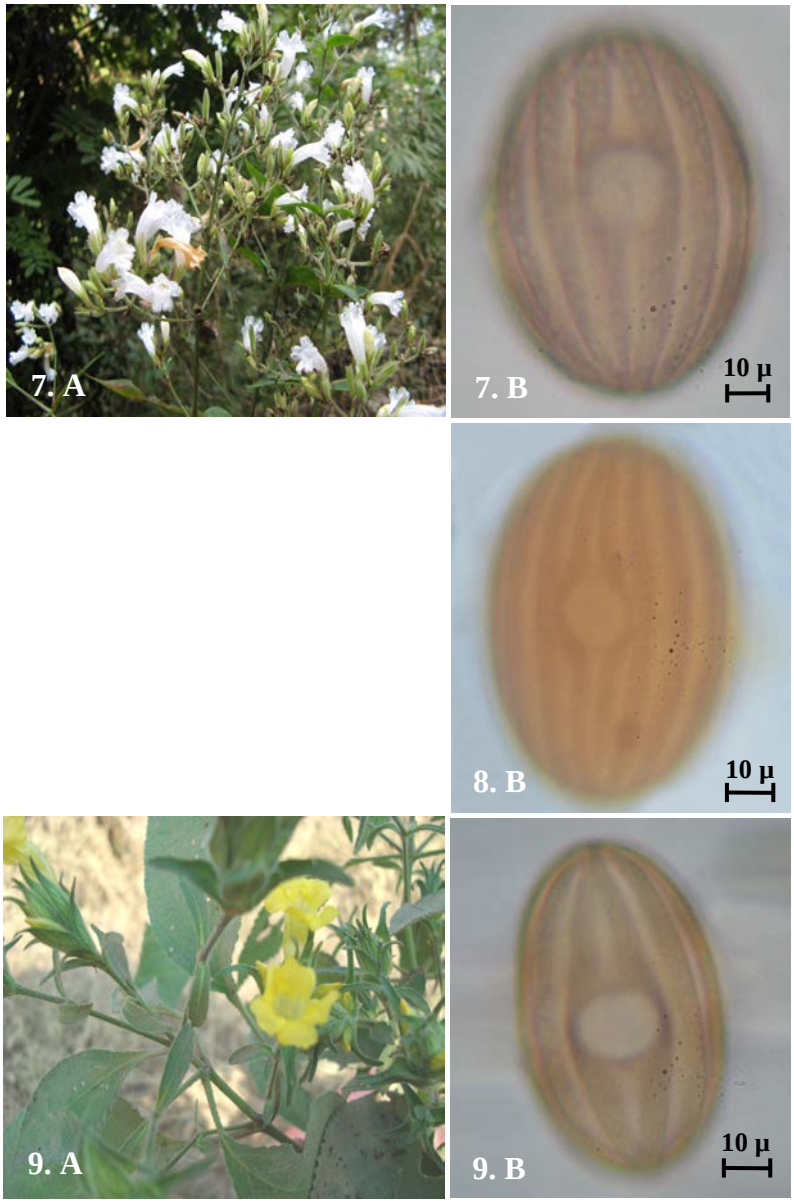

Figure 7. *Strobilanthes helictus* T.Anders.
A. Habit B. Equatorial view
Figure 8. *Strobilanthes papillosus* T.Anders.
A. Habit B. Equatorial view
Figure 9. *Strobilanthes phyllostachyus* Kurz
A. Habit B. Equatorial view

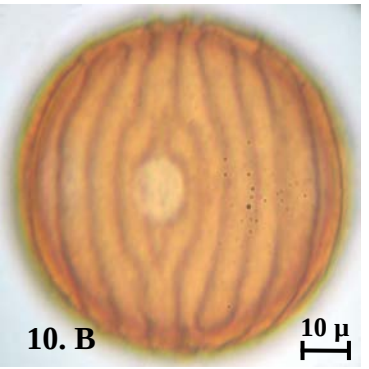

Figure 10. *Strobilanthes walkeri* Arn.
A. Habit B. Equatorial view

An Artificial key to the Pollen Morphology

1. Pseudocolpi more than 14 ---------- 2
1. Pseudocolpi less than 13 ----- ---------- 5
   2. Perprolate---------- 3
   2. Prolate or oblate ---------- 4
3. Pori lalongate ---------- *Strobilanthes auriculatus*
3. Pori circular ---------- *S. glabratus*
   4. Sculpture distinctly reticulate---------- *S. papillosus*
   4. Sculpture wavy flanges---------- *S. walkeri*
5. Pseudocolpi 9 ---------- 6
5. Pseudocolpi 12 ---------- 7
   6. Lumina more than 2.5µ width ---------- *S. helictus*
   6. Lumina less than 1.2µ width---------- *S. phyllostachyus*
7. Pori lalongate----------8
7. Pori circular---------- 9
   8. Lumina up to 6.0µ in width ---------- *S. asper*
   8. Lumina up to 2.5µ in width ---------- *S. dupeni*
9. Grain more than 100µ in length---------- *S. helicoides*
9. Grain less than 85µ in length ---------- *S. divaricatus*

Discussion and Conclusion

The pollen morphology of ten species of genus *Strobilanthes* from family Acanthaceae has been collected and studied. All the collected species are wild from Pyin Oo Lwin Township (Mandalay Region) and Ywa ngyn Township (Southern Shan State).

Erdtman (1952) stated that, Acanthaceae is eurypalynous family. This pollens are di-polyaperturate and colporate grains sometimes provided with pseudocolpi; peroblate to perprolate shape. In this study, the type of colporate pollen grains were found and then all *Strobilanthes* pollen grains with pseudocolpi present.

Benneth and Scotland (2003) proposed that, Strobilanthes pollens are ellipsoidal and spheroidal and tricolporate and triporate with pseudocolpus. The pseudocolpi divide the exine into 12- 21 longitudinal ribs. The present results, the number of pseudocolpi are 9, 12, 15 or 18. Nine pseudocolpi were found in two species (*Strobilanthes helictus* T.Anders. and *S. phyllostachyus* Kurz);Twelve pseudocolpi were found in four species (*Strobilanthes asper* Wight, *S. divaricatus* T.Anders, *S. dupeni* Bedd. and *S. helicoides* (Nees)T.Anders.; fifteen pseudocolpi were found in three species (*Strobilanthes auriculatus* (Wall.)Nees, *S. glabratus* Nees and *S. walkeri* Arn.); eighteen pseudocolpi were found in *Strobilanthes papillosus* T.Anders.

Carine & Scotland (1998) recorded that in some species of *Strobilanthes* have long and narrow ectoaperture, circular, lalongate endoaperture ; these pollen grains has logitudinal ribs, each rib with a ladder like reticulum. In this study, all species of *Strobilanthes* pollen grains have pseudocolpi between the ladder like ribs forming reticulum. The circular edoaperture was found in four species: *Strobilanthes divaricatus* T.Anders, *S. glabratus* Nees and *S. walkeri* Arn. while the remaining species are lalongate endoaperture type.

In addition to the exine sculpture, two types of exine sculpture have been recorded. The wavy flanges exine ornamentation found in *Strobilanthes walkeri* Arn., the flanges run longitudinally around each a short colpus. The distinctly reticulate exine sculpture were found in the remaining species. The lumina are heterobrochate and the muri are simplibacculate thus the size of muri and lumina are variable.

It was concluded that the study of pollen morphology is very important and unique in the classification and identification of plants. It is hoped that genus *Strobilanthes* pollen characters could provide the morphological information for further studies in various field of research.

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I am greatly indebted to Dr Tint Moe Thuzar, Rector and Dr U Khin Myot, Dr Khin Maw Maw Soe and Dr Myint Myint Oo, Pro-rectors of Yadanabon University for their permission to submit this research paper. I would like to express my sincere thanks to Dr Khin Pale', Professor and Head, Department of Botany, Yadanabon University for her kindness permission to carry out this research work. I wish to express thanks to Dr Khin Myo Win and Dr Win Naing, Professors, Department of Botany, Yadanabon University for their valuable advice to carry out this research work.

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Pollen Morphology of Ten Species of Subfamily Rauvolfioideae

Hnin Hnin Yu[1], Swe Swe Lin[2] & Nwe Nwe Yi[3]

Abstract

Pollen Morphology of 10 species belonging to 7 genera of Subfamily Rauvolfioideae were studied. All the species collected from Mandalay Region and Southern Shan State from 2016 – 2017. The types of pollen grains were monads. In the present study, the aperture types of pollen grains were porate and colporate. The shape of monad grains was suboblate, oblate and oblate spheroidal. The size of pollen grains was small, medium and large. The smallest pollen (18.8 – 22.0 × 20.0 – 23.8 µm) was found in *Leuconotis griffithii* Hook. f. and the largest pollen (50.0 – 52.5 × 52.5 – 56.5 µm) in *Alstonia angustiloba* Miq. The pollen photomicrographs of each species were presented by equatorial view and polar view.

Keywords : Rauvolfioideae, pollen grains

Introduction

Apocynaceae plants are often poisonous, containing rich alkaloids or glycoside, especially in the seeds and latex. Nonetheless, some plants are valuable sources of medicine, insecticides, fibers and rubber (Li *et al*. 1995; Smitinand & Larsen 1999 as cited in Prinya 2015) and have significant economic value. The gynoecium usually consists of two carpels, syncarpous or secondarily apocarpous. Fruit types include berries, drupes and follicular and seed features to be found in the family include wings, comas, and arils. (Sennblad & Bremer 1996).

Within the Apocynaceae, the degree of fusion of the gynoecium and the androecium provide significant characters, as do structures associated with pollen transport. Rauvolfioideae (84 genera about 1,000 spp.) are trees, shrubs, woody lianas, and vines, with alternate, opposite, or whorled leaves; rarely herbs in Old and New world. Anthers are mostly fertile and free from the stylar head; pollen shed as monads Nine tribes are recognized: Alstonieae, Alyxieae, Carisseae, Hunterieae, Melodineae, Plumerieae,

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[3] Professor and Head, Dr., Department of Biology, Sagaing University of Education

Tabernaemontaneae, Vinceae, and Willughbeae (Heywood *et al*. 2007). Most angiosperms release pollen as monads at anther dehiscene, however, various forms of aggregated pollen have arisen independently several times during the evolution of flowering plants.

Type 1 is found in five species of section *Alstonia* (Kuijt & Raymond 1997).The pollen grains of *Melodinus* are usually colporate, medium- sized monads, and their shape varies from suboblate to oblate spheroidal. They are mostly 3- aperturate, while four species have pollen with both three and four apertures, and one species has exclusively 4 - aperturate pollen (Van de ven & Van der ham 2006).

Materials and Methods

All of the specimens were collected from Mandalay Region and Shan States from July 2015 to October 2017. Morphology of pollen from mature flowers of 10 species belonging to 7 genera were examined at Department of Botany, University of Mandalay. Identification of specimens were carried out by referring to the literature such as Hooker (1882), Small (1933), Backer & Brink (1965), Myanmar names were referred to Hundley & Chit Ko Ko (1987) and Kress *et al.* (2003).

Methods for Acetolysis of Pollen grains

The pollen samples were acetolysed by the standard method of Erdtman (1960). The samples in the glass vials were put into a test tube, then crushed with a glass rod. The acetolyis solution was mixed using a measuring cylinder; 9 parts of glacial acetic acid was added, and then 1 part of concentrated sulphuric acid was added. The acid was dropped gently down the side of the tube. 1 cc of acetolysis mixture was poured into the test tube containing the pollen samples and stirred with a glass rod. The test tube containing the pollen samples stirred with a glass rod. The test tube containing the pollen sample was transferred to a water bath 70°- 80°C for 20-30 minutes. The test tube was diluted with distilled water and the test tubes were put to an electric centrifuge for 20-30 minutes at 3000 rpm. This was repeated twice decanting the water each time. After centrifuging and decanting, in a drops of dilute glycerin solution was added to the residue, then transferred and stored in air tight glass vials. Pollen grains were measured and recorded on their polar length (P); equatorial diameter (E); length and breadth of the colpi; diameter and length of pores and exine

thickness. These measurements were based on 10 grains per sample. The terminology used in the identification of pollen is according to (Erdman 1952 & 1954; Moore *et al.* 1991; Hoen 1999; Paldat 2015 & Hesse 2009).

Results

1. *Alstonia angustiloba* Miq., Fl. Ind. Bat. 2: 438. 1856. (Figure 1 A)

Myanmar name : Unknown

English name : Common pulai, Pulai

Flowering period : June to August

Outstanding Characters

Perennial tree with milky latex; stems and branches terete, bark brown or whitish grey. Leaves simple, whorls, petiolate; blades elliptic, oblong or obovate. Inflorescences terminal, umbelliform cymes, many - flowered, clustered. Flowers white, about 2.0 cm in diameter at anthesis. Calyx campanulate, 5 - lobed; tubes short; lobes ovate, apex acute to rounded. Corolla salver form, 5 - lobed; tubes long; lobes triangular. Stamens 5, free, inserted in upper half of corolla tube; filaments short; anthers sagittate, disk very small.

Pollen Morphology (Figure 1 B & C)

Tricolporate, oblate spheroidal, large, 50.0 – 52.5 μm × 52.5 – 56.5 μm in length and breadth; amb rounded triangular; colpi longicolpate, 46.3 – 47.5 × 8.8 – 12.5 μm in length and breadth, pseudocolpi present; pori lolongate, 10 – 15 × 8.8 – 11.3μm in length and breadth; exine about 1.3 μm thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate 1.5 – 3.8 μm; the muri simplibaculate about 1.3 μm wide.

2. *Alstonia rupestris* Kerr, Kew Bull. Misc. Inform. Kew. 43.1937.

(Figure 1 D)

Myanmar name : Unknown

English name : Unknown

Flowering period : June to September

Outstanding Characters

Perennial shrubs with milky latex; stems and branches lenticellate, brown. Leaves simple, whorls, exstipulate, sessile; blades narrowly elliptic, cuneate at the base. Inflorescences terminal, umbelliform cymes, many - flowered. Flowers creamy white, about 0.3 cm in diameter at anthesis. Calyx campanulate, 5- lobed; tubes very short; lobes ovate, apex obtuse, ciliate. Corolla salver form, 5- lobed; tubes very short; lobes oblong, apex rounded. Stamens 5, free, inserted at the mouth of the tube; filaments short; anthers dithecous, basifixed, disk 2 - lobed.

Pollen Morphology (Figure 1 E, F)

Tricolporate, suboblate, medium to large, 38.8 – 41.3 µm × 51.3 – 53.8 µm in length and breadth; amb rounded triangular, colpi ½ way to the pole, 18.8 – 21.3 × about 12.5 µm in length and breadth, pseudocolpi present; pori lolongate, 8.8 – 11.3 × 7.5 – 10.3 µm; annuli about 2.5 µm in width; exine about 1.3 µm thick, sexine thicker than nexine; sculpturing distinctly reticulate; the lumina heterobrochate, about 1.5 – 1.8 µm in width; the muri simplibaculate, about 1.3 µm wide.

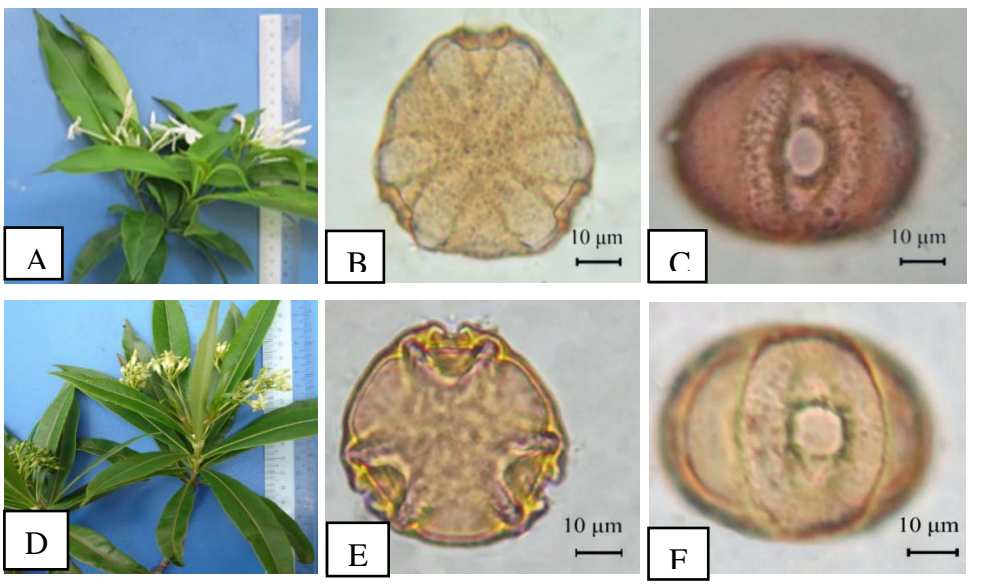


Figure 1.

A. Inflorescences of *Alstonia angustiloba* Miq.
B. Polar view of *A. angustiloba* Miq.
C. Equatorial view of *A. angustiloba* Miq.
D. Inflorescences of *A. rupestris* Kerr
E. Polar view of *A. rupestris* Kerr
F. Equatorial view of *A. rupestris* Kerr

3. ***Alstonia scholaris*** **(**L.) R. Br., Asclepiadeae 75. 1810. (Figure 2 A)

Echites scholaris L., Mant. Pl. 1: 53. 1767.

Myanmar names : Taung ma yo; Taung meoak

English name : Devil tree

Flowering period : September to December

Outstanding Characters

Perennial, deciduous trees with milky latex; stems and branches terete, creamy - brown. Leaves simple, whorled, exstipulate, petiolate; blades oblong, attenuate at the base. Inflorescences terminal, clustered cymes, many - flowered; peduncles long. Flowers greenish - yellow, 1.3 cm in diameter at anthesis, sessile. Calyx tubular, 5 - lobed; tubes short; lobes truncate. Corolla salver form, 5 - lobed; tubes cylindrical; lobes obovate, overlapping to the left. Stamens 5, free, epipetalous, included; sessile; anthers dithecous, sagittate.

Pollen Morphology (Figure 2 B & C)

Tricolporate, oblate spheroidal, small, 20.0 – 21.3 × 22.5 – 23.8 μm in length and breadth; amb rounded; colpi ½ way up to the pole, 8.8 – 10.0 × about 1.3 μm in length and breadth, pseudocolpi present; pori lolongate, 7.5 – 10.0 × 2.0 – 3.8 μm in length and breadth; exine about 1.3 μm thick, sexine thicker than nexine; sculpturing obscularly reticulate.

4. ***Alyxia reinwardtii*** Blume, Catalogus 43. 1823. (Figure 2 D)

A. lucida Wall. Roxb., Fl. Ind. ed. Carey 2 : 540. 1824.

Myanmar name : Unknown

English name : Selender climbing or scandent

Flowering period : May to August

Outstanding characters

Perennial, small tree with watery juice; stems and branches terete, pale brown. Leaves simple, opposite to whorls, exstipulate, petiolate; blades, elliptic to obovate, cuneate to obtuse at the base. Inflorescceces axillary or terminal, paniculate cymes, many-flowered. Flowers orangish white, about 0.3 cm in diameter at anthesis. Calyx cup - shaped, 5 -lobed;

tubes short; lobes ovate to lanceolate, apex acuminate to obtuse. Corolla tubular, 5-lobed; tubes short; lobes ovate to oblong, apex acute to rounded. Stamens 5, free, inserted, filaments short, anthers basifixed.

Pollen Morphology (Figure 2 E & F)

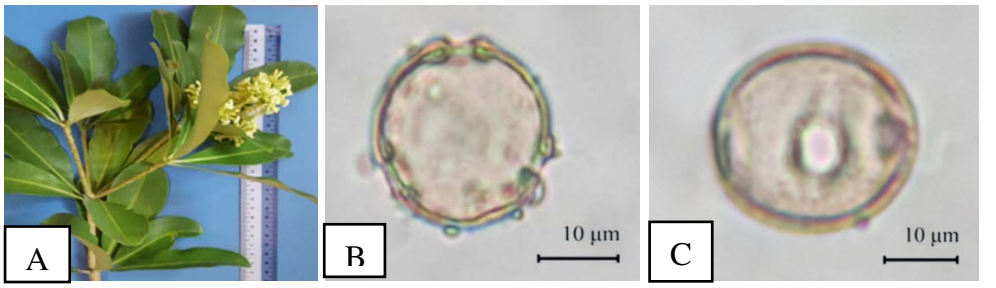
Diporate, oblate, medium to large, 37.5 – 41.3 × 56.3 – 61.3 µm in length and breadth; amb rounded; pori lolongate, about 35 × 15 – 20 µm in length and breadth; exine 1.3 – 1.8 µm thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate 2.5 – 3.8 µm width; the muri simplibaculate, about 1.3 µm wide.

5. *Carissa carandas* L., Mart, Pl. 1:52. 1767. (Figure 3A)

Myanmar Name	:	Taw khan
English name	:	Bengal currant
Flowering Period	:	April to June

Outstanding Characters

Perennial small trees with milky latex; stems and branches terete, green, spines boated. Leaves simple, opposite and decussate, exstipulate, petiolate; blades ovate to obovate, cuneate to rounded at the base. Inflorescences dichasial cymes, 2- to 3- flowered. Flowers white, about 1.5 cm in diameter at anthesis. Calyx campanulate, 5 - lobed; lobes ovate to narrowly ovate. Corolla salver form, 5 – lobed; tubes short. Stamens 5, free, inserted, attached to the tube; filaments short; anthers oblong.



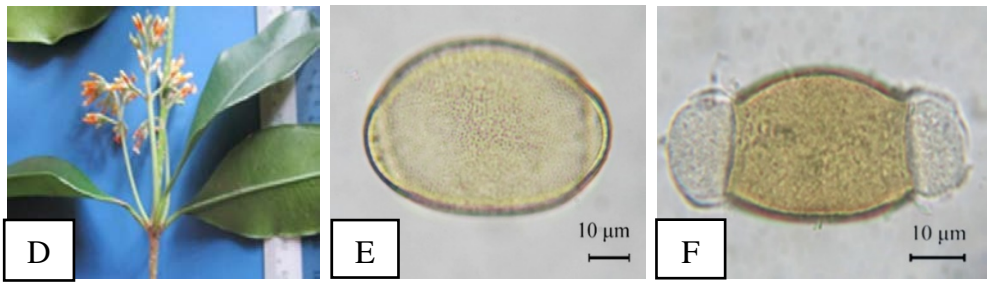


Figure 2.

A. Inflorescences of *Alstonia scholaris* (L.) R. Br. D.Inflorescences of *Alyxia reinwardtii* Blume
B. Polar view of *A. scholaris* (L.) R. Br. E. Polar view of *A. reinwardtii* Blume
C. Equatorial view of *A. scholaris* (L.) R. Br. F. Equatorial view of *A. reinwardtii* Blume

Pollen Morphology (Figure 3 B & C)

Tricolporate, oblate spheroidal, medium, 25.8 – 28.8 × 26.3 – 31.3 µm in length and breadth; amb rounded; colpi ½ way to the pole, 12.5 – 13.8 × 3.8 – 7.5 µm in length and breadth, pseudocolpi present; pori lolongate, 3.8 – 5.0 × 2.5 – 6.3 µm in length and breadth; annuli about 1.3 µm in diameter; exine about 1.3 µm thick, sexine thicker than nexine; sculpturing obscurely reticulate.

6. *Carissa spinarum* L. Mant. Pl. 2:559.1771. (Figure 3 D)

Myanmar name	:	Khan pin
English name	:	Bush plum
Flowering period	:	June to September

Outstanding Characters

Perennial, small trees with milky latex; stems and branches terete, green; spines boated. Leaves simple, opposite and decussate, exstipulate, petiolate; blades broadly ovate, rounded at the base. Inflorescences axillary, dichasial cymes, 2- to 3- flowered. Flowers pink, about 1.0 cm in diameter at anthesis. Calyx campanulate, 5 - lobed; lobes lanceolate, pubescent. Corolla salver form, 5 - lobed; tubes slightly swollen at the apex, reddish at the apex; lobes ovate, overlapping to the right in bud. Stamens 5, free, inserted, attached to the tube; filaments short; anthers oblong.

Pollen Morphology (Figure 3 E & F)

Tetracolporate, oblate spheroidal, medium, 28.8 – 31.3 μm × 31.3 – 35. 0 μm in length and breadth; amb quadrangular; colpi ½ way up to the pole, 13.8 – 16.3 μm × 1.3 – 5.0 μm in length and breadth; pori circular, 3.8 – 6.3 μm in diameter; exine 1.3 – 2.5 μm thick; sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate 1.3 – 2.5 μm width; the muri simplibaculate, about 1.3 μm wide.

7. *Catharanthus pusillus* (Murr.) G. Don, Gen. Hist. 4: 95.1837.(Figure 4 A)

Lochnera pusilla (Murr.) Schum in Pflanzenfam. 4(2): 145. 1895.

Myanmar name : Taw nga yoke

English name : Tiny periwinkle

Flowering period : June to November

Outstanding Characters

Annual, erect herbs with watery juice; stems and branches acutely 4 - angled, green. Leaves simple, opposite, exstipulate, petiolate; blades lanceolate, cuneate at the base. Inflorescences terminal cymes, 2- to 3-flowered. Flower white, about 2.0 cm in diameter at anthesis. Calyx campanulate, 5 – lobed; tubes very short; lobes filiform. Corolla salver form, 5 - lobed, hairy at the throat of the corolla-tube; tubes short; lobes obovate.Stamens attached to the corolla-tube in the inflated portion near the mouth; anthers distint, narrowly ovate-lanceolate, disk absent.

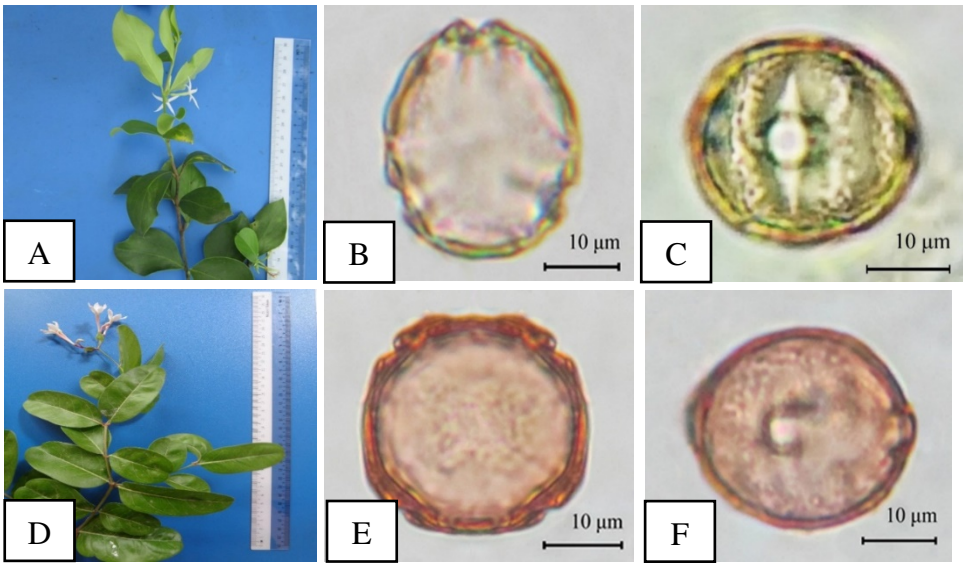


Figure 3.

A. Inflorescences of *Carissa carandas* L. D. Inflorescences of *C. spinarum* L.
B. Polar view of *C. carandas* L. E. Polar view of *C. spinarum* L.
C.Equatorial view of *C. carandas* L. F. Equatorial view of *C. spinarum* L.

Pollen Morphology (Figure 4 B & C)

Tricolporate, oblate spheroidal, medium, 38.8 – 43.8 × 41.3 – 46.3 µm in length and breadth; amb rounded triangular; colpi ½ way up to the pole, 20.0 – 22.5 × 3.8 –7.5 µm in length and breadth; pori circular, 6.3 – 7.5 µm in diameter; exine about 2.5 µm thick, sexine thicker than nexine; sculpturing reticulate; lumina heterobrochate 1.3 – 2.5 µm width; muri simplibaculate, about 0.6 µm wide.

8. *Leuconotis griffithii* Hook.f., Fl. Br. Ind. 3: 628. 1882. (Figure 4 D)

Myanmar name : Unknown
English name : Unknown
Flowering period : March to June

Outstanding Characters

Perennial shrubs with milky latex; stems and branches lenticellate, brown. Leaves simple, opposite and decussate; exstipulate, petiolate; blades

elliptic to oblong, rounded to cuneate at the base. Inflorescences paniculate cymes, many - flowered. Flowers yellow, about 0.3 cm in diameter at anthesis. Calyx campanulate, 5 - lobed; tubes short; lobes ovate, apex obtuse to rounded. Corolla salver form, 5 - lobed; tubes short; lobes ovate, apex rounded. Stamens 5, free, inserted at the top of the tube; filaments short; anthers dithecous, basifixed.

Pollen Morphology (Figure 4 E& F)

Triporate, oblate spheroidal, small, 18.8 – 22.0 × 20.0 – 23.8 μm in length and breadth; amb rounded; pori circular, 1.8 – 3.1 μm in diameter; annuli 1.3 μm in width; exine about 1.3 μm thick, sexine thicker than nexine; sculpturing obscurely reticulate.

9. *Melodinus cochinehinensis* (Lour.) Merr., Trans. Amer. Phil. Soc. 24: 310. 1935. (Figure 5 A)

Oncinus cochinchinenisis Lour. Fl. Cochinich. 123.1790

Myanmar name : Unknown

English name : Unknown

Flowering period : April to June

Outstanding Characters

Perennial small trees with milky latex; stems and branches terete, green. Leaves simple, opposite or whorled, exstipulate, petiolate; blades narrowly ovate, elliptic, oblong or narrowly obovate, rounded to cuneate at the base. Inflorescences axillary or terminal, peniculate cymes, many - flowered. Flowers creamy white, about 0.9 cm in diameter at anthesis. Calyx campanulate, 5 - lobed; lobes ovate, obtuse to round at the apex. Corolla salver form, 5 - lobed, yellow; tubes about 2.0 mm long; lobes obovate. Corolline corona, small, fleshy, 2 - lobed. Stamens 5; anthers dithecous, basifixed, sagittate at the base, glabrous.

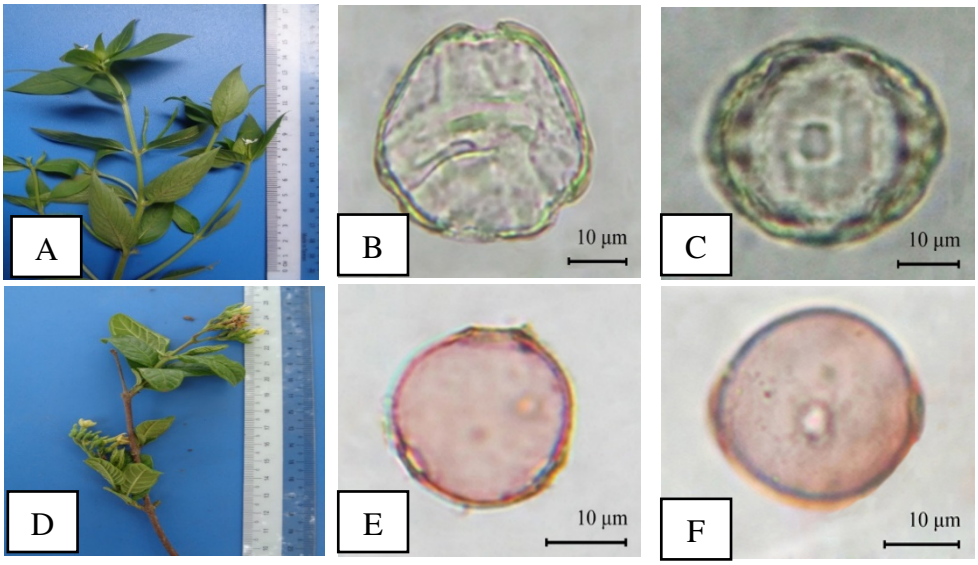


Figure 4

A. Inflorescences of *Catharanthus pusillus* (Murr.) G.Don
B. Polar view of *C. pusillus* (Murr.) G. Don
C. Equatorial view of *C. pusillus* (Murr.) G. Don
D. Inflorescences of *Leuconotis griffithii* Hook.f.
E. Polar view of *L. griffithii* Hook.f.
F. Equatorial view of *L. griffithii* Hook.f.

Pollen Morphology (Figure 5 B & C)

Tetracolporate, oblate spheroidal, medium, 25.0 – 27.5 × 27.5 – 30.0 µm in length and breadth; amb quadrangular; colpi ½ way to the pole, 11.3 – 13.9 × 2.5 – 3.8 µm in length and breadth; pori circular, 2.5 – 3.8 µm in diameter; annuli 1.3 µm in width; exine about 2.5 µm thick; sexine thicker than nexine; sculpturing obscurely reticulate.

10. *Rauvolfia serpentina* (L.) Benth. ex Kurz, Fl. Burm. 2: 171. 1877.

(Figure 5D)

Ophioxylon serpentioum L. Sp. Pl. 1043. 1753.

Myanmar name : Bonma yaza

English name : Indian snakeroot or devil papper

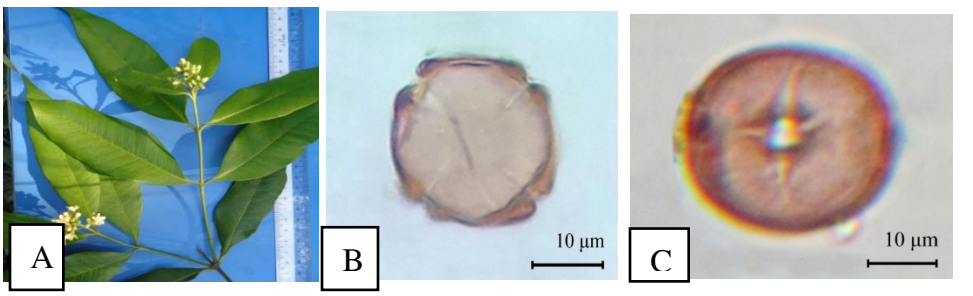
Flowering period : May to July

Outstanding Characters

Perennial low growing shrubs with milky latex; stems and branches terete, brown. Leaves simple, whorl, exstipulate, petiolate; blades broadly obovate, cuneate at the base. Inflorescences terminal, irregular corymbose cymes, many - flowered. Flowers reddish- white, about 0.4 cm in diameter at anthesis. Calyx cup - shaped, 5-lobed; tubes short; lobes lanceolate. Corolla tubular, 5 - lobed; tubes slender; lobes ovate. Stamens 5, free, inserted, adnate in the middle of the corolla tube; filaments short; anthers dithecous, basifixed.

Pollen Morphology (Figure 5 E & F)

Tricolporate, oblate, medium to large, 36.3 – 45.0 × 57.5 – 66.3 µm in length and breadth; amb rounded triangular; colpi longicolpate, 33.8 – 40.0 × 12.5 – 16.3 µm in length and breadth, pseudocolpi present; pori lolongate, 12.5 – 15.0 × 6.3 – 11.3 µm in length and breadth; exine about 1.8 µm thick, sexine thicker than nexine; sculpturing reticulate, lumina heterobrochate 0.8 – 1.3 µm; muri simplibaculate about 0.8µm wide.



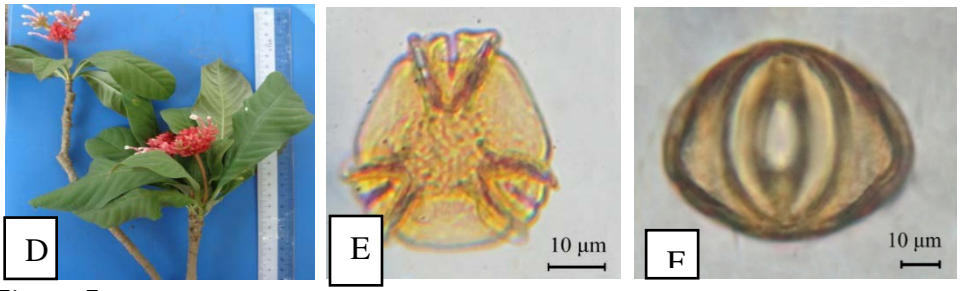


Figure 5.

A. Inflorescences of *Melodinus cochinehinensis* (Lour.) Merr.
B. Polar view of *M.cochinehinensis* (Lour.) Merr.
C.Equatorial view of *M. cochinehinensis* (Lour.) Merr.
D.Inflorescences of *Rauvolfia serpentina* (L.) Benth. ex Kurz
E. Polar view of *R. serpentina* (L.) Benth. ex Kurz
F. Equatorial view of *R. serpentina* (L.) Benth. ex Kurz

Discussion and Conclusion

Pollen Morphology of 10 species belonging to 7 genera under Subfamily Rauvolfioideae were studied in this research. This collected species were classified and identified according to the types of pollen grains that the monad. In this present study, tetracolporate pollen grains were found in *Carissa spinarum* L., *Melodius cochinenhinensis* (Lour.) Merr. and the remaining species were tricolporate. Diporate in *Alyxia reinwardtii* Blume. and *Leuconotis griffithii* Hook.f. were triporate. These characters were agreed with those described by Endress and Bruyns (2000).

The size of the pollen grains was different from each other. The small size of the pollen grains was (20.0 – 21.3 × 22.5 – 23.8 µm) in *Alstonia scholaris* (L.) R. Br., and (18.8 – 22.0 × 20.0 – 23.8 µm) in *Leuconotis griffithii* Hook.f. and the large size was *Alstonia angustiloba* Miq. and the remaining species were medium and medium to large. These characters were agreed with those described by Hesse *et al*. (2009).

The oblate spheroidal shape of pollen grains was found in *Alstonia angustiloba* Miq. These characters agreed with those stated by Kuijt and Raymond (1997). Moreover, the rounded triangular shape of amb was found in *Alstonia* genus, the pollen grains of pori were lolongate and exine thickness 1.8 – 2.5 µm in *Rauvolfia serpentina* (L.) Benth. ex Kurz. These characters were agreed with those described that Endress *et al.* (1996). The

pollen grains of *Melodius cochinenhinensis* (Lour.) Merr., were oblate spheroidal, medium size and amb was quadrangular.

The pseudocolpi were present in *Alstonia angustiloba* Miq., *A. rupestris* Kerr. *A. scholaris* (L.) R.., and *Rauvolfia serpentina* (L.) Benth. ex Kurz. These characters were agreed with those described by Hesse *et al*. (2009). The annuli pollen grains of *Alstonia rupestris* Kerr., *Carissa carandas* L., *Leuconotis griffithii* Hook.f., *Melodius cochinenhinensis* (Lour.) Merr., *Rauvolfia serpentina* (L.) Benth. ex Kurz. These characters were agreed with those stated by Hoen (1999).

According to the results, pollen morphology of different genera in subfamily Rauvolfioideae were variable from each other. It is hoped that these differences of palynological characters will be supported the classification and identification of Rauvolfioideae and give some information for the further study of researchers.

Acknowledgements

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Screening of Endophytic Fungi from *Premna corymbosa* Rottle & Willd for Antimicrobial Activities

Taik Paing[1] & Mya Htet Htet Aung[2]

Abstract

In this study, six fungi were isolated from the leaves of *Premna corymbosa* Rottle &Willd (Pyae-sone) collected at Pathein area. The isolation of endophytic fungi were done by surface sterilization method. Their morphological characters of isolated endophytic fungi were studies. . In the study of test for starch hydrolyzing activity of six endophytic fungi, it was found that one strain PF-4 showed the starch hydrolyzing activity. In the investigation of antimicrobial activities of isolated endophytic fungi, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus* and *Candida albicans* were used for the test throughout the research studies. However, one isolated fungus (PF-2) showed excellent activity against *Staphylococcus aureus*, two isolated fungi (PF-4 and PF-6) against good activities of *E.coli* and one isolated fungus (PF-5) showed weak activity against *Candida albicans*, two strains (PF-2 and PF-3) exhibited antimicrobial activities against *Staphylococcus aureus* and one strain PF-1 showed against *Bacillus subtilis*. Among them, fungus PF-2 showed more highly selective antibacterial activity against *Staphylococcus aureus* than other isolated fungi. Therefore, this strain PF-2 was selected for further investigations. The distinct characters of selected endophytic fungus PF-2 was studied by using microscope.

Keywords: isolation, endophytic fungi, antimicrobial activities

Introduction

Microorganisms have significant functions in ecosystems and are found in all kinds of habitats. They are immensely diverse with respect to their habitats, materials production, and genetic information and so on. Life on earth would have been impossible without microorganisms in nature (Harayama & Isono 2002). Numerous varieties of microorganisms are living on earth and are deeply involved with human life. It is very hard to substrata not isolated any microbes in nature. Therefore, any substrata

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collected in nature are useful materials for isolating microorganisms. Different materials have been reported as the substrata or the samples for microorganisms. The typical materials are soil, living and fallen leaves, leaf litters, dung, insect, fresh water, marine water, and so on (Ando & Inaba 2002).

Many of natural, semi-synthesized and synthesized antibacterial and antifungal metabolites have been reported in clinical and agricultural uses. However, some pathogenic microbes are resistant to antibiotics agents today are required to have potent activity and be safe to animals, humans and ecosystems. Microbial metabolites are biodegradable in nature, giving less stress to the ecosystem, are more likely to meet the above requirements than synthetic one (Phay 1997).

Endophytes represent a promising source of biologically active metabolites for pharmaceutical and agricultural application. Endophytic fungi are considered as potential sources of antimicrobial compounds. Endophytes are microbes that colonize the internal plant tissues beneath the epidermal cell layers without causing any apparent harm or symptomatic intention to their host endophytic fungi are known to contribute to their host plants by producing excessively substances that provide protection and survival value to the plant (Adeleye 2002).

Endophytic fungi are unexplored group of organisms that has enormous potentials for new pharmaceutical substances. They play an essential role to provide protection to their host against attack by other pathogens and environmental factors. An endophyte is a bacterial (including actinomycetes) or fungal microorganisms, which spends the whole or part of its life cycle colonizing inter- and/or intra-cellularly inside the healthy tissues of the host plant, typically causing no apparent symptoms of disease (Tan & Zou 2001).

Endophytes are an extremely host-specific and specialized subset of endophytes with their own peculiar life cycles. The isolation and identification methods for endophytes was last reviewed in 1986 in which be presented a useful table list and surface sterilization protocols for various kind of plants and plant organ (Petrini 1986). Of the 300,000 plant species that exist on the earth, each individual plant is host to one or more endophytes, providing a rich reservoir of microorganisms (Strobel & Daisy 2003).

Antibiotics (metabolites) may be more useful than synthetic chemicals in the treatment and control of diseases. These metabolite are produced from microorganisms such as fungi, bacteria and actinomycetes . Microorganisms are not simply additional elements to the biological variation on Earth; they are an indispensable part of life 5n the biosphere, for they a function role in any ecosystem. They are the major component of soil biomass, they accelerate rock weathering and biological decaying, and they interact with other living organisms through many different kinds of association. Microorganisms are ubiquitous and morphologically diverse, and they have unique physiological and biochemical properties (Subramanian 1986).

Plants as a possible natural habitat, particularly in the tropics, has yielded endophytic microorganisms. Isolation techniques for isolating effectively new or interesting microorganisms from natural substrata are expected to emerge from the field research. There is no rule for isolating microbes. Indeed to think creatively and come up with new isolation techniques constituents the real flair of microbiologists and the charm of microbiology (Moncalvo 1997).

The objectives of the research are to isolate the different endophytic fungi from *Premna corymbosa* Rottle &Willd, to investigate the antibacterial activities of endophytic fungi and to study the distinct characters of selected endophytic fungus.

Materials and Methods

Collection of plant samples

The leaves plant sample, *Premna corymbosa* Rottle &Willd was utilized for the isolation of endophytes. These plant sample was collected at Pathein University Campus, Pathein Township, Ayeyarwady Region.

Figure 1. Habit and leaves of *Premna corymbosa* Rottle &Willd

Scientific name - *Premna corymbosa* Rottle &Willd

Myanmar Name - Pyae-sone
Family - Lamiaceae
Flowering and fruiting periods – August to October

Outstanding characters of *Premna corymbosa* Rottle & Willd

Small trees or large scandent shrubs. Leaves simple, opposite and decussate; blades ovate – oblong, pubescent on both surfaces, rounded at the base, entire and ciliate at the margin, acuminate at the apex. Inflorescences axillary and terminal dichasial corymbiform cymes with few flowers. Flowers creamish white, bisexual, zygomorphic, subssile. Calyx campanute. Corolla infundibuliform. Stamens 4, didynamous, slightly exerted; filaments glandular, filiform. Styles as same length as the stamens. Fruit drupaceous, obovoid, smooth, with black coloured pericarp; fruiting calyx saucer - shaped.

Isolation procedure of endophytes from plants (Tomita, 1998)

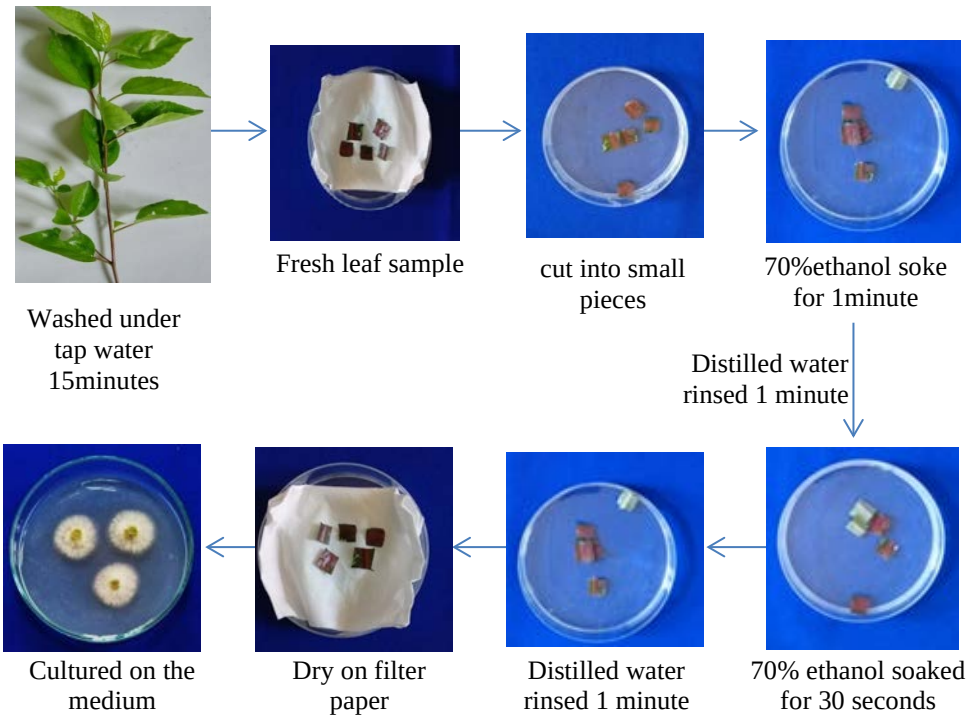


Figure 2. Isolation procedure of endophytes from plants

Isolation procedure of endophytes from plants (Tomita 1998)

1. The plants were washed in running tap water for 15 mins.
2. The plant leaves were cut into about 1 cm pieces.
3. Sterile the surface of plant part by soaking it in 75% ethanol for 2 mins.
4. These parts were dried on sterilized paper and then were placed on agar plate containing medium.
5. The plates were incubated for 3 days to 1 week at room temperature

Medium Used for the isolation of fungi

Low Carbon Agar medium (Ando 2004)

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

Potato Glucose Agar medium (Ando 2004)

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

(after autoclaving chloramphenicol and Penicillin-G were added to the medium)

Test for Starch Hydrolyzing Activity

The isolated endophytic fungi were inoculated in 10 ml liquid medium (soluble starch 1.5%, K_2HPO_4 0.2%, $MgSO_4$ 0.15% and Distilled water 100 mL) incubated for 3 days. Iodine solution (drop by drop 0.1 mL to 0.3 mL) was poured slowly into the liquid culture medium. The color was also done.

After adding iodine solution, the culture color is purple, so the microorganism cannot hydrolyze the starch. If the culture color is not change to purple, the microorganisms can hydrolyze the starch. This study was focused for amylase enzyme production.

Screening or preliminary study for antimicrobial activities by paper disc diffusion assay (Tomita 1998)

The isolated fungi were grown at 25˚C for 5 days on PGA medium. The isolated fungi were inoculated into seed medium and incubated at 25˚C for 3 days. 25 mL of seed culture was transferred into the fermentation medium. The fermentation was carried out for 10 days. After the end of fermentation, the fermented broth (20 µL) was used to check the antimicrobial activity against test organisms by paper disc diffusion assay.

Paper disc having six millimeter diameter were utilized for antimicrobial assays.

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

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

No.	Test organisms	Infections
1.	*Bacillus subtilis*	Fever, food poisoning and tissue necrosis
2.	*Micrococcus luteus*	Skin disease, pneumonia, urinary tract, meningilis and peritionilis
3.	*Escherichia coli*	Food spoilage, diarrhoea and urinary tract infections
4.	*Candida albicans*	Typhoid and candidosis
5.	*Staphylococcus aureus*	Boils and food poisoning, blood stream infections and bone and joint infections

Seed Medium (Ando 2004)

Glucose	2.0%
Sucrose	0.3%
Yeast extract	0.3%
KNO_3	0.1%
K_2HPO_4	0.01%
DW	100 mL
pH	6.5

Fermentation Medium (Ando 2004)

Glucose	1.0 g
Soluble starch	0.5 g
Yeast extract	0.5 g
NZ amine type A	0.5 g
K_2HPO_4	0.001 g
$MgSO_4$	0.001 g
$CaCO_3$	0.1 g
DW	100 mL
pH	6.5

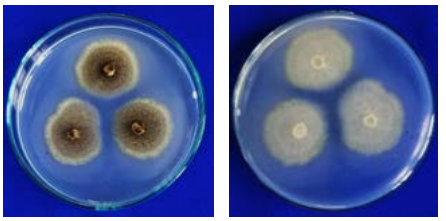
These seed medium and fermentation medium were employed in the studies for antimicrobial activities.

Morphological, photomicrograph and distinctive characters of PF-2

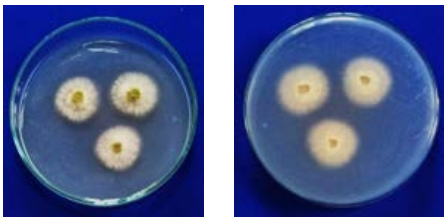
For the study of morphology and macroscopical characters, fungus PF-2 was cultured at 25˚C on Potato Glucose Agar (PGA) medium and Low Carbon Agar (LCA) medium. Then, these plates were incubated at room temperature for 3 to 7 days. Colony forms, surfaces and reverse pigments of isolated selected strains were studied for morphology at Microbiology Lab, Department of Botany, Pathein University. Photomicrograph were investigated by using high magnification of microscope at Botany Department, Pathein University.

Results

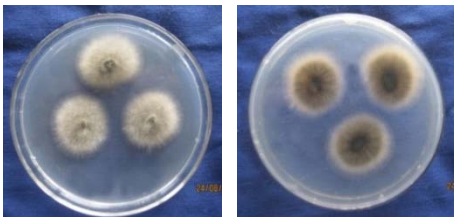
In the isolation of endophytic fungi, six different endophytic fungi (PF-1 to PF-6) from the leaves of *Premna corymbosa* Rottle &Willd (Pyae-sone) as shown in figure 3.



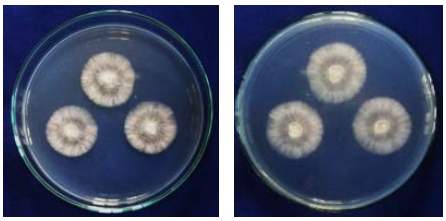
Front and reverse view of PF-1



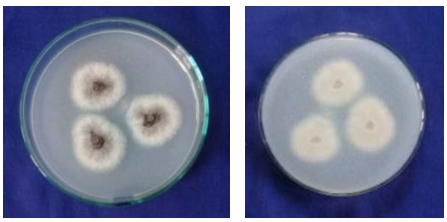
Front and reverse view of PF-2



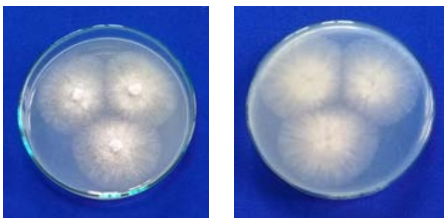
Front and reverse view of PF-3



Front and reverse view of PF-4



Front and reverse view of PF-5



Front and reverse view of PF-6

Figure 3. Morphological characters of isolated fungi on PGA media(5 days old culture)

Test for starch hydrolyzing activity

In this study, it was found that one endophytic fungus (PF- 4) can hydrolyze the starch, but other strains cannot hydrolyze the starch. Therefore this fungus may produce amylase enzyme (Figure 4).

Before reaction of iodine solution **After reaction with add iodine solution**

(Control and PF-1 to PF-6) **(Control andPF-1 to PF-6)**

Figure 4. Starch hydrolyzing activities of PF-1 to PF-6

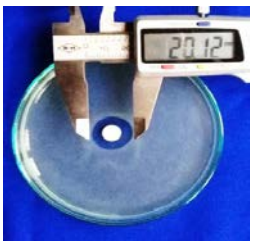
Screening of Effective Microorganisms Isolated from Soil by Paper Disc Diffusion Assay

In the course of the investigation of fungi, six endophytic fungi were isolated from the leaves of *Premna corymbosa* . During the study of antimicrobial activities of these isolated endophytic fungi, one isolated fungus (PF-2) showed excellent activity against *Staphylococcus aureus*, two isolated fungi (PF-4 and PF-6) against good activities of *E.coli* and one isolated fungus (PF-5) showed weak activity against *Candida albicans*, two strains (PF-2 and PF-3) exhibited antimicrobial activities against *Staphylococcus aureus* and one strain PF-1 showed against *Bacillus subtilis* . Among them, fungus PF-2 showed more highly selective antibacterial activity against *Staphylococcus aureus* than other isolated fungi. Therefore, this strain PF-2 was selected for further investigations.

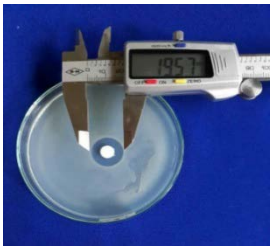
Table 2. Antimicrobial activities of isolated endophytic fungi (PF-1 to PF-6)

Strain No.	***Micrococcus luteus***	***Bacillus subtilis***	***Staphylococcus aureus***	***E.coli***	***Candida albicans***
PF-1	-	20.12mm	-	-	-
PF-2	19.57mm	-	**24.14mm**	-	-
PF-3	19.23mm	-	-	-	-

Strain No.	*Micrococcus luteus*	*Bacillus subtilis*	*Staphylococcus aureus*	*E.coli*	*Candida albicans*
PF-4	-	-	-	23.50mm	-
PF-5	-	-	-	-	18.76mm
PF-6	-	-	-	21.73mm	-

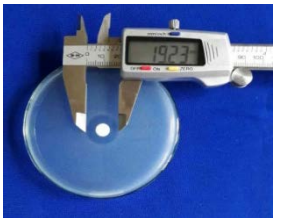


PF-1 against *Bacillus subtilis*



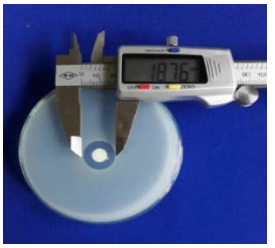
PF-2 against *Micrococcus luteus*

PF-2 against *Staphylococcus*

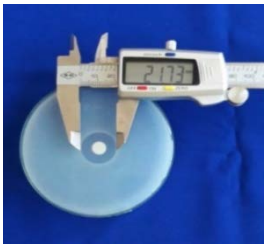


PF-3 against *Micrococcus luteus*

PF-4 against *E.coli*



PF-5 against *Candida albicans*



PF-6 against *E.coli*

Figure 5. Antimicrobial activities of isolated endophytic fungi (PF-1 to PF-6)

Morphological and Microscopical characters of PF-2

The surface color of PF-2 was white and its reverse color of was pale yellow. After 5 days of cultivation, it was observed that white colonies reach 1.7 cm diameter at 25°C on PGA medium. Distinctive characters of endophytic fungus PF-2 was found that conidia lacking septa, globose, smooth, pale brown color, conidiophores without septa, elongated not branched, hyaline to gray color (Figure 6).

Front View of PF-2 Reverse View of PF-2 Photomicrograph (X-400)

Figure 6. Morphology and photomicrograph of PF-2

Discussion and Conclusion

In the course of the screening for antibacterial metabolite producing fungi, six fungi were isolated from the leaves of *Premna corymbosa* Rottle &Willd (Pyae-sone), these plant samples were collected at Pathein University Campus, Pathein Township, Ayeyarwady Region. According to Ando(2004), different microbes can be found under different environments. Endophytic fungi that live in healthy plants are different although they are the same genus or species. Moreover one strain PF-4 showed the starch hydrolyzing activity. Therefore this fungus may produce amylase enzyme.

During the study of antimicrobial activities of these isolated endophytic fungi, one isolated fungus (PF-2) showed excellent activity against *Staphylococcus aureus* (24.14mm), two isolated fungi (PF-4 and 6) against good activities of *E.coli* and one isolated fungus (PF-5) showed weak activity against *Candida albicans*, two strains (PF-2 and PF-3) exhibited antimicrobial activities against *Staphylococcus aureus* and one strain PF-1 showed against *Bacillus subtilis* . Among them, fungus PF-2 showed more highly selective antibacterial activity against *Staphylococcus aureus* than other isolated fungi. Therefore, this strain PF-2 was selected for further investigations. Distinctive character of soil fungus PF-2 was found

that conidia lacking septa, globose, smooth, pale brown color, conidiophores without septa, elongated not branched, hyaline to gray color.

In conclusion, the present study found on the isolation and activities of endophytic fungus PF-2 from the leaves of *Premna corymbosa* and screening them for antibacterial activity against *Staphylococcus aureus.* This endophytic fungus PF-2 may lead to the production of antibacterial metabolites.

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Antibacterial Activities of Endophytic Fungi Isolated from *Andrographis paniculata* (Burm. f.) Nees.

Kyaw Linn[1] & Htar Htar[2]

Abstract

In the present study, the endophytic fungi were isolated from the leaves, stems and roots of *Andrographis paniculata* (Burm. f.) Nees. These plants were collected from Monywa. During this study, five kinds of endophytic fungi were isolated. Two kinds of endophytic fungi were isolated from the leaf, only one kind of endophytic fungus was isolated from the stem and two kinds of endophytic fungi were isolated from the root. In this study, their macroscopial and microscopical characters of endophytic fungi and their antibacterial activity have been undertaken.

Keywords: Endophytic fungi, macroscopial characters, microscopical characters, antibacterial activity

Introduction

The term “endophyte” is derived from the Greek, endo = within and phyte = plants. It was first introduced in 1866 by De Bary. It was used broadly to refer to any organism found within tissues of living plants. Mycologists generally agree that endophytes are organisms that colonize internal plant tissues without causing apparent harm to their host. Different groups of organisms such as fungi, bacteria, actinomycetes and mycoplasma are reported as endophytes of plants. Endophytic fungi have proven they are a promising source of bio-control agents. These organisms are present in the internal healthy plant tissues during a part or/all of their life cycle without causing apparent harm to their hosts. They influence greatly the physiological activities of their host plants. Fungal endophytes enhance their host resistance against abiotic stress, disease, insects and mammalian herbivores by producing a broad range of fungal metabolites. Indeed several interesting metabolites isolated from endophytic fungi belong to diverse chemical classes, including: alkaloids, steroids, flavonoids, terpenoids, quinones and phenols (Arnold 2007).

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Fungal endopytes have attracted a great interest to microbiologists, chemicals and ecologists as a treasure of biological resource, because they play diverse indispensable roles in the ecosystem for stress tolerance, eco-adaptation, and promoting growth and development. Recently, endophytic fungi have drawn a particular attention, due to their considerable biodiversity, unparalleled metabolic pathways and unique habitats. Therefore, they were considered as an unusual source of novel secondary metabolites, exhibiting a variety of biological activity, which are in use in modern agriculture, pharmaceutical and biotechnological industry. Fungal metabolites are diverse including those associated with proteins synthesis and respiration. Several secondary metabolites have been isolated and frequently, chemically defined. Some of these are waste products while others such as pigments, toxins, and antibiotics clearly have biological functions. Because of their synthetic abilities, fungi are used in industry for the production of alcohol, citric acid and other organic acids, various enzymes, riboflavin (Krik *et al*. 2008).

A major feature of industrial antibiotic production is directed to screening programming for new potent antibiotic producing organisms either from natural sources or from established cultures. Screening for antibiotics producing microorganisms, can be detected and isolated by the use of highly selective procedure which allows detection and isolation of only those microorganism of interest from a large population is possible (Waksman 1961).

Over five thousand antibiotics have been identified from the culture of gram-positive, gram-negative and filamentous fungi but only hundred antibiotics have been commercially used to treat human, animal and plant disease (Bulock 1997).

A. paniculata (Burm. f.) Nees.is found throughout the plains of India and it is also utilized in Chinese medicine. The leaf juice is a household remedy for many ailments of the alimentary tract. A number of researchers have described the isolation of flavonoids, sesquiterpens, lactones and other groups of compound from the plant. The active antihepatotoxic principle is probably the diterpenelactone andrographolide which has been shown to protect against alcohol and carbon tetrachloride induced hepatic microsomal lipid peroxidation (Trease & Evans 2002).

The *Cladosporium* sp. is parasitic on higher plants or saprophytic on plant material. The *Fusarium* sp. is parasitic on higher plants or sporophytic

on decaying plant material .The *Trichonderma* sp. is saprophytic in soil or on wood, very common, some species reported as parasites on other fungi. The *Cephalosporium* sp. is saprophytic or parasitic, some species causing vascular wilts of trees. Microspores of certain species of *Fusarium* are similar and may be confused. The *Penicillium* sp. is parasitic and saprophytic species (Barnett 1956).

The need for new bioactive compounds used in medicine, industry, and agriculture has increased. Historically, many compounds have been isolated from the natural environment, particularly plants. Therefore, many of drugs available commercially are derived from plant-based chemicals. While plants have been a major source of new compounds for drug discovery, attention has more recently turned to endophytes as these microorganisms demonstrate great potential sources for new bioactive compounds (Strobel 2003).

From the above point of views, the isolation of endophytic fungi from the plant *A. paniculata* (Burm. f.) Nees. Their macroscopical and microscopical characters and their antimicrobial activity were tested in this study.

Materials and Methods

Collection of plant samples

Healthy and mature leaves, stems and roots of *A. paniculata* (Burm. f.) Nees. were collected from Monywa. The collected plants were identified according to the morphological characters shown in the literatures of Backer 1968, Hooker 1885, Dassanayake 1998.

Isolation of Endophytic fungi

The endophytic fungi were isolated by the method of Ando and Inaba 2004. The stems, leaves, and roots of the plant were washed in running tap water for 15 minutes. They were separated and cut into small pieces 1 cm in length. They were successively washed with 95% ethanol for 15 second, 1% NaOCl for 5 minutes and 95% alcohol for 15 second. After rinsing with sterile water, they were dried on sterile tissue paper and placed on agar plate. They were incubated for 3 days to one week.

Preparation of culture medium

The SLNA medium (Synthetic Low Nutrient Agar) medium (Glucose 0.2g, Sucrose 0.2g, KH_2PO_4 0.1g, $MgSO_4 + 7H_2O$ 0.5g, KNO_3 0.1g, KCL 0.05g, Agar 1.8 – 2.0g, Distilled water 100ml, pH 6.5) was prepared for the isolation of endophytic fungi.

Macroscopical and Microscopical Characters

The PGA medium (Potato Glucose Agar medium) was composed of potato powder 0.5g, Glucose 1.6 – 1.8g, Agar 1.8 – 2.0g, Distilled water 100ml, pH 6.5 for the macroscopical characters of isolated endophytic fungi.

The WGA medium (Water Glucose Agar medium) was prepared glucose 1.6 – 1.8g, Agar 1 .8 – 2.0g, Distilled water 100ml, pH 6.5 for the microscopical characters of isolated endophytic fungi.

Screening of Effective Endophytic Fungi by Paper Disc Diffusion Assay

The screening of antimicrobial activities was carried out by the method of Applied Microbiology Lab. Hokkaido University, Japan, 1998. The isolated endophytic fungi were grown on PGA agar medium and were inoculated into seed medium (Glucose 20.0g , Soluble starch 3.0g,Yeast extract 3.0g, KNO_3 1.0g , K_2HPO_4 0.1g, Distilled water 1000 ml, pH 6.5) and incubated for 3 days at 27°C.

Seed culture (5 ml) was transferred to the identical fermentation medium(Glucose 20.0g, Glycerol 10.0ml, Yeast extract 3.0g, Peptone 3.0g, $CaCO_3$ 1.0g, Distilled water 1000ml , pH 6.5).The fermentation was carried out for 7 days.

After the end of fermentation, the fermented broth (20 µl) was used to check the antibacterial activity against test organisms by paper disc diffusion assay. Paper disc having 12.5cm were utilized for antibacterial assays.

The assay medium (Glucose 10.0g, Polypepton 3.0g, KNO_3 1.0g, Agar 18.0g, Distilled water 1000 ml, pH 6.5 – 7.0) was used for the antimicrobial activity test.

One percent of test organism was added to assay medium, then poured into plates. After solidification, paper disc impregnated with samples (fermented broth) were applied on the agar plates and incubated for

24 – 36 hr. at 28 to 30°C. Clear zones (inhibitory zones) surrounding the test disc indicate the presence of bioactive metabolites which inhibit the growth of test organisms.

The test organisms *Bacillus subtilis* KY–327, *E coli* AHU–5436, *Saccharomyces cerevisiae* NITE–52847, *Salmonella typhi* AHU–7943 and *Staphylococcus aureus* AHU–8465 were used in paper disc diffusion assay. These test organisms were supported by NITE (National Institute of technology and Evaluation, Japan) and Faculty of Agriculture, Hokkaido University, Japan for the cooperation research.

Result

Collection of plant samples

Scientific name : *Andrographis paniculata* (Burm. f.) Nees.

Family : Acanthaceae

Myanmar name : Saykhagyi

Flowering period : October to February

Annual erect herbs; stems and branches sharply quadrangular, glabrous. Leaves simple, opposite and decussate, exstipulate; petioles glabrous; blades ovate-elliptic, attenuate at the base, entire along the margin acuminate at the apex, glabrous on both surfaces. Inflorescences terminal and axillary paniculata racemes with many flowered; peduncles, glabrous. Flowers white; bracts linear lanceolate, bracteoles, bisexual, zygomorphic, pentamerous, hypogynous. Calyx campanulate, 5-partite; lobes linear lanceolate, glabrous within. Corolla bilabiate, 5-lobed, white; upper lip 2-lobed, white; lower lip 3-lobed, oblong, white violet spots within. Stamens 2, exserted, free, epipetalous; filaments flattened; anthers dithecous, introrse, basifixed longitudinal dehiscence. Ovary oblong, bilocular with many ovules in each locule on the axile placentae; style filiform, stigma slightly bifid. Capsule linear-oblong, compressed, furrowed on opposite sides, mucronate, 12-seeded. Seeds brown, glabrous as shown in Figure 1.

Figure 1. Inflorescence of *A. paniculata* (Burm. f.) Nees.

Isolation of endophytic fungi

Five kinds of endophytic fungi species were isolated from the Leaves, stems and roots of *A. paniculata* (Burm. f.) Nees. using SLNA medium (Ando & Inaba 2004).

Macroscopic and microscopic characters of endophytic fungi

Macroscopical characters of KL 01

After 3 – 7 days cultivation, it was observed that the mycelium were whitish gray color in colonies at 25°C on PGA medium as shown in Figure 2(A).

Microscopical characters of KL 01

The conidiophores are dark. They are branched variously near the upper or middle portion. They are clustered. The conidia are dark, 1- celled, variable in shape and size, ovoid to cylindrical and irregular as shown in Figure 2(B).

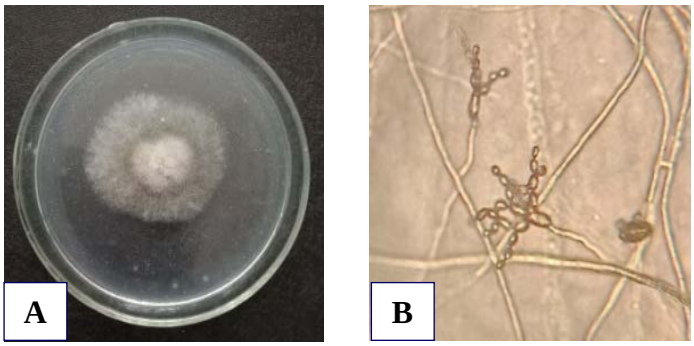

Figure 2. Macroscopical and microscopical characters of KL 01
(A) Macroscopical character of KL 01
(B) Microscopical character of KL 01

Macroscopical character of KL 02

After 3 – 7 days cultivation, it was observed that the mycelium were pale whitish black color in colonies at 25°C on PGA medium as shown in Figure 3(A).

Microscopical character of KL 02

Mycelium was extensive and cottony in culture. The conidiophores are variable, slender and simple, short. The conidia are hyaline, variable, macroconidia are several celled, slightly curved or bent at the pointed ends, 2-3-celled, ovoid or oblong, as shown in Figure 3(B).

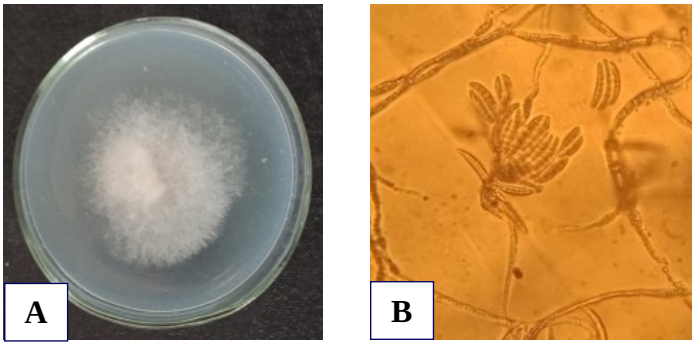

Figure 3. Macroscopical and microscopical characters of KL 02
(A) Macroscopical character of KL 02
(B) Microscopical character of KL 02

Macroscopical character of KL 03

After 3 – 7days cultivation. it was observed that the mycelium were whitish gray color in colonies at 25°C on PGA medium as shown in Figure 4(A).

Microscopical character of KL 03

The conidiophores are arising singly from the mycelium, branched near the apex to from a brush–like conidial bearing apparatus; ending in phialides which pinch off. The conidia are dry chains; hyaline or brightly colored in mass, 1-celled, mostly spherical, produced basipetally as shown in Figure 4(B).

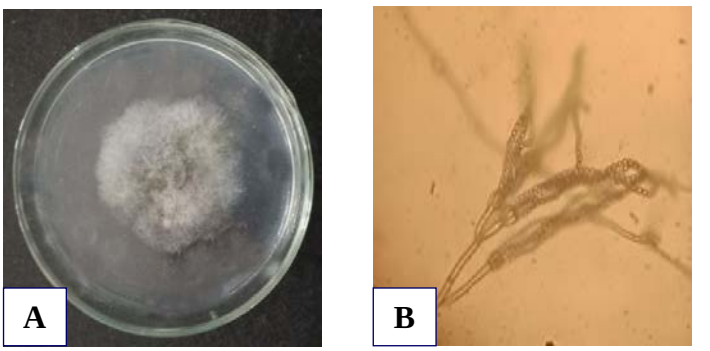

Figure 4- Macroscopical and microscopical characters of KL 03
(A) Macroscopical character of KL 03
(B) Microscopical character of KL 03

Macroscopical character of KL 04

After 3 – 7 days cultivation, it was observed that the mycelium were gray color in colonies at 25°C on PGA medium as shown in Figure 5(A).

Microscopical character of KL 04

The conidiophores are hyaline, upright, much branched. The conidia are hyaline, 1-celled, ovoid, borne in small terminal cluster as shown in Figure 5(B).

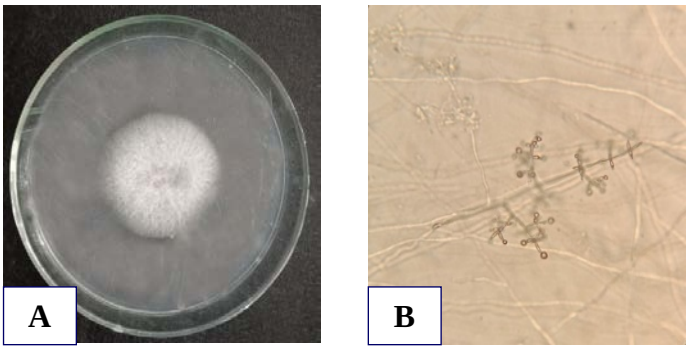

Figure 5. Macroscopical and microscopical characters of KL 04
(A) Macroscopical character of KL 04
(B) Microscopical character of KL 04

Macroscopical character of KL 05

After 3 – 7 days cultivation, it was observed that the mycelium were whitish gray color in colonies at 25°C on PGA medium as shown in Figure 6(A).

Microscopical character of KL 05

The conidiophores are slender and simple. The conidia are hyaline, 1-celled, produced successively at the tip and collecting in a slime drop, produced endogenously as shown in Figure 6(B).

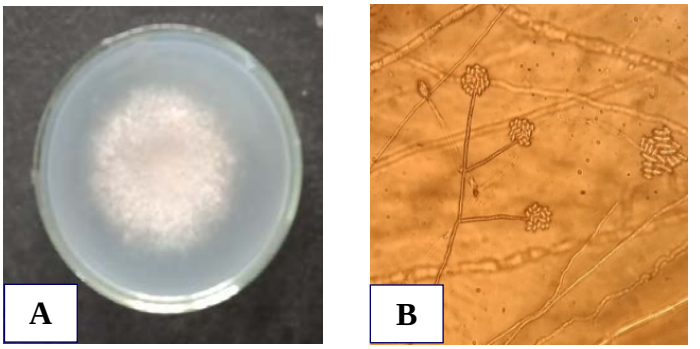

Figure 6. Macroscopical and microscopical characters of KL 05
(A) Macroscopical character of KL 05
(B) Microscopical character of KL 05

Screening of Effective Endophytic Fungi by Paper Disc Diffusion Assay

In the investigation of their antibacterial activities, the endophytic fungus KL 01, *Cladosporium* sp. showed the antibacterial activities against the *Bacillus subtilis* (16.95 mm), the KL 02, *Fusarium* sp. against the *Bacillus subtilis* (21.54 mm) and the KL 03, *Penicillium* sp. against the *E. coli* (30.11 mm) as shown in Figure 7.

Figure 7. Antibacterial activities

A. The activity of endophytic fungus KL 01 against *B. subtilis* (16.95 mm)
B. The activity of endophytic fungus KL 02 against *B. subtilis* (21.54 mm)
C. The activity of endophytic fungus KL 03 against *E. coli* (30.11 mm)

Discussion and Conclusion

In the present study, for the isolation of antimicrobial metabolite producing endophytic fungi, five kinds of endophytic fungi were isolated from the leaves, stems and roots *of A. paniculata* (Burm. f.) Nees.

According to Trease and Evans (2002), *A. paniculata* (Burm. f.) Nees. as found throughout the plains of India and it is also utilized in Chinese medicine. The leaf juice is a household remedy for many ailments of the alimentary tract. A number of researchers have described the isolation of flavonoids, sesquiterpens, lactones and other groups of compound from the plant.

According to the macroscopical and microscopical characters which based on the reference keys of Barnett (1956), the strain KL 01 is the fungus *Cladosporium* sp, the strain KL 02 is the fungus *Fusarium* sp, the strain KL 03 is the fungus *Penecillium* sp, the strain KL 04 is the fungus *Trichoderma* sp, and the strain KL 05 is the fungus *Cephalosporium* sp.

Barnett (1956) stated that the *Cladosporium* sp. was parasitic on higher plants or saprophytic on plant material. The *Fusarium* sp. was parasitic on higher plants or saprophytic on decaying plant material. Widely distributed in soil and associated with plants. Most species are harmless saprobes, and are relatively abundant members of the soil microbial community. The *Penicillium* sp. are parasitic and saprophytic species. The *Trichoderma* sp. are saprophytic in soil or on wood, very common, some species reported as parasites on other fungi. The *Cephalosporium* sp. are saprophytic or parasitic, some species causing vascular wilts of trees. This fungus spreads through the soil and enters the plant through wounds in its roots.

In the present study, *Cladosporium* sp., *Fusarium* sp., *Penicillium* sp., *Trichoderma* sp. and *Cephalosporium* sp. were occurred as endophytic fungi isolated from the A. paniculata (Burm. f.) Nees.

The antibacterial activities of the endophytic fungus KL 01, *Cladosporium* sp. showed the antibacterial activities against the *Bacillus subtilis* (16.95 mm), the KL 02, *Fusarium* sp. against the *Bacillus subtilis* (21.54 mm) and the KL 03, *Penicillium* sp. against the *E. coli* (30.11 mm).

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A Study on Pollen Morphology of Sapindales Found in Mandalay, Sagaing Region and Kachin State

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Abstract

The pollen morphology of 8 species belonging to 7 genera of Sapindales was studied. The specimens were collected from Mandalay, Sagaing Region and Kachin State from 2018 to 2019. The collected plants include 1 species of Burseraceae, 2 species of Anacardiaceae, 4 species of Sapindaceae and 1 species of Rutaceae. The examined of pollen grains were found in monad type. The morphological characters of each grain were studied. The aperture types of all the pollen grains are colporate and tricolporate. The pollen shapes were found in oblate spheroidal, subprolate, prolate spheroidal, oblate to prolate. The sizes of pollen grains were small and medium. The small sizes of pollen grains was found in 6 species and medium size of pollen grains was found in 2 species. The sculpture patterns of 8 species are varied from psilate, reticulate, obscurely reticulate, striate to rugulae.

Keywords: Sapindales, Palynology, Kachin State.

Introduction

Pollen is Latin and means “fine dust” or “flour”. Its first use as a scientific word to describe the male sperm carrying units of flowering plants is credited to Carl Linnaeus in *Sponsalia Plantarum* published in 1747. Pollen is the dust of vegetable, which will burst when moistened with the appropriate liquid, and propulsively explode a substance which is not discernable by the naked senses (Kesseler 2009).

Palynology was introduced by Hyde and Williams, Cardiff, in the 1940’s. It is derived from the Greed verb *palynein*, which mean the spread or strew around-pollen grains and spores are indeed often dispersed by the wind, or by insects or other animals (Erdtman 1969).

According to the classification system of Byng *et al.* (2016), Sapindalesis an order of flowering plants. However, only six out of nine

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family are found in Myanmar. The remaining three family, namely, Biebersteiniaceae, Nitrariaceae, Kirkiaceae are not found in Chit Ko Ko (1961) and Kress *et al.* (2003). Sapindales is about 278 species (Hundley and Chit Ko Ko 1961) and is about 285 species (Kress *et al.*2003).

Many researchers had given an account on the classification and identification of order Sapindales in various localities in Myanmar. However, pollen morphology of the order Sapindales has not been mentioned in Myanmar. Therefore, a research on pollen morphology of order Sapindales still needed to be studied and recorded.

The aims and objectives of this research were to identify and classify the morphological variation in pollens of Sapindales, to study and record the collected species systematically from the palynological point of views and to provide the different pollen characters.

Materials and Methods

Collection of Plants

The specimens of the order Sapindales were collected from Mandalay, Sagaing Region and Kachin State from 2018 to 2019. The collected species were photographed to record their inflorescences and flowers. Identification of genera and species were carried out by referring to Backer (1965), Dassanayake (1980), Hooker (1881). Myanmar names were referred to Hundley and Chit Ko Ko (1961) and Kress and Daw Yin Yin Kyi (2003) in Myanmar.

Acetolysis of Pollen Grain

The pollen samples were acetolysed by the standard method of Erdtman (1960). The acetolysis solution was mixed using a measuring cylinder; 9 parts of glacial acetic acid were added, and then 1 part of concentrated sulphuric acid was added. 1 cc of acetolysis mixture was poured into the test tube containing the pollen samples and stirred with a glass rod. The test tube containing the pollen sample was transferred to a water bath at 75°C for 30 minutes. The test tube was diluted with distilled water and the test tubes were put in an electric centrifuge tube for 30 minutes at 3000 rpm. After centrifuging and decanting, a few drops of dilute glycerin solution was added to the residue, then transferred and stored in air tight glass vial.

Slide Preparation

A drop of sample was taken from sample bottle with a glass rod and placed on a slide, then covered with a cover-slip. These measurements were based on 10-15 grains per sample. The pollen terminology used in identification is according to Moore & Webb (1978), Hoen (1999) and Hesse (2009).

Results

In this research, pollen morphology of 8 species belonging to 7 genera in four family of Sapindales were studied. The list of collected species were arranged according to classification system of Byng *et al.* (2016) and listed in alphabetically as shown in Table 1.

Table 1. List of the collected specimens

Order	Family	No.	Scientific Name	Myanmar Name
Sapindales	Burseraceae	1	*Garuga pinnata* Roxb.	Chinyok
	Anacardiaceae	2	*Lannea coromandeli* (Houtt.) Merr.	Nabe
		3	*Rhus semiaata* Murr.	Chying ma
	Sapindaceae	4	*Aesculus punduana* Wall.	Yemyaw
		5	*Sapindus emarginata* Vahl.	Kinpadi
		6	*Sapindus rarak* DC.	Magyibauk, Kala kimmum
	Rutaceae	7	*Schleichera oleosa* (Lour.) Merr.	Gyo
		8	*Clausena emarginata* C.C.Huang	Unknown

1. Family – Burseraceae

***Garuga pinnata* Roxb., Hort. Bengal.33;Pl. Corom iii.5.t. 1819.**

(Figure 1 A)

Myanmar name : Chinyok

English name : Teasap

Flowering period : March to April

Outstanding characters

Perennial trees, bark gray-brown, rough; stems and branches terete, glabrous. Leaves imparipinnate, alternate, exstipulate. Inflorescences terminal and axillary paniculate cymes. Flowers bisexual, actinomorphic, pentamerous, hypogynous. Sepals 5, free. Petals 5, free, oblong. Fruit drupaceous, globose, yellow when ripe.

Specimens examined: Mandalay Region, Pyin Oo Lwin Township, Yae Chan Owe village; 21°40'26"N and 96°26'30"E; March 16, 2019; Hnin Yu Maw, collection no. 25.

Pollen morphology (Figure 1 B, C)

Tricolporate, subprolate, medium, 12.0 – 31.2 × 12.0 – 26.4 µm in length and breadth; amb triangular; colpi ¾ way up to the pole, 9.6 – 27.6 × 3.6 – 6.0 µm in length and breadth; pori lolongate, 4.8 – 9.6 × 3.6 – 8.4 µm in length and breadth; exine about 1.5 µm thick, sexine thicker than nexine; sculpturing rugulae, the lumina heterobrochate, about 2.4 µm width; the muri simplibaculate, about 0.6 µm wide.

2. Family –Anacardiaceae

***Lannea coromandelica* (Houtt.) Merr., J. Arnold. Arbor. 19. 353. 1938.(Figure 1 D)**

Myanmar name : Nabe

English name : Indian ash tree, Mandhol, Modhad

Flowering period : December to March

Outstanding characters

Perennial, deciduous trees; stems and branches terete. Leaves imparipinnate, alternate, exstipulate. Inflorescences apically crowded, paniculate spikes in the axils of fallen leaves on the branchlets. Flower unisexual, actinomorphic, tetramerous, hypogynous. Calyx campanulate, 4 partite, pale green. Petals 4 or 5, free ovate-oblong. Fruits drupaceous,1-seeded, obliquely oblongoid. Seeds compressed, endospermic.

Specimens examined: Mandalay Region, Kyaukse Township, along the road-side of Yeywa area; 21°41'21" N and 96°25'44" E; 31 March, 2018; Hnin Yu Maw, collection no.11.

Pollen morphology (Figure 1 E, F)

Tricolporate, prolate spheroidal, small, 21.6 – 24.0 × 20.4 – 21.6 µm in length and breadth; amb rounded triangular; colpi¾ way up to the pole, 18.0 – 21.6 × 7.2 – 8.4 µm in length and breadth; pori lalongate, 7.2 – 9.6 × 9.6 – 10.8 µm in length and breadth; exine about 2.4 µm thick, sexine thicker than nexine; sculpturing obscurelyreticulate.

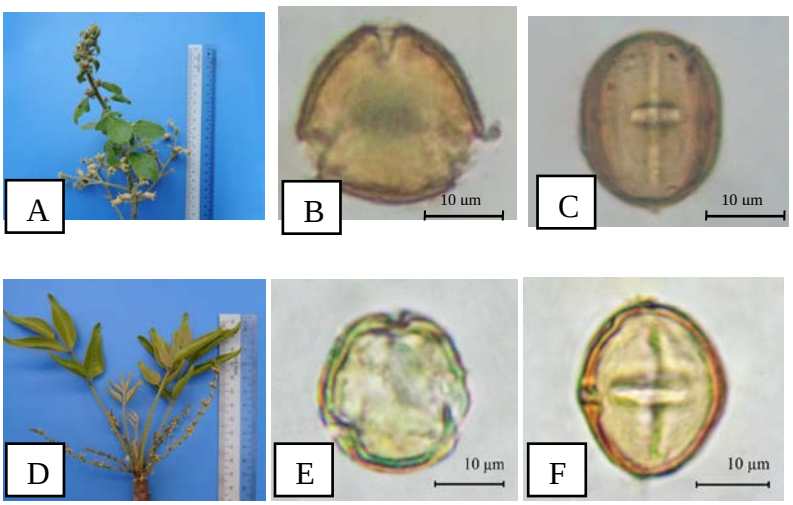

Figure 1. A. Inflorescences of *Garuga pinnata* Roxb.
B. Polar view pollen grain of *G. pinnata* Roxb.
C. Equatorial view pollen grain of *G. pinnata* Roxb.
D. Inflorescences of *Lannea coromandelica* (Houtt.) Merr.
E. Polar view pollen grain of *L. coromandelica* (Houtt.) Merr.
F. Equatorial view pollen grain of *L. coromandelica* (Houtt.) Merr.

3. *Rhus semialata* Murr., Commentat. Soc. Regiae Sci.Gott.vi. 1784. (Figure 2A)

Myanmar name : Chyingma

English name : Unknown

Flowering period : April to September

Outstanding characters

Perennial small trees; stems and branches terete, grayish brown, branchlets covered with brownish gray. Leaves imparipinnate, alternate, exstipulate; racheae winged. Inflorescences axillary or terminal large panicle, many-flowered. Flowers bisexual, actinomorphic, pentamerous, hypogynous. Calyx 5-lobed, whitish-green, pubescent. Petals 5, free, oblong. Fruits drupaceous, orbicular, 1seeded, glabrous. Seeds ovoid, pale brown.

Specimens examined: Mandalay Region, Pyin Oo Lwin Township, Yae Chan Owe village; 21° 40' 26"N and 96°26' 30"E; Aug 17, 2019; Hnin Yu Maw, collection no. 36.

Pollen morphology (Figure 2 B, C)

Tricolporate, oblate, small, 12.0 – 14.4 × 16.8 – 19.2 µm in length and breadth; amb rounded triangular; colpi ¾ way up to the pole, 10.8 – 14.4 × 3.6 – 6.0 µm in length and breadth; pori lalongate, 3.6 – 6.0 × 4.8 – 7.2 µm in and length breadth; exine 1.2 µm thick, sexine thicker thannexine; sculpturing striate, the lumina heterobrochate, 2.4 – 3.6 µm width; the muri simplibaculate, about 0.6 µm wide.

4. Family – Sapindaceae

***Aesculus punduana* Wall., Numer. List (Wallich) nm1189.1829. (Figure 2 D)**

Myanmar name : Yemyaw

English name : Horse chestnut

Flowering period : March to May

Outstanding characters

Perennial, every green trees; stems and branches terete, stout, glabrous. Leaves palmately compound, opposite, exstipulate, glabrous on both surfaces. Inflorescences terminal, many-flowered. Flowers bisexual, zygomorphic, pentamerous, hypogynous. Sepals 5, elliptic-oblong. Petals 5, orbicular, glabrous. Fruits capsule, yellowish brown. Seeds oblongoid, black, glabrous.

Specimens examined: Kachin State, Naw Lann Village; 25° 23' 39"N and 97º 53° 33'E; March 18, 2019; Hnin Yu Maw, collection no. 30.

Pollen morphology (Figure 2 E, F)

Tricolporate, subprolate, medium, 27.6 – 32.4 × 25.2 – 27.6 µm in length and breadth; amb triangular; colpi ¾ way up to the pole, 21.6 – 24.0 × 4.8 – 7.2 µm in length and breadth; pori lalongate, 2.4 – 4.8 × 3.6 – 6.0 µm in length and breadth; exine 1.2 – 2.4 µm thick, sexine thicker than nexine; sculpturing reticulate, the lumina heterobrochate, 1.2 – 2.4 µm width; the muri simplibaculate, about 1.2 µm wide.

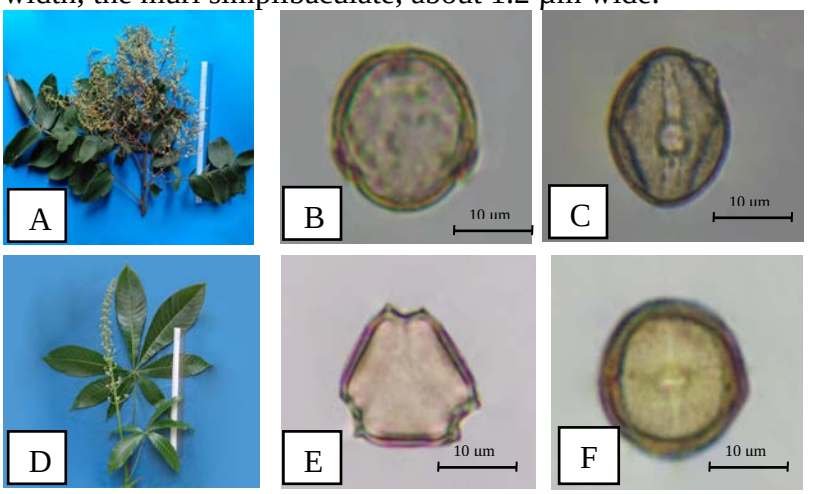

Figure 2. A. Inflorescences of*Rhus semialata* Murr.
B. Polar view pollen grain of*R. semialata* Murr.
C. Equatorial view pollen grain of *R. semialata* Murr.
D. Inflorescences of *Aesculus punduana* Wall.
E. Polar view pollen grain of *A. punduana* Wall.
F. Equatorial view pollen grain of *A. punduana* Wall.

5. *Sapindus emarginata* Vahl. Symb. Bot. 3:54. 1794. (Figure3 A)

Myanmar name : Kinpadi

English name : Soapnut

Flowering period : July to January

Outstanding characters

Medium to large-sized tree with much branched spreading crown; stems and branches terete, densely silky. Leaves paripinnate, opposite or subopposite, Inflorescences terminal paniculate, crowded at the base of young shoot. Flowers bisexual, white, polygamous. Sepals 5, 2 - seriate, ovate, apex strigose. Petals clawed, with woolly scales above the claw. Berry 3-lobed, obovoid. Seed globular, with a hard testa, black.

Specimens examined: Mandalay Region, Mandalay Hill; 22º 00' 50" N and 96º 06' 38" E; 13 January, 2019; Hnin Yu Maw, collection no. 24.

Pollen morphology (Figure 3 B, C)

Tricolporate, oblate spheroidal, small, 12.0 – 20.4 × 14.4 – 21.6 µm in length and breadth; amb rounded triangular; colpi longicolpate, 12.0

18.0 ×4.8 – 7.2 µm in length and breadth; pori circular, 6.0 – 7.2 µm in diameter; annuli present, 1.2 – 3.6 µm in diameter; exine about 1.2 µm thick, nexine thicker than sexine; sculpturing psilate.

6. *Sapindus rarak* DC. Prodr.(DC.) 1:608. 1824. (Figure 3D)

Myanmar name : Magyi bauk, Kala kimmum

English name : Soapnut

Flowering period : March to June

Outstanding characters

Perennial, deciduous trees; stems and branchesstrong, grooved. Leaves paripinnate, alternate. Inflorescences terminal, erect. Flowers bisexual, zygomorphic, hypogynous, pentamerous. Sepals 5, free,ovate,

pubescent. Petals 4, free, ovate, glabrous. Fruits schizocarps, globose, 3-seeded. Seeds nonendospermic.

Specimens examined: Kachin State, Bamaw Township; 21°56'11 "N and 95°58'49" E; March 16, 2019; Hnin Yu Maw, collection no. 26.

Pollen morphology (Figure 3 E, F)

Tricolporate, oblate, small, 12.0 – 14.4 × 18.0 – 20.4 µm in length and breadth; amb triangular; colpi ¾ way up to the pole, 9.6 – 13.2 × 3.6 – 6.0 µm in length and breadth; pori lalongate, 2.4 – 6.0 × 3.6 – 4.8 µm in length and breadth; exine 0.6 – 2.4 µm thick, sexine thicker than nexine; sculpturing obscurely reticulate.

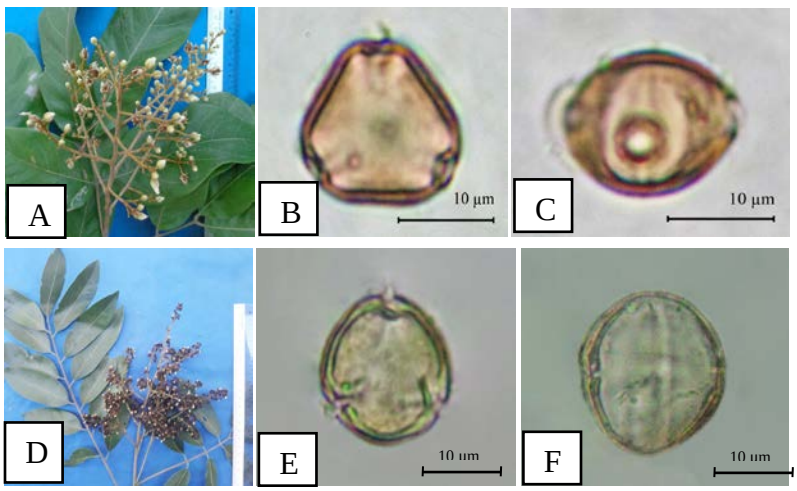

Figure 3. A. Inflorescences of *Sapindus emarginata* Vahl.
B. Polar view pollen grain of *S. emarginata* Vahl.
C. Equatorial view pollen grain of *S. emarginata* Vahl.

7. *Schleichera oleosa*(Lour.) Merr., Interpr. Herb. Amboin. 337. 1917.

(Figure 4A)

Myanmar name : Gyo

English name : Ceylon oak, Lac tree

Flowering period : March to April

Outstanding characters

Perennial deciduous tree; stems and branches terete, glabrous. Leaves paripinnate, alternate, exstipulate. Inflorescences axillary lax panicles. Flowers bisexual or unisexual, actinomorphic, hypogynous, pentamerous. Male flowers; perianth lobe 5, triangular; female flowers; carpels 3; ovary superior, pubescent. Fruits drupaceous, ovoid to subglobular. Seeds 1- or 2 seeded, ovoid.

Specimens examined: Kachin State, Momauk Township, Stone Village Resort; 21°57'53"N and 96°12'53"E; March 18, 2019; Hnin Yu Maw, collection no. 31.

Pollen morphology(Figure 4 B, C)

Tricolporate, prolate spheroidal, small, 19.2 – 22.8 × 16.8 – 20.4 µm in length and breadth; amb triangular; colpi longicopate, 18.0 – 21.6 × 2.4 – 4 .8 µm in length and breadth; pori lalongate, 2.4 – 4.8 × 3.6 – 6.0 µm in length and breadth; exine 1.2 – 2.4 µm thick, sexine thicker than nexine; sculpturing reticulate, the lumina heterobrochate, 0.6 – 2.4 µm width; the muri simplibaculate, about 1.2 µm wide.

8. Family – Rutaceae

***Clausena emarginata* C.C. Huang, Acta Phytotax. Sin.8: 93. 1959**

(Figure 4D)

Myanmar name : Unknown

English name : Unknown

Flowering period : April to May

Outstanding characters

Trees; stems and branches terete, grayish black. Leaves imparipinnate, alternate, exstipulate. Inflorescences axillary or terminal paniculate raceme. Flowers bisexual, actinomorphic, pentamerous, hypogynous. Calyx 5-partite, broadly ovate, green. Petals 5, free, oblong, glabrous. Fruits baccate, globoid to ovoid, one seeded. Seeds solitary, oblong, non-endospermic.

Specimens examined: Mandalay Region, Pyin Oo Lwin Township, Yae Chan Owe village; 21° 40' 26"Nand 96° 26' 30"E; May 11, 2019; Hnin Yu Maw, collection no. 33.

Pollen morphology(Figure 4 E, F)

Tricolporate, prolate , small, 18.0 – 24.0 × 14.4 – 16.8 µm in length and breadth; amb triangular; colpi ¾ way up to the pole, 14.4 – 18.0 × 2.4 – 6 .0 µm in length and breadth; pori lalongate, 3.6 – 6.0 × 4.8 – 7.2 µm in length and breadth; exine 1.2-2.4 µm thick, sexine thicker than nexine; sculpturing reticulate, the lumina heterobrochate, 2.4 – 3.6 µm width; the muri simplibaculate, about 2.4 µm wide.

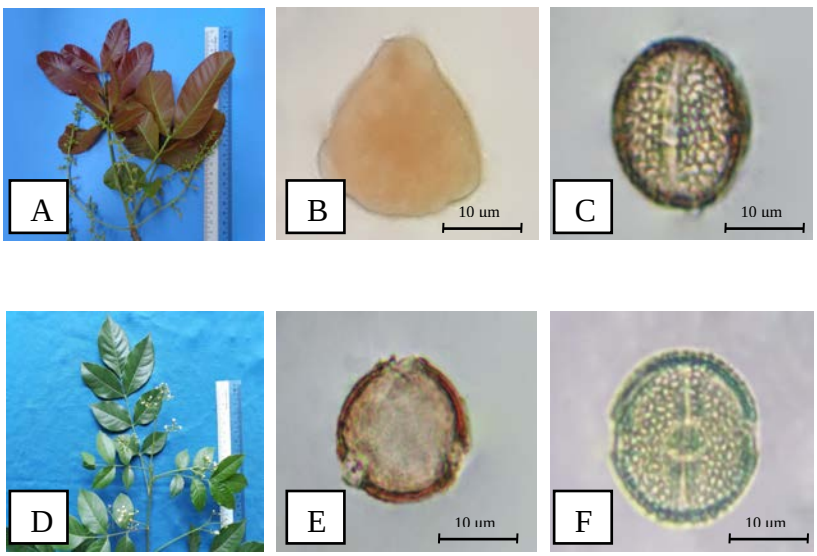

Figure 4. A. Inflorescences of *Schleichera oleosa* (Lour.) Merr.
B. Polar view pollen grain of *S. oleosa* (Lour.) Merr.
C. Equatorial view pollen grain of *S. oleosa* (Lour.) Merr.
D. Inflorescences of *Clausena emarginata* C.C. Huang
E. Polar view pollen grain of *C. emarginata* C.C. Huang
F. Equatorial view pollen grain of *C. emarginata* C.C. Huang

Discussion and Conclusion

The pollen morphology of 8 species and7genera belonging to the order Sapindales found in Mandalay, Sagaing Region and Kachin State was examined. The order Sapindales consists of nine families, namely Anacardiaceae, Biebersteiniaceae, Burseraceae, Kirkiaceae, Meliaceae, Nitrariaceae, Rutaceae, Sapindaceae and Simaroubaceae. Among them, six families such as Anacardiaceae, Sapindaceae, Rutaceae, Simaroubaceae, Burseraceae and Meliaceae are found in Myanmar. In this paper, four families, Burseraceae, Anacardiaceae, Sapindaceae and Rutaceae were presented.

In the present study, all species are trees and the family Burseraceae contain one species of *Garuga pinnata* Roxb., Anacardiaceae including 2 species and 2 genera of *Lannea coromandelica* (Houtt.) Merr. and *Rhus semialata* Murr. were recorded. Four species and 3 genera of Sapindaceae was found in *Aesculus punduana* Wall., *Sapindus emarginata* Vahl., *Sapindus rarak* DC. and *Schleichera oleosa* (Lour.) Merr.; one species of *Clausena emarginata* C.C. Huang belong to family Rutaceae.

According to the aperture types, tricolporate pollen grain were found in all studied species. The shape of pollen grains are prolate spheroidal, oblate spheroidal, prolate, oblate and subprolate. Prolate spheroidal were found in *Lannea coromandelica* (Houtt.) Merr. and *Schleichera oleosa* (Lour.) Merr. Oblate spheroidal were found in *Sapindus emarginata* Vahl., prolate pollen grain were occurred in *Clausena emarginata* C.C. Huang., oblate pollen grain are found in *Rhus semialata* Murr. and *Sapindus rarak* DC. Subprolate pollen were described in *Garuga pinnata* Roxb. and *Aesculus punduana* Wall.

In the study area, 6 species of pollen grains are small and 2 species are medium grains. The sculptures of pollen grains are psilate, reticulate, rugulae, striate and obscurely reticulate. 2 species are obscurely reticulate, 3 species are reticulate, and rugulae, striate and psilate are one species respectively.

According to the results, different types of pollen characters were investigated and recorded. The important pollen morphological

characteristics that are useful for classification and identification of flowering plants are pollen shape and type of exine sculpturing. These morphological features of pollens will support the identification and classificatioin of order Sapindales. Therefore, these pollen characters are very important and beneficial for the future researchers.

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Optimization Parameters of Fermentation of Selected Soil Bacterium KM-39 on *Escherichia coli*

Khin Min Min Kyaw[1] & Zar Zar Yin[2]

Abstract

The present study was focused on the fermentation conditions of selected bacterium (KM-39), on *Escherichia coli.* Different fermentation parameters of KM-39 were studied and included fermentation period, inoculum size, age of inoculum, and antibacterial activity of KM-39 on various carbon and nitrogen sources, fermentation medium (FM), pH, temperature and agitation condition. In the fermentation period, KM-39 showed the highest antimicrobial activity (26.66 mm) on *Escherichia coli* at 2 days old culture. In the size of inoculum, 5%, 10%, 15%, 20%, 25% and 30% were used and 20% was suitable conditions. In the age of inoculum, 24 hrs, 36 hrs, 48 hrs, 60 hrs, 72 hrs, 84 hrs, 96 hrs, 108 hrs, 120 hrs, 132 hrs, 144 hrs, 156 hrs were used and 72 hrs were found to be the optimum age of inoculum. In the investigation of different carbon and nitrogen sources, excellent growth and the best antimicrobial production of selected bacterium KM-39 were observed both in carbon and nitrogen sources. The addition of lactose as a carbon source and yeast extract as nitrogen source resulted the excellent growth of KM-39. The highest antibacterial activity was obtained by using the sucrose and maltose in the carbon source and potassium nitrate in the nitrogen source. Twelve kinds of fermentation media (FM) were utilized and the highest antibacterial activity was obtained in FM-5. And maximum bioactive metabolite production occurred in pH 8 (23.63 mm) and temperature 40ºC was found to be the best activities (22.41 mm) on *Escherichia coli.* In the comparison of shaking culture and static culture, the diameter of inhibitory zone was more higher activity (26.51 mm) on *Escherichia coli* than the static culture.

Keywords: fermentation conditions, growth of bacteria, antibacterial activity

Introduction

Bacteria are present in most habitats on Earth, growing in soil, acidic, radioactive waste, (Fredrickson JK, *et al.*, 2004). Water, and deep in the Earth's crust, as well as in organic matter and the live bodies of plants and animals, providing outstanding examples of mutualism in the digestive

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tracts of humans, termites and cockroaches. There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a milliliter of fresh water, in all, there are approximately five nonillion (5×10^{30}) bacteria on Earth (Whitman WB, 1998). Most bacteria have not been characterized, and only about half of the phyla of bacteria have species that can be grown in the laboratory (Rappe MS, Giovannoni SJ, 2003).

E. coli a member of the bacterial family of Enterobacteriaceae, is the most prevalent commensal inhabitant of the gastrointestinal tracts of humans and warm-blooded animals, as well as one of the most important pathogens (Kaper, J.B. *et al.,* 2004). *E. coli* is used in a wide variety of applications both in the industrial and medical area and its is the most used microorganism in the field of recombinant DNA technology (Yoo, S.H, *et al.,* 2009).

This research work was carried out by the optimum fermentation conditions of selected bacterium (KM-39).

The aim and objectives of this research were to investigate the utilization of carbon and nitrogen sources of the selected bacterial growth and to optimize the fermentation condition of selected bacterium (KM-39).

Materials and Methods

The effects of fermentation period size and age of inoculum for fermentation of KM-39

The fermentation period (24, 48, 72, 96 and 120 hrs) were employed for the production of antimicrobial metabolite.

In the investigation of sizes of inoculum (5%, 10%, 15%, 20%, 25% and 30%) were used for the antimicrobial activity of KM-39. Seed culture was inoculated at room temperature. In the study of ages of inoculum, the incubation of seed culture times (24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144 and 156 hrs) were used and transferred into the fermentation media. Fermentation were carried out for 7 days and antimicrobial activity was tested by agar well diffusion method.

Preparation of Agar Well Method

Isolated strains were subjected with antimicrobial activities by agar well method for antimicrobial activity. Cork borer was used to make the wells (8 mm in diameter) in the autoclaved basal antimicrobial test-medium. Well impregnated with 24, 48 and 72 hours fermented broth (20 µL) were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured.

Therefore, the diameter of clear zones has observed as potent activity shown by respective strain. Clear zones surrounding the test wells were indication of the presence of antimicrobial activities which inhibit the growth of the test organisms selectively (Collins, 1965).

Test organisms

Escherichia coli AHU 5436, *Bacillus subtilis* IFO 90571, *Bacillus pumilus* IFO 12092, *Candida albicans* NITE 09542, *Pseudomonas fluorescens* IFO 94307, *Staphylococcus aureus* AHU 8465, *Agrobacterium tumefaciens* NITE 09678 and *Malassezia furfur* AUV 0255.

Carbon and Nitrogen Utilization

Optimal fermentation conditions are very important for maximal productivity of metabolites. In this study, carbon and nitrogen sources were employed in the fermentation for the production of antimicrobial metabolites. Carbon sources such as arabinose, dextrose, fructose, galactose, lactose, maltose, sucrose, xylose, glycerol, mannitol and soluble starch were used. Nitrogen sources such as asparagine, casein, gelatin, peptone, urea, yeast extract, ammonium chloride, ammonium sulphate, ammonium nitrate, potassium nitrate, sodium nitrate and malt extract were also used.

Media used in fermentation study (NITE, 2005)

Fermentation was undertaken with suitable conditions of 20% sizes and 72 hrs ages of inoculum with twelve different media. Fermentation was carried out 5 days and antimicrobial activity test was carried out every 24 hrs.

Effect of incubation pH and temperature on KM-39

Effects of different pH were used for antimicrobial activity of pH 4, 5, 6, 7, 8, 9 and 10. These different pH were adjusted by NaOH and HCl.

The selected bacterium KM-39 was inoculate and incubated at five different temperature by using 25ºC, 30ºC, 35ºC, 40ºC and 45ºC.

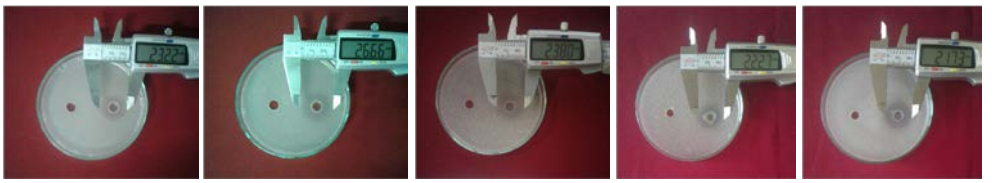
Effect of aeration upon the secondary metabolite

100 mL conical flask containing 50 mL of the best fermentation medium was incubated on the shaker (100 rpm) for 2 days. At the same time, another those fermentation medium was incubated under static condition without shaking. These shaking culture and static culture were compared by using agar well diffusion assay method.

Results

Table 1. Antibacterial activity on fermentation period of selected bacterium KM-39 against *E. coli*

Fermentation period (day)	Antimicrobial activity (mm)
1 day	23.22
2 days	**26.66**
3 days	23.80
4 days	22.27
5 days	21.73



1 day 2 days 3 days 4 days 5 days

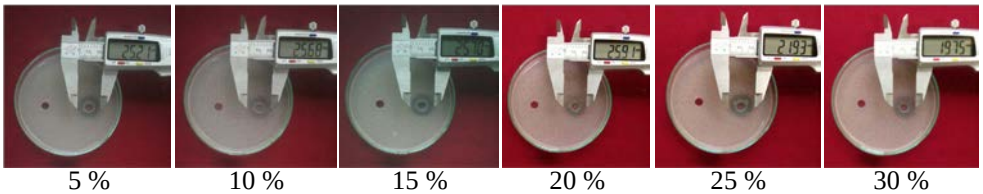
Figure 1. Antibacterial activity of selected bacterium KM-39 against *E. coli*

Table 2. Effect of size of inoculum for selected bacterium KM-39

Size (%)	Inhibitory zone (mm)
5%	25.21
10%	25.68
15%	25.70
20%	**25.91**

Size (%)	Inhibitory zone (mm)
25%	21.93
30%	19.75

Test organism was *E. coli* (fermentation 2 days)



5 % 10 % 15 % 20 % 25 % 30 %

Figure 2. The effects of sizes of inoculum on *E. coli* for KM-39

Table 3. The effect of ages of inoculum of the fermentation against *E. coli*

Age of Inoculum (hrs)	Antibacterial activity inhibitory zone (mm)
24 hrs	22.90
36 hrs	26.89
48 hrs	27.30
60 hrs	28.77
72 hrs	**31.38**
84 hrs	26.08
96 hrs	25.30
108 hrs	24.05
120 hrs	23.80
132 hrs	23.64
144 hrs	21.73
156 hrs	20.68

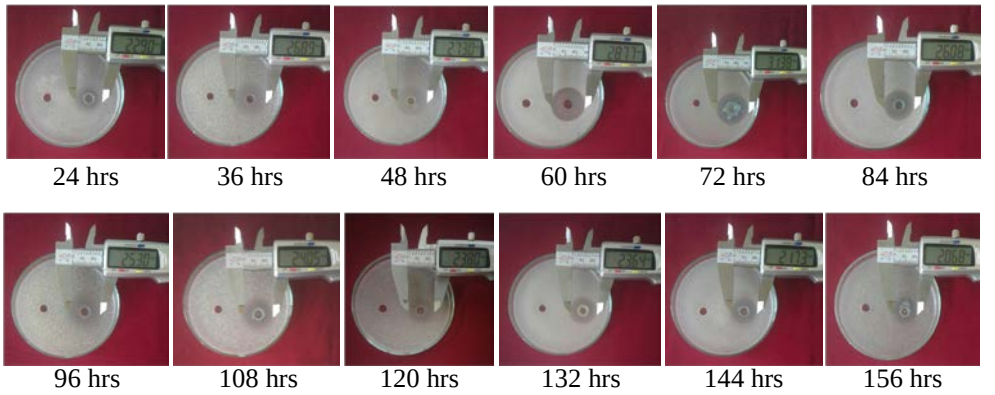


Figure 3. The effects of age of inoculum on *E. coli* for KM-39

Investigation of carbon sources utilization

The effects of different carbon sources were observed for antibacterial maximum metabolites production. In the antibacterial activity, the highest activity of KM-39 was obtained by using the sucrose (25.18 mm) on *E. coli*.

Table 4. Antibacterial activity on different carbon sources of selected bacterium KM-39

No.	Carbon sources	Inhibitory zone (mm)
1	Arabinose	23.73
2	Dextrose	22.05
3	Fructose	21.89
4	Lactose	20.44
5	Galactose	20.69
6	Xylose	24.46
7	**Sucrose**	**25.18**
8	Maltose	21.20
9	Glycerol	21.91
10	Mannitol	20.92
11	Soluble starch	21.73

Agar well = 8 mm

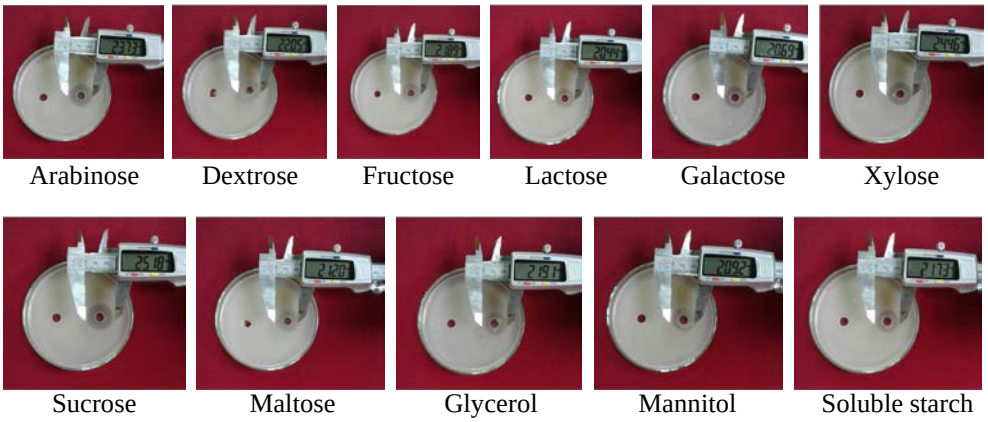


Figure 4. Effects of carbon utilization of fermentation against *E. coli*

Investigation of nitrogen sources utilization

In this study, the highest antibacterial activity was obtained by using potassium nitrate (29.07 mm) on *E. coli.*

Table 5. Antibacterial activity on different nitrogen sources of selected bacterium KM-39

No.	Nitrogen sources	Inhibitory zone (mm)
1	Asparagine	22.00
2	Casein	17.79
3	Gelatin	19.92
4	Peptone	17.46
5	Urea	19.48
6	Yeast extract	22.08
7	Ammonium chloride	20.36
8	Ammonium sulphate	18.90
9	Ammonium nitrate	24.01
10	**Potassium nitrate**	**29.07**
11	Sodium nitrate	28.39
12	Malt extract	19.65

Agar well = 8 mm

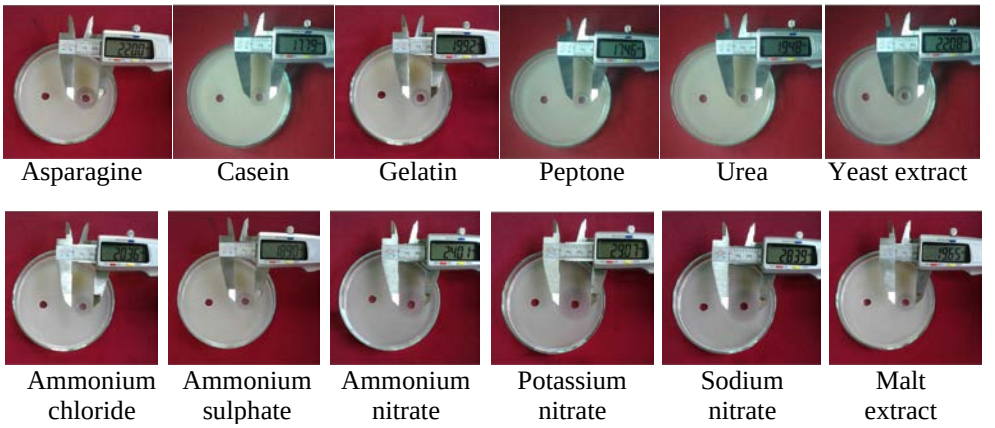
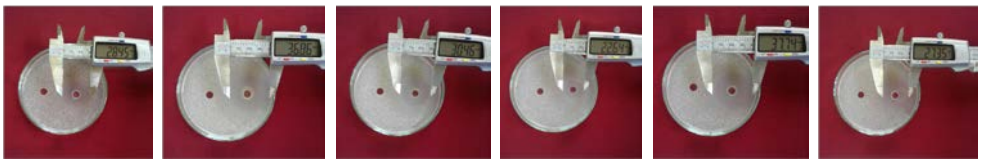


Figure 5. Antibacterial activity on different nitrogen sources of selected bacterium KM-39

Table 6. Selection of fermentation medium based on the results of antibacterial activity of KM-39

Fermentation media	Antibacterial activity (mm)
FM-1	25.10
FM-2	32.56
FM-3	29.31
FM-4	37.11
FM-5	**43.95**
FM-6	38.02
FM-7	28.45
FM-8	36.86
FM-9	30.46
FM-10	22.64
FM-11	37.74
FM-12	27.35

FM-1 FM-2 FM-3 FM-4 FM-5 FM-6

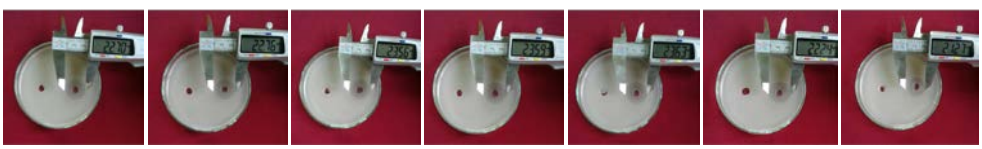


FM-7 FM-8 FM-9 FM-10 FM-11 FM-12

Figure 6. Selection of fermentation medium based on the results of antibacterial activity of KM-39

Table 7. Effects on pH utilization of KM-39 against *E. coli*

pH range	Inhibitory zone (mm)
4	22.70
5	22.76
6	23.56
7	23.59
8	**23.63**
9	22.24
10	21.27



pH-4 pH-5 pH-6 pH-7 pH-8 pH-9 pH-10

Figure 7. Effects of pH on the fermentation of KM-39 on *E. coli*

Table 8. Effect of different temperature utilization of fermentation against *E. coli*

Temperature range	Inhibitory zone (mm)
25ºC	18.86
30ºC	20.52
35ºC	20.58
40ºC	**22.41**
45ºC	-

25ºC 30ºC 35ºC 40ºC

Figure 8. Effects of different temperature utilization of fermentation against *E. coli*

Comparison between shaking culture and static culture

In this study, it was observed that the comparison between shaking culture and static culture. The shaking culture of KM-39 showed the inhibitory zone (26.51 mm) and the static culture showed (22.96 mm) for 2 days fermentation against *E. coli.*

Shaking culture on *E. coli* Static culture on *E. coli*

Figure 9. Comparison of shaking culture and static culture against *E. coli*

Discussion and Conclusion

The present study was focused on the fermentation conditions of selected bacterium (KM-39), on *Escherichia coli.* In the fermentation period, KM-39 showed the highest antimicrobial activity (26.66 mm) on *E. coli.*

Fermentation time is a very important factor, which affect yield and quality of metabolites (Breidt *et al.,* 1995). Fermentation technology is widely used for the production of various economically important compounds which have applications in the energy production, pharmaceutical, chemical and food industry. Various microorganisms have been reported to produce an array of primary and secondary metabolites, but in a very low quantity (Dubey *et al.,* 2011).

In the investigation to optimize the fermentation, it was found that 72 hrs age of inoculum and 20% of size of inoculum were suitable for fermentation.

Shakeela, 2017 reported that effect of size of inoculums by selected bacterial isolates was significantly affected by varying concentration of inoculum (1%, 5%, 10% and 15%) at 35ºC after 72 hrs of incubation.

In the investigation of different carbon and nitrogen sources, excellent growth and the best antimicrobial production of selected bacteria KM-39 were observed both in carbon and nitrogen sources.

Carbon source is an important parameter for active proliferation of organisms and production of organic acids and nitrogen source is important for the production of organic acids (Kucey, 1989).

Glucose, usually an excellent carbon source for growth, interferes with the biosynthesis of many antibiotics such as bacitracin (Haavik, 1974) and actinomycin (Gallo and Katz, 1972).

The nitrogen sources appear to have the most relevance for microbial nitrogen mass in soil (M.AI-Kaisi Mahdi, *et al.,* 2008).

Twelve kinds of fermentation media (FM) were utilized and the highest antimicrobial activity was obtained in FM-5.

A balanced ingredient in the medium as nutrition for bacterial growth and production of antimicrobial substances is important. Their synthesis can be influenced by manipulating the type and concentration of nutrients formulating the culture media. Among them, the effect of the carbon source has been the subject of continuous studies by both industry and research groups (Sanchez, *et al.,* 2010).

And maximum bioactive metabolite production occurred in pH 8 and temperature 40ºC.

The production of antimicrobial substances depends upon the substrate medium for their optimal growth, temperature, pH and the concentration of nutrients in the medium (Leifert *et al.*, 1995).

In the comparison of shaking culture and static culture, the diameter of inhibitory zone was more higher activity (26.51 mm) on *E. coli* than the static culture.

Bala, 2012 obtained that the antimicrobial activity was higher in the combination of yeast extract and fructose and highest activity of this combination was recorded against *P. expansum* (46.5 ± 2.12 mm) and *E. coli* (42 ± 1.41 mm).

In conclusion, the current fermentation conditions that regulate antimicrobial substances metabolism allows to choose fermentation process that minimize, moderate or maximize antimicrobial substances productivity.

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Palynological Study on Some Species of Family Malvaceae in Shwedaung Township

Myint Myint Khaing[1] & Sanda Myint[2]

Abstract

The pollen morphology of twelve species belonging to nine genera of Malvaceae family were studied in the present paper. The pollen grains of all specimens were collected from Shwedaung Township, Pyay District, Bago Region (west). The pollen morphological characteristics of all species were studied. The aperture type and sculpture pattern of each grain were examined by electric microscope. Two types of aperture (tricolporate and porate) and two types of exine sculpture echinate and reticulate are found in this study. The outline of pollen images for each species were presented by surface view, polar and equatorial views and then were recorded by photomicrographs of clear cut pollen images and types of habit and flower.

Keywords: pollen grains, exine sculpture, echinate, reticulate.

Introduction

Palynology is the scientific study of plant pollens, spores, microscopic planktonic organism, in both living and fossil form. Pollen morphology is one of the most important and fundamental branches of palynology. The study of pollen morphology helps in the confirmation of relationship and affinities between the related taxa. Pollens of related families and genera are usually of more or less the same type (Nair *et al*., 1964).

The palynological features have provided a wealth of characters that are important in inferring phylogenetic relationship of plants (Simpson, 2006).

The examination of pollen grains, both recent and ancient can be of value in a range of scientific study (Moore *et al*., 1991). The morphological studies of pollens are very important. It is also used in the field of agriculture, forestry, archaeology and plant geography (Aftab & Perveen, 2006).

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Malvaceae family of about 199 genera, and 3180 species are mainly distributed in tropical and subtropical regions; some species are cultivated for fibre, food or ornamental purposes (HU Qi-ming, 2007). In Myanmar, about 52 genera and 202 species (Kress *et al*. 2003).

The aims of this research are to study the pollen morphology of the collected species, to support some information into the features use for pollen identification and to provide the valuable pollen characters that can be used in plant classification and identification.

Materials and Methods

The specimens were collected from Shwedaung Township, Pyay District, Bago region (west). Shwedaung Township is situated on the eastern bank of Ayeyarwady River and it is in western part of Bago Region. It lies between 18°20' 10" and 18°45' 00" north latitudes and 95°02' 20" and 95°23' 00" east longitudes.

All the specimens were recorded during the flowering period. Describing and classifying the species were used fresh specimens. Identification of specimens were accomplished in accordance with the taxonomic procedures. By using floristic literatures of Lawrence (1968), Backer (1963-1968), Ali and Nasir (1979-1990), Gilbert (1994), Dassanayake (1983-2000) and HU.Qi-ming & WU Delin (SCBG), (2007-2009). Myanmar names were referred to Hundley and Chit Ko Ko (1987) and Kress *et al*. (2003).

For the pollen study, pollen samples of the specimens were freshly collected from the anthers in blooming flowers. Pollens of each species were stored in glass vials with 1cc of glacial acetic acid/glass bottle with 99.9% alcohol and the specimen was labelled with its specific name. The pollen sample in glacial acetic acid were acetolysed by the standard acetolysis method of Erdtman (1952). The anther specimen in aglass vial were crushed with a glass rod and 1cc of glacial acetic acid were added and then transferred into a test tube and 5-9 drops of concentrated sulphuric acid were added, depending on the amount of pollen materials. The test tubes were put in a water-bath for 15-30 minutes at 70-80˚C.

The fluid in the test tubes were stirred frequently and after boiling, it was centrifuged with distilled water and decant the clear parts. These were carried out repeatedly for three or more times. Then glycerine jelly with

safranin were added to the polliniferous materials according to the method of Kisser formula (Erdtman, 1952).

For the pollen study, the storage bottles were warmed in water bath and a drop of polliniferous jelly were taken out and placed on the glass slide and then covered with a glass coverslip. A glass slide mounted with pollen sample was examined under electric light microscope with (x400) and photomicrograph. The samples of pollen grains for each species were measured and studied. The shape, size, and exine sculpture of the pollens were studied and recorded by microphotographs.

Results

In pollen morphology, 12 species belonging to the 9 genera of family Malvaceae were identified and the morphological characteristics of pollen grains were studied.

Pollen Morphology of Study Species

***1. Abelmoschus ficulneus* (L.) Wight & Arn. Pl. 14 1833. Fig (1)**

Myanmar Name : Taw-yonbade

English Name : White wild musk mallow

Flowering period : September to November

Pollen Morphology

Polyporate, (about 35), pantoporate, spheroidal, very large, 82.5-117.5 µm in diameter; amb circular; pori circular, about 2.0 µm in diameter, the inter poral spaces 10.0-12.5 µm in width; exine 3.7-5.0 µm thick, sexine thicker than nexine; sculpturing echinate, spines 8.7-12.5 µm in length, rounded, straight.

***2. Abelmoschus moschatus* (L.) Medik. Sp. Pl. 1: 696. 1753. Fig (2)**

Myanmar Name : Taw-wah

English Name : Musk mallow

Flowering period : September to January

Pollen Morphology

Polyporate, pantoporate, spheroidal, large-very large, 85-105 µm in diameter; amb circular; pori circular, 3 µm in diameter; exine about 1.5 µm

thick, sexine thicker than nexine; sculpturing echinate, spine 6-7 µm in length, curved, pointed.

***3. Abutilon indicum* (L.) Sweet., FTW ed 2, 3: 379. 1993. Fig (3)**

Myanmar Name : Tha-mu-chok

English Name : Country mellow

Flowering period : October to December

Pollen Morphology

Triporate, zonoporate, spheroidal, medium-large, 42.5 - 55 µm in diameter; amb rounded; pori circular, 3.7-5.0 µm in diameter; exine 2.5-3.7 µm thick, sexine thicker than nexine; sculpturing echinate, spine 3.7-5.0 µm in length, very pointed, straight.

4. Corchorus capsularis L. **Sp. Pl. 529. 1753. Fig (4)**

Myanmar Name : Gonshaw pilaw, pilaw yin

English Name : White jute

Flowering period : September to October

Pollen Morphology

Tricolporate, zonocolporate, subprolate, small, 25-26.2 x 20-22.5 µm in length and breadth; amb rounded; colpi longicolpate with apocolpia, 18.7-20 x 2.5 µm in length and breadth; pori lolongate, 5.0-6.2 x 3.7 µm in length and breadth; exine 1.25 µm thick, sexine thicker than nexine; sculpturing reticulate.

5. Hibiscus cannabinus L., **Syst. Nat., ed. 10. 2: 1149. 1759. Fig (5)**

Myanmar Name : Shwe bo chin baung

English Name : Indian-hemp.

Flowering period : June to August

Pollen Morphology

Polyporate, (about 200), pantoporate, spheroidal, very large, 120 - 145 µm in diameter; amb circular; pori circular, 3.7-5.0 µm in diameter, inter poral spaces 6.2-7.5 µm in width; exine 3.7-5.0 µm thick, sexine

thinner than nexine; sculpturing echinate, spines 7.5-12.5 µm in length, pointed, straight.

***6. Hibiscus sabdariffa* L., Sp. Pl. 2:695. 1753. Fig (6)**

Myanmar Name : Chin-paung

English Name : Roselle

Flowering period : October to December

Pollen Morphology

Polyporate, (about 32), pantoporate, spheroidal, very large, 110.0-117.5 µm in diameter; amb circular; pori circular, 5.0-7.5 µm in diameter; exine about 6.2 µm thick, sexine thicker than nexine; sulpturing echinate; spines 12.5 - 15.0 µm in length, pointed, straight.

***7. Malachra capitata* (L.) L. Syst. Nat. ed. 122:458 1767. Fig (7)**

Myanmar Name : Sinma-hmwe-sok

English Name : Wild okra; Malva

Flowering period : August to November

Pollen Morphology

Polyporate, (70-80), pantoporate, spheroidal, large-very large, 82.5-117.5 µm in diameter; amb circular; pori circular, about 2.5µm in diameter, inter poral spaces 7.5-8.7 µm in width; exine 5.0-7.5 µm thick, sexine thicker than nexine; sculpturing echinate, spines 12.5-15 µm in length, pointed, straight.

***8. Melochia corchorifolia* L. FGD 1: 143. 1987. Fig (8)**

Myanmar Name : Pilaw

English Name : Red weed

Flowering period : October to December

Pollen Morphology

Tricolporate, zonocolporate, prolate spheroidal, medium, 32.2-35.0 x 27.5-31.2 µm in length and breadth; amb rounded; colpi brevicolpate, 17.5-20 x 0.7-1.7 µm in length and breadth; pori lalongate, 3.7 x 8.7-10.0

µm in length and breadth; exine 1.25 µm thick, sexine thicker than nexine; sculpturing reticulate.

***9. Sida acuta* Burm.f. FTW ed. 2, 3: 748. 1993. Fig (9)**

Myanmar Name : Thabyetsi-bin

English Name : Broomweed

Flowering period : August to November

Pollen Morphology

Polyporate, (about 12), pantoporate, spheroidal, large, 60.0 – 65.0 µm in diameter; amb circular; pori circular, about 3.7-5.0 µm in diameter, inter poral spaces 15 and 30 µm in width; exine about 5.0 µm thick, sexine thicker than nexine; sculpturing echinate, spines about 5.0 µm in length, pointed, straight.

***10. Thespesia lampas* Dalz & Gibs. Bombay Fl. 19. 1861. Fig (10)**

Myanmar Name : Thinn paung shaw

English Name : Mallow

Flowering period : Throughout the year

Pollen Morphology

Polyporate, (about 35), pantoporate, spheroidal, very large, 105-120 µm in diameter; amb circular; pori circular, about 3.7-5.0 µm in diameter, the inter poral spaces 11.2-17.5 µm in width; exine about 5.0 µm thick, sexine thicker than nexine; sculpturing echinate, spines about 15.0 µm in length, pointed, straight.

***11. Urena lobata* L. FTW ed. 2, 3:752, pl. 377. 1993. Fig (11)**

Myanmar Name : Kat-se-ne-gale

English Name : Congo jute

Flowering period : August to November

Pollen Morphology

Polyporate (about 36), pantoporate, large, 67.5-75.0 µm in diameter; amb circular; pori circular, 7.5 µm in diameter; exine about 5.0 µm thick, sexine

thicker than nexine; sculpturing echinate; spine 4.5 - 6 µm in length, pointed, straight.

***12. Urena* sp. Fig (12)**

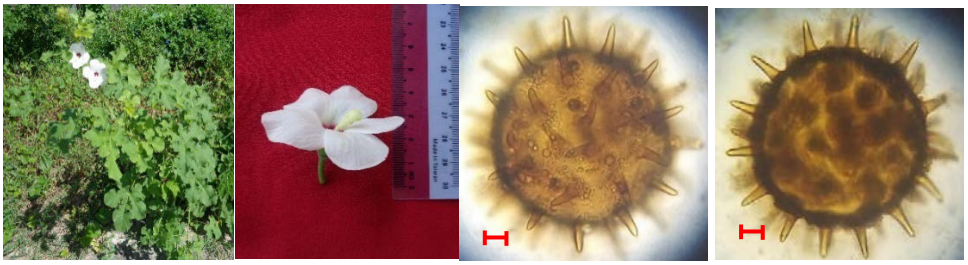
Myanmar Name : Not known

English Name : Not known

Flowering period : June to August

Pollen Morphology

Polyporate, (about 30), pantoporate, spheroidal, large-very large, 95-110 µm in diameter; amb circular; pori circular, 2.5-5.0 µm in diameter, inter poral spaces 10 - 12.5 µm in width; exine 3.7-5.0 µm thick, sexine thicker than nexine; sculpturing echinate, spines 10.0-12.5 µm in length, rounded, slightly curved.

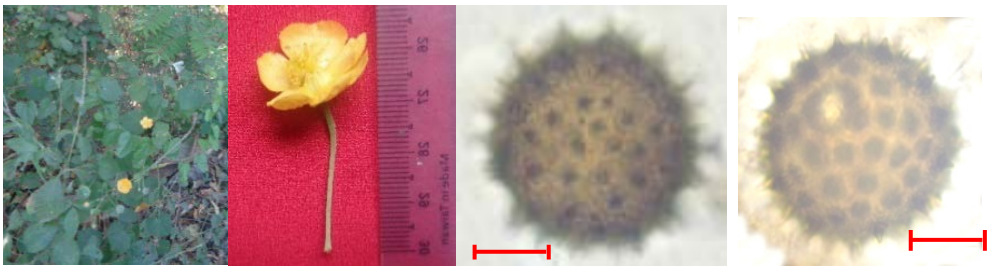


Habit Flower Surface view Surface view

Figure 1. *Abelmoschus ficulneus* (L.) Wight & Arn.

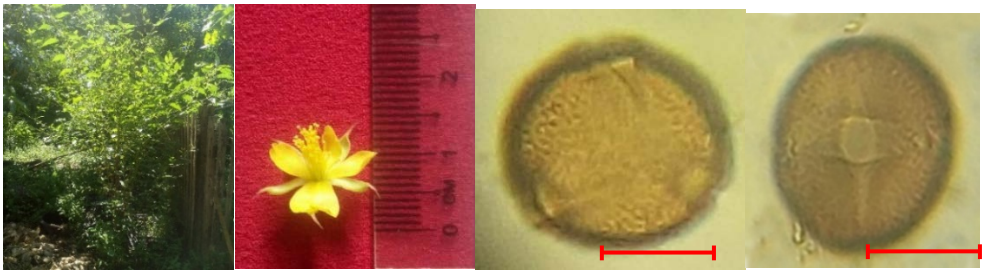
Habit Flower Surface view Surface view

Figure 2. *Abelmoschus moschatus* (L.) Medik.



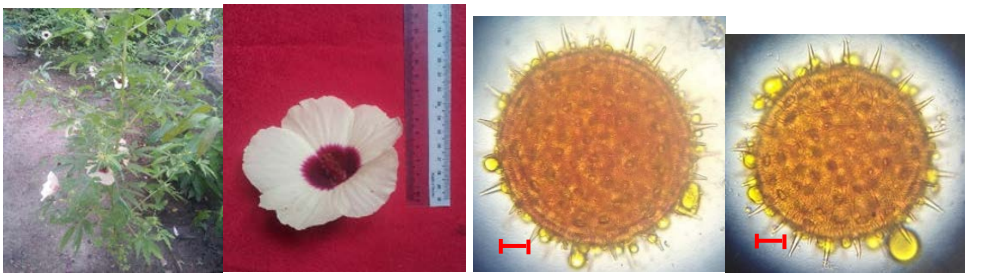
Habit Flower Polar view Equatorial view

Figure 3. *Abutilon indicum* (L.) Sweet.



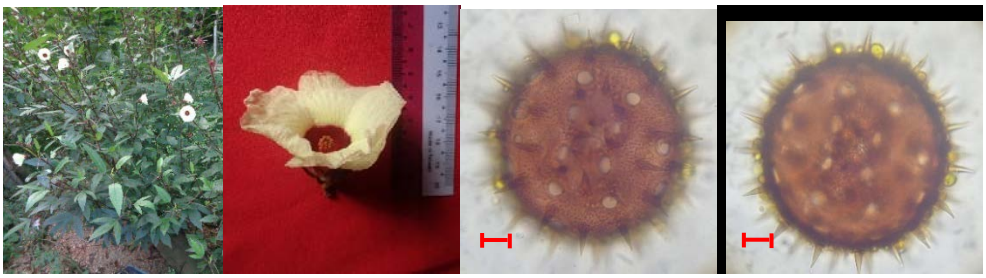
Habit Flower Polar view Equatorial view

Figure 4. *Corchorus capsulari*



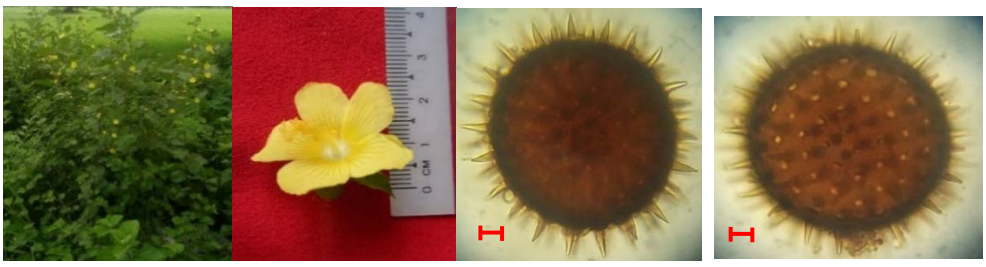
Habit Flower Surface view Surface view

Figure 5. *Hibiscus Cannabinus* L.



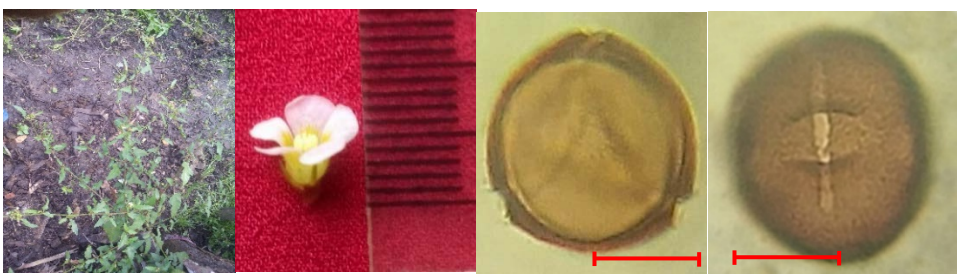
Habit Flower Surface view Surface view

Figure 6. *Hibiscus sabdariffa* L.



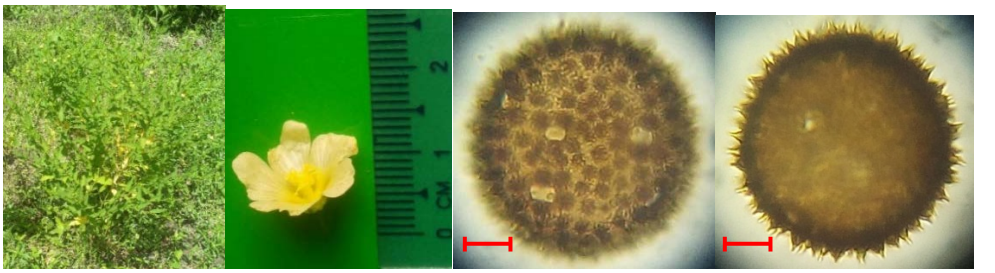
Habit Flower Surface view Surface view

Figure 7. *Malachra capitata* L.



Habit Flower Polar view Equatorial view

Figure 8. *Melochia corchorifolia* L.

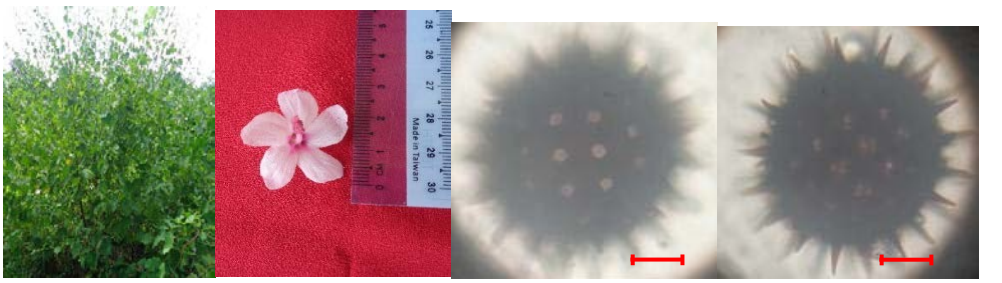


Habit Flower Surface view Surface view

Figure 9. *Sida acuta* Burm.f.

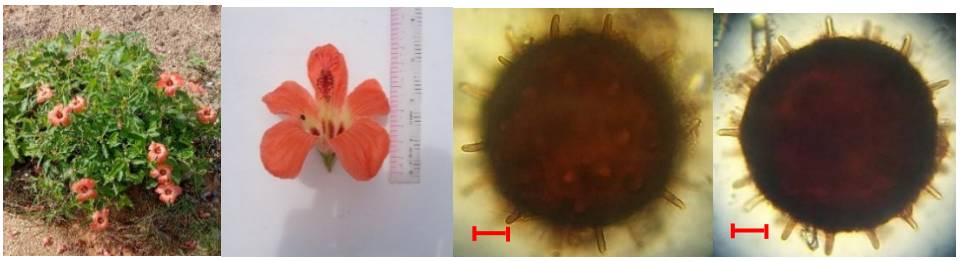
Habit Flower Surface view Surface view

Figure 10. *Thespesia lampas* Dalz & Gibs.



Habit Flower Surface view Surface view

Figure 11. *Urena lobata* L.



Habit Flower Surface view Surface view

Figure 12. *Urena* sp.

Discussion and Conclusion

The family Malvaceae (Mallow family) include 39 genera and 800-900 species distributed over the world (Bailey, 1939). Malvaceae, the hibiscus, or mallow family (order Malvales) containing some 243 genera and at least 4,225 species of herbs, shrubs, and trees. Genera now included in Malvaceae were thought to be very closely related to the DNA studies were done at the end of the 20 century, they were placed in four different families; Malvaceae, Bombacaceae, Tiliaceae, and Sterculiaceae, this group of families alone constituting Malvales for some earlier botanists (Website). In the present paper, pollen morphology of 12 species belonging to 9 genera of family Malvaceae has been studied.

In the classification of taxa, taxonomic characters of pollen morphology were apertures type, number, position, sculpture, shapes and grain size. In the Malvaceae (Mallow) family, the species of pollen grains were poly porate (panto porate), the pores number about 50-100, spheroidal and the exine were spinose (Erdtman *et al*., 1961).

In the present paper, the types of pollen grains were the colporate and porate. The tricolporate grains were found in ***Corchorus capsularis* L.** and ***Melochia corchorifolia* L.** The rest of all species were porate grains. In the porate grains, ***Abutilon indicum* (L.) Sweet**. was triporate and the remaining species were polyporate grains.

Hesse *et al*., (2009) stated that pollen size varies less than 10µm to more than 100 µm. In this research, the smallest pollen, 25-26.2 x 20-22.5 µm in length and breadth was found in ***Corchorus capsularis* L.** and the

largest pollen, 120-145 µm in diameter was found in ***Hibiscus cannabinus* L.**

In this study, the sculpture patterns of the pollens were observed as reticulate and echinate. The reticulate sculpture was found in ***Corchorus capsularis* L.** and ***Melochia corchorifolia* L.** The others were echidnae sculpture.

The echinate sculpture was the distinct character of pollen grains in the family of Malvaceae. The length and shape of spines in echinate sculpture varies according to the species. In the present study, the spines were within the range of 3.7- 15.0 µm in length. Among them, the largest spine of ***Thespesia lampas* Dalz & Gibs.** was about 15.0 µm in length and the smallest spine 3.7-5.0 µm length in ***Abutilon indicum* (L.) Sweet.**

In the present study, the exine range was found between 1.25 µm and 6.2 µm in thick. The thinnest exine was found in ***Corchorus capsularis* L.** and ***Melochia corchorifolia* L.** (about 1.25 µm in thick), the thickness exine found in ***Hibiscus sabdariffa* L.** (about 6.2 µm in thick).

According to present research, these characters were in agreement with Endtman *et. al* (1961). Therefore, aperture and exine sculpture forms useful taxonomical characters in systematic study of Malvaceae. The present result will provide valuable information for the further studies of palynology. It is believed that the palynological study will be useful in phylogenetic relationship.

Acknowledgements

We are deeply indebted to Dr. Thwe Linn Ko, Pro-Rector, Pyay University, for her permission and encouragement to do this research paper. We wish to express our deep gratitude to Dr. Nyo Nyo Thaung, Professor and Head, Department of Botany, Pyay University, for her encouragement and suggestions.

Glossary of Pollen and Spore Terminology

Amb : The outline of a pollen grain or spore seen in polar view

Aperture : In living pollen grain or spores the apertures usually function as site of germination

Colporus : Compound aperture composed of a colpus combined with an endoaperture of variable size and shape.

Echinate	:	Describing pollen and spores with an ornamentation comprising spines longer than 1µm.
Equatorial view	:	The view of a pollen grain or spore where the equatorial plane is directed toward the observer.
Exine	:	The outer layer of the wall of a palynomorth.
Nexine	:	The inner, non- sculptured part of the exine which lies below the sexine.
Palynology	:	The study of pollens grains or spores.
Panto	:	Pollen grain with pores distributed more or less regularly over the whole surface.
Polar view	:	A view of a pollen grain or spore in which the polar axis is directed towards the observer.
Reticulate	:	A net-work like pattern consisting of lumina and muri.
Sculpture	:	The surface of a pollen grain.
Sexine	:	The outer, sculptured layer of the exine.
Spheroidal	:	Describing the shape of a pollen grain or spore in which the polar axis and equatorial diameter is approximately equal.
Zono	:	A prefix indicating features located equatorially.

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Isolation of Endophytic Microorganisms from Six Plants of Fabaceae and their Antimicrobial Activity

Thinzar Myint[1] & Khin Min Min Phyo[2]

Abstract

The leaves of six medicinal plants grown in Pathein were collected for the isolation of endophytic microorganisms. Thirtysix endophytic microorganisms including 6 bacteria (TZB-01 to TZB-06) and 30 fungi (TZF-01 to TZF-30) were isolated with the different media from six medicinal plants belonging to Fabaceae. The isolated endophytic microorganisms were tested the antimicrobial activity on 9 different test organisms by paper disc diffusion method. Among them, TZF-15 from *Mucuna pruriens* Roxb. (Khwele-ya) showed the best antibacterial activity on *Bacillus pumilis* (28.84 mm of inhibitory zone).

Keywords: endophytic microorganisms, antimicrobial activity

Introduction

Endophytes found ubiquitous in all plant species in the world, contribute to their host plants by producing plenty of substances that provide protection and ultimately survival value to the plant. Many researches have proven that endophyte is a new and potential source of novel natural products for exploitation in modern medicine, agriculture and industry. A great number of novel natural products possessing antimicrobial activities have been isolated from endophytes (Yu *et al.*, 2009).

Endophytes are bacterial or fungal microorganisms that colonize healthy plant tissue intercellularly and/or intracellularly without causing any apparent symptoms of disease (Wilson, 1995). Endophytes are viewed as an outstanding source of bioactive natural products because there are so many of them occupying literally millions of unique biological niches (higher plants) growing in so many unusual environments. Thus, it appears that these biotypical factors can be important in plant selection since they may govern the novelty and biological activity of the products associated with endophytic microbes (Strobel and Daisy, 2003).

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The microbial pathogens, fungi, bacteria, phytoplasmas, viruses and viriods have some common distinguishing characteristics based on which they are classified into class, order, family, genus and species. The taxonomic characters may be studied using various traditional methods involving light microscopy or electron microscopy. Light microscope techniques are employed for the study of fungal. Fungal pathogens infect different plant parts such as roots, stem, leaves, flowers and seeds. Fruits and vegetables may be affected either in the field or during storage (Scott, 1996). The aim and objectives of this research were to study the outstanding characters of medicinal plants, to isolate the endophytic microorganisms from the leaves of medicinal plants belonging to Fabaceae and to investigate the antimicrobial activities of the endophytic microorganisms on different test organisms.

Materials and Methods

Isolation procedure of endophytic microorganisms

Six medicinal plants grown in Pathein were collected for the isolation of endophytic miroorganisms. These six plants belong to Fabaceae by outstanding characters according to the literatures of Botany Department at Pathein University. Leaves of six medicinal plants were used to isolate the endophytic microorganisms.

Plant part were washed in running tap water for 10 min. Plant parts were cut into small pieces. The surface of cut plant pieces were sterilized by socked in 95% alcohol for 15 seconds. Next, the surface of cut plant pieces into smaller pieces. The samples were dried on sterilized tissue paper. Then, the samples were cut pieces were incubated on nutrient agar plate for 2 days to 1 week at room temperature.

Preliminary study for the endophytic microorganisms

The endophytic microorganisms grown on the four sterilized growth media (Medium-I, Glucose 1g, Polypeptone 0.3 g, Agar 1.8 g and DW 100 mL, Medium-II, Glucose 1 g, PDA 0.3 g, Agar 1.8 g and DW 100 mL, Medium-III, Glucose 1 g, Yeast extract 0.3 g, agar 1.8 g and DW 100 mL, Medium-IV, Glucose 1 g, Malt extract 0.3 g, Agar 1.8 g and DW 100 mL) were inoculated in 25 mL of seed medium (Glucose 1 g, Polypeptone 0.3 g, $CaCO_3$ 0.01 g and DW 100 mL) and then incubated for 3 days.

After incubation, the seed culture (1%) was transferred into the fermentation medium (Glycerol 1.0 mL, Yeast extract 0.3 g, $CaCO_3$ 0.01 g and DW 100 mL) and carried out for 3-12 days. Then, the fermentation broth was used to check the antifungal activity by the paper disc diffusion assay (Suto, 1999).

Paper disc diffusion assay

Isolated endophytic bacteria and fungi were tested the antimicrobial activity on 9 different test organisms by paper disc diffusion method (Ando, *et al.*, 2004). The assay medium (glucose 1g, polypeptone 0.3 g, agar 1.8g at pH 7.0) was utilized for the test organisms (Table - 2). The test organisms were inoculated in 20 mL of assay broth in conical flasks respectively and incubated overnight. One percent of test organism was added to assay medium and then poured into petridishes. After solidification, the paper discs impregnated with the samples were applied on the agar plates and the plates were incubated for 24-36 hrs. Clear zones (inhibitory zones) surrounding the paper discs indicate the presence of the metabolites which inhibit the growth of the test organisms.

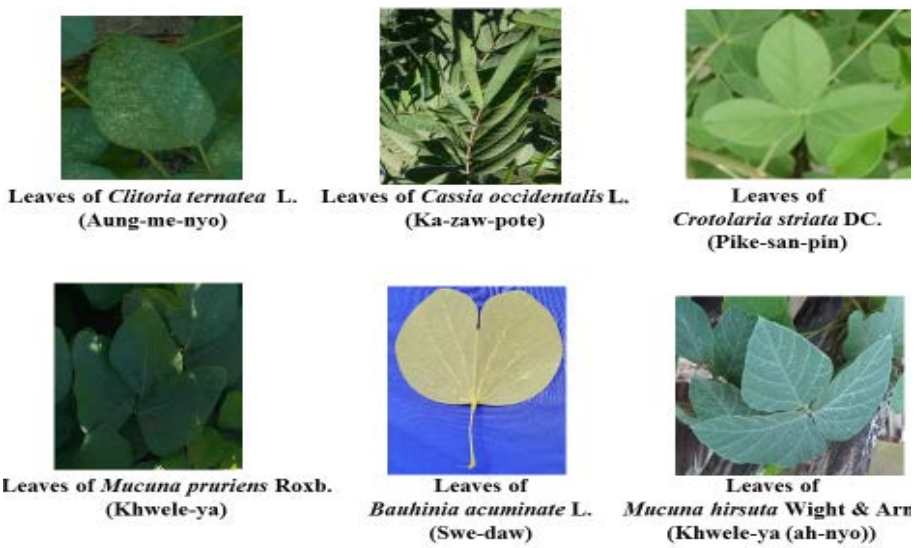


Figure 1. Used the leaves for isolation of endophytic microorganisms

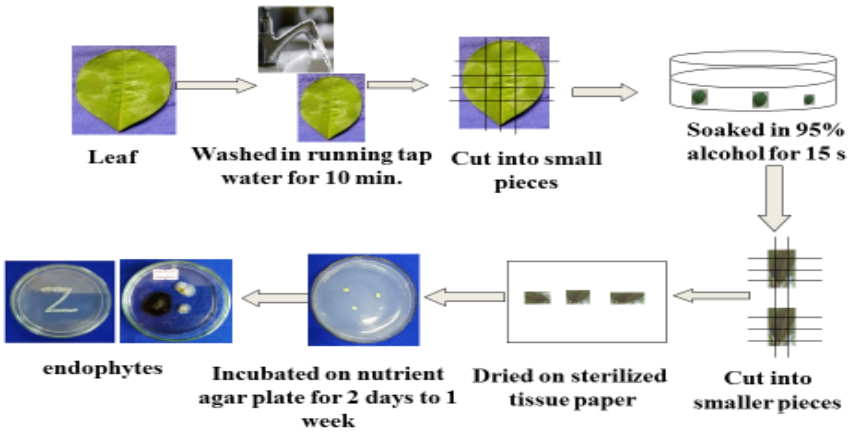


Figure 2. Isolation procedure for endophytic microorganisms (Suto, 1999)

Table 1. Six medicinal plants used for screening of endophytic microbes

No	Scientific Name	Myanmar Name	Family
1	*Clitoria ternatea* L.	Aung-me-nyo	Fabaceae
2	*Cassia occidentalis* L.	Ka-zaw-pote	Fabaceae
3	*Crotolaria striata* DC.	Pike-san-pin	Fabaceae
4	*Mucuna pruriens* Roxb.	Khwele-ya	Fabaceae
5	*Bauhinia acuminate* L.	Swe-daw	Fabaceae
6	*Mucuna hirsuta* Wight & Arn.	Khwele-ya (ah-nyo)	Fabaceae

Table 2. Test organisms used in antimicrobial activities

No	Test organisms	Sources	Infections
1	*Escherichia coli*	AHU 5436	Diarrhoea
2	*Agrobacterium tumefaciens*	NITE 09678	Crown gall disease

No	Test organisms	Sources	Infections
No	Test organisms	Sources	Infections
3	*Candida albicans*	NITE 09542	Candidasis
4	*Bacillus subtilis*	IFO 90571	DNA topoisomerase I
5	*Staphylococcus aureus*	AHU 8465	Food poisoning, Methicillin Resistance
6	*Pseudomonas fluorescens*	IFO 94307	Rice disease
7	*Malasseia furfur*	AVU 0255	Danddruff, Seborrhoeic dermatitis
8	*Bacillus pumilis*	IFO 12092	Wound and burn infection, Fever
9	*Micrococcus luteus*	NITE 83297	Skin disease

Results

Outstanding Characters of *Clitoria ternatea* L. (Aung-me-nyo) (Fabaceae)

Annual, twining herbs. Leaves unipinnate, stipule linear lanceolate. Inflorescence axillary and solitary cymes. Flowers bisexual, lanceolate, pubescent. Calyx tubular, sparsely pubescent, green, lobes subequal. Corolla papilionaceous, standard ovate, bright blue with pale yellow blotch. Stamens 10, diadephous, filaments filiform, anther dithecous, uniform, yellow. Carpels one, ovary superior, unilocular with few ovules in each locule on the marginal placenta, style filiform. Pods linear, flat, dehiscent. Seeds reiform, pale brown.

Figure 3. Habit and flower of *Clitoria ternatea* L. (Aung-me-nyo)

Outstanding Characters of *Crotolaria striata* DC. (Pike-san-pin) (Fabaceae)

Shrubs, silky pubescent. Leaves alternate, trifoliolate palmately compound, stipule linear. Inflorescence lateral elongate raceme. Flower yellow, the bracteoles present near the base of calyx. Calyx campanulate, lanceolate, valvate. Petals 5, the standard yellow, the wings smaller than the keel, yellow, the keel falcate, yellow with red stripes. Stamens (5+5), the anther dimorphous, the stigma minute capitate. Pod oblongoid, turgid, aseptate. Seeds many, reniform.

Figure 4. Habit and inflorescence of *Crotolaria striata* DC. (Pike-san-pin)

Outstanding Characters of *Mucuna pruriens* Roxb. (Khwele-ya) (Fabaceae)

Annual climbing Shrub. Leaves trifoliolate, pubescent. Inflorescences axillary, racemes, pendulous. Calyx campanulate-asymmetrical, sericeous-pubescent, the wings purple. Corolla papilionaceous, standard ovate, wings oblique-oblong. Stamens 10, diadelphous, filaments filiform, anthers dithecous. Carpel 1, ovary oblong, unilocular, style filiform, capitate. Pods oblong, covered with orange hairs. Seeds ellipsoid, brown with black spots.

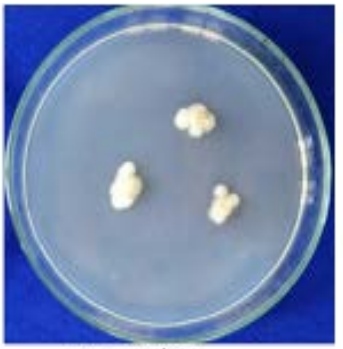
Figure 5. Habit and inflorescence of *Mucuna pruriens* Roxb. (Khwele-ya)

Outstanding Characters of *Mucuna hirsuta* Wight & Arn. (Khwele-ya (ah-nyo)) (Fabaceae)

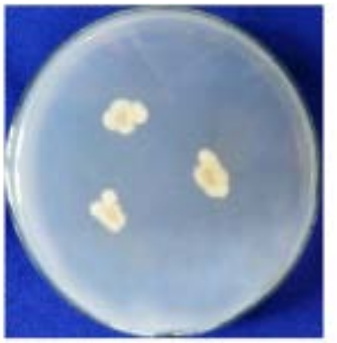
Herbs. Leaves pinnately trifoliolate compound, alternate, pubescent. Inflorescences axillary, racemes, pendulous. Flowers bisexual, dark violet. Calyx campanulate. Corolla papilionaceous, standard ovate, wings oblique-oblong, keels oblong, purple. Stamens 10, diadelphous, filaments filiform, anthers dithecous. Carpel 1, ovary oblong, unilocular, style filiform, stigma small, capitate. Pods oblong, densely orange brown hairs. Seeds oblong black.

Figure 6. Habit and inflorescence of *Mucuna hirsuta* Wight & Arn.
(Khwele-ya (ah-nyo)

Thirtysix endophytic microorganisms were isolated from the leaves of six medicinal plants belonging to Fabaceae. Among them, 6 endophytic bacteria were designated as TZB-01 to TZB-06 and 30 endophytic fungi as TZF-01 to TZF-30 on the different 4 media in table 3. The morphological characters of isolated endophytic fungi for antimicrobial activity were showed in figure 7 to 10. According to the antimicrobial activity on 9 different test organisms, the isolated endophytic bacteria and fungi in the fermentation of seed culture were showed the 3 classes as the best, second best and good inhibitory zones in table 4.



Front view



Reverse view

Figure 7. Isolated endophytic fungus (7 days old culture of TZF-01) from *Clitoria ternatea* L.,

Front view

Reverse view

Figure 8. Isolated endophytic fungus (7 days old culture of TZF-03) from *Crotlaria striata* L.,

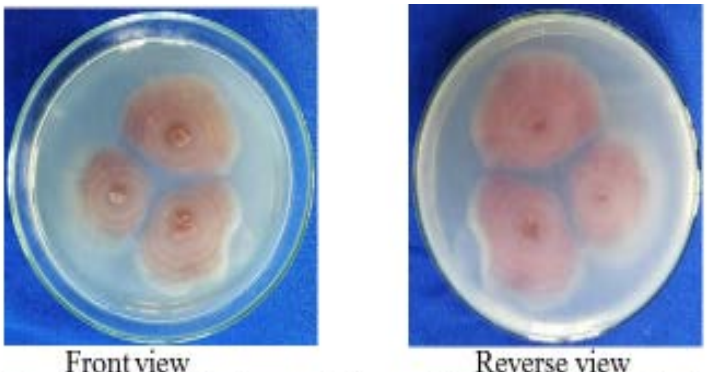


Figure 9. Isolated endophytic fungus (7 days old culture of TZF-15) from *Mucuna pruriens* Roxb,

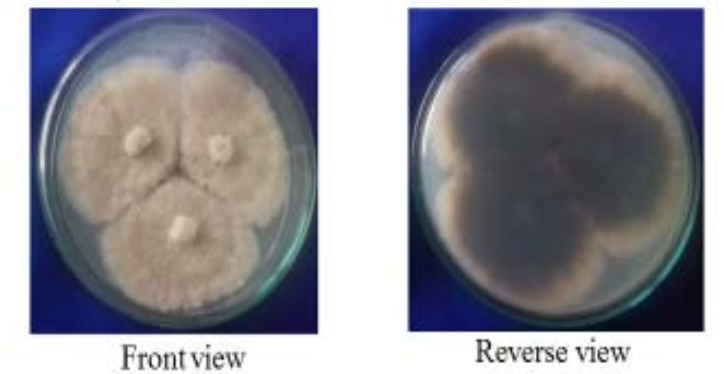


Figure 10. Isolated endophytic fungus (7 days old culture of TZF-30) from *Mucuna hirsute* Weight & Arn,

Table 3. No. of isolated of endophytic microorganisms from six medicinal plants

Scientific Name	M-I	M-I	M-II	M-III	M-IV	Total
Clitoria ternatea L.	TZB-1	TZF-1	TZF-9, TZF-10	TZF-19	TZF-25	6
Cassia occidentalis L.	TZB-2	TZF-2	TZF-11	TZF-20	TZF-26	5
Crotolaria striata DC.	TZB-3	TZF-3, TZF-4	TZF-12	TZF-21	TZF-27	6

Scientific Name	M-I	M-I	M-II	M-III	M-IV	Total
Mucuna pruriens Roxb.	TZB-4	TZF-5, TZF-6	TZF-13, TZF-14, TZF-15	TZF-22	TZF-28	8
Bauhinia acuminate L.	TZB-5	TZF-7, TZF-8	TZF-16, TZF-17	TZF-23	TZF-29	7
Mucuna hirsuta Wight & Arn.	TZB-6	TZF-18	TZF-18	TZF-24	TZF-30	4
Total	6	8	10	6	6	36

Table 4. Antimicrobial Activity of Endophytic Microorganisms on 9 Test Organisms

Used plants	Isolates	*Escherichia coli*	*Agrobacterium tumefaciens*	*Candida albicans*	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas fluorescens*	*Malasseia furfur*	*Bacillus pumilus*	*Micrococcus luteus*
Clitoria ternatea L.	TZB-1	+	-	+	-	-	14 mm	-	-	-
	TZF-1	25.62 mm	-	-	-	15 mm	20.17 mm	-	-	12.04 mm
	TZF-9	20 mm		-	-	-	-	-	15 mm	+
	TZF-10	+	-	-	-	-	-	-	+	-
	TZF-19	-	-	-	-	-	-	-	-	-
	TZF-25	-	-	-	-	-	-	-	-	-
Cassia occidentalis L. .	TZB-2	+	-	-	-	-	15 mm	-	-	-
	TZF-2	+	-	-	-	-	-	-	-	-
	TZF-11	+	-	-	+	+	+	-	+	-

Used plants	Isolates	*Escherichia coli*	*Agrobacterium tumefaciens*	*Candida albicans*	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas fluorescens*	*Malasseia furfur*	*Bacillus pumilus*	*Micrococcus luteus*
	TZF-20	-	-	-	-	-	-	-	-	-
	TZF-26	-	-	-	-	-	-	-	-	-
***Crotolaria striata* DC.**	TZB-3	+	-	-	-	-	+	-	-	-
	TZF-3	26.03mm	-			16 mm	27.99 mm			25 mm
	TZF-4	19 mm	-	+	+	14 mm	+	-	-	+
	TZF-12	+		-	+	+	+	-	+	-
	TZF-21	14.02 mm	-	+	15.09 mm	-	-	-	+	-
	TZF-27	+	-	+	+	-	+	-	+	+
Mucuna pruriens Roxb. .	TZB-4	+	-	-	-	-	+	-	-	-
	TZF-5	+	-	-	+	+	+	-	-	-
	TZF-6	+	-	-	+	+	+	-	-	-
	TZF-13	+	-	-	+	+	-	-	+	-
	TZF-14	+	-	-	+	+	-	-	+	-
	TZF-15	24.90 mm	-	-	15.50 mm	+	-	-	28.84 mm	-
	TZF-22	+	-	+	+	+	+	+	+	-
	TZF-28	+	-	-	+	+	+	+	+	-
***Bauhinia acuminate* L.**	TZB-5	+	-	-	-	-	+	-	-	-
	TZF-7	+	-	-	+	-	+	-	-	-
	TZF-8	+	-	-	+	-	+	-	-	-

Used plants	Isolates	*Escherichia coli*	*Agrobacterium tumefaciens*	*Candida albicans*	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas fluorescens*	*Malasseia furfur*	*Bacillus pumilus*	*Micrococcus luteus*
	TZF-16	+	-	+	+	+	-	+	+	-
	TZF-17	+	-	+	+	+	-	+	+	-
	TZF-23	+	-	+	+	-	-	+	+	-
Mucuna hirsuta Wight & Arn	TZF-29	+	-	+	+	-	-	+	20.72 mm	-
	TZB-6	+	-	-	-	-	+	-	-	-
	TZF-18	+	-	+	+	+	+	-	+	-
	TZF-24	+	-	-	+	-	-	+	+	-
	TZF-30	+	-	+	+	+	-	+	28.10 mm	-

+ = 10 mm of inhibitory zone, size of paper disc = 6 mm, - = no activity

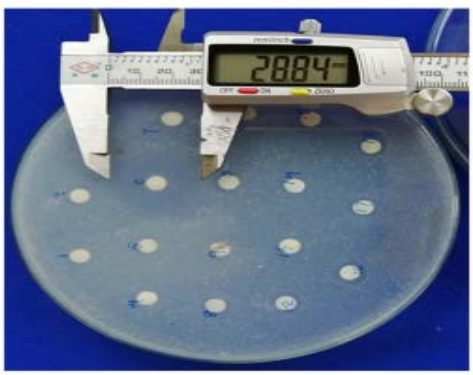


Figure 11. The best antimicrobial activity of TZF-15 from *Mucuna pruriens* Roxb. On Bacillus pumilis (28.84 mm of inhibitory zone)

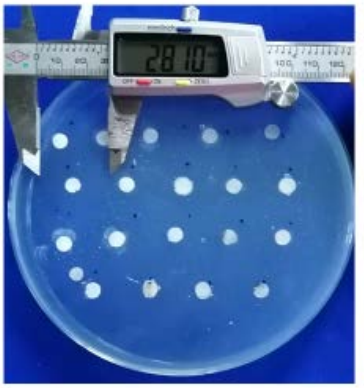


Figure 12. The second best antimicrobial activity of TZF-30 from *Mucuna hirsuta Weight & Arn,* On Bacillus pumilis (28.10 mm of inhibitory zone)

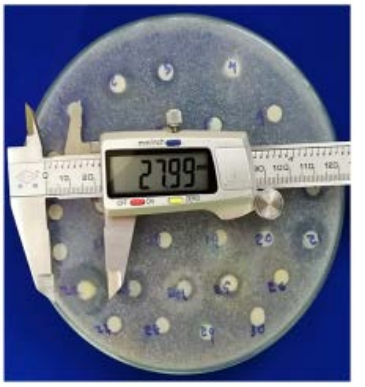


Figure 13. The second best antimicrobial activity of TZF-3 from *Crotlaria striata* DC, On *Pseudomonas fluorescens* (27.99 mm of inhibitory zone)

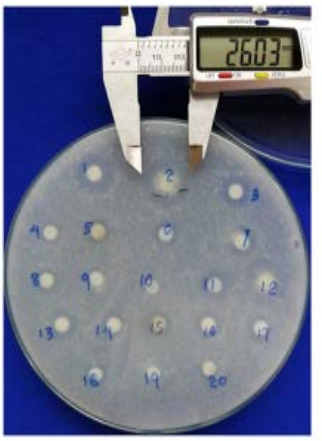


Figure 14. The good best antimicrobial activity of TZF-3 from *Crotlaria striata* DC, On *E. coli* (26.03 mm of inhibitory zone)

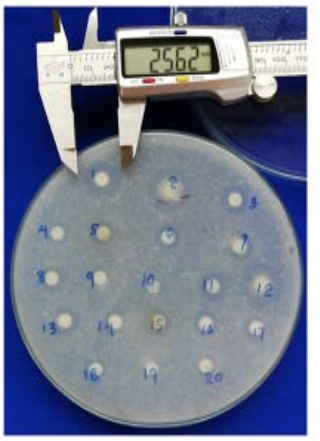


Figure 14. The good best antimicrobial activity of TZF-1 from *Crotlaria ternatea* L, On *E. coli* (25.62 mm of inhibitory zone)

Discussion and Conclusion

Endophytes are a poorly investigated group of microorganisms that represent an abundant and dependable source of bioactive and chemically novel compounds with potential for exploitation in a wide variety of medical, agricultural and industrial areas. The mechanisms through which endophytes exist and respond to their surroundings must be better

understood in order to be more predictive about which higher plants to seek, study and spend time isolating microfloral components (Strobel and Daisy, 2003).

The endophytic microorganisms including six bacteria and thirty fungi were isolated from six medicinal plants belonging to Fabaceae grown in Pathein. There were one bacterium and five fungi from *Clitoria ternatea* L., one bacterium and four fungi from *Cassia occidentalis* L., one bacterium and five fungi from *Crotolaria striata* DC., one bacterium and seven fungi from *Mucuna pruriens* Roxb., one bacterium and six fungi from *Bauhinia acuminate* L. and one bacterium and three fungi from *Mucuna hirsuta* Wight & Arn. The endophytic bacteria and endophytic fungi in the fermentation of seed culture 3 days were tested the antimicrobial activity on 9 different test organisms by paper disc diffusion method. According to the antimicrobial activity, fermentation 9 days of TZF-15 from *Mucuna pruriens* Roxb. showed the best antimicrobial activity (28.84 mm of inhibitory zone) on *Bacillus pumilis.* Fermentation 9 days of TZF-30 from *Mucuna hirsuta* Wight and Arn. and TZF-3 from *Crotolaria striata* DC. showed the second best antimicrobial activities (28.10 mm and 27.99 mm of inhibitory zones) on *Bacillus pumilis* and *Pseudomonas fluorescens.* Fermentation 9 days of TZF-3 from *Crotolaria striata* DC. and TZF-1 from *Clitoria ternatea* L. showed the good antimicrobial activity (26.03 mm and 25.62 mm of inhibitory zones) on *E. coli.* All endophytic bacteria and fungi showed no activity on *Agrobacterium tumefaciens.* Others endophytes showed a little antimicrobial activity on the respective 8 test organisms as in table 4. Life on earth would have been impossible without microorganisms in nature. Man has taken advantages of the activities of microorganisms to his benefit. The contributions of beneficial microbes to the welfare of mankind and the role of harmful microbes in infections and epidermics are well understood and established. Numerous varieties of microorganisms are living on earth and are deeply involved with human life. They are immensely diverse with respect to their habitats material production and genetic information and so on (Ando, et al., 2004). According to the best antibacterial activity (inhibitory zone), TZF-15 from *Mucuna pruriens* Roxb. will be selected for further investigation of kinetic growth and substitution of the growth medium.

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I am sincerely grateful to Dr Than Htun Rector, Pathein University, for his permission to do my research. I am also greatful to thanks Dr Myint Myint Khaing and Dr Nay Aung, Pro-rectors, Pathein University, for their suggestions and advices. I would like to express my best gratitude to Dr Min Min Soe, Professor and Head, for her encouragements in this research. I would like to express my best gratitude to my supervisor, Dr. Khin Min Min Phyo, Professor, Department of Botany, University of Yangon, for her guidance and invaluable suggestion throughout my study. Finally, special thanks to my parents and elder brother for their support and understanding.

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Isolation of Soil Fungi from Three Villages and their Antimicrobial Activities

Tin May Htwe[1] & Zar Zar Yin[2]

Abstract

In the present work, soil samples were collected from six different places of Katha Township, Sagaing Region, during July 2019. Soil fungi were isolated by the serial dilution method from these samples. The media used for the isolation included Potato Dextrose Agar (PDA) medium Blakeslee's Malt Extract Agar (BMEA) medium, and Low Carbon Agar (LCA) medium, Czapek-Dox Agar (CZA) medium and incubated for 3-7 days at room temperature. Pure colonies were preserved into slant culture containing PDA medium. Sixteen fungal strains were obtained. The surface color of all isolated fungi were white, black, cream, brown, blue, pale yellow, pink, yellow and greenish yellow and their reserve color were red, cream, brown, pale yellow, pink and yellow. The antimicrobial activity of all fungal strains were tested by agar well diffusion method on eight test organisms. Among them, four fungal strains showed the antimicrobial activity on all test organisms. Especially, TM-1 showed the highest antibacterial activity (29.35 mm) on *Agrobacterium tumefaciens* and TM-9 showed the strong antimicrobial activity (28.19 mm) on *Candida albicans* and (25.23 mm) on *Bacillus pumilus*. TM-6 exhibited the antibacterial activity (22.76 mm) on *Escherichia coli*. These results suggested that the soil fungi may be utilized for screening the antimicrobial substances and to treat the diseases caused by pathogenic bacteria.

Keywords: Soil Fungi, Colony Morphology, Antimicrobial Activity

Introduction

Soil is considered one of the most suitable environments for microbial growth (Cavalcanti *et al*., 2006)[1]. Fungi are an important component of the soil microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Anisworth, 1995 & Bissett J and Parkinson D, 1979)[2][3]. Fungi are one

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of the dominant groups present in soil, which strongly influence ecosystem structure and functioning and thus plays a key role in many ecological services (Seth *et al*, 2016)[4].

Several fungal species produces bioactive compounds, secondary metabolites and chemical metals having pharmaceutical importance. There are about 23000 known secondary metabolites, 42% of which are produced by actinobacteria, 42% by fungi (eg. *Penicillium* spp.) and 16% by other bacteria. Antibiotics can be classified according to their made of actions (Lambert, 1977)[5].Therefore, the aim of the research work was to produce antimicrobial compounds by isolated fungi from six different places soil in Katha Township. To achieve this aim, the present work was to analyze physicochemical properties of soil from Katha Township, to isolate fungi from different soil samples of Katha Township and to study the colony morphology and preliminary antimicrobial activity of isolated fungi on eight test organisms.

Materials and Methods

Collection of soil samples

Soil samples were collected from Meik Tha Lin (24.202 N 96.320 E), Kyauk Htone Gyi (24.194211 N 96.334251E) and Lan Gwa (24.233627 N 96.371258 E) villages of Katha Township, during July, 2019. These samples were collected from different places (up to 15 cm depth) into sterilized polyethylene bags after removing the surface soil for the isolation of fungi and brought to the laboratory of Biotechnology and Development Center of Pathein University.

Physicochemical analysis of Soil Samples

The collected soil samples were characterized for its physicochemical properties. Physicochemical parameters include organic carbon, nitrogen, pH, moisture content and temperature etc. Temperature and colour of soil samples were recorded. The physicochemical parameters of the soil samples were analyzed at Department of Agricultural Research, Yezin, Myanmar.

Serial Dilution Method (Dubey, 2002)[6]

One gram of soil sample was introduced into a conical flask containing 99 mL of distilled water. The flask was 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 separated test tubes and 1 mL each of the above dilution was separately transferred into sterile petri dishes under aspetic condition. The sterilized medium in conical flask was cooled down to about 45°C and separately poured into each of the petri dish containing the respective soil dilutions. The inoculated plates were shaken clockwise and anti-clockwise direction for about 5 minutes so as to make uniform distribution of the fungi inoculums. When the agar was solidified, the inoculated plates were inverted and incubated at 27°C-30°C for 3-7 days. Isolated pure fungi were preserved into slant culture containing PDA medium for further experimentations.

Agar Well Method (Collins, 1965)[7]

Isolated strains were tested by agar well method for the preliminary antimicrobial activities. Cork borer was to make the wells (8 mm in diameter) in the autoclaved basal antimicrobial test medium. Wells impregnated with 3-6 days old culture fermented broth (20 μL) were incubated at room temperature for 24-28 hours. After 24-28 hours of incubation, the clear zones were measured. Therefore, the diameter of clear zones had been observed as potent activity as shown by representative strain. Clear zones surrounding the well indicated the presence of antimicrobial activities which inhabit the growth of the test organism selectively.

Eight kinds of Test Organisms used for Antimicrobial Activity were *Escherichia coli* AHU 5436, *Bacillus subtilis* IFO 90571, *Bacillus pumilus* IFO 90571, *Candida albicans* NTTE 09542, *Pseudomonas fluorescens* IFO 94307, *Staphylococcus aureus* AHU 8465, *Agrobacterium tumefaciens* NITE 09678, *Malassezia furfur*. These test organisms were obtained from NITE (National Institute of Technology Evaluation, Japan) and PRD (Pharmaceutical Research Department, Yangon, Myanmar).

Results

In present research work, fungi were isolated from three different samples collected from Katha Township, Sagaing Region. The results of the

physicochemical properties of soil samples showed that soil environment of Meik Tha Lin and Kyauk Htone Gyi were Sandy Clay Loam while the sample from Lan Gwa village was Sandy Loam. The pH values of the soil samples showed that moderately acidic and neutral between 5.1 to 7.18. The temperature of soil environments of Katha Township at the time of this investigation (rainy season) showed that the soil environment of Katha Township at temperature range between 30ºC to 34ºC with great variation in present moisture content (4.6-19.3 %), organic carbon (0.26-0.96%), organic nitrogen (41-87 mg/kg) and potassium (50-383 mg/kg).

In the present research work, 16 fungal isolates were obtained and 5 strains from Meik Tha Lin, 6 strains from Kyauk Htone Gyi and 5 strains from Lan Gwa village. These strains were isolated by three culture media. (Table 1)

Table 1. Isolation of soil fungi on four different media

Soil Sample	Place	PDA	BMEA	LCA	CZA	Total
1	Meik Tha Lin	TM-1, 2, 3	TM-4	TM-5	-	5
2	Kyauk Htone Gyi	TM-6, 7	TM- 8	TM-9	TM-10, 11	6
3	Lan Gwa	TM-12, 13, 14	TM-15	TM-16	-	5
		8	3	3	2	16

The total of 16 fungal strains were symbolized as TM-1 to TM-16. The results showed that the colonies of those isolated strains were medium and large in size, entire in margin, raised, flat and convex in elevation and form in circular and irregular (Fig-1).Their antimicrobial activities were also tested (Table2-9 & Figure 2-5).

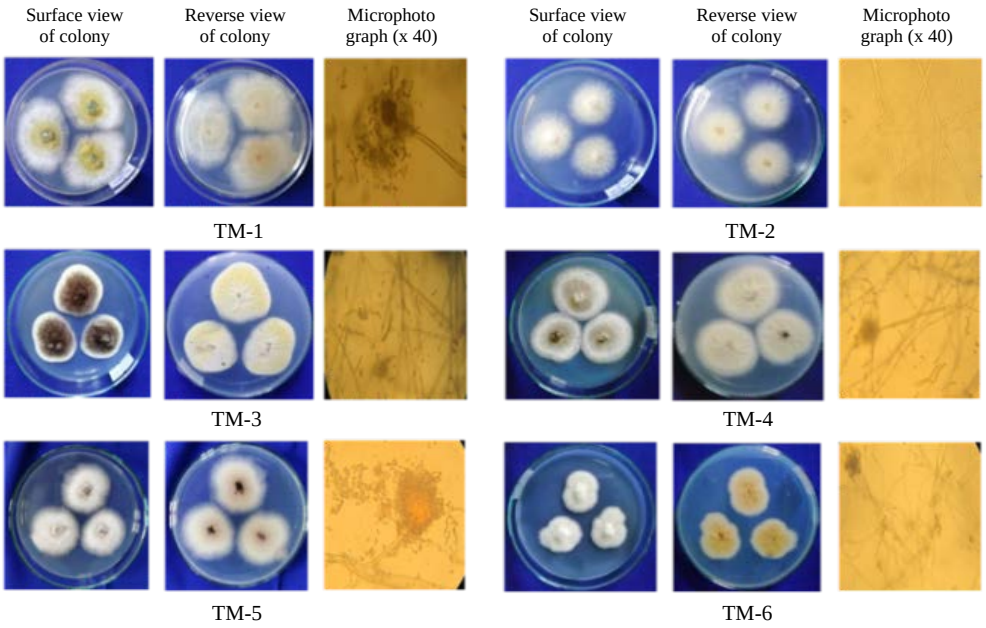


Figure 1.(a) Morphology and their microscopical characters of isolated fungi (TM-1 to TM-6)

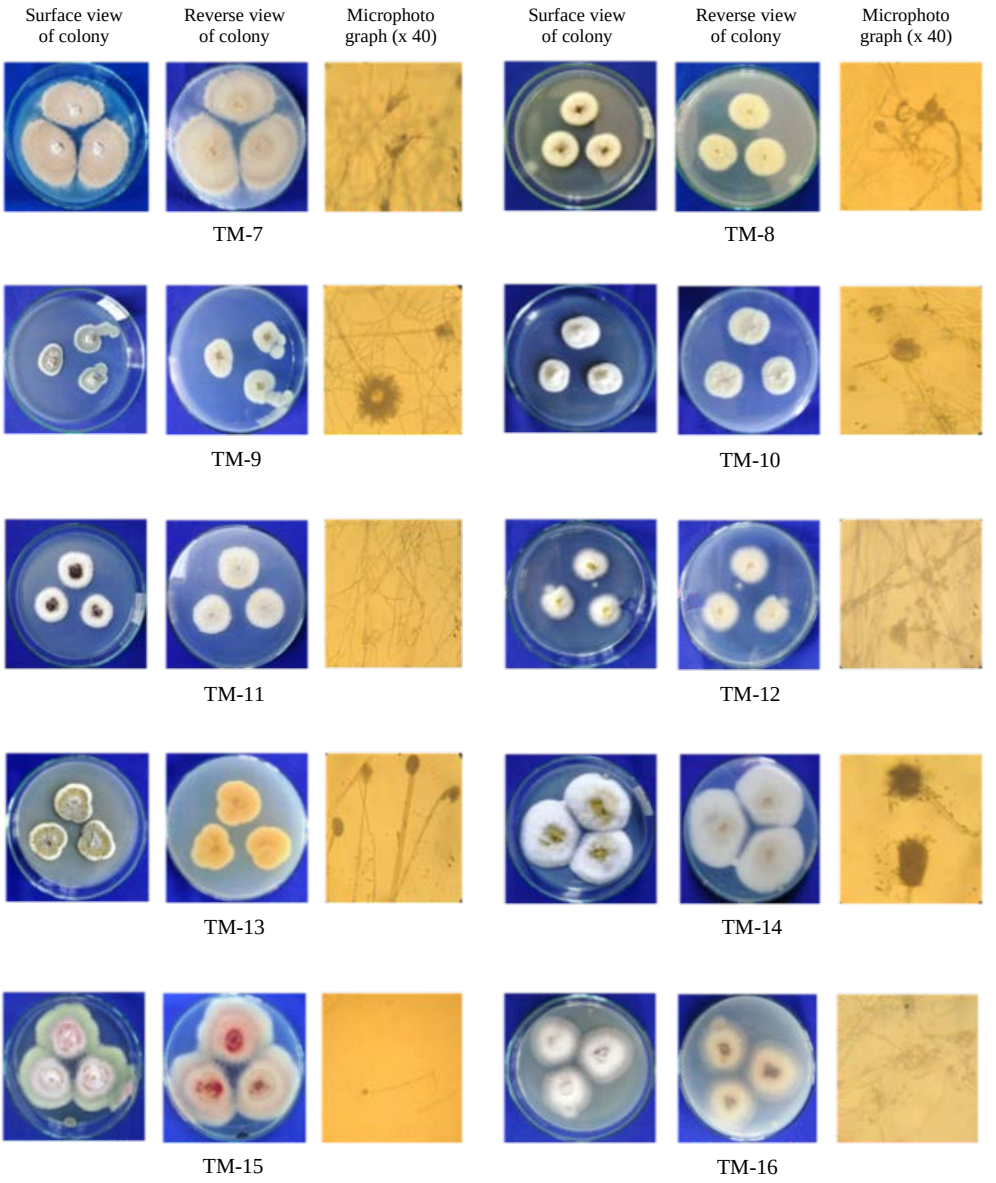


Figure 1.(b) Morphology and their microscopical characters of isolated fungi (TM-7 to TM-16)

Table 2. Antibacterial Activity of Isolated Fungal strains against *Escherichia coli*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)				
		3rd day	4th day	5th day	6th day	7th day
1	TM-1	+	19.11	+	+	-
2	TM-2	-	12.34	14.55	+	-
3	TM-6	+	**22.76**	20.11	19.23	+
4	TM-9	-	-	**25.23**	18.52	+

(+) present (-) no activity Agar well = 8 mm

Table 3. Antibacterial Activity of Isolated Fungal strains against *Bacillus subtilis*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)				
		3rd day	4th day	5th day	6th day	7th day
1	TM-1	+	+	19.22	+	+
2	TM-2	+	+	+	18.84	+
3	TM-6	+	**+**	19.22	+	-
4	TM-9	+	+	22.69	18.30	+

(+) present (-) no activity Agar well = 8 mm

Table 4. Antibacterial Activity of Isolated Fungal strains against *Bacillus pumilus*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)				
		3rd day	4th day	5th day	6th day	7th day
1	TM-1	-	18.55	19.88	+	-
2	TM-2	-	-	+	18.34	+
3	TM-6	-	17.89	+	-	-

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)				
		3rd day	4th day	5th day	6th day	7th day
4	TM-9	22.94	**25.23**	21.88	18.22	+

(+) present (-) no activity Agar well = 8 mm

Table 5. Antibacterial Activity of Isolated Fungal strains against *Candida albicans*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)				
		3rd day	4th day	5th day	6th day	7th day
1	TM-1	-	+	17.45	+	-
2	TM-2	-	23.66	20.12	+	+
3	TM-6	+	+	20.11	18.11	+
4	TM-9	+	19.25	**28.19**	20.12	+

(+) present (-) no activity Agar well = 8 mm

Table 6. Antibacterial Activity of Isolated Fungal strains against *Pseudomonas fluorescence*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)				
		3rd day	4th day	5th day	6th day	7th day
1	TM-1	-	+	19.35	17.51	+
2	TM-2	+	18.28	19.22	+	+
3	TM-6	+	17.89	+	+	-
4	TM-9	+	**20.60**	18.23	+	-

(+) present (-) no activity Agar well = 8 mm

Table 7. Antibacterial Activity of Isolated Fungal strains against *Staphylococcus aureus*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)				
		3rd day	4th day	5th day	6th day	7th day
1	TM-1	-	+	+	17.22	+
2	TM-2	+	17.88	+	-	-
3	TM-6	-	19.39	+	+	-
4	TM-9	18.91	+	+	-	-

(+) present (-) no activity Agar well = 8 mm

Table 8. Antibacterial Activity of Isolated Fungal strains against *Agrobacterium tumefaciens*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)				
		3rd day	4th day	5th day	6th day	7th day
1	TM-1	-	**29.35**	19.25	+	-
2	TM-2	-	15.52	18.27	+	-
3	TM-6	-	14.59	16.57	+	+
4	TM-9	-	22.35	20.59	19.19	+

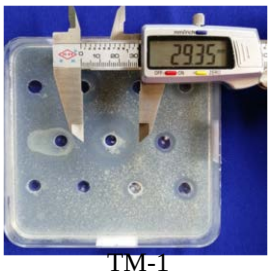
(+) present (-) no activity Agar well = 8 mm

Table 9. Antibacterial Activity of Isolated Fungal strains against *Malassezia furfur*

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)				
		3rd day	4th day	5th day	6th day	7th day
1	TM-1	+	18.86	17.75	+	-
2	TM-2	-	+	18.92	+	-
3	TM-6	16.44	**17.66**	17.51	+	-

No.	Isolated Fungal	Fermentation Period (Days) and Inhibitory Zone (mm)				
		3rd day	4th day	5th day	6th day	7th day
4	TM-9	+	18.33	19.25	+	-

(+) present (-) no activity Agar well = 8 mm

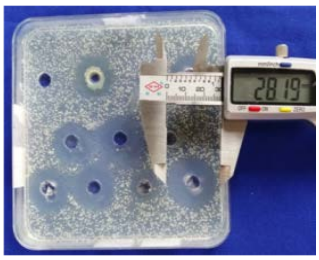


TM-1

Figure 2. Antibacterial Activity of Isolated Fungal strains against *Agrobacterium tumefaciens*

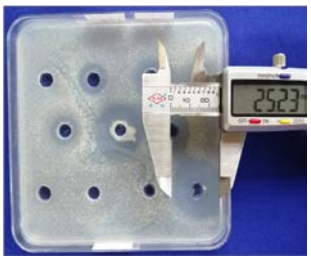
TM-6

Figure 3. Antibacterial Activity of Isolated Fungal strains against *Escherichia coli*



TM-9

Figure 4. Antifungal Activity of Isolated Fungal strains against *Candida albicans*



TM-9

Figure 5. AntibacterialActivity of Isolated Fungal strains against *Escherichia coli*

Discussion and Conclusion

Fungi are important components of the soil microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions. Physicochemical analysis showed that pH of the soil is moderately acidic and is rich nutrients which is favourable for the growth of fungi. Fungal diversity of any soil depend on a large number of factors of the soil such as pH, organic content, moisture and soil texture (Rangaswai, 1998)[8].

There are many sources where antibiotics can be discovered of novel antibiotics since long ago (Dulmage and Rivas, 1978)[9].

The colour of soil samples were brown with variation in pH (5.11-7.18). The temperature of soil environments of Katha Township at the time of this investigation (rainy season) showed that the soil environment of Katha Township at temperature ranged between 30ºC to 35ºC with great variation in present moisture content (4.6-19.3 %), organic carbon (0.26-0.96 %), organic nitrogen (41-87 mg/kg) and potassium (50-383 mg/kg). Total number of colonies obtained from Kyauk Htone Gyi village and its pH had 5.23 with (moisture 12.0 %). Other two samples from Meik Tha Lin and Lan Gwa village had pH 5.44 with (moisture 12.1) and 7.18 (moisture 4.6) respectively.

The results showed that low pH and optimum moisture content favour the growth of fungi. Normal soil contains enormous number of microbes and substantial quantities of microbial biomass. Ramann *et al*., 1899[10] also reported that due to the accumulation of more liter in scrub and deciduous forest more percentage of fungi are presented in the soil for the purpose of recycling of dead organic matter.

A total of 16 fungi were isolated from three different soil samples of Katha Township by using four different media including BMEA, PDA, CZA and LCA medium. The isolated fungi were designated as TM-1 to TM-16.

The surface color of TM-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12,13,14,15 and 16 are pale yellow, cream, white, pink, black, greenish yellow, brown, yellow, greenish blue and their reverse color were cream, brown. red, pale yellow, brown and yellow respectively.

Stevens, 1981[11] also reported that fungi grew on decrease habitats in nature and that fungi are cosmopolitan in distribution requiring several

specific elements for growth and reproduction. A wide range of media are used for growing fungi. Media will affect colony morphology and colour.

Fennell, 1965[12] reported that after 7 days of incubation, all strains on plates were observed for macroscopic characteristics such as colony diameter, surface and reverse colour of colony.

All fungal strains were tested by eight test organisms for preliminary study of antimicrobial activities. Among them, 4 strains showed different level of antimicrobial activities.

In conclusion, the results showed that acidic pH and optimum moisture content and rich mineral content is favourable conditions for the growth of fungi. These results were drawn by the isolation of Fungi occurring in the soil and their colony morphology and preliminary study of antimicrobial activity was performed by using eight test organisms. The future studies are expected fermentation condition by using eight test organisms with selected soil fungi.

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Comparison on Types of Achenes Found in Eighteen Species of Family Asteraceae

Tin Win Kyi[1], Nu Nu Yee[2] & Thi Thi Htun[3]

Abstract

The achene studies on the family Asteraceae from Sagaing Region were undertaken. In the present study, 18 species belonging to 16 genera of family Asteraceae were collected, studied and identified during December 2018 to December 2019. One species each from the genera *Acanthospermum, Ageratum, Blainvillea, Centratherum,Galinsoga, Guizotia, Lagascea, Melampodium***,** *Parthenium, Praxelis, Synedrella, Sigesbeckia, Tridax, Tithonia, Xanthium* and three species from genera *Biden* were collected. Achenes of five species were absent pappus and the remaining 13 species were present the various pappus. The characteristics of the family and detail descriptions of individual species are described with relevant photographs. The collected species were systematically arranged tribes by Jeffery and Kadereit 2007.

Keywords: Asteraceae, different achenes.

Introduction

The Asteraceae (Compositae) is known by the aggregated flowers ofter occurring at the ends of branches or stems. The technical term for such a group of flowers is capitulum which is macroscopic (Visible to the naked eye). Aggregation of flowers occurs on usually flat surface called receptacle. It is also referred to as the banner of the family Asteracea (Tadesse, 2014).

The compositae is generally a temperate family, and it is therefore no surprise that the majority of species, about 75% grow in the montane zones of the island generally above 3000 ft (Dassanayake, 1980).

A single-seeded indehiscent dry fruit with the seed free from the pericarp except at the placenta; the fruit in almost all Compositae. Generally breaks free from the receptacle at maturity and often falls together with pappus elements which are borne at distal end of the achene or epappose.

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Unite of dispersal in which one or more achenes are dispersed together with adherent phyllaries and paleas and sometimes with non-fruiting florets. In some Compositae, each ray achene falls together with a subtending phyllary (Monika, 2014).

The genus *Melampodium* L. of Asteraceae-Heliantheae is represented by 45 species distributed in tropical and subtropical regions, and Central America (Jagtap, 2017). *Parthenium hysterophorus* L. is widely occurring and occupied almost all the parts of world such as in Asia, Africa, Australia and the Pacific (Monika, 2014).

The aim is to fulfill the information of taxonomical distinct characters found in Asteraceae for future research works. The objectives of the present study are to classify and identify the achenes of family Asteraceae, to record the differences between achene characters of collected species.

Materials and Methods

Specimens of Asteraceae were collected from Sagaing and Shwebo District during December 2018 to October 2019. Plant parts including leaves, inflorescence, flowers and fruits were collected and recorded in field notes. Achenes of the specimens were recorded by photographs.

Identification of genera and species were carried out by comparison with Hooker (1881), Backer (1965), Dassanayake (1980), Jeffery and Kadereit (2007), Bean and Pruski (2009), Wu (2011), Monika (2014), Titiek *et al.* (2015) and Wilson (2015). Myanmar names were checked by Hundley, H. G and Chit Ko Ko (1987) and Kress *et al.* (2003).

All the collected specimens were identified and described with their characters. The species of Asteraceae are arranged according to Jeffery and Kadereit (2007).

Results

Taxonomic Description

Centratherum intermedium Less.

Outstanding Characters

Perennial herbs stem erect, terete, branching foliate. Leaves alternate, simple. Capitula terminal, homogamous, discoid; involucre leafy bracts, unequal in size, 3-4 seriate, the phyllaries oblong, receptacle convex. Florets bisexual, tubular, corolla with 5 lobed; stamens 5, anthers bases truncate; acute at the apex; ovary obovoid, style exserted.

Achene Morphology

Achenes of *Centratherum intermedium* Less. have obovoid, 1.0 mm to 2.0 mm long, 8-1 ribbed, brown. Pappus scales 8- 9, linear 1.0 mm long, dirty white, pubescent.

Bidens biternatus **(**Lour**.)** Merr. & Scherff .

Outstanding Characters

Annual erect herb, stems tetragonous, angular. Leaves bipinnately 5 foliolate to bipinnatifidly compound, opposite, petiolate; blade lateral leaflets ovate-lanceolate, attenuate at the base, deeply dentate along the margin, acute at the apex, heterogamous, radiate; involucres campanulate, receptacle flat, paleaceous. Ray florets, neuter, ligule, yellow. Disc florets, bisexual, corolla with 5 lobed, yellow, stamens 5, anthers bases sagittate acute at the apex, ovary oblong, dorsally compressed, sparsely 3 haires, along the angles; styles exserted.

Achene Morphology

Narrowly fusiform achenes of *Bidens biternatus* **(**Lour**.)** Merr. & Scherff . linear 0.5 mm to 1.0 mm long, blackish, furrowed, without spines on the margin, glabrous or pubescent. Pappus bristles with retrorsely barbed awns, 3.0 mm to 4 mm long, whitish, persistent.

Bidens pilosa var. ***pilosa***

Outstanding Characters

Annual erect herbs, stem tetragonous, glabrescent. Leaves decompond pinnatifid compound, opposite, petiolate; blade linear. Capitula heterogamous, radiate; involucre campanulate, 2 seriate, receptacle flat. Ray florets, neuter, ligule, whitish, with deeply 3- 4 lobed tuber corolla. Disc florets bisexual, corolla with 5 lobed, yellow; stamens 5, anther base sagittate, obtuse at the apex; ovary dorsally compressed, triquetrous, pale brown, sparsely 3 haires, along the angles, glabrous; styles inserted, yellow.

Achene Morphology

Obcompressed achenes of *Bidens pilosa* var. *pilosa* bearing marginal prickles and oblongoid, 0.6 mm to 1.5 cm long, blackish, with a few pale stiff hairs on the angles in the upper part, glabrous. Aristate pappus of bristle 3 retrorsely bared awns, unequal, persistent.

Bidens pilosa var. ***minor.***

Outstanding Characters

Annual erect herbs, stem solid, terete, glabrous. Leaves trifoliolate or pentapinnate compound, petiolate; Capitula heterogamous, radiate; involucres campanulate, involucre 2 seriate, receptacles flat. Ray florets, neuter, ligulate, without lobed, creamy white. Disc florets, bisexual, corolla with 5 lobed, yellow; stamens 5, anther base truncate, obtuse at the apex. Ovary oblongoid, more or less compressed, pale brown, dorsally compressed, triquetrous, glabrous.

Achene Morphology

Obcompressed achenes of *Bidens pilosa* var. *minor* bearing marginal prickles oblongoid, 7 mm to 8 mm long, black, with a few pale stiff hairs on the angles in the upper part. Pappus bristles 2 retrorsely bared awns, 2 mm to 3.5 mm long, whitish, persistent.

Parthenium hysterophorus L.

Outstanding Characters

Annual erect herbs, stem and terete. Leaves simple, alternate, pinnatifid, petiolate, blades oblong- lanceolate. Capitula heterogamous, radiate; involucre ovoid- oblong, 5 series, receptacle convex. Ray florets, female, corolla with 2 lobed, white or creamy white; ovary obovoid, flattened, dorsally compressed and slightly concave, margin with white broad and soft wings; style exserted. Disc floretsmale, 4 - lobed; creamy white, stamens 4, inserted; anthers base sagittate, obtuse at the apex.

Achene Morphology

Achenes of *Parthenium hysterophorus* L. 2 spiny, oblong or elliptic-oblong, 0.5 mm to 1.5 mm long, flattened, triangular and dark brown- black with two thin, white, spoon- shaped appendages, pale brown. Pappus 0.5 mm long, pale brown, persistent.

Xanthium indicum Koenig in Roxb.

Outstanding Characters

Annual erect herbs, stem stout and hairy, Leaves simple, alternate, petiolate; blades broadly ovate, 3-5 lobed. Capitula unisexual, monoecious; capitula of two kinds; male and female florets in separate capitula, staminate capitula involucral 1-2 seriate, stamens 5, strongly exserted, the filament connate, the anthers separate, Pistillate capitula ovoid, covered with spines; ovary oblong-ovoid, style terminal, filiform, the spines.

Achene Morphology

Achenes of *Xanthium indicum* Koenig in Roxb. usually 2 erect or diverging, retained inside conceptacle, enclosed in the hardened involvcral cells, narrowly obovoid or elliptic, greyish black, thick. Pappus absent.

Blianvillea acmella (L.)

Outstanding Characters

Annual erect herbs, stems dichotomously branched, pilosa. Leaves simple, opposite, blade ovate. Capitula heterogamous, radiate, involucre campanulate, receptacles convex. Ray florets, female, white, 2 or 3 lobed.

Disc florets, bisexual, corolla with 5 lobed, stamens 5, anther bases sigittate, ovate at the apex; ovary oblong, slightly compressed, styles inserted.

Achene Morphology

Achenes of *Blianvillea acmella* (L.) laterally compressed, truncate at the apex with weak, blackish. Pappus minute, unequal, bristles or sometimes small scales, whitish.

Synedrella nodiflora (L.)

Outstanding Characters

Annual erect herbs, stem and branches, terete. Leaves simple, opposite and decussate, petiolate blades ovate. Capitula heterogamous, radiate; involucre ovoid- oblong, 2-3 seriate, receptacle convex. Ray florets, female, 2 lobed, yellow. Disc florets, bisexual, 4- lobed, stamens 5, anthers base sagittate, obtuse at the apex, ovary oblong- cuneate, flat or subtrigonous, black or blackish brown; style inserted.

Achene Morphology

Achenes of ray florets of *Synedrella nodiflora* (L.) were dorsiventrals, compressed, margin with broad lacerate hard wings; 2-6 pairs, upward directed spines, oblong or elliptic- oblong, 2.5 mm to 4.5 mm long, pale brown. Those of disc florets have oblong- cuneate, flat or subtrigonous, 3.0 mm to 4.5 mm long, black or blackish brown, 3-4 spines, Pappus 2.5 mm long, pale brown, persistent.

Lagascea mollis Cav.

Outstanding Characters

Annual erect herbs, stems terete. Leaves simple, alternate, petiolate; blades ovate. Capitula homogamous, discoid; involucre leafy bracts, 2 seriate, receptacle small convex. Florets all tubular, bisexual, corolla 5-lobed; stamens 5, anthers base sagittate, acute at the apex, ovary oblong, style exserted.

Achene Morphology

Achenes of *Lagascea mollis* Cav. were oblong, ribbed, with hairy,

green. Pappus 4, linear, green, pubescent.

Tithonia rotundifolia (Mill) Blake.

Outstanding Characters

Perennials erect shrubs, stem terete. Leaves simple, alternate, petiolateblade ovate or broadly ovate, 3-nerved, 3-7 lobed. Capitula heterogamous, radiate; involucral broadly campanulate, 2 seriate, receptacle convex. Ray florets, neuter, corolla with 3 lobed. Disc florets, bisexual, corolla with 5 lobed, orange colored; stamens 5, anther base attenuate, obtuse at the apex; ovary oblong- conical, style exserted.

Achene Morphology

Achenes of *Tithonia rotundifolia* (Mill) Blake. were oblong, 1.5 mm to 2.0 mm long, pubescent, dark brown, sericeous. Pappus of ligulate florets minutely scaly, those of tubular florets, 2 awns 4.0 mm to 5.0 mm long with about 6 short broad scales connate ate the base, linear- lanceolate, margin fimbriate, scarious, white, persistent.

Tridax procumbens L.

Outstanding Characters

Perennial procumbent herbs, stem terete, decumbent. Leaves simple, opposite and decussate, petiolate; blade ovate and lanceolate. Capitula heterogamous, radiate, involucre campanulate, 3 seriate, receptacles concave. Ray florets, female, deeply 3 lobed, cremy-white. Disc florets, bisexual, corolla with 5 lobed; stamens 5, anthers sagittate base, obtuse at the apex; ovary oblongoid, style exserted.

Achene Morphology

Achenes of *Tridax procumbens* L. were conical, 1.5 mm to 2.0 mm long, blackish. Pappus filiform, unequal in length, 4.0 mm to 6.5 mm long, bristles.

Acanthospermum hispidium DC.

Outstanding Characters

Annual erect, stems terect. Leaves simple, opposite, sessile, blade elliptic-ovate. Capitula heterogamous, radiate; involucral campanulate, 2 seriate; receptacle convex. Ray florets, female, 2 to 3 lobed. Disc florets, bisexual, corolla with 5 lobed; stamens 5, anther base obtuse, ovate at the apex. Ovary oblong, style exserted.

Achene Morphology

Achenes of *Acanthospermum hispidium* DC. were triangular, spinescent with 2 large horn like erect, divergent apical spine, 4.5 mm long, black, strainght or uncinate prickles. Pappus absent.

Guizotia abyssinica (L.f.) Cass.

Outstanding Characters

Annual erect herbs, stems terete. Leaves simple, opposite, sessile, blade, linear-lanceolate. Capitula heterogamous, radiate, involucre campanulate, 2 seriate, receptacle conical. Ray florets, female, 3 lobed, brightly yellow. Disc florets bisexual, corolla with 5 lobed; stamens 5, anthers base sagittate, acute at the apex; ovary obovoid, triquetrous, dorsally flattened, styles exserted.

Achene Morphology

Achene of *Guizotia abyssinica* (L.f.) Cass. were obovoid, angular-compressed, with round apex, 1.5 to 3 mm long, ciliated at the top. Pappus absent.

Sigesbeckia orientalis **L.**

Outstanding Characters

Annual erect herb; stems terete. Leaves simple, opposite and decussate, petiolate; blade ovate-lanceolate to triangular ovate. Capitula heterogamous, radiate; involucre hemispherical, 2 seriate, receptacle convex. Ray florets, female, corolla with 3 lobed, bright yellow. Disc florets, bisexual, corolla with 5 lobed; stamens 5, anther base sagittate,

acute at the apex; ovary oblong-obovoid, tetragonous, curved; style exserted.

Achene Morphology

Achenes of *Sigesbeckia orientalis* L. were conical, slightly curved, 4 angular, tipped by a rim, glabrous, 3 mm to 4 mm long, black. Pappus absent.

Melampodium divaricatum (Rich. Ex Pers) DC.,

Outstanding Characters

Annual erect herbs. Leaves simple, opposite, petiolate; blades broadly elliptic or ovate, Capitula heterogamous, radiate; involucre campanulate, 2 seriate; receptacle oblong. Ray florets, female, without lobed, yellow. Disc florets many, bisexual, corolla with 5 lobed; stamens 5, anthers base obtuse, acute at the apex, ovary oblong, style exserted.

Achene Morphology

Achenes of *Melampodium divaricatum* (Rich. Ex Pers) DC. were obovoid, or trigonous, with a short point on the posterior angle, top flattened, 1.5 mm to 3.0 mm long; black. Pappus absent.

Galinsoga parviflora Cav.

Outstanding Characters

Annual erect aromatic herbs, stem, terete, pubescent. Leaves simple, opposite and decussate, petiolate. Capitula heterogamous, radiate; involucre hemispherical, 1- 2 seriate; receptacle conical. Ray florets, female, 3 lobed, white. Disc florets bisexual, corolla with 5 lobed; stamens 5, anthers base sagittate, acute at the apex, ovary oblong, style exserted.

Achene Morphology

Achenes of *Galinsoga parviflora* Cav. were obovate, 0.5 mm to 1.0 mm long, 3- ribbed, pubescent, black. Pappus, linear- lanceolate, margin fimbriate, scarious, white.

Ageratum conyzoides L.

Outstanding Characters

Annual erect herbs, stems terete. Leaves simple, alternate while young, opposite in age, petiolate; blade deltoid-ovate or broadly ovate, Capitula homogaamous, discoid; involucre campanulate, 3 to 4 seriate, Receptacle slightly convex, naked. All florets, bisexual, corolla with 5 lobed, purple; stamens 5, anther bases attenuate, obtuse at the apex; ovary oblongoid, 5 angled, sparsely pubescent; style slightly exserted.

Achene Morphology

Achenes of *Ageratum conyzoides* L. were linear-oblong, angular, 1.5 mm long, slightly curved, brownish black. Pappus scales 5, 2.0 mm long, slightly longer than the corolla acuminate into bristle-like point.

Praxelis clematidea (Griesb.)

Outstanding Characters

Perennial erect herbs, stems bright green, terete. Leaves simple, opposite, subsessile or shortly petiolate; blades ovate. Capitula terminal or subterminal, homogamous, discoid; involucre broadly cylindric campanulate, 4- 5 seriate; receptacle highly conical. Florets all tubular, bisexual, corolla with 5- lobed, purple, stamens 5; anthers base sagittate, obtuse at the apex, ovary oblong; style exserted.

Achene Morphology

Achenes of *Praxelis clematidea* (Griesb.) were oblong, ribbed, black. Pappus hair capillary, white, persiste.

B

Figure 1**.** A. Achene of *Centratheruminter medium* Less.

B. Achene of *Bidens biternatus* **(**Lour**.)** Merr. & Scherff .,
C. Achene of *Bidens pilosa* var. *pilosa.,*

D. Achene of *Bidens pilosa* var. *minor.*

E. Achene of *Parthenium hysterophorus* L.

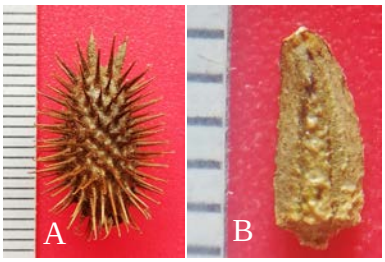

Figure 2. A. Achene of *Xanthium indicum* Koenig in Roxb.

B. Achene of *Blianvillea acmella* (L.)
C. Achenes of *Synedrella nodiflora* (L.)
D. Achene of *Lagascea mollis* Cav.

E. Achenes of *Tithonia rotundifolia* (Mill) Blake.

Figure 3. A. Achene of *Tridax procumbens* L.
B. Achene of *AcanthospermumhispidiumDC.*
C. Achene of *Guizotia abyssinica* (L.f.) Cass.
D. Achene of *Sigesbeckia orientalis* L.

Figure 4. A. Achene of *Melampodium divaricatum* (Rich. Ex Pers) DC.
B. Achene of *Galinsoga parviflora* Cav.
C. Achene of *Ageratum conyzoides* L.
D. Achene of *Praxelis clematidea* (Griesb.)

Discussion and Conclusion

The present research work deals with the achene study of Asteraceae growing in Sagaing Region. The species were collected from December 2018 to December 2019 and 18 species belonging to 16 genera of family Asteraceae were recorded. The collected species of this family were classified and identified according to the achene types. The genera and species of this family Asteraceae have been arranged tribes.

Most species of Asteraceae such as *Blianvillea acmella* (L.), *Guizotia abyssinica* (L.f.) Cass., *Melampodium divaricatum* (Rich.ExPers) DC., *Sigesbeckia orientalis* L. and *Xanthium indicum* Koenig in Roxb. were

absent pappus and the remaining 13 species of Asteraceae were present pappus. Among them, the six species, *Bidens biternatus* **(**Lour**.)** Merr. & Scherff., *B. pilosa* var. *pilosa., B. pilosa* var. *minor., Xanthium indicum* Koenig in Roxb., *Blianvillea acmella* (L.) and *Synedrella nodiflora* (L.) were present as spiny pappus.

The three genus *Biden* in Asteraceae family were found different characters of achenes. The achenes of *Bidens biternatus* **(**Lour**.)** Merr. & Scherff . and *B. pilosa* var. *minor.* were absent spines on the margins and the another one species was found with spines on the margins and then bearing prickles and aristate pappus of three antrorsely barbed awns. Therefore, the characters of this species were agreed with Bean and Pruski (2009), Wu *et al.* (2011), Monika (2014) and Wilson (2015).

Achenes of ray florets of *Synedrella nodiflora* (L.) were dorsiventrals, compressed and have margins with broad lacerate hard wings; 2-6 spiny pairs, oblong or elliptic- oblong, 2.5 mm to 4.5 mm long, pale brown. Pappus have 2-3 spines.

The present studied area was found two species. *Xanthium indicum* Koenig in Roxb. has unisexual capitula, monoecious plant, capitula of two kinds; male and female in separate capitula and the achenes of this species were to be pistillate capitula. *Parthenium hysterophorus* L. has unisexual disc florets (male florets) and ray florets (female florets) on the same receptacle. The achenes of this species were to be ray florets. The characters of these species were agreement with, Rahman (2008), and Muhammad *et al*. (2017) .

The species of family Asteraceae are seed dispersal, they grow rapidly and distribute enormously. The present study can give valuable information about some species of the family Asteraceae.

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Morphological Studied on Some Species of Family Asteraceae from Natmouk Township

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Abstract

In this research, there is an emphasis on some species from family Asteraceae scientific studies carried out to find information about morphologically, the characters of stems, leaves, flower, fruits and seeds were observed in 10 selected plants from Natmouk Township. These plants are taken from collection period 2018-2020. In the present study, totally of 10 species under 10 genera belonging to the family Asteraceae were recorded from this area. The morphological characters of habit and inflorescence have been studied and documented by photograph. For each species scientific name with author citation, Vernacular name, flowering period and their morphological character of each plant have been identified in this study. All the species were Global Positioning System are also provided.

Keywords: Angiosperms, flowering period.

Introduction

Asteraceae are widely distributed family among the angiosperms. Most of the member of the Asteraceae show flowers and fruits in winter and summer, especially between the months of December and March. The family Asteraceae placed under the order Asterales includes 20000 species and abundant everywhere. The flowers are small, but they are arranged in heads, each of which seems at first glance to be an individual flower, although actually it consists of several flowers. The head is surrounded by a series of involucral bracts. The flowers are produced on a compound receptacle formed by coalescence of the individual receptacles and the entire head is supported by a peduncle. Mostly the flowers are of two types: the flowers in the centre of the head are disc flowers; usually those of the outermost single series are specialized as ray flowers. There is great variation among members of this family with respect to the types of flowers on the receptacle. Some bearing both ray and disc flowers, some only with

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disc flowers. Typically ray flowers are unisexual and zygomorphic, disc ones are bisexual and actinomorphic. All types of flowers are epigynous. This family is characterized by its compact heads, the absence of the normal calyx, which is replaced by the pappus, the presence of the corolla of ordinary coloration and texture, epipetalous stamens with the anthers forming a tube around the style an inferior ovary with one locule, containing the basal ovule and ripening into a achene.

In the present research deals with the morphological study on the family Asteraceae from Natmouk Township which is located in Central Myanmar of Magway District. It is situated between North Latitude (20° 17’ 44.222” to 20° 46’ 20.86”) and East longitude (95° 6’ 47.09” to 95° 43’ 31.994”).

The aims and objectives of this present research are to study family Asteraceae from Natmouk Township and to clarify, identify, to get distinguished morphological characters and to record some species of family Asteraceae from Natmouk Township.

Materials and Methods

Specimens are properly collected from 2018 to 2020. All the collected specimens were recorded by photographs. Field notes were made of detail plants description, habitat types and precise location by using GPS. Identification of species was carried out by referring the books of Flora of British India (Hooker 1879), Flora of Java (Baker 1965), Flora of Ceylon (Dassanayake 1980) and Flora of China (2011). All of the nomenclatural studies were finalized by referring to the web site of Plants of the World, Kew Science (http://www.kew. org/science). Local names were recorded by Hundley & Chit Ko Ko (1987) and Kress *et al*. (2003). Most of the plant specimens have been air dried, pressed and mounted according to reference (Lawrance 1964). The genera and species arrangement under the families were arranged in alphabetical order. All studied species were described with taxonomic descriptions and were also constructed their artificial key.

Results

1. *Acanthospermum hispidum* DC. Candolle, Prodr. 5: 522. 1836

Vernacular name : Kut sine;
Flowering period : September to January

Annual, erect herbs; stems much branched, terete, covered with spreading hirsute hairs and smaller glandular hairs. Leaves simple, opposite and decussate, sessile; blades elliptic, oblanceolate or obovate, 1.3-7.5 cm by 1.0-2.0 cm, green, whitish pilose on both surfaces, attenuate at the base, shallowly serrate along the margins, acute or obtuse at the apex. Capitula or head heterogamous, axillary, yellow, 0.4-0.5 cm in diameter; peduncles 0.2-0.5 cm long. Involucral bracts 1-seriate, 5 or 6 elliptic or ovate, 0.3-3.5 mm long, pubescent without. Receptacles conic, paleaceous; palea about 2.5 mm long, lacerate-ciliate, obtuse at the tip, glandular- hairy without. Ray florets peripherial, ligulate, zygomorphic, epigynous, pentamerous, 1.5 mm long, pale yellow, uniseriate. Corolla of ligulate florets, 3-lobed, about 0.1 cm long; Disc florets 5-lobed, actinomorphic, epigynous, pentamerous, 1.7-2.0 mm long, yellow, with a pistillose, white. Stamens 4, epipetalous; filament filiform; anthers syngenesious, obtuse at the apex, black. Ovary inferior, unilocular, with solitary basal ovule, oblong; styles filiform, obtuse. Achenes oblong-obconic, many-seeded.

Specimen examined: Natmouk Township, 2018; (N Lat 20° 29′ 48″ and E Lon 95° 38′ 13″).

2. *Blainvillea acmella* (L.) Philipson, Blumea 6: 350. 1950.

Vernacular name : Hnan-sa;
Flowering period : January to April

Annual herbs, much branches; stem and branches cylindrical, with longitudinal striation, pilose. Leaves simple; upper leaves alternate, lower ones opposite; petioles about 1.5 cm long, blades ovate, 2.0-4.5 cm by 2.0-3.0 cm, slightly oblique at the base, dentate along the margin, acute at the apex. Inflorescences axillary or terminal; peduncles 1.0-2.5 cm long, stout,

dichotomous branches. Head heterogamous, cup-shaped or broad campanulate. Involucre cup-shaped; bracts 1-2 seriate, leafy green, lanceolate, about 0.4-0.6 cm by 0.2-0.4 cm, acute at the apex. Receptacles flat or slightly convex, paleaceous. Outer ray florets linguliform, bisexual, zygomorphic, epigynous, pentamerous, white; Inner disc florets white. Stamens 5, inserted, brown; filament short; anthers obtuse or slightly sagittate at the base, pappus 2 or 3, caducous. Ovary inferior, triquetrous truncate at the apex; styles filiform, slightly curve, obtuse at the apex.

Specimen examined: Natmouk Township, 2018; (N Lat 20° 29′ 54″ and E Lon 95° 37′ 36″).

3. ***Chromolaena odorata*** (L.) R. M. King & H. Rob: Syst. Ed. 1012051759.

Vernacular name : Bizat;
Flowering period : December to February

Annual, erect fetid shrubs: stems and branches terete, sparsely pubescent. Leaves simple, opposite and decussate, exstipulate; petioles 2.0-3.5 cm long, canaliculated, pubescent; blades deltoid-ovate, 4.5-10 cm by 2.5-5.0 cm, pubescent on both surfaces, glandular beneath, cuneate at the base, dentate along the margin, acuminate at the apex. Inflorescences terminal, corymbose-heads; Peduncles 2.0-3.0 cm long, pubescent. Heads homogamous, pale blue purple, Involucre tubular, 7.0-9.0 cm long; bracts 4-5 seriate. Receptacles convex; pitted. Disc forets 25 to 30, tubular, bisexual, actinomorphic, epigynous, pentamerous; outer florets about 2.5 mm long: inner ones 5.0-7.0 mm long; corolla infundibuliform, puple; tubes 4.0-4.5 mm long; lobes deltoid acute, bout 0.5 mm long, recurved; pappus capillary, numerous, uniseriate. 5.0-6.0 mm long, pale brown, spinulose, persistent. Stamens 5, epipetalous, inserted; filaments filiform, 1.0-2.0 mm long; anthers syngenesious, obtuse at the

base, appendages acute, white. Ovary inferior, 5- angled, linear or elliptic-lanceolate, about 4.5 mm long, styles 3.0-4.0 mm long; stylar arms 2, linear-filiform, purple. Achenes 1-seeded, oblongoid, 5-angled, about 4.5 mm long, brown, hairy. Pappus unequal, filiform, spinulose.

Specimen examined: Natmouk Township, 2019; (N Lat 20° 30′ 54″ and E Lon 95° 37′ 40″).

4. ***Cyathocline purpurea*** (Buch. Ham. Ex D.Don) Kuntze in DC, Prod. 53741836

Vernacular name : Unknown;
Flowering period : February to April

Annual herbs, about 1.0 m high; stems slender, whitish pilose. Leaves simple, alternate, exstipulate, pinnatifid to pinnatisect; blades ovate, 5.0-9.0 cm by 4.0-6.0 cm, cuneate at the base, dentate along the margin, acute at the apex, puberulent on both surfaces. Inflorescences terminal corymbose, 2.5-3.5 mm in diameter. Head heterogamous, purple, disciform. Involucre cupulate, biseriate, 2.0.3.0 mm long, phyllaries linear, 1.0-2.0 mm long, pubescent. Receptacles naked. Peripheral florets numerous, many series, tubular, bisexual, actinomorphic, epigynous, pentamerous, fertile; corolla-tube filiform, 1.0-1.5 mm long; central florets 15- to 25, infundibuliform, bisexual, actinomorphic, epigynous, pentamerous, 1.0-2.0 mm in diameter; corolla tube 1.0-2.0 mm long; lobes deltoid, pubescent. Stamens 5; anther dithecous, basifixed. Carpel 1; ovary inferior, obovate; stylar arms with acute tip. Achenes 1-seeded, ovate, glabrous.

Specimen examined: Natmouk Township, 2019; (N Lat 20° 30′ 54″ and E Lon 95° 37′ 40″).

5. ***Eclipta prostrata*** L. Mant. 2: 2861771.

Vernacular Name : Kyeik-man;

Flowering period : Throughout the year

Perennial, erect or procumbent herbs; stems and branches cylindrical, reddish, appressed-pubescent. Leaves simple, opposite and decussate, subsessile, exstipulate; blades elliptic-oblong or oblong-lanceolate, 2.5-10.5 cm by 1.0-2.5 cm, cuneate at the base, entire or spinulose-crenate along the margin, acute at the apex, appressed-pubescent on both surfaces. Inflorescences solitary or geminate, in the upper leaf-axils or terminal; peduncles 6-10 mm long, longer after anthesis. Heads heterogamous, radiate; involucre campanulate; involucral bract 2-seriate, elliptic, 5-7 mm long, appressed-pubescent without; receptacle convex, paleaceous. Marginal flowers 1- to 2-scriate, liguliform, pistillate, fertile: inner ones numerous, disciform, bisexual, fertile, not exceeding the involucre. Corolla of ray floret ligulate, shallowly or deeply 2-toothed, white, about 2 mm long; corolla of disc floret tubular, 4- or 5-lobes, white; tube about 1.5 mm long. Stamens 4, epipetalous; filaments short, free; anthers syngenesious, acute or obtuse base and apiculate apex. Ovary inferior, triquetrous, thinly hirsute, unilocular with solitary basal ovule; style filiform, style arms short, flat, obtuse. Achenes triquetrous, oblongoid, black, white-hairy at the top.

Specimen examined: Natmouk Township, 2019; (N Lat 20° 31′ 54″ and E Lon 95° 37′ 40″).

6. *Grangea maderaspatana* (L.) Poir, Encycl. Suppl. 2: 825. 1812.

Vernacular name : Taw ma hnyo lon;
Flowering period : January to August

Annual erect herb, 0.7- 0.21 cm high; stem cylindrical, villous. Leaves simple, alternate, exstipulate, sessile, blade oblong- obovate, 1-5 m by 0.5- 2.8 cm, cuneate at the bases, pinnatifid to pinnatisect along the margins, acute at the apex, the both surfaces tomentose, heads terminal and subglobose pedunculate, 0.6-1.4 by 0.4-1.2 cm, heterogamous, yellow, the involucral bracts lanceolate, 0.1-0.2 cm by 0.05-0.07 cm ;ray florets

pistillate, zygomorphic, light yellow, 0.3-0.4 by 0.1-0.2 cm at the anthesis, numerous , pappus hairs minute, membranous , ciliate , persistent, corolla 2 to 4- lobed, ligulate , the lobes 0.15- 0.2 cm by 0.1-0.15 cm, puberulous without, glabrous within; disc florets bisexual, actinomorphic , yellow , 0.1-0.15cm by 0.05-0.1 cm at anthesis, pentamerous, epigynous, pappus hairs minute, ciliate , corolla 4 or 5-lobed, infundibuliform , 0.1-0.2 cm by 0.05-0.1 cm , glabrous. Stamens 4 or 5, free, exserted, epipetalous, the filaments 0.2-0.3 cm long, anther dithecous, syngenesious, basifixed , longitudinally dehiscing. Ovary inferior, obovoid, 0.01-0.04 cm by 0.1-0.2 cm, unilocular.

Specimen examined: Natmouk Township, 2018;(N Lat 20° 29′ 23″ and E Lon 95° 37′ 44″).

7. *Mikania scandens* (L.) Willd., Prodr. 5.199.1836.

Vernacular Name : Be zut nwe;
Flowering Period : September to January

Annual twining herbs. Stems and branches with faint longitudinal striations, flaccid, pubescent. Leaves simple, opposite; petioles 5.0-6.5 cm long, glabrous, blades rhomboid-ovate, 7.0-9.0 cm by 6.5-7.5 cm, cordate at the base, dentate at the margin, acuminate at the apex, Inflorescences axillary or terminal, corymbose-head, 4 florets per head; the flarets white, bisexual, actinomorphic cyclic, pentamerous, epigynous. Involucre tubular, 3.5-4.0 mm long, the bracts 4, elliptic or elliptic-ovate, 3.5-4.0 mm long, receptacles flat about 1.0 mm in diameter. Pappus white, numerous, one whorl, hairy, filiform, 3.5-4.5 mm long, spinulose, persistent. Corolla tubular with 5 teeth, glabrous; the tubes 2.0-3.5 mm long; the lobes ovate-acute. Stamens 5, epipetalous, inserted; filaments filiform, about 0.5 mm long: anthers dithecous, basifixed. Ovary linear-oblongoid, 1.5-2.0 mm long, glabrous, bicarpellary, unilocular, one ovule in each locule, basal placentation; styles exserted, 2.5-3.5 mm long; stylar arms linear, 1.0-1.5 mm long. Achenes straight, 5-angled, oblongoid, 25-3.0 mm long, glabrous, glandular, brownish-black.

Specimen examined: Natmouk Township, 2018; (N Lat 20° 29′ 5″ and E Lon 95° 37′ 33″).

8. ***Tridax procumbens*** L., Sp. Pl. 900. 1753.

Vernacular name : Hmwezok ne gya;

Flowering period : Throughout the year

Perennial, procumbent and hirsute herbs; stems and branches pubescent. Leaves simple, opposite and decussate; exstipulate; petioles 1.0-2.0 cm long, pubescent; blades ovate or lanceolate,2.0-7.0cm by 0.8-3.8 cm, scabrid hirsute on both surfaces, cuneate at the base, serrate to coarsely incised-dentate or trilobed at the margin, acute or acuminate at the apex. Inflorescences terminal or solitary head, yellow, 1.0-2.0 cm in diameter, heterogamous, many-flowered; peduncles long, 10.0-20.0 cm long. Involucre campanulate, 2-seriate; outer bracts ovate, 0.4-0.6 cm long; inner bracts oblong,about 0.5 cm long. Receptacles convex, paleaceous. Outer ray florets unisexual, zygomorphic, epigynous, pentamerous, creamy white. Inner disc floret bisexual, actinomorphic, epigynous, pentamerous, numerous per head, pale white. Pappus about 20, white, feathery. Stamens 5, epipetalous, inserted; filaments filiform, short; anther syngenesious, base sagittate, appendages acute, ovate. Ovary inferior, obovate, , unilocular with solitary basal ovule; stylar arms exserted, about 0.1 cm long; stigma bifid.

Specimen examined: Natmouk Township, 2018; (N Lat 20° 29′ 2″ and E Lon 95° 37′ 28″).

9. ***Vicoa indica*** (L.) DC, in Wight, Contrib. 10. 1834 et Prod. 5: 474.1836.

Vernacular Name : Taung lone kyaw;

Flowering period : November to March

Annual erect herbs, stems and branches reddish, slender, rigid, 1 m height, with spreading branches, sparsely pubescent. Leaves simple, alternate, exstipulate, sessile; blades linear-lanceolate, 6.0-7.5 cm by 5.0 mm- 15.0 mm; hastate at the base, entire and revolute at the margin, acuminate at the apex, scabrid or glabrous above, glandular punctate beneath. Inflorescences

terminal or axillary solitary, often combined into a corymbs at the terminal portion; peduncles reddish, pubescent. Heads bright yellow, 1.3-1.7cm in diameter, heterogamous, on long slender spreading peduncles: peduncles 2.0-4.0 mm long, bearing small leaves, spirally arranged, involucral bracts, many-seriate, linear lanceolate, about 3.0 mm long; ray florets bright yellow, bisexual, fertile, actinomorphic, epigynous; Pappus hairs white, persistent. Corolla of ray florets yellow, ligulate; corolla of disc florets yellow, tubular, with 5 teeth; tube 3.0-4.0 mm long. Stamens 5, free, epipetalous; filaments 1 mm long; anthers dithecous, basifixed, syngenesious, dehiscing by longitudinal slit. Ovary oblongoid, pubescent, unilocular with solitary basal ovule: style filiform, 40-3.0 mm long; stylar arms obtuse. Achenes small, ribbed, oblong, about 0.8 mm long, brown, pubescents.

Specimen examined: Natmouk Township, 2019; (N Lat 20° 29′ 9″ and E Lon 95° 37′ 31″).

10. ***Xanthium strumarium*** L., Sp. Pl. 9871753.

Vernacular Name : Katsine;
Flowering period : June to December

Annual erect herbs, 1-1.5 m tall, hispidulous or glabrescent. Leaves simple, alternate, petiolate, exstipulate, broadly ovate, 3-5 lobed, petioles 2-12 cm long; blade 3-15 by 2.5-15 cm, rounded or cordate at the base, irregularly serrate along the margins, acute or acuminate at the apex, hispidulous on both surfaces, glandular beneath. Male capitula globose, 5 mm across; phyllaries 1-2 seriate, lanceolate, 2.0-2.5 mm long, pubescent; corolla 2.5 mm long. Stamens 5, united, exserted 1mm beyond corolla mouth; paleae oblong, obtuse, 2.5 mm long, pubescent near the apex. Famale capitula ovoid, 5-6 mm long, covered with spines and glands; flowers 2. Ovary inferior, 2.0-2.5 mm long, and style 2.5-4.0 mm long, divided almost to the base. Fruiting capitulum 15-18 mm long including rostra, spines 3 mm long, glandular and hispidulous. Rostra thick, divergent, hooked at the apex, as long as the spines. Achenes narrowly ovoid or elliptic, 1.3-1.5 mm long, 3-4 mm broad, grayish black, smooth, tapering to a fine point at the apex.

Specimen examined: Natmouk Township, 2019; (N Lat 20° 29′ 16″ and E Lon 95° 37′ 35″).

Artificial Key to the Species

1. Plants Shrub----------**3. *Chromolaena odorata***
1. Plants herbaceous----------2
   2. Plants perennial ----------3
   2. Plants annual ----------4
3. Leaves blade oblong; stamens 4----------**5. *Eclipta prostrata***
3. Leaves blade ovate; stamens 5----------**8**. ***Tridax procumbens***
   4. Leaves opposite----------5
   4. Leaves alternate----------6
5. Stems and branches hirsute; leaves blade elliptic-obovate; stamens 4----------**1. *Acanthospermum hispidum***
5. Stems and branches pubescent; leaves blade ovate; stamens 5----------**7. *Mikania scandens***
   6. Leaves pinnatisect ----------7
   6. Leaves lanceolate- ovate ----------8
7. Stems and branches pilose; ovary ovate---------**4. *Cyathocline purpurea***
7. Stems and branches villous; ovary obovoid----------**6. *Grangea maderaspatana***
   8. Leaves lanceolate----------**9**. ***Vicoa indica***
   8. Leaves ovate----------9
9. Head cup-shaped----------**2. *Blainvillea acmella***
9. Head ovoid or globose----------**10. *Xanthium strumarium***

Discussion and Conclusion

In this research, some species from family Asteraceae growing in Natmouk Township are described. There are 10 species, 10 genera from family Asteraceae in this paper. The family Asteraceae show a remarkable diversity in habit and includes annual and perennial herbs. Some plants are shrubby herbs and woody at the base. Not many of them are trees and only a few are aquatic. The species described in this paper are belonging to Tribes Astereae, Eupatorieae, Heliantheae, Inulae.

The members of the Tribe Astereae can be distinguished by its alternate or radial leaves, heterogamous heads in corymb or panicle, concave or convex pitted receptacle, basally obtuse and never sagittate anthers. Grangea possess disciform capitula with inconspicuous ray flowers.

Convex receptacle is found in Grangea. It can be easily distinguished by marginal female florets tubular; disk florets bisexual; inflorescences solitary capitula or laxly corymbiform. Cyathocline can be distinguished by leaves pinnatifid or pinnatisect; receptacles cup-shaped or funnel-shaped; marginal female floret lamina none (outer) or short, narrowly funnelform (inner), 2-lobed, purple; disk florets 5-lobed.

The members of the Tribe Eupatorieae can be distinguished by its simple opposite leaves, rhomboid to ovate lamina, homogamous disciform heads, flat naked receptacles, basally obtuse or acute anthers, and ribbed or angled achenes. The species of Mikania are twiner and that of Chromolaena are erect. The species of Chromolaena can be distinguished by its herbaceous or shrubby stem, campanulate or tubular involucre.

The Tribe Heliantheae can be distinguished by its mostly opposite leaves, heterogamous and radiate capitula, obtuse or sagittate base of anther, palaceous receptacles, and aristate achenes. Achenes enveloped and enclosed by prickly inner phyllaries in Acanthospermum. Blainvillea can be distinguished by pappus elements 2–5, unequal, spinelike or squamalike, persistent, base connate; ray florets female, ray floret lamina short or very short, apex 2–4-dentate; capitula small. Ray florets of eclipta are white; achene body tuberculate. Tridax can be distinguished by pappus of plumose setae. Unisexual head is found only in the genus Xanthium and the rest genera possessing bisexual heads. The nature of style, involucral bracts, achenes and pappus can be used in identification for the members of the rest genera.

The Tribe Inuleae can be distinguished by its alternate leaves, heterogamous and radiate or disciform capitula, several-seriate involucral bracts, basally sagittate anther, and naked receptacles. Radiate capitula are found only in Vicoa and disciform capitula in the rest genera.

The present research work can provide the valuable information and beneficial knowledge for the students, other researchers and local people in various ways. This research will also be partial fulfillment for systematic Botany of Natmouk Township and its surrounding area.

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Antimicrobial Metabolite Producing Actinomycetes Isolated from Soil in Myitkyina Township

Zin Moe Hlaing[1], Moe Moe Aye[2] & Nyunt Phay[3]

Abstract

In the course of the isolation of actinomycetes, twelve actinomycetes were isolated from the three soil samples collected in Myitkyina Township. The isolation of actinomycetes was undertaken by the method of Chemical Treatment Dilution Method and Physical Treatment Serial Dilution Method. Soil actinomycetes were tested the antimicrobial activities with nine test organisms by using paper disc diffusion method. In the studies of antimicrobial activities, actinomycete ZM-01 showed the highest antibacterial activity (26.30 mm clear zone) against *Micrococcus luteus*. Therefore, this actinomycete was selected for further investigation. This selected actinomycete ZM-01 was isolated from the soil collected in the Pamati Village. In the study of the age and size of inoculum, it was observed that 50 hrs seed culture and 2.5% sizes of inoculum were optimized for the ZM-01 fermentation.

Keywords: Actinomycetes, Dilution method, Antimicrobial activity, Fermentation

Introduction

Microbial pathogens are increasing their efficiency and resistance to many drugs day by day and becoming more dangerous to the living forms of life, therefore new and potential antibiotics are needed through proper development strategies (Chaudhary *et al*., 2013).

Soil is the most extensively studied ecological niche. Organisms such as bacteria present in those environments are good sources of bioactive metabolites. One of the most important and well acknowledged groups of microorganisms in the soil is the actinomycete. Actinomycetes are proved to be most promising strains for production of various bioactive secondary metabolites, especially the genera *Streptomyces* holds a prominent position of producer of different classes of antibiotics and other pharmaceutically and industrial important compounds (Pereira and Kamat, 2013).

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Search for new antibiotics effective against multi-drug resistant pathogenic bacteria is presently an important area of antibiotic research. It is anticipated that the isolation, characterization and the study on actinomycetes can be useful in the discovery of antibiotics and novel species of actinomycetes. The aim and objectives of this study were to isolate the different actinomycetes from soil samples of Myitkyina Township and to investigate their antagonistic properties against test organisms.

Materials and Methods

Collection of soil samples

The soil samples were collected from different places in Myitkyina Township, Kachin State during July, 2017. The top layer of the soil was removed for about 5-6 cm with clean spade. The samples were taken up from a depth of 6 inches and the soil was collected by using clean, dry plastic bags along with sterile spatula.

After collection, the soil samples were labeled indicating the dates and places of sample collected. Three different places of soil samples were used for the isolation of actinomycetes and their location, soil pH and soil types are shown in Table 1 and 2.

Isolation of microorganisms from different soil samples

The collected soil samples were air-dried at room temperature in the laboratory. Isolation of soil actinomycetes was undertaken by the methods of Chemical Treatment Dilution Method (Phay and Yamamura, 2005) and Physical Treatment Serial Dilution Method (Phay and Yamamura , 2005).

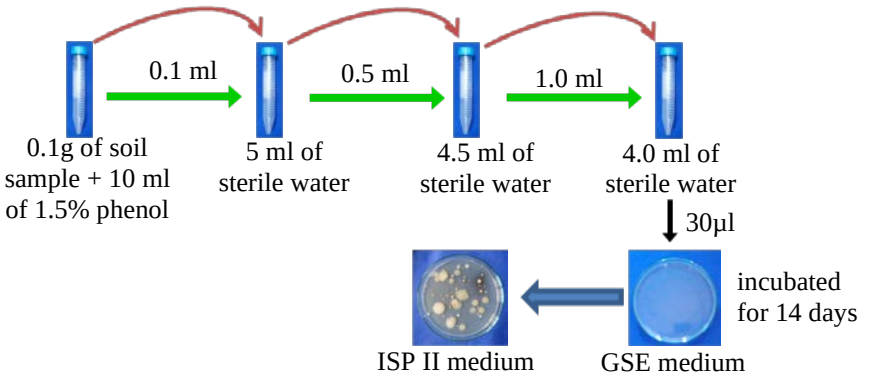


Figure 1. Chemical treatment dilution method

(Phay and Yamamura, 2005)

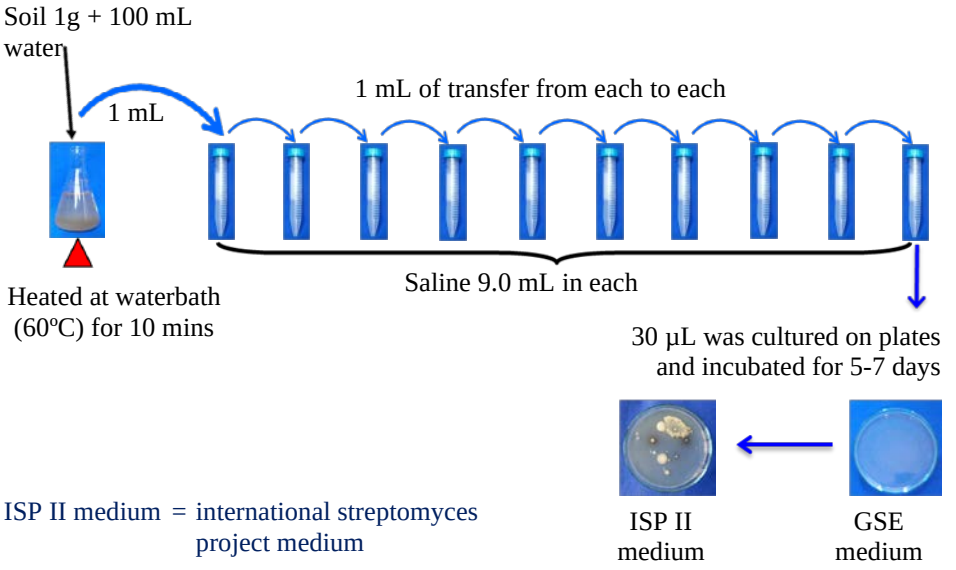


Figure 2. Physical treatment serial dilution method
(Phay and Yamamura, 2005)

Medium Used for Screening of Actinomycetes

GSE Agar medium (NITE, 2005)

Glucose	1.0 g
Soy bean flour	0.5 g
Soil extract	50 ml

Nalidixic acid	0.01 g
Humic Acid	0.001 g
$CaCO_3$	0.02 g
NaH_2PO_4	0.5 g
KCl	1.7 g
$FeSO_4.\ 7H_2O$	0.01 g
Agar	1.8 g
DW	50 ml
pH	7.2

After autoclaving, cycloheximide was added to this medium.

Preliminary study for antimicrobial activities by paper disc diffusion assay (NITE, 2004)

The isolated actinomycetes were grown at 27ºC for 14 days on ISP II medium. And then actinomycetes were inoculated on seed medium and incubated at 27ºC for 3 days. Seed culture (2.0%) was transferred into the fermentation medium and incubated at 27ºC for 3 days. After three days, 20 µL sample was put on paper disc and dry. And then placed on assay agar plate containing test organism (Paper disc size = 8 mm).

Test organisms used in antimicrobial activities

Nine kinds of test organisms *Saccharomyces cerevisiae* NITE 52847, *Candida albicans* NITE 09542, *Bacillus subtilis* IFO 90571, *Micrococcus luteus* NITE 83297, *Xanthomonas oryzae* IFO 93517, *Staphylococcus aureus* AHU 8465, *Pseudomonas fluorescens* IFO 94307, *Salmonella typhimurium* AHU 7943 *and Escherichia coli* AHU 5436 were used in paper disc diffusion method.

Medium for antimicrobial activities

Seed medium (NITE, 2004)		**Fermentation medium (NITE, 2004)**	
Glucose	2.0 g	Glucose	2.0 g
Yeast extract	1.0 g	Glycerol	1.0 mL

Peptone	0.5 g	Yeast extract	1.5 g
KNO_3	0.1 g	Polypeptone	1.2 g
K_2HPO_4	0.001 g	K_2HPO_4	0.001 g
DW	100 mL	$MgSO_4$	0.001 g
pH	7.0	$CaCO_3$	0.1 g
		DW	100 mL
		pH	7.2

Study on the age and sizes of inoculum for the fermentation

The selected actinomycetes ZM-01 was inoculated into the seed medium and then seed culture (1.5%) was transferred to fermentation medium 4 hrs intervals. Age of culture with (38 hrs, 42 hrs, 46 hrs, 50 hrs, 54 hrs, 58 hrs) were used for fermentation. In the study of sizes of inoculum, 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0% of seed culture at 50 hrs were transferred into the flasks containing the fermentation medium. Fermentation was carried out 7 days and antibacterial activity was tested by paper disc diffusion assay method (Omura, 1985).

Results

Collection of soil samples

Three different soil samples were collected in Myitkyina Township, Kachin State as shown in Table 1.

Table 1. Soil samples collected at different places (Myitkyina Township)

Soil No.	Collected place		Texture	pH
S-1	Pamati village	N 25º 22′31.112″ E 97º 20′ 21.487″	Silt Loam	7.3
S-2	Mawhpaung village	N 25º 21′53.422″ E 97º 17′ 29.799″	Silt Loam	7.8

Soil No.	Collected place		Texture	pH
S-3	Loikhaw village	N 25º 19′55.704″ E 97º 08′ 27.184″	Silty Clay Loam	7.8

Isolation of microorganisms from different soil samples

In this study, 12 kinds of soil actinomycetes were isolated from three different soil samples in Myitkyina Township, Kachin State as show in Table 2.

Table 2. Actinomycetes isolated from three different soil samples by chemical and physical treatment methods

Soil sample No.	Collected Places	Total Isolated Actinomycetes			
		Chemical Treatment	Physical Treatment	Total	No.
S-1	Pamati village	1	3	4	ZM-01,02,03, 04
S-2	Mawhpaung village	3	2	5	ZM-05,06,07,08, 09
S-3	Loikhaw village	1	2	3	ZM-10, 11, 12
Total Isolated Actinomycetes		5	7	12	

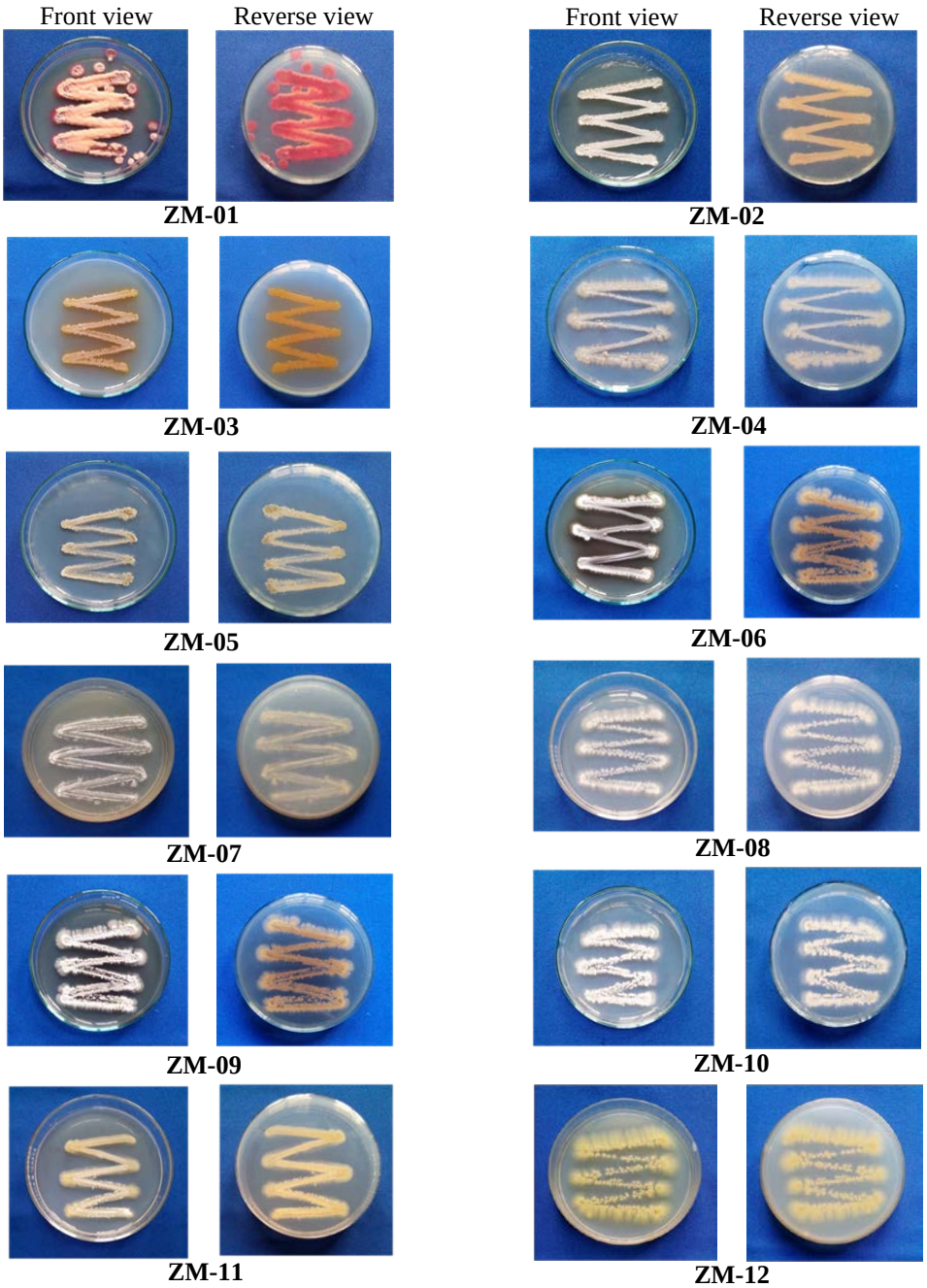


Figure 3. Mycelium colour of actinomycete strains on ISP II medium (14 days old culture)

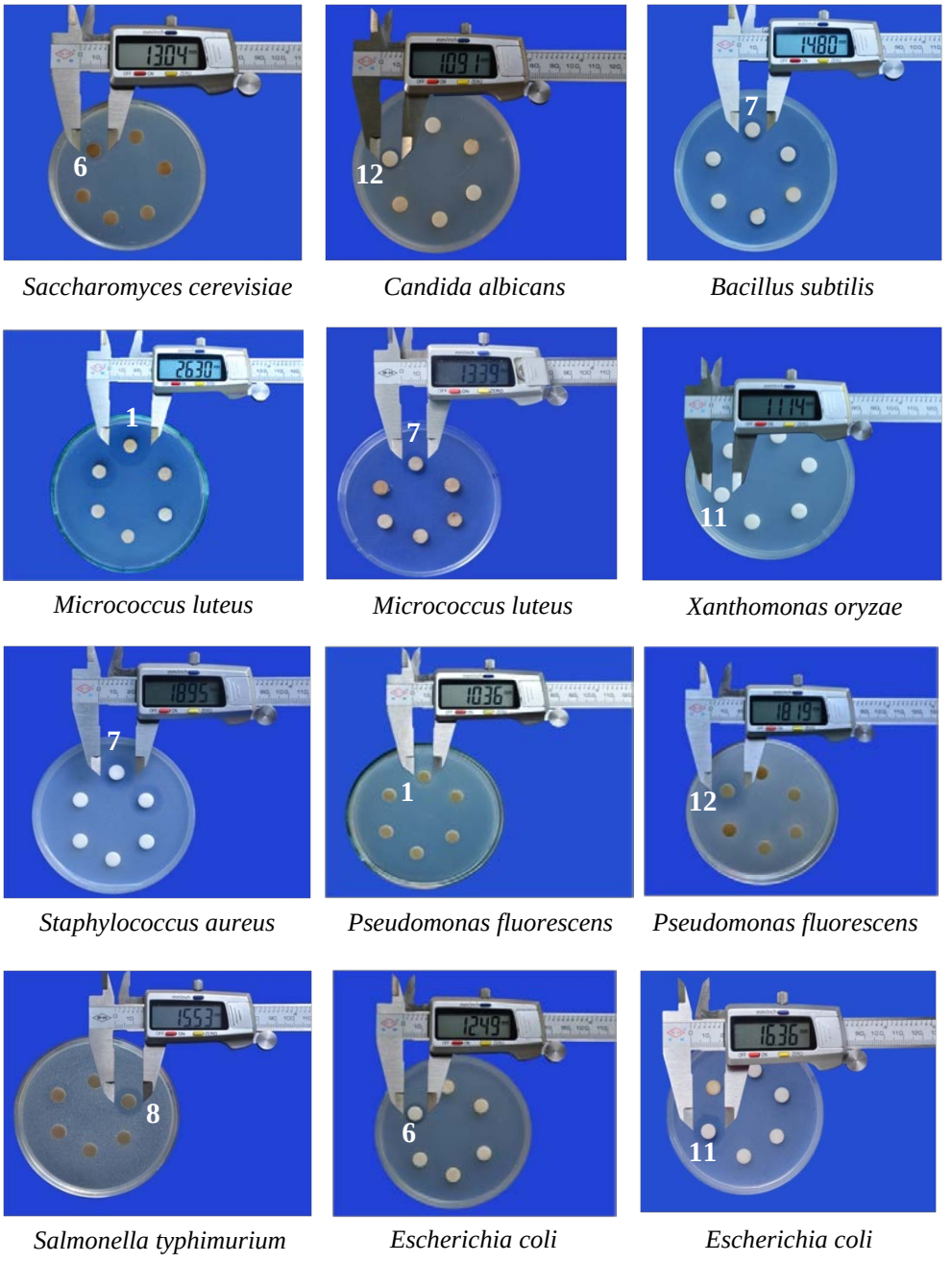
Preliminary study for antimicrobial activities by paper disc diffusion assay (NITE, 2004)

In this study, eight soil actinomycetes exhibited distinct clear zone against 9 kinds of test organisms (Table 3). Among them, it was found that strain ZM-01 and ZM-06 showed highly antibacterial activity against *Micrococcus luteus*. However, actinomycete ZM-01 (26.30 mm) showed more highly antibacterial activity than ZM-06 (23.17 mm). Therefore, ZM-01 was selected for further investigation.

Table 3. Antimicrobial activities of isolated actinomycetes (At 3 days fermentation)

Isolated	Test Organisms and Inhibitory Zone (mm)								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
ZM-1	-	-	-	**26.30**	-	-	10.36	-	-
ZM-2	-	-	-	-	-	-	-	-	-
ZM-3	-	-	-	12.67	-	-	-	-	-
ZM-4	-	-	-	-	-	-	-	-	-
ZM-5	-	-	-	-	-	-	-	-	-
ZM-6	13.04	-	-	**23.17**	-	-	-	-	12.49
ZM-7	-	-	14.80	13.39	-	18.95	-	-	-
ZM-8	-	-	13.72	-	-	17.21	-	15.53	-
ZM-9	-	-	-	-	-	-	-	-	-
ZM-10	-	-	13.49	-	-	-	-	-	-
ZM-11	-	-	-	-	11.14	-	-	-	16.36
ZM-12	-	10.91	-	-	-	-	18.19	-	-

1 = *Saccharomyces cerevisiae*
2 = *Candida albicans*
3 = *Bacillus subtilis*
4 = *Micrococcus luteus*
5 = *Xanthomonas oryzae*
6 = *Staphylococcus aureus*
7 = *Pseudomonas fluorescens*
8 = *Salmonella typhimurium*
9 = *Escherichia coli*
(Paper disc = 8 mm)



Saccharomyces cerevisiae *Candida albicans* *Bacillus subtilis*

Micrococcus luteus *Micrococcus luteus* *Xanthomonas oryzae*

Staphylococcus aureus *Pseudomonas fluorescens* *Pseudomonas fluorescens*

Salmonella typhimurium *Escherichia coli* *Escherichia coli*

Figure 4. Antimicrobial activities of isolated soil actinomycetes

Table 4. The effects of ages of inoculum on the fermentation of ZM-01 (Test organism- *Micrococcus luteus*)

Culture Times (Ages of Culture, hrs)	**Antibacterial activity (Clear zone, mm)**
38	19.37
42	20.62
46	22.13
50	**24.90**
54	23.07
58	20.16

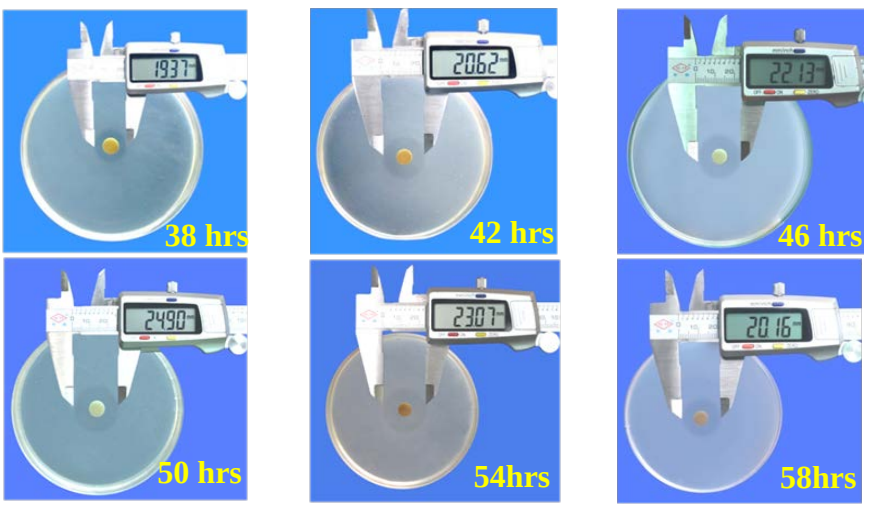


Figure 5. Antibacterial activity of ages of inoculum on *Micrococcus luteus*

Table 5. The effects of sizes of inoculum on the fermentation of ZM-01 (Test organism-*Micrococcus luteus*)

Culture Times (Sizes of Culture, %)	**Antibacterial activity (Clear zone, mm)**
0.5%	20.27
1.0%	22.48
1.5%	23.92
2.0%	25.62

Culture Times (Sizes of Culture, %)	Antibacterial activity (Clear zone, mm)
2.5%	**26.51**
3.0%	25.07

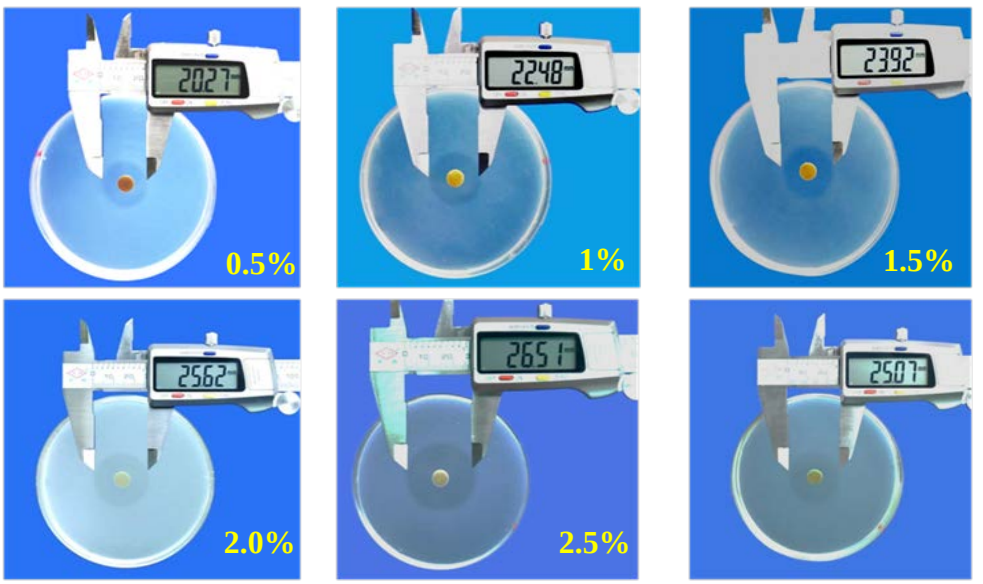


Figure 6. Antibacterial activity of sizes of Inoculum on *Micrococcus luteus*

Discussion and Conclusion

Soil microorganisms provide an excellent resource for the isolation and identification of therapeutically important products. Among them, actinomycetes are potential source of many bioactive compounds which have diverse clinical effects and important application in human medicine (Watve *et al*., 2001). It has been estimated that approximately one-third of the thousands of naturally occurring antibiotics have been obtained from actinomycetes (Takizawa *et al*., 1993).

In the course of the investigation for antimicrobial metabolite producing actinomycetes, 12 different actinomycetes were isolated from the three soil samples collected in Myitkyina Township. Morphological character and reverse color of isolated actinomycetes were found to be different. Actinomycetes ZM- 01, 02, 03 and ZM-04 were isolated from

Pamati village soil sample. ZM-05, 06, 07, 08 and 09 were isolated from Mawhpaung village soil sample. ZM-10, 11, and 12 were isolated from Loikhaw village soil sample. There are numbers of bacteria which are capable of producing antibiotics which include Bacillus (Waites *et al.*, 2008), Actinomycetes (Abdulkadir and Wliyu, 2012; Tiwari and Gupta, 2013), Pseudomonas (Cartwright *et al.*,1995) and Streptomyces (Willey *et al*., 2008). In the studies of antimicrobial activities, actinomycete ZM-06 showed antifungal activity against *Saccharomyces cerevisiae.* ZM-12 showed antifungal activity against *Candida albicans.* Actinomycetes ZM-07, ZM-08 and ZM-10 showed antibacterial activity against *Bacillus subtilis.* Actinomycetes ZM-01, ZM-03, ZM-06 and ZM-07 showed antibacterial activity against *Micrococcus luteus.* Actinomycete ZM-11 showed antibacterial activity against *Xanthomonas oryzae.* ZM-07 and ZM-08 showed antibacterial activity against *Staphylococcus aureus.* Actinomycetes ZM-01 and ZM-12 showed antibacterial activity against *Pseudomonas fluorescens*. Actinomycete ZM-08 showed antibacterial activity against *Salmonella typhimurium*. Actinomycetes ZM-06 and ZM-11 showed antibacterial activity against *Escherichia coli.* Among them ZM-01 and ZM-06 showed the highest antibacterial activity against *Micrococcus luteus*. However, isolated soil actinomycete ZM-01 (26.30 mm) showed more highly antibacterial activity than ZM-06 (23.17 mm). Therefore, this strain ZM-01 was selected for further studies such as ages of culture and sizes of inoculum. This actinomycete ZM-01 was isolated from the Pamati village soil (Silty Loam, pH-7.3) of Myitkyina Township, Kachin State.

Optimal fermentation conditions are very important for maximal productivity of antibiotics (bioactive compounds or metabolites). The proper cultivation and transfer of inoculum are crucial to produce primary and secondary metabolites (Cruger, 1989). Therefore, the proper size and age of inoculum were investigated. In the study of the effects of ZM-01 ages and sizes of inoculum, it was found that 50 hrs of ages of culture and 2.5% sizes of inoculum were optimized to produce the antibacterial metabolite during fermentation. In conclusion, the resulting optimum conditions for antibacterial metabolites will be aimed to utilize in further more investigations.

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A Screening for a Potent Antimicrobial Compound of Selected Soil Fungus in Patheingyi Township, Mandalay Region

Zin Nwe Aye*

Abstract

In the study of isolation of soil fungi, ten different fungi were isolated from two different soil samples in Patheingyi Township in Mandalay Region. The isolation of soil samples were undertaken by soil plating method and chemical treatment dilution method. In the study of starch hydrolyzing activity, it was observed that four isolated soil fungi ZN-01, ZN-02, ZN-03 and ZN-05 showed starch hydrolyzing activities. The morphology of ten isolated fungi the front view of five colour and reverse view of seven colour. In the preliminary study of antimicrobial activities of ten isolated soil fungi, seven fungi showed the antibacterial activity against *Bacillus subtilis*, two fungi against *Escherichia coli* and two fungi against *Pseudomonas fluorescens*, two fungi against *Xanthomonas oryzae.* According to the results of assay, it was observed that three fungi showed antifungal activities against *Candida albican*. So it was observed that eight fungi showed antibacterial and two showed antifungal activities. Among them, fungus ZN-03 exhibited the highest antibacterial activity against *B.subtilis*. Therefore the selected fungus ZN-03 showed both starch and antibacterial activities.

Keywords: soil fungi, starch hydrolyzing, antimicrobial activities

Introduction

Soil are the foundation of all terrestrial ecosystems and are home to a vast of bacteria, archaea, fungi, insects and other invertebrates as well as plant and algae. Microorganisms are found in all kind of habitats as have significant functions in ecosystem (Subramanian, 1992). Soil sample is a primary source of microorganism. Soil bacteria and fungi play a significant and an important role in antibiotic discovery (Atals and Bartha, 1998). The typical materials for microbial sources are soil, living and fallen leaves, leaf litters, dung, insect, fresh water and marine water. The success of screening program depends upon the isolation of appropriate tests as well as appropriate microorganism to be tested (Crueger, 1989).

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Natural products have a key role in the discovery and development of many antibiotics. Natural products continue to be an important source of new pharmaceutical products (Newmen and Cragg, 2007). Enzymes are proteins that are biocatalysts produced by living cells. They accelerate the rate of chemical reactions without themselves undergoing any chemical change at the end of the reactions (Lilly and Barnett, 1951).

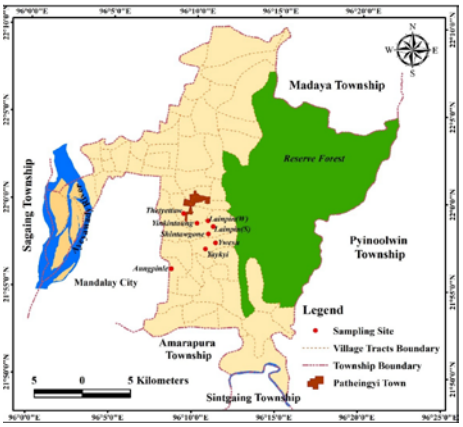
Antibacterial and antifungal antibiotics produced by microorganisms still do not have the required quality in some cases to be used as safe and effective antifungal agents (Phay, 1997). One of the best approaches to the discovery of new antimicrobial agent from natural sources has been to use folklore or historical records to guide the collection of samples or a good research work on the soil of that area (Cordell *et. al.*, 1994).

The aims and objectives of this research are to investigate the varieties of fungi in different soil samples; to study the morphological characters of isolated fungi and screen the antimicrobial activity possessing fungi from soil samples.

Materials and Methods

Collection of Soil Samples

The soil samples were collected from two different places of Patheingyi Township in Mandalay Region (Figure 1 and Table 1).The soil samples were taken and the experiments were carried out at the microbiology laboratory of Botany Department, Yadanabon University from July to September 2018.



Source-Department of Geography, Yadanabon

Figure 1. Soil samples collected area (Patheingyi Township map)

Table 1. The collection of soil sample at Patheingyi Township

Soil No.	Collected at		Soil Type	Soil pH	Collected Date
	Places	Location			
S-1	Yan kin taung Village	N 21º 58'40.880" E 96º 10' 10.488"	Sandy Loam	9.07	7.7.2018
S-2	West Lain pin Village	N 21º 58' 49.438" E 96º 10' 51.61"	Clay	8.43	7.7.2018

Method -1

Soil Plating Method (Ando and Inaba, 2004)

Soil samples were air dried at room temperature for a week. Soil samples were ground and sieve in 2 mm screen. 20 mL of LCA medium was poured into the plate and which was rotated. These culture plate were incubated at room temperature 2-5 days. Low Carbon Agar (LCA) medium was weighed as medium composition Agar: 1.8g, Glucose: 2.0g, Sucrose: 2.0g, K_2HPO_4:1.0g, KCl: 0.5g, $MgSO_4.7H_2O$:0.5g, DW: 100ml, pH:6.5 as shown in (Figure 2).

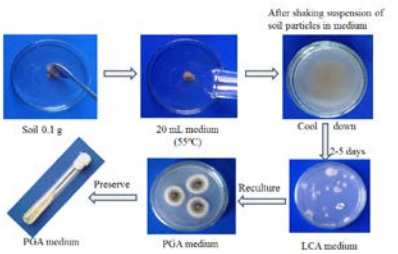


Figure 2. Soil plating method

Method -2

Chemical Treatment Dilution Method (Hayakawa and Kobayashi, 2005)

Soil samples were air- dried at room temperature for a week. Soil samples were ground and sieved in 2 mm screen. 2 g of sieve soil was put into the test tube. 4 mL of sterile water was put into the test tube containing soil and settle for 6 hours to germinate early germinating soil microorganisms. 14 mL of 70 % ethanol was added into the test tube containing soil suspension and shaken for 1 minute and dilution with sterile water. The dilution series were cultured on Low Carbon Agar medium as shown in (Figure 3).

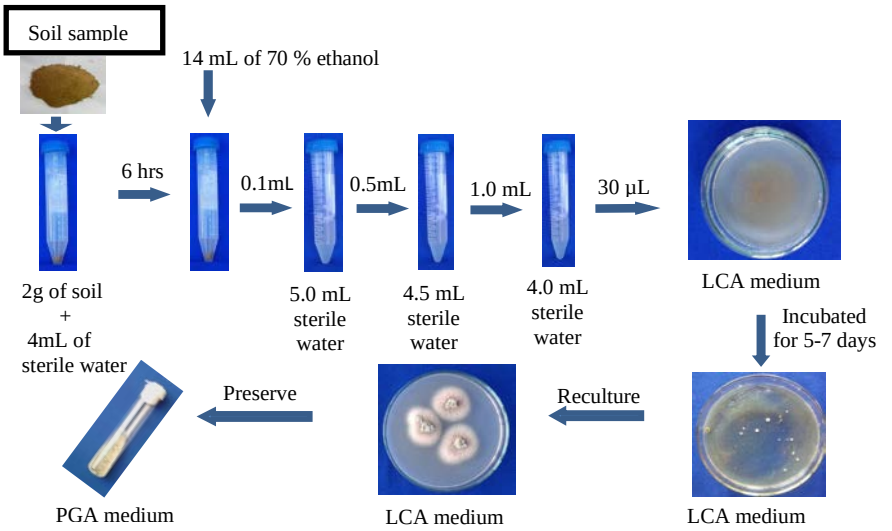


Figure 3.Chemical treatment dilution method

Procedure for starch hydrolyzing activity (NITE, 2004)

The isolated fungi were inoculated in 10mL liquid medium (Soluble starch:1.0g, K_2HPO_4:0.1g, NaCl:0.1g, $(NH_4)_2SO_4$:0.1g, $CaCO_3$:0.01g, DW:100ml: pH-6.5) containing soluble starch and incubated for 3 days at 27 ºC. Iodine solution was poured slowly into the liquid culture medium drop by drop. The control test tube without fungi was also done. If the culture medium is purple color, it indicates that the fungi cannot hydrolyze the starch. If the culture medium changes to colorless, it indicates that the fungi can hydrolyze the starch.

Screening of Antimicrobial Activity (NITE, 2005)

The isolated fungi were grown for 7 days on PGA medium at room temperature. The isolated fungi were inoculated on seed-Glucose:2.5g, Yeast extract:0.8g, $MgSO_4$:0.02g, K_2HPO_4:0.01g, DW:100ml, pH-6.5 (Nakagawa, 1995) and incubated at room temperature for 3 days.5mL of seed culture was transferred into the fermentation medium Glucose:1.5g, Yeast extract: 0.6g, Soluble Starch:0.3g, K_2HPO_4:0.01g, $MgSO_4$:0.02g, DW:100ml, pH-6.5 and incubated at room temperature for 3-7 days.20 µL of fermented broth was put on paper disc and placed on assay plate containing test organisms. Assay medium-Glucose: 1.0g, Polypeptone 0.3g, KNO 3:0.01g, Agar: 1.8gm DW: 100ml, pH-6.5 (Tomita, 1988) as shown in **(**Table 2 and Figure 4).

Figure 4. Procedure for antimicrobial activity test

Table 2. Test Organisms used in Antimicrobial Activities

No	Test organism	Infection
1	*Bacillus subtilis* IFO 905771	Fever
2	*Escherichia coli* AHU 5436	Chlorea, Diarrhea and vomiting,

No	Test organism	Infection
		urinary tract infections
3	*Pseudomonas fluorescens* IFO 94307	Rice disease
4	*Xanthomonas oryzae* NITE 09582	Leaf blight
5	*Candida albicans* NITE 09542	Candidosis

Results

Isolation of Soil Fungi

In the course of investigation of fungi, ten fungi were isolated from two different soil samples by using two methods which collected from Patheingyi Township, Mandalay Region. Four fungi were isolated from yan kin taung village and six fungi were isolated from West Lain pin village respectively as shown in (Table 3).

Table 3. Isolated Fungi from Two Different Soil Samples

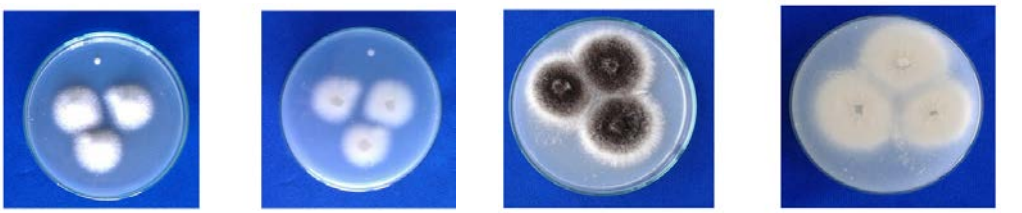
	Collected Place	Soil pH	Isolation Methods		Isolated Fungi
			Soil Plating	Chemical Treatment Dilution	
S-1	Yan kin taung village	9.07	2	2	ZN-01,02,03,04
S-2	West Lain pin village	8.43	3	3	ZN-05,06,07,08,09,10
Total isolation soil fungi			5	5	10

Morphological character of isolated fungi

Morphological character of isolated fungi ZN-01 and ZN-10 on PGA medium (7 days old culture) as shown in Table 4 and Figure 5 to 9.

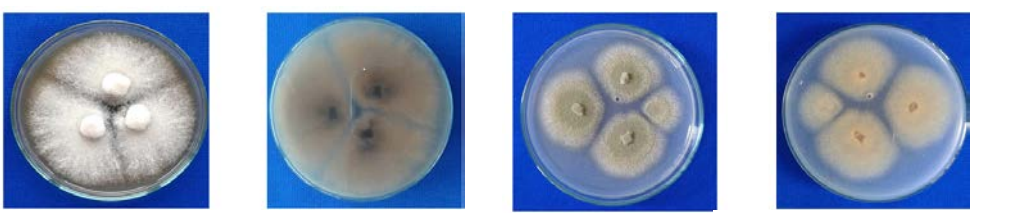
Table 4. Macroscopical Characters of Isolated Soil Fungi (ZN-01 to ZN-10)

Isolated Fungi	Front View	Reverse View
ZN-01	White	White
ZN-02	Center black, edge white	Cream
ZN-03	White	Cream
ZN-04	Center green, edge pale green	pale green
ZN-05	Center green, edge white	Greenish yellow
ZN-06	Pale pink	Center pink, edge cream
ZN-07	White	Center cream, edge white
ZN-08	Center black, edge white	White
ZN-09	Pale green	Center red,edge white
ZN-10	Center black, edge white	White



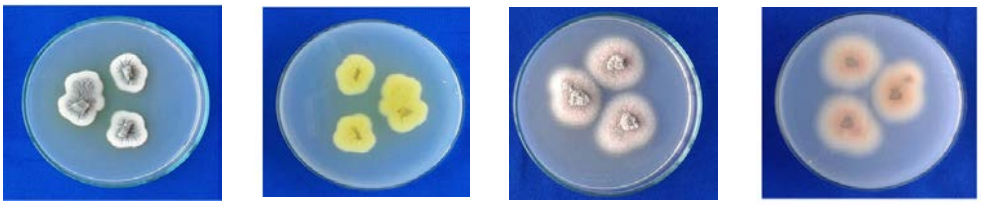
Fungus ZN-01(Front view) Fungus ZN-01(Reverse view) Fungus ZN-02(Front view) Fungus ZN-02(Reverse view)

Figure 5. Morphological character of isolated fungi ZN-01 and ZN-02 on PGA medium (7 days old culture)



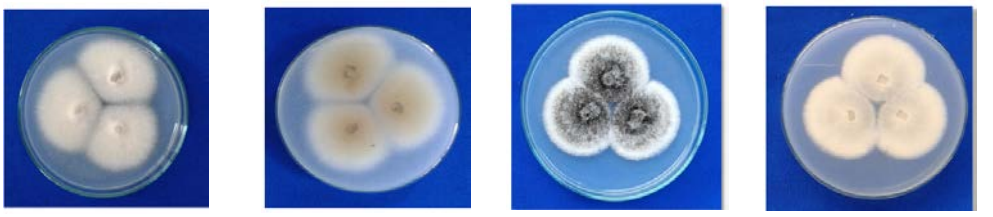
Fungus ZN-03(Front view) (Fungus ZN-03Reverse view) Fungus ZN-04(Front view) Fungus ZN-04(Reverse view)

Figure 6. Morphological character of isolated fungi ZN-03 and ZN-04 on PGA medium (7 days old culture)



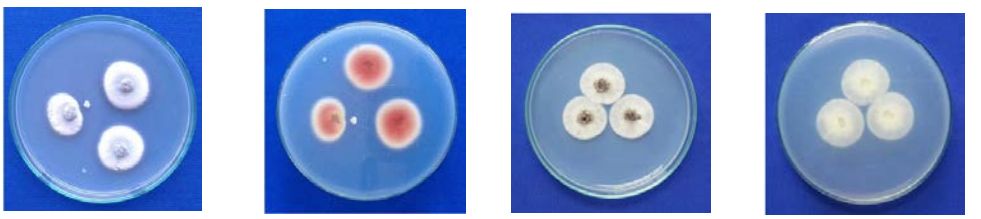
Fungus ZN-05(Front view) Fungus ZN-05(Reverse view) Fungus ZN-06(Front view) Fungus ZN-06Reverse view)

Figure 7. Morphological character of isolated fungi ZN-05 and ZN-06 on PGA medium (7 days old culture)



Fungus ZN-07(Front view) Fungus ZN-07(Reverse view) Fungus ZN-08(Front view) Fungus ZN-08(Reverse view)

Figure 8. Morphological character of isolated fungi ZN-07 and ZN-08 on PGA medium (7 days old culture)



Fungus ZN-09(Front view) Fungus ZN-09 (Reverse view) Fungus ZN-10(Front view) Fungus ZN-10 (Reverse view)

Figure 9. Morphological character of isolated fungi ZN-09 and ZN-10 on PGA medium (7 days old culture)

Starch hydrolyzing activity of isolated soil fungi

In this study, it was found that four strains can be hydrolyze the starch, but other strains did not hydrolyze the starch as shown in (Table 5 and Figure 10).

Table 5. Starch hydrolyzing activity of isolated soil fungi (ZN-01 to ZN-10)

Isolated Fungi	Starch Hydrolyzing Activity
ZN-01	+++
ZN-02	+++
ZN-03	+++
ZN-04	-
ZN-05	+++
ZN-06	-
ZN-07	-
ZN-08	-
ZN-09	-
ZN-10	-

+++ = immediately reaction, - = no reaction

Screening for Starch Hydrolyzing Activity (NITE, 2004)

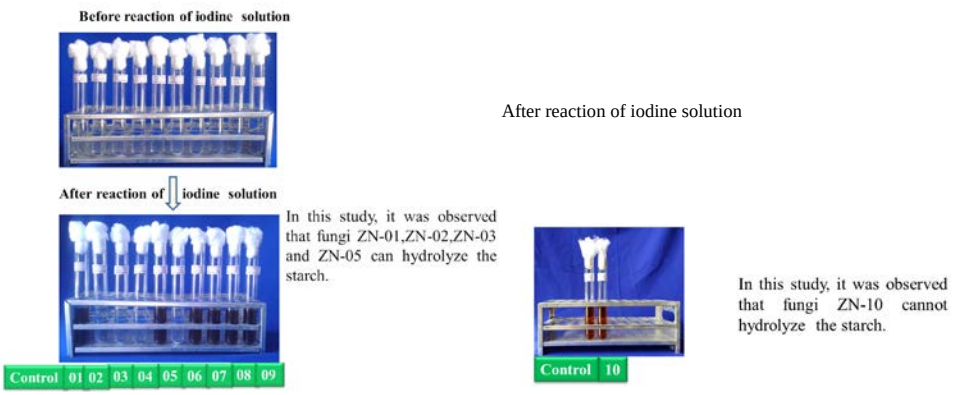


Figure 10. Starch hydrolyzing activities of isolated soil fungi (ZN-01 to ZN-10)

Antimicrobial Activities of Isolated Soil Fungi

All fungal strain were tested by five test organisms for preliminary study of antibacterial activities. Among them ZN-09 exhibited the smallest, ZN-10 are medium and ZN-03 exhibited the highest activities as shown in (Table 6).

Table 6. Antimicrobial Activities of Isolated Soil Fungi (ZN-01 to ZN-10)

Isolated	Antimicrobial Activity (mm)				
Fungi	Antibacterial				Antifungal
	Bacillus subtilis	*Escherichia coli*	*Pseudomonas fluore scens*	*Xanthomonas oryzae*	*Candida. albicans*
ZN-01	-	-	16.89	16.46	-
ZN-02	19.06	-	-	-	15.16
ZN-03	24.15	-	-	-	-
ZN-04	22.04	-	-	-	-
ZN-05	20.04	19.13	-	-	17.30
ZN-06	-	15.38	-	-	16.19
ZN-07	17.21	-	-	-	-
ZN-08	-	-	-	-	-
ZN-09	20.11	-	14.58	16.35	-
ZN-10	18.82	-	-	-	-

(-) = no activity, paper disc = 8mm, 10 to 15 = smallest activity,

15 to 20 = moderate activity, 20 to 25= highest activity

Screening for Antibacterial Activity (NITE, 2005)

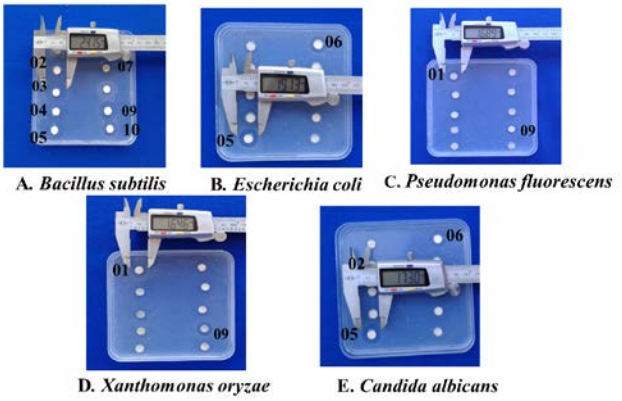


Figure 11. Antibacterial activity of ten isolated soil fungi against test organisms

Biological Properties of Isolated Soil Fungi

In the study of biological properties of isolated fungi, ZN-03 showed both starch hydrolyzing and antibacterial activities. ZN-03 showed more significant antibacterial activity against *B.subtilis* than other. Therefore this fungus ZN-03 was selected for further investigation.

Table 7. Biological Properties of Isolated Soil Fungi

Isolated Fungi	Antimicrobial Activity		Starch Hydrolyzing Activity
	Antibacterial	Antifungal	
ZN-01	+	-	+++
ZN-02	+	-	+++
ZN-03	++	-	+++
ZN-04	+	+	-
ZM-05	+	-	+++

Isolated Fungi	Antimicrobial Activity		Starch Hydrolyzing Activity
	Antibacterial	Antifungal	
ZN-06	+	-	-
ZN-07	+	+	-
ZN-08	+	-	-
ZM-09	+	-	-
ZN-10	+	-	-

++ = highest activity +++ = immediately reaction,
+ = antimicrobial activity - = no activity
- = no reaction

Discussion and Conclusion

In the study of isolation of soil fungi, 10 different fungi were isolated from two different soil samples collected at Patheingyi Township in Mandalay Region. The isolation of soil fungi were undertaken by two methods throughout the study. In this study, five fungi were isolated by soil plating method and five fungi were isolated by chemical treatment dilution method.

Four fungi were isolated from soil sample collected at Yan kin taung village and six fungi from West lain pin village. In the study of starch hydrolyzing activity, it was found that four isolated soil fungi showed starch hydrolyzing activities. So these isolated soil fungi may produce amylase enzymes, primary metabolites. In the investigation of antimicrobial activities of isolated 10 different soil fungi, seven fungi showed against *B. subtilis*, two fungi against *E.coli*, two fungi against *P. fluorescens*, two fung against *X.oryzae*, three fungi against *C.albicans*.

Hlaing Myint Thu, (2020) reported that HMT-21 isolated from soil, Homalin Township, Sagaing Region, that exhibited antibacterial activities against *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens.* The results show that ZN-01 to ZN-04 were isolated from the soil, Yan kin taung village, Patheingyi Township, Mandalay Region, that exhibited antibacterial

against *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens.* This is a agreement with the observation of Hla Myint Thu (2020).

Yee Yee Thin (2019) reported that YTF-8 isolated from soil, Kan Gyi Daung Township, Ayeyarwady Region, that exhibited antimicrobial activities against, *Escherichia coli, Xanthomonas oryzae, Candida albicans,* The result show that ZN-05, ZN-06 and ZN-09 isolated from soil West Lain pin Village, Patheingyi Township, Mandalay Region, that exhibited antibacterial against *Escherichia coli, Xanthomonas oryzae, Candida albicans.* The morphological character of YTF-08 is similar to ZN-05.

In conclusion, it was found that the concentration of soil fungi in two places the soil samples were in thousands per gram soil. The selected soil fungi show that effective against the five bacterial tested. Majority of fungi produces secondary metabolites which may be beneficial towards pharmaceutical chemist as these metabolites are widely used in medicine. Therefore the results seen in the present study also support the medicinal usage as antibacterial agents in new drugs for therapy infection diseases caused by pathogens and undergo further pharmacological screening that can be used as sources for new drugs.

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Morphological and Anatomical Characters of Leaves of *Murraya koenigii* (L.) Spreng.

Khin Mar Kyu[1] & Tin Tin Mar[2]

Abstract

In this research, the specimens of *Murraya koenigii* (L.) Spreng. (Pyin daw thein) belonging to the family Rutaceae were collected from Pauk-pin Village, Monywa Township, Sagaing Region. The identification of the plant was carried out by refering by Hooker (1885) and Dassanayake (1980). Morphology of vegetative and reproductive parts, anatomical structure of leaves were studied and described. Hand sections of the specimens were made and stained with safranin. In the petiole, vascular bundle was lying concentric, amphicribral, xylem surrounded by phloem, phloem surrounded by discontinuous ring of sclerenchymatous cells. Vascular tissue of midrib composed of 1 smaller and 1 larger bundles forming a widely elliptic shaped. In lamina, palisade parenchyma observed 2-layered and spongy parenchyma 4 to 8 layered,

Keywords: The petiole, vascular bundle concentric, amphicribral; vascular tissue of midrib, widely elliptic shaped; lamina, palisade parenchyma 2-layered and spongy parenchyma 4 to 8 layered, oil gland present.

Introduction

Murraya koenigii (L.) Spreng. belongs to the family Rutaceae, commonly known as curry leaf plant is a highly valued plant for its medicinal value and characteristic aroma.

The family Rutaceae distributed in tropical, sub-tropical regions and also warm temperate zones. Nearly all the members of this family possess aromatic and purgent properties. Moreover Rutaceae is generally characterized by the presence of smooth, gland-dotted and exstipulate leaves which produce the aromatic smell when crushed.

Murraya koenigii (L.) Spreng. is a tropical to sub-tropical tree which is native to India and Sri-Lanka. The family Rutaceae has more than 150 genera and 1000 species (Krishnaiah *et al*, 2009).

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The leaves of *Murraya koenigii* (L.) Spreng. are used in many dishes as curry leaves. They are highly aromatic. Curry leaves have many medicinal properties. It stimulates digestive enzymes and helps in breakdown of food more easily good remedy for nausea and indigestion. It also improves eye-sight and prevents cataract. Eating curry leaves lowers blood glucose level (Salomi and Manimekalai, 2016).

Health benefits of *Murraya koenigii* leaves are anti-diabetic, antioxidant, anti-inflammatory (Ningappa *et al.,* 2008) and antimicrobial (Vandana *et al.,* 2012).

The medicinal value of the leaf has been reported as antibacterial. Green leaves are eaten raw for cure of dysentery, and for checking vomiting, diarrhoea. Leaves and roots are also used traditionally as analgesic, bitter, anthelmintics curing piles, inflammation, itching and are useful in blood disorders and leucoderma (Nadkarni *et al.* 1976 and Kirtikar *et al.* 1981).

In this paper, the morphological characteristics of vegetative and reproductive organs and anatomical characteristics of the leaf of *M. koenigii* (L.) Spreng. were studied, described, discussed and their photographs and photomicrographs are also presented.

This research is carried out by the aims of providing more detailed information of morphological and anatomical characteristics of the *M. koenigii* (L.) Spreng..

Materials and Methods

The vegetative and reproductive parts of *Murraya koenigii* (L.) Spreng. were collected from Pauk-pin village, Monywa township, Sagaing Region. Both of the vegetative and reproductive parts of fresh specimens were immediately studied, measured and recorded in detail for taxonomic description. The specimens were identified by the help of the literature of Hooker (1885) and Dassanayake (1980).

For anatomical study, some of the fresh petioles, middle portion of the midribs and lamina were cut into thin sections by using razor blades. The free hand sections were cleared in chloral hydrate solutions and stained with safranin and temporarily mounted by glycerin. They were observed under a light microscope and recorded by a digital camera, then photographs and photomicrographs of the specimens were presented.

Results

Morphological studies

Taxonomic description of *Murraya koenigii* (L.) Spreng.

Family - Rutaceae

Local name - Pyin daw thein

English name - Curry-leaf tree

Flowering period - February to March

Unarmed, evergreen shrubs or small trees, about 4 m in height, the stems cylindrical, glabrous, the barks about 1 mm thick, smooth, greyish dark, the branches cylindrical and stout, young branches pubescent, the branchlets cylindrical, pubescent. Leaves, alternate, unipinnate, imparipinnately compound, exstipulate, the petioles cylindrical, 0.6 – 4.0 cm long, reddish to greenish, pubescent, the petiolules cylindrical, 0.2 – 0.3 cm long, reddish, pubescent, the leaflets 7 – 15 unequal, ovate or lanceolate or falcate, the distal leaflets larger than the proximal ones, 3.7 – 7.3 cm long 1.4 – 3.6 cm wide, the bases cuneate or oblique, the margins slightly crenate, the tip acuminate, with pellucid dotted, subcoriaceous, dark green above, green beneath, the upper surface glabrous, the lower surface pubescent. Inflorescence terminal corymbs, 3.5 – 4.0 cm long, 4.0 – 9.0 cm wide, the penducles cylindrical, densely pubescent. Flowers small dense, 0.8 – 1.0 cm long and 1.0 – 1.2 cm wide at anthesis, actinomorphic, complete, bisexual, pentamerous, hypogynous, white, fragrant, bracteate, the pedicels cylindrical, 0.2 – 0.6 cm long, green, pubescent. Calyx 5, fused, dentate, minute, deeply 5 lobed, 1.2 – 1.5 mm long, green, densely pubescent. Petals 5, free, oblong, 0.2 – 0.4 cm long 0.1 – 0.2 cm wide, thick, imbricate, oil gland present, greenish white above, white beneath, glabrous. Stamens 10, unequal, free, 5 long and 5 short alternately arranged, inserted, around the disk, the filaments linear or subulate, 0.2 – 0.4 cm long, white, broadened towards the base, glabrous, the anthers dithecous, sub-dorsifixed, ovoid, 0.9 – 1.0 mm long, longitudinal dehiscence, introrse, brown. Ovary superior, oblongoid, 1.5 – 2.0 mm long 1.0 – 1.3 mm wide, bilocular, one ovule in each cell, axile placentation, glabrous, the style 1, stout, cylindrical, 0.2 – 0.4 cm long, pale green, glabrous, caducous, the stigma capitate, persistent, green, disc present. Fruits drupe, oblong, 1.0 – 1.7 mm in diameter, purple black when ripe, glabrous. Seed solitary, oblong, testamembranous.

Figure 1. A. Habit B. Inflorescence C. Flowers D. L.S of Flower
E. Stamens F. Pistil G. T.S of Ovary H. Fruits

Anatomical studies

Microscopical characters of the leaves of *Murraya koenigii* (L.) Spreng.

Petioles

In transverse section, the petiole of ***Murraya koenigii* (L.) Spreng.** studied are circular in outline, 4500 – 5500 µm in diameter, distinguishable into dermal, ground and vascular tissue systems.

Dermal tissue system: Composed of epidermal parenchymatous cells and trichomes. In transverse section, epidermis one-layered, compact, cells parenchymatous, rectangular or barrel shaped, 10 – 20 µm long and 10 – 30 µm wide, both outer and inner walls convex, crystal containing cells larger than the other; cuticle thin; non-grandular trichomes unicellular and uniseriate.

Ground tissue system: Composed of cortex and pith. The cortical cells collenctymatous; the collenchymatous cells, 10 to 15 layered, the layers 120 – 150 µm thick, the cells oval or rounded in shape, the cells 20 – 50 µm long and 25 – 60 µm wide, secretory cavity present; pith like parenchymatous cells present at the center of vascular tissue, intercellular space and crystal present in some cells.

Vascular tissue system: Vascular bundles embedded in inner ground tissue, more or less circular in outline in transverse section, 800 – 900 µm in diameter collateral, concentric, amphicribral, xylem surrounded by phloem; phloem surrounded by discontinuous ring of pericyclic sclerenchymatous cells, 2 – 5 layered, polygonal in shape; cambium lying between the phloem and xylem; xylem towards the center, xylem rows 50 – 250 µm in thickness, 1 – 7 cells in each row, composed of vessel elements, tracheids, fiber tracheids, fibers and xylem parenchyma; phloem towards the peripheral, 4 – 6 layered, the layers 40 – 100 µm in thickness, phloem composed of sieves tubes, companion cells, phloem parenchyma and phloem fiber; pith large, parenchymatous; intercellular space and crystal present in some cells.

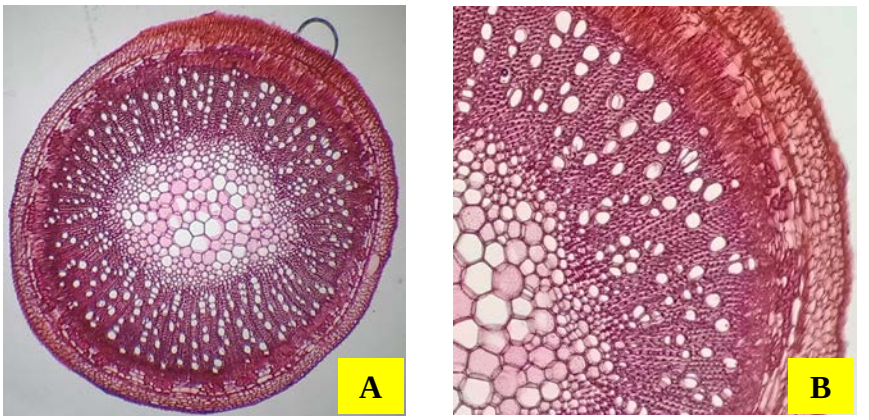

Figure 2. A. T.S of a petiole showing tissue system and an outline
B. T.S of a portion of petiole showing vascular bundle

Midrib

In transverse section, the midrib of *Murraya koenigii* (L.) spreng. studied, are more or less circular in outline with slightly convex at adaxial side, 2000 – 2300 µm long and 2200 – 2300 µm in wide; distinguishable into dermal, ground and vascular tissue systems.

Dermal tissue system: Composed of epidermal parenchymatous cells and oil gland. In transverse section, both upper and lower epidermal cells 1-layered, rounded or barrel shaped; adaxial epidermal cell 20 – 40 µm in length, 15 – 40 µm in breath, abaxial epidermal cell 20 – 50 in length and 20 – 40 µm in breadth; both outer and inner walls convex, anticlinal walls straight; trichomes non-glandular, unicellular and uniseriate, cuticle thin.

Ground tissue system: In transverse section, lying internal to epidermis, made of two type of cells chlorenchyma and parenchyma, chlorenchymatous cells subepidermal and parenchymatous cells internal to chlorenchymatous cells; at adaxial side chorenchymalous cells 2 or 3 layered, the cells 20 – 30 µm long and 15 – 40 µm wide, polygonal in shaped, chloroplast abundant, lysigenous oil cavities and crystal present; inner parenchymatous layer internal to collenchymatous cells, those at adaxial side 2 – 3 layered, the layers 100 – 120 µm thick, the cells oval or rounded, 20 – 24 µm in length and 30 – 70 µm in breadth, crystal present.

Vascular tissue systems: Vascular bundles embedded in ground tissue, in transverse section, more or less circular in outline with slightly convex at

adaxial side, vascular tissues composed of 1 smaller and 1 larger bundles forming a widely elliptic in shape, smaller bundle lying at the adaxial side and separated by parenchymatous cells, the larger bundle 400 – 440 µm long and 300 – 350 µm wide, phloem lying at abaxial size, xylem at adaxial side; phloem 1 – 2 layered, the layers 20 – 40 µm in thick, the cell polygonal in shaped, phloem composed of sieve tubes, companion cells, phloem parenchyma and phloem fibers; xylem 20 – 26 rows , 2 – 8 cells in each row; xylem composed of vessel elements, tracheids, fibers and xylem parenchyma; crystals present in many cells.

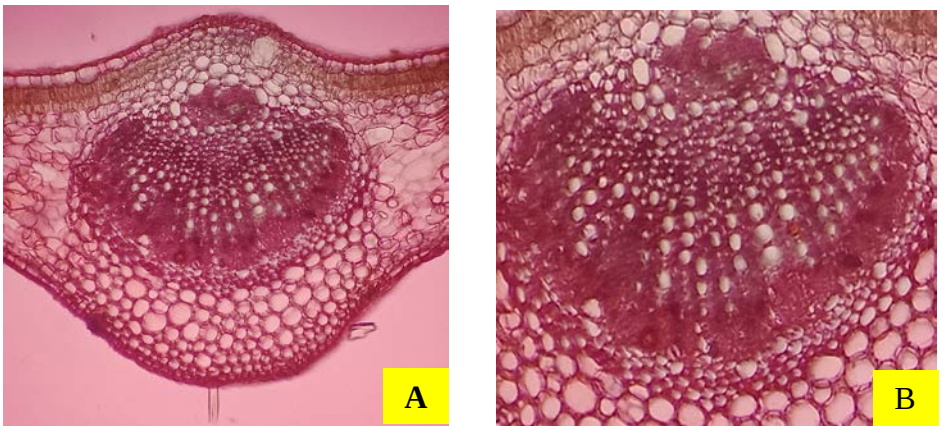

Figure 3. A. T.S of a midrib showing an outline and tissue system
B. T.S of a portion of midrib showing vascular bundle

Lamina

The lamina of *Murraya koenigii* (L.) Spreng. studied, are 300 – 350 µm in thickness, typically dorsiventral and venation reticulate, distinguishable into dermal, ground and vascular tissue systems in transverse section.

Dermal tissue system: Composed of epidermal cells, guard cells of stomata and oil gland. Epidermal cells of both surfaces were parenchymatous; in surface view, cell walls slightly thick at adaxial side and thin walled at abaxial side; stomata present only on the lower surface, of anomocytic type, the guard cells reniform, oil glands present on both surfaces. In transverse section, both upper and lower epidermal cells 1 layered, rectangular or barrel shaped, at adaxial epidermal cells 1- layered, rectangular or barrel shaped, 30 – 60 µm in length, 20 – 40 µm in breadth; at abaxial epidermal

cells 20 – 50 µm in length,10 – 20 µm in breadth, both outer and inner walls convex, anticlinal walls straight; cuticle thick.

Ground tissue system: In transverse section, mesophyll cells differentiated into lying palisade and spongy parenchyma; palisade parenchyma present only at adaxial side, 2 layered, the layers 80 – 100 µm thick, the cells vertically elongated, columnar, thin-walled, straight, intercellular space abscent, chloroplast abundant; spongy parenchyma at abaxial side, 4 – 8 layered, the layers 200 – 300 µm thick, the cells oval or rounded in shape, intercellular space large.

Vascular tissue systems: Vascular bundles embedded in mesophyll cells in a single row, collateral type, the bundles 15 – 40 µm in diameter, bundle sheath 1- layered, parenchymatous, oval or rounded, thin walled; phloem towards the lower epidermis, composed of sieve tubes, companion cells and phloem parenchyma; xylem towards the upper epidermis, composed of vessel elements, tracheids, fibers and xylem parenchyma.

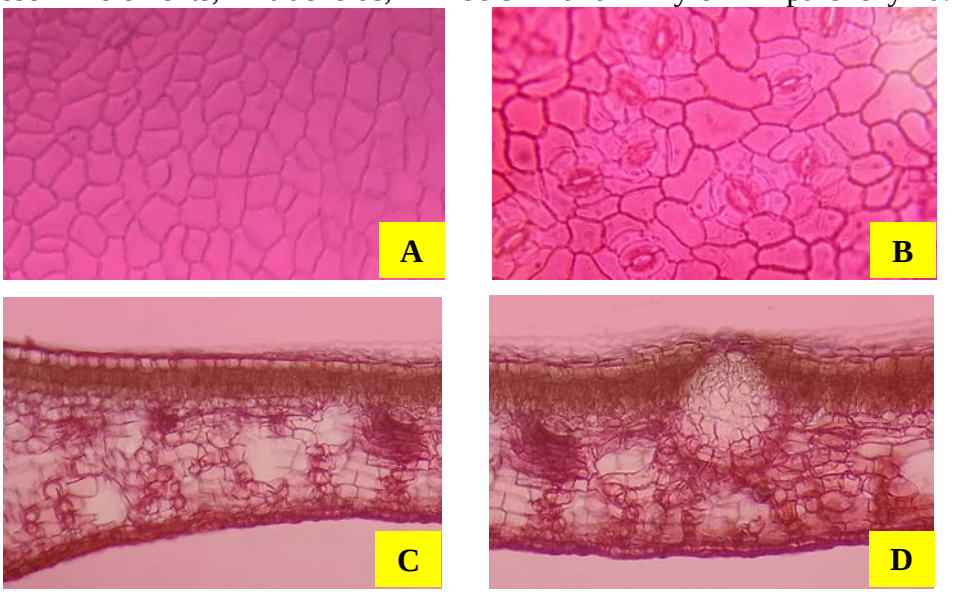

Figure 4. A. Upper surface layer
B. Lower surface layer showing stomata
C. T.S of lamina showing an outline and tissue system
D. T.S of a portion of lamina showing oil gland

Discussion and Conclusion

In this research, *Murraya koenigii* (L.) Spreng. belonging to the family Rutaceae was studied their morphological and anatomical characteristics were important and useful in identification of the studied species and in effective use for medicinal value.

In this present study, the plant of *Murraya koenigii* (L.) Spreng is unarmed, evergreen and small trees or shrubs which agree with Ohnmar Khin (1997). The inflorescence of this species is terminal corymbs. This finding agrees with Hooker (1872) and Tin Tin Htay (1976).

The fruits of *M. koenigii* (L.) Spreng are drupe, oblong and glabrous in this study, although Hooker (1872) and Tin Tin Htay (1976) described that they are berries and globose.

In this present work, the transverse section of petiole was circular in outline. Pith like parenchymatous cells is present at the center of vascular tissue. The vascular bundles are found to be concentric, amphicribral and collateral type. Crystals are present in some cells.

In transverse section, the shape of midrib was more or less circular in outline with slightly convex at adaxial side. The vascular bundles of midrib were widely elliptic shaped, of collateral type. *M. koenigii* (L.) Spreng was found one smaller and one larger bundles. Smaller bundle and the larger bundle are separated by parenchymatous cells. Smaller bundle observed at the adaxial side and the larger bundle at abaxial size in this study. Sreekala *et al* (2016) stated that the vascular bundle forms arc shape with adaxial xylem and abaxial phloem.

The lamina of *M. koenigii* (L.) Spreng. studied in this work was of dorsiventral types. The stomata are anomocytic type and found only on the lower surface. Oil glands present on both surfaces. The type of stomata was agreed on that of Handral and Shruthi (2016).

In transverse section, the ground tissue of lamina was differentiated into palisade parenchyma and spongy parenchyma. Palisades parenchymatous cells were two layered at adaxial side and spongy cells 4 – 8 layered at abaxial side. These characters agree with that of Sreekala *et al* (2016).

The morphological characteristics of *Murraya koenigii* studied present in this work are useful in identification of the flowering plants. Its

anatomical characteristics may also support for other researchers in traditional medicine.

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Extraction and Comparison of Genomic DNA from Plants and Ex-plants

Khin Min Min Phyo*

Abstract

The leaves of five plants grown in Miyazaki University Farm of Japan were collected for the isolation of genomic DNA. They were *Allium tuberosum* Roxb. Hort. Beng. (Chinese-chive) belonging to Amaryllidaceae, *Brassica oleracea* L. (cabbage) and *Arabidopsis thaliana* (L.) Heynh. (Arabidopsis) belonging to Brassicaceae and the other two plants were unknown ex-plants. Amplification of DNA was undertaken by polymerase chain reaction (PCR) and verified with the bands for the comparison by 2.5% agarose gel electrophoresis. The bands of the amplified PCR products from the three plants and the two ex-plants of intergeneric hybrids were compared within 500 and 1000 base pairs by UV illuminator.

Keywords: DNA, PCR, amplification, gel electrophoresis

Introduction

A cruciferous vegetable with a high level of vitamins and functional compounds beneficial to health and wellness has become widely used in the industry, a precise method for quality control of vegetable species is necessary. To select suitable parents for hybrid breeding, thoroughly analyzed the genetic diversity of Cruciferae (Etoh, 2003).

Eukaryotic cells in general are bigger and more elaborate than prokaryotic cells and their genomes are bigger and more elaborate too. Deoxyribonucleic acid (DNA) is the substance of which genes are made. Each DNA molecule is packaged in a separate chromosome and the total genetic information stored in the chromosome of an organism is said to constitute its genome. DNA molecule is a helical polymer composed of two strands. All of the bases of the DNA molecule are on the inside of the double helix with the sugar phosphate on the outside. Furthermore, complementary base pairs form between adenine and thymine and between guanine and cytosine. Amplification of target DNA by PCR which consists

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of three steps including heat denaturation, primer hybridization and extension. Polymerase chain reaction allows the DNA from a selected region to be amplified several-billion fold over a short period of time. This technique was invented by Dr Kary B. Mulis who won the Nobel Prize in Chemistry 1993. The impact of the PCR upon molecular biology has been profound. The reaction is easily performed and leads to the amplification of specific DNA sequences by an enormous factor. In the identification of plant species by PCR, DNA was isolated from the unknown samples. Using them, the target DNA was amplified by PCR. This PCR gives the species specific DNA amplification pattern. By comparing these data with known samples, those unknown samples may be identified. DNA is polar due to its highly charged phosphate backbone. Its polarity makes it water-soluble. If enough ethanol is added, the electrical attraction between phosphate groups and any positive ions present in solution becomes strong enough to form stable ionic bonds and DNA precipitation. This usually happens when ethanol composes over 64% of the solution. DNA is less soluble in isopropanol so it precipitates faster even at low concentrations. The downside however is that salt will also precipitate in isopropanol. With ethanol, DNA needs to be at a higher concentration to flocculate but the salt tends to stay soluble, even at colder temperatures. Agarose gel electrophoresis is convenient for separating DNA fragments ranging in size from a few hundred to about 20 kb. Each nucleotide in a nucleic acid molecule carries a single negative charge (on the phosphate group) such that DNA molecules move uniformly toward the positive electrode. In general, the migration rates of the DNA molecules were inversely proportional to the logarithms of the molecular weights (Inaba, 2019).

The objective of this research was to extract the genomic DNA with a simple and fast procedure for PCR, enzyme digestion and comparison of the genomic DNA bands from plants and ex-plants for breeding and transformation of desirable traits.

Materials and Methods

The leaves of *Allium tuberosum* Roxb. Hort. Beng. (Chinese-chive) belonging to Amaryllidaceae, *Brassica oleracea* L. (cabbage) and *Arabidopsis thaliana* (L.) Heynh. (Arabidopsis) belonging to Brassicaceae and the other two unknown ex-plants grown in Miyazaki University Farm of Japan were collected to Plant Physiology Laboratory of Miyazaki

University for extraction of genomic DNA in 2019. The leaves are cut between 0.5 cm^2 and 1.0 cm^2 and put it into a tube and then grind without extraction buffer with homogenizer (figure 1). Then, 400 µL of extraction buffer (200 mM Tris 7.5-8.0, 250 mM NaCl, 25 mM EDTA and 0.5% SDS) were added and grinded again and then mixed by tapping-agitate and spinned for 2 minutes at 13000 rpm at room temperature. After that 300 µL of supernatant were added to 300 µL of isopropanol in a new tube. After vortexing, it was spinned at 13000 rpm for 5 minutes at room temperature and removed the supernatant and kept the precipitate. It was washed with 300 µL of 70% ethanol and spinned at 13000 rpm for 3 minutes. The supernatant was discarded and the precipitate was dried in vacuum centrifuge for 5 minutes and added 50 µL of TE buffer (10 mM Tris-HCl pH 8.0 and 1 mM EDTA) for the extraction of genomic DNA as the template for PCR.

PCR involves two oligonucleotide primers, 17-30 nucleotides in length which flank DNA sequence that is to be amplified. The primers hybridize to opposite strands of DNA sequence after it has been denatured and oriented so that DNA synthesis by the polymerase proceeds through the region between the two primers. The extension reactions create two double-stranded target regions, each of which can be denatured ready for a second cycle of hybridization and extension. The third cycle produces two double-stranded molecules that comprise precisely the target region in double stranded form. By repeated cycles of heat denaturation, primer hybridization and extension there follows a rapid exponential accumulation of the specific target fragment of DNA. After 22 cycles, an amplification of about 10^6 fold is expected and amplifications of this order are actually attained in practice (figure 2).

Template DNA (2 µL) was added into a tube containing reaction mixture (2 µL of 10x ExTaq buffer, 1.6 µL of 2.5 mM dNTP, 0.4 µL of primer 1 (B49873), 0.4 µL of primer 2 (A50272), 0.1 µL of ExTaq and 13.5 µL of distilled water). This should be done on ice. Reaction mixture was mixed by tapping and set a tube onto thermal cycler. Reaction condition for PCR protocol used to amplify the gene fragment involved a preheating step at 94°C for 2 minutes, followed by 40 cycles of denaturation of DNA at 94°C for 30 seconds, primer annealing at 55°C for 1 minute and DNA extension at 72°C for 1 minute and finally a terminal extension step at 72°C for 5 minutes. Amplified DNA was verified by 2.5% agarose gel electrophoresis (figure 3).

The marker (20 µL) was applied onto the agarose gel. The bands in the agarose gel were stained with the intercalating dye, ethidium bromide. PCR reaction mixture was mixed with 4 µL of the dye by pipetting and 20 µL of mixture was applied onto the gel. Those bands can be detected as visible fluorescence when the gel was illuminated with ultraviolet light (figure 4). The agarose gel electrophoresis was analyzed by UV illuminator and taken the picture (Inaba, 2019).

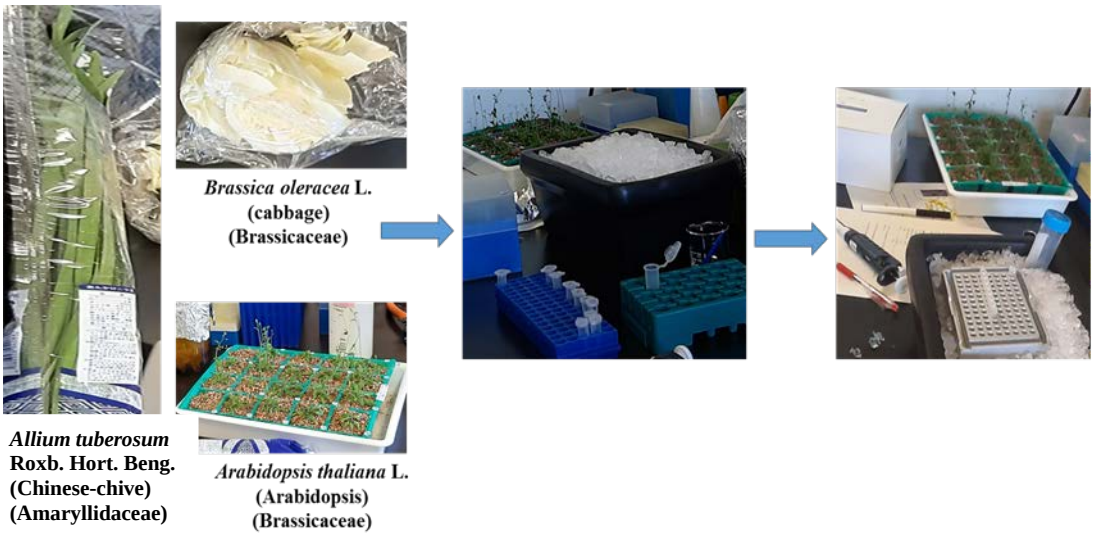


Figure 1. Isolation of genomic DNA from plants

Figure 2. Amplification of genomic DNA by Polymerase Chain Reaction

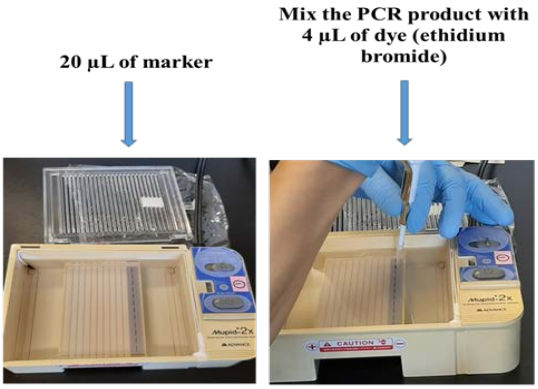


Figure 3. Verification of genomic DNA by agarose gel electrophoresis

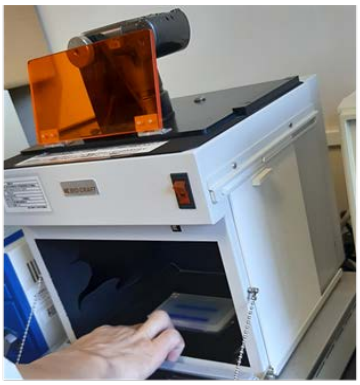


Figure 4. Detection of genomic DNA bands by illuminated ultraviolet light

Results

Outstanding characters of *Allium tuberosum* Roxb. Hort. Beng. (Chinese-chive) (Amaryllidaceae)

Herbs, bulbs coated, elongate, cylindric with white fleshy root-fibres, scales grey, fibrous; Leaves narrow-linear, flat, tall. Head-flowered, spathes small, pedicel ascending, much longer than the small white stellate flowers; sepal oblong-lanceolate; filament simple, linear, connate and

perigynous, inserted on the bases of the sepals, gradually dilated from below the middle to the base, outer shorter, broader; style short. Ovary globosely obovoid, deeply 3-lobed, stigma obscurely 3-toothed; cells 3-ovuled. Capsule obcordate (figure 5).

Figure 5. Habit and leaves of *Allium tuberosum* Roxb. Hort. Beng.

Outstanding characters of *Brassica oleracea* L. (cabbage) (Brassicaceae)

Herbs, stem branching in the upper part, obtusely angular, quite glabrous, branchlets suberect, leaves fleshy, large, lower and basal petiolate with a large terminal lobe, rounded at the apex, entire, inflorescence long in fruit, racemes, panicle, buds raised far above highest expanded flowers, pedicel long in fruit, spreading, sepals long, petals yellow, long, clawed, stamens erect, anther long, midvein of valves prominent, ovules more than 30, pods erect, seeds globose, dark brown, finely reticulate (figure 6).

Figure 6. Habit and Inflorescence of *Brassica oleracea* L.

Outstanding characters of *Arabidopsis thaliana* (L.) Heynh. (Arabidopsis) (Brassicaceae)

Herbs, stems erect, mostly branched, covered at the base with simple hairs, glabrescent above, branches ascending, basal leaves rosulate, rotund at apex, mostly forked, stem-leaves smaller, sessile, oblong, mostly entire, covered with forked and simple hairs on both sides and on margin, racemes-flowered, pedicels long, capillary, sepals erect-patent, long, glabrous, sometimes violet, petals white, long, stamens lateral nectariferous glands semi-globose, ovary with many ovules, fruiting pedicels long, filiform, pods linear with a short style, valves thinly 1-nerved, seeds minute, ovoid, pale brown (figure 7).

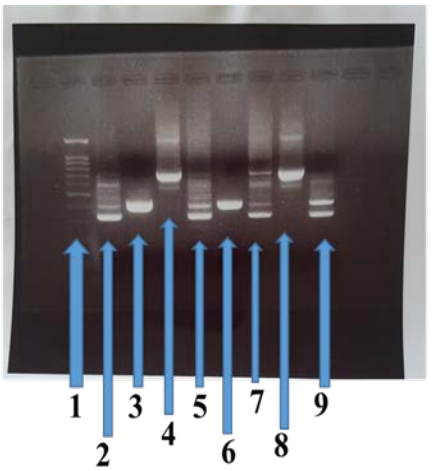
Figure 7. Habit and Inflorescence of *Arabidopsis thaliana* (L.) Heynh.

Figure 8. Visualization of genomic DNA bands on agarose gel electrophoresis

Figure 9. Analysis of genomic DNA bands by illuminator

The genomic DNA was extracted from *Allium tuberosum* Roxb. Hort. Beng. (Chinese-chive) belonging to Amaryllidaceae, *Brassica oleracea* L. (cabbage) and *Arabidopsis thaliana* (L.) Heynh. (Arabidopsis) belonging to Brassicaceae and the two unknown ex-plants by crushing the leaves with buffer. The extracted genomic DNA was amplified by PCR with primers. The PCR products were detected for the comparison of the bands by agarose gel electrophoresis with ultraviolet illuminator (figure 8 and 9).

According to the agarose gel electrophoresis spectrum, the bands of the amplified PCR products from *Allium tuberosum* Roxb. Hort. Beng. (Chinese-chive) and *Brassica oleracea* L. (cabbage) were detected within 500 base pairs and the band of the amplified PCR products from *Arabidopsis thaliana* (L.) Heynh. (Arabidopsis) was detected within 1000 base pairs. The bands (no. 7) of the amplified PCR products from the ex-plant of intergeneric hybrids (*Allium tuberosum* Roxb. Hort. Beng. (Chinese-chive) and *Arabidopsis thaliana* (L.) Heynh. (Arabidopsis)) were detected within 500 and 1000 base pairs. The bands (no. 9) of the amplified PCR products from the ex-plant of intergeneric hybrids (*Allium tuberosum* Roxb. Hort. Beng. (Chinese-chive) and *Brassica oleracea* L. (cabbage)) were also detected within 500 and 1000 base pairs (figure 10).



1. **Marker (1000 bp)**
2. ***Allium tuberosum* Roxb. Hort. Beng. (Chinese-chive)**
3. ***Brassica oleracea* L. (cabbage)**
4. ***Arabidopsis thaliana* (L.) Heynh. (Arabidopsis)**
5. ***Allium tuberosum* Roxb. Hort. Beng. (Chinese-chive)**
6. ***Brassica oleracea* L. (cabbage)**
7. ***Allium tuberosum* Roxb. Hort. Beng. + *Arabidopsis thaliana* (L.) Heynh.**
8. ***Arabidopsis thaliana* (L.) Heynh. (Arabidopsis)**
9. ***Allium tuberosum* Roxb. Hort. Beng. (Chinese-chive) + *Brassica oleracea* L. (cabbage)**

Figure 10. Comparison of genomic DNA bands

Discussion and Conclusion

The identification of genetic markers that strictly differentiate single cultivars is helpful for effective conservation of plant material in gene banks and for breeders have investigated genetic variation within cabbage cultivars. Furthermore, characterization of diversity in genetic resources and genotype fingerprinting are important for managing gene banks. The randomly amplified polymorphic DNA (RAPD) markers generated by PCR is technically the simplest, less expensive, fast and does not require prior knowledge of the target sequences for the design of primers. The RAPD markers have already been used in *Brassica oleracea* L. for the assessment of genetic variability, diversity and fingerprinting of cabbage genotypes (Lal *et al*., 2013).

Cardoza and Stewart Jr. (2004) in an invited review stated that considerable progress has been accomplished in the cellular and molecular biology of *Brassica* species in the past few years. Plant regeneration has been increasingly optimized via organogenesis and somatic embryogenesis using various explants with tissue culture improvements focusing on factors such as age of the ex-plant, genotype and media additives. The production of haploids and doubled haploids using microspores has accelerated the production of homozygous lines in the crop species.

This protocol was for extracting genomic DNA from the leaf tissues of plant that is applicable to a variety of organisms regardless of the complexity of their genomes. This procedure was not only very simple but also time and cost effective. In addition, a very small sample was required for DNA extraction. There were many advantages in using the genomic DNA extraction method to obtain the template for PCR amplification. The genomic DNA of many different plants could be amplified using the same DNA extraction method and the same PCR protocol. Somatic cell fusion has facilitated the development of interspecific and intergeneric hybrids in the sexually incompatible species (Inaba, 2019).

Therefore, the bands of the amplified PCR products from *Allium tuberosum* Roxb. Hort. Beng. (Chinese-chive), *Brassica oleracea* L. (cabbage) and *Arabidopsis thaliana* (L.) Heynh. (Arabidopsis) and the two ex-plants of intergeneric hybrids (*Allium tuberosum* Roxb. Hort. Beng. (Chinese-chive) and *Arabidopsis thaliana* (L.) Heynh. (Arabidopsis)) (*Allium tuberosum* Roxb. Hort. Beng. (Chinese-chive) and *Brassica oleracea* L. (cabbage)) were compared for the further investigation of the desired intergeneric hybrid crops.

In conclusion, this protocol proves the possibility of the use of molecular markers in marker-assisted selection, breeding and transformation technology for the introduction of desirable traits.

Acknowledgements

I would like to acknowledge to all of the staffs from Ministry of Education, Myanmar especially Department of Higher Education for this research work which was supported by grants from Japan Science and Technology Agency of Sakura Exchange Program at Miyazaki University of Japan. I would like to express my sincere thanks to Dr Si Si Hla Bu, Rector (Retired) of Pathein University for her valuable advice and guidance and also to Dr Than Htun, Rector of Pathein University for his interests. I would like to thank to Dr Min Min Soe, Professor and Head, Department of Botany, Pathein University

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Phytochemical Investigation and Nutritional Value of *Coccinia grandis* (L.) Voigt. Leaves

Khin Myo Win*

Abstract

The medicinal plant *Coccinia grandis* (L.) Voigt. known as Kin-mon and its belonging to the family of Cucurbitaceae.. It was collected from Tha-ka-ta Township during flowering and fruiting period. The morphological characters were studied in detail to characterize according to the literature of many manuals. The preliminary phytochemical examination and test for nutritional value of the plant has been undertaken. In microscopical studies, the anomocytic type of stoma present. Vascular bundles of leaf are bicollateral type.The preliminary phytochemical investigation of leaves revealed that the presence of alkaloid, glycoside, phenolic compound, α-amino acid, saponin, flavonoid, steroid, terpenoid, reducing sugar and starch. Nutritional value showed leaf contains energy (135 kcal), proteins (20%) and carbohydrates (7%). It is vegetable food for human especially it provides rich in protein contents;

Keywords: *Coccinia grandis* (L.) Voigt., Phytochemical, Nutritional Value

Introduction

The World Health Organization (WHO) estimates that about 80% of the populations living in the developing countries rely almost on traditional medicine for their primary health care needs. Plants have played a significant role in maintaining human health and improving the quality of human life (Tamilselvan, 2011). *Coccinia grandis* (L.) Voigt. belongs to the family Cucurbitaceae and known as Kin-mon in Myanmar. The Cucurbitaceae are representing about 100 genera and 850 species, primarily of pantropical and subtropical distributions with range extensions into the temperate northern and southern hemispheres (Lawrence, 1951).

Kraemer (1910), stated that the plant is climbing or prostrate and rooting mostly perennial herbs with tuberous roots. The leaves are alternate, being opposite the tendrils, petioleate, and palmately- lobed. Metcalfe and Chalk (1950), described that the transverse section of petiole exhibits circular vascular bundles with bicollateral type. Cortex includes large

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number of collenchymas especially in the ribs. Phytochemical analysis of leaves extract of *Coccinia grandis* indicated the presence of alkaloids, phenols, saponins, terpenoids, glycosides, flavonoids, carbohydrates and proteins (Harborne, 1998; Kirtikar and Basu, 1975).

Alkaloids are widely distributed and naturally occurring in plants. Most of the alkaloids from plants are used for medicinal purpose as analgesic, antispasmodic and bacterial effects (Johnson *et al.*, 2008). Saponins helps as an antiinflammatory (Just *et al.,* 1998). Tincture of leaves is used to treat gonorrhea, paste of leaves is applied to the skin diseases. *Coccinia indica* are used in the treatment of dizziness, sickness and asthma (Ashin-Nagathein, 1976).

In the present investigation, microscopically studies, preliminary phytochemical test and nutritional quantity of fresh leaves have been emphasized and undertaken which can be used for the standardization of traditional medicine.

The main objectives are to investigate the phytochemical constituents and to examine the nutritional value from leaves of this plant. Therefore, the plant leaves might be used as nutritional source as well as chemotherapeutic agent. This is an ongoing study and further work is being carried to investigate its biological activities.

Materials and Methods

Sample collection and preparation

Coccinia grandis (L.) Voigt. were collected from Tha-ke-ta Township, Yangon Division during the flowering period of December-April, 2019. After the collection, the morphological characters of the specimens were identified by the available literatures such as Hooker 1879, Backer and Bakhu 1963, Lawrence 1951, and Dassanayake, 1997.

The samples were washed and then dried at room temperature until it reaches constant weight. After that, the samples were ground into powder and stored in air tight containers for phytochemical and nutritional examination.

Anatomical examination from leaves of *Coccinia grandis* (L.) Voigt.

For the anatomical structures, the leaf was observed by free hand section by using microscope. Microscopically examinations were conducted according to the method of Metcalfe and Chalk (1950), Esau (1953), and Trease and Evans (1978).

Phytochemical test from leaves of *Coccinia grandis* (L.) Voigt.

Phytochemical test was carried out to determine the presence or absence of phytoconstituents from the powdered leaves of *Coccinia grandis* (L.) Voigt. The powdered leaves were tested qualitatively according to the method of Marini Bettolo *et al.,* (1981) and Trease and Evans (2002).

Examination of Nutritional value from leaves of *Coccinia grandis* (L.) Voigt.

Nutritional value of powdered specimens such as protein, crude fat and carbohydrate were carried out in Food Industries Development Supporting Laboratory (FIDSL), Lanmadaw Township, Yangon. The powdered samples have been determined according to the procedures given in the methods of Association of Official Analytical Chemists (AOAC) Horwitz (1980).

Result

Morphological characters of *Coccinia grandis* (L.) Voigt.

Family	-	Cucurbitaceae
Scientific name	-	*Coccinia grandis* (L.) Voigt.
Common name	-	Kin-mon
English name	-	Ivy gourd

A perennial climber herb is up to 2 meters with tuberous rootstock producing annual stem. Stems are solid, glabrous and green when young, brown in mature. Tendrils are axillary, spirally coiled. Leaves simple and palmately 3 - 5 lobed, alternate, bright green on the upper surface, light green on the lower surface, the bases deeply cordate, the margins seriate-dentate, the tips acuminate, petiolate; inflorescence axillary, solitary cyme and dioecious flowers; male flower 4 - 6 cm long, 3 - 4 cm wide, pedicel 3 - 5 cm long, pentamerous, epigynous; calyx 5, synsepalous, valvate, sepaloid,

superior; corolla 5, sympetalous, companulate, petaloid, superior; stamen 5, synandrous arranged in a group (2+2+1), filament short, extrorse, superior; ovary absent: female flower 6 - 7 cm long and 4 - 5 cm wide, pedicel 3.5 - 4.5 cm long, 0.4 - 0.5 cm width, pentamerous, superior; calyx 5, synsepalous, valvate, sepaloid, superior; corolla 5, sympetalous, companulate, petaloid, superior; stamen absent; ovary tri-carpellary, syncarpous with numerous ovules, parietal placentation; style short, stigma tri-fid, inferior; fruits ovoid to ellipsoid in shaped, pepo, red on ripening; seeds numerous, ovoid, small, hard, the tips acute, the base round, smooth, surrounded by yellowish pulpy arils. Flowering and fruiting period is from December-April (Figure 1-2)

Morphological characters of *Coccinia grandis* (L.) Voigt.

Figure (A) Figure (B) Figure (C)

Figure 1. (A) Habit, (B) Leaves with tendril and (C) Inflorencece of male flower

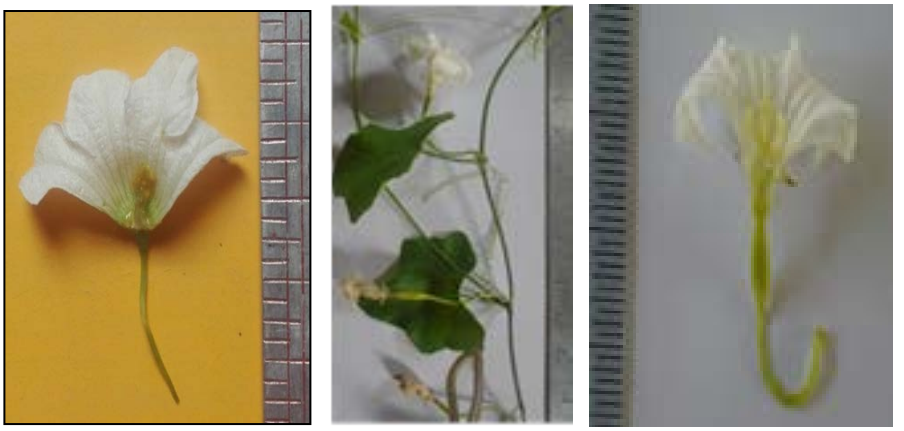


Figure (A) Figure(B) Figure (C)

Figure 2. (A) L.S. of male flower (B) Inflorencece of female flower and (C) L.S. of female flower

Histological characters for leaves of *Coccinia grandis* (L.) Voigt.

Lamina

In surface view, the epidermal cells of both surfaces were thin-walled parenchymatous cell, compactly arranged. The anticlinal walls of upper epidermal cells were slightly wavy whereas lower ones were deeply wavy. Stomata were abundant found on the lower surface. They were anomocytic type.

In transverse section of lamina, the cuticle layer was fairly thick with striations. The epidermal cell was one layered on both sides. They were irregular in shape and compactly arranged. The mesophyll layer composed of palisade and spongy parenchyma. Below the epidermis, one layer of palisade parenchyma cells was located, cylindrical in shape with numerous chloroplasts. Below the palisade cells, spongy cells were 3 - 5 layered, loosely arranged and oval to rounded parenchymatous cells. They contain numerous chloroplasts. Vascular bundles are bicollateral type. (Figure 3. A, B and C)

Midrib

In transverse section, the basal portion of the midrib was convex in the upper surface and protrudes in the lower surface. Epidermal cells were slightly oval in shape and tightly arranged, covering with thick cuticle. Small collenchymatous patch lied under the upper epidermis and 2 - 4 layers of collenchymatous cells were found on the bundle. Below the bundle, 3 - 6 layers of parenchymatous cells were present, thin walled and rounded in shape. Vascular bundles were bicollateral type, semicircular in shape. (Figure 4. A)

Petiole

In surface view, epidermal cells are thin-walled, irregular in shape and stomata were anomocytic type. In transverse section, single layered epidermis with covering thick cuticle. The petiole was basically circular in shape and concave in the upper surface in outline. Below the epidermis, 2 - 5 layered collenchymatous and 2 - 6 layered circular, thin walled, chlorenchymatous cells with intercellular spaces were observed. Bicollateral vascular bundles were seven in number and arranged in a single ring. In centre very widely pith was observed which was composed of large parenchymatous cells. (Figure 4. B and C)

Histological characters for leaves of *Coccinia grandis* (L.) Voigt.

Figure (A) Figure (B) Figure (C)

Figure 3. (A) Surface view of upper epidermal cells, (B) Surface view of lower epidermal cells and (C) Transverse Section of lamina

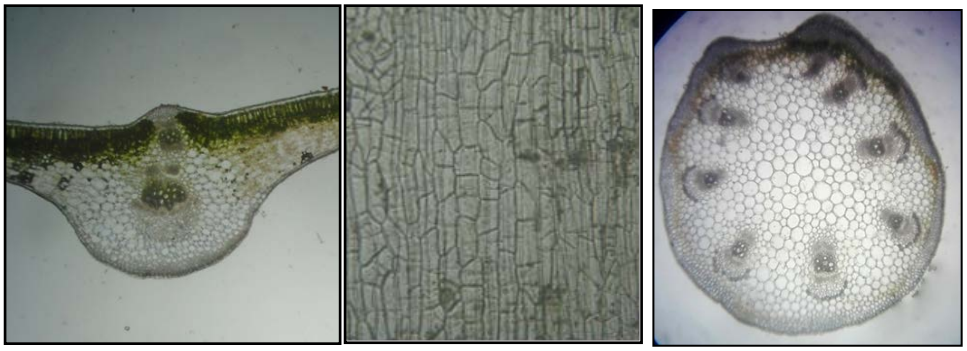


Figure (A) Figure (B) Figure (C)

Figure 4. (A) Transverse Section of midrib (B) Surface view of petiole and (C) Transverse Section of petiole

Phytochemical investigation of leaves of *Coccinia grandis* (L.) Voigt.

The investigation of these test observed that the presence or absence of alkaloid, glycoside, phenolic compound, α-amino acid, saponin, tannin, flavonoid, steroid, terpenoid, reducing sugar, starch and cyanogenic glycoside were shown in Table (1).

Table 1. Preliminary phytochemical test for leaves of *Coccinia grandis* (L.) Voigt.

No.	Type of compound	Extract	Reagent used	Observation	Results
1.	Alkaloid	1%HCL	Mayer's reagent	Cream colour (turbid)	+
			Wagner's reagent	Reddish brown ppt.	
			Dragendorff 's reagent	Yellowish brown ppt.	
			Hager's reagent	Yellow colour (turbid)	
2.	Carbohydrate	H_2O	10% α-naphthol & $H_2SO_{4(Conc:)}$	red ring	+
3.	Glycoside	H_2O	10% Lead acetate solution	White ppt.	+
4.	Phenol	H_2O	5% $FeCL_3$solution	Brownish green	+

No.	Type of compound	Extract	Reagent used	Observation	Results
				ppt.	
5.	α-amino acid	H_2O	Ninhydrin reagent	Pink colour	+
6.	Saponin	H_2O	H_2O	Persistent foam	+
7.	Tannin	H_2O	1% Gelatin & 10% NaCL solution	No ppt.	-
8.	Flavonoid	70%EtOH	Mg ribbon & Conc; HCL	Pink colour.	+
9.	Steroid	Petroleum Ether	Acetic anhydrite & Conc; H_2SO_4	Bluish green	+
10.	Terpenoid	Petroleum Ether	Acetic anhydrite & Conc; H_2SO_4	Pink	+
11.	Reducing sugar	H_2O	Fehling's solution NaOH dilute	Yellow ppt.	+
12.	Starch	H_2O	Iodine solution	Brown	+
13.	Cyanogenic glycoside	H_2O	Conc; H_2SO_4 sodium picrate paper	No colour change	-

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

According to the preliminary phytochemical test for leaves of *Coccinia grandis* (L.) Voigt., the presence of alkaloid, glycoside, phenolic compound, α-amino acid, saponin, flavonoid, steroid, terpenoid, reducing sugar and starch were observed. However, tannin and cyanogenic glycosides were absent.

Nutritional Value of *Coccinia grandis* (L.) Voigt.

The experimental work for the nutritional value was carried out at the Food Industries Development Supporting Laboratory (FIDSL). According to the experiment, moisture, ash, protein, crude fiber, crude fat, carbohydrate and energy value were found. The results were shown in Figure (5, 6) and Table (2).

Table 2. Analysis of the nutrients in leaves *Coccinia grandis* (L.) Voigt.

Sr. No.	Test Parameter	Results
1.	Moisture	10.68%
2.	Ash	42.15%
3.	Crude Protein	19.76%
4.	Crude Fiber	17.54%
5.	Crude Fat	3.32%
6.	Carbohydrate	6.55%
7.	Energy value (Kcal /100gm)	135

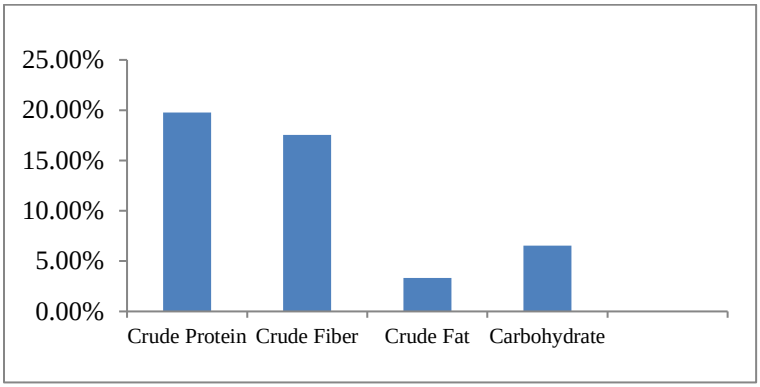
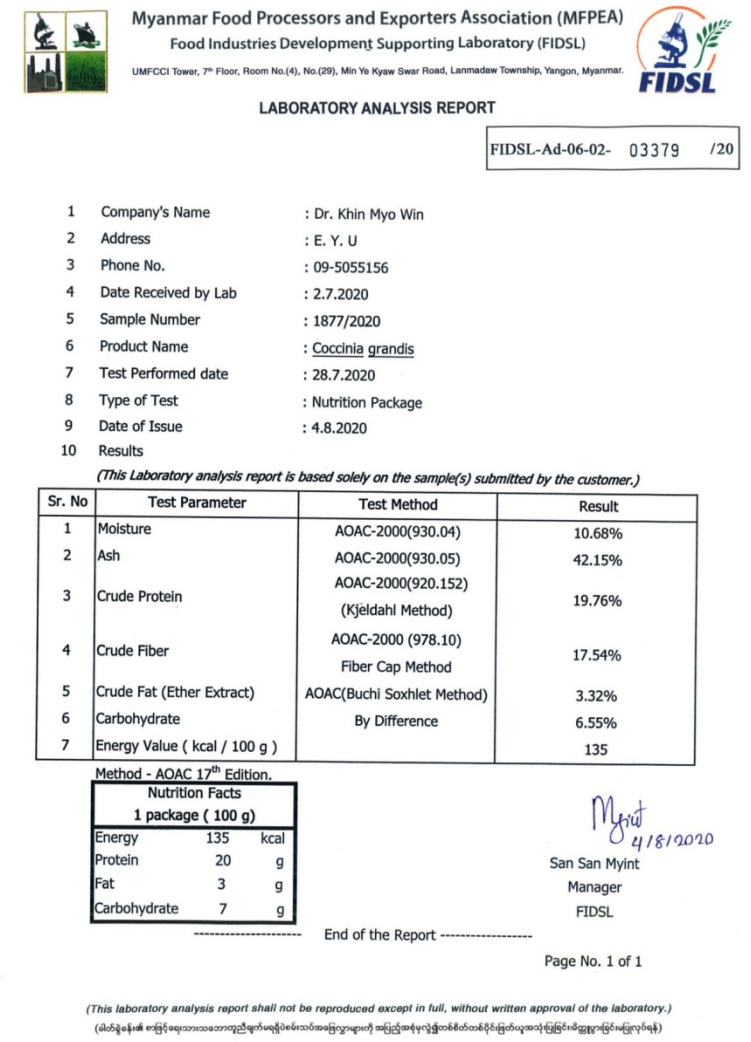


Figure 5. Nutritional contents in leaves of *Coccinia grandis* (L.) Voigt.



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

FIDSL

LABORATORY ANALYSIS REPORT

FIDSL-Ad-06-02- 03379 /20

1	Company's Name	: Dr. Khin Myo Win
2	Address	: E. Y. U
3	Phone No.	: 09-5055156
4	Date Received by Lab	: 2.7.2020
5	Sample Number	: 1877/2020
6	Product Name	: Coccinia grandis
7	Test Performed date	: 28.7.2020
8	Type of Test	: Nutrition Package
9	Date of Issue	: 4.8.2020
10	Results	

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

Sr. No	Test Parameter	Test Method	Result
1	Moisture	AOAC-2000(930.04)	10.68%
2	Ash	AOAC-2000(930.05)	42.15%
3	Crude Protein	AOAC-2000(920.152) (Kjeldahl Method)	19.76%
4	Crude Fiber	AOAC-2000 (978.10) Fiber Cap Method	17.54%
5	Crude Fat (Ether Extract)	AOAC(Buchi Soxhlet Method)	3.32%
6	Carbohydrate	By Difference	6.55%
7	Energy Value (kcal / 100 g)		135

Method - AOAC 17th Edition.

Nutrition Facts 1 package (100 g)		
Energy	135	kcal
Protein	20	g
Fat	3	g
Carbohydrate	7	g

4/8/2020
San San Myint
Manager
FIDSL

---------- End of the Report ----------

Page No. 1 of 1

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

Figure 6. Laboratory analysis report of nutritional package of tested at Food Industries Development Supporting Laboratory (FIDSL) Yangon.

According to this result, the content of ash was higher than the other; crude protein, crude fiber and moisture moderately presence; crude fat and carbohydrate were found as small amount in the leaves of *Coccinia grandis* (L.) Voigt.

Discussion and Conclusion

According to the result, *Coccinia grandis* (L.) Voigt. has perennial climber herb is up to 2 meters with tuberous rootstock producing annual stem. The leaves are alternate, being opposite the tendrils, petiolate, and palmately- lobed. These characters were in agreement with those given by Backer, 1963, Kraemer, 1910 and Gopalan, 1976.

Inflorescence axillary, solitary cyme and dioecious flowers; male flower pedicellate, pentamerous, epigynous; calyx 5, synsepalous,, superior; corolla 5, sympetalous, superior; stamen 5, synandrous arranged in a group (2+2+1), filament short, extrorse, superior; female flower, pedicellate, pentamerous, superior; calyx 5, synsepalous, superior; corolla 5, sympetalous, superior; stamen absent; ovary tri-carpellary, syncarpous with numerous ovules, parietal placentation, style short, stigma tri-fid, inferior. These characters were in accordance with those mentioned by Pandey and Chadha, 2016.

In the present study, upper epidermal cells of leaves were slightly wavy whereas lower ones were deeply wavy. Stomata were abundantly found on the lower surface only. They were anomocytic type. In transverse section of midrib was small collenchymatous patch lied under the upper epidermis. According to these results were in agreement with those stated by Gopalan, 1976. In transverse section of petiole was basically circular in shape, vascular bundles were bicollateral type. According to these results were in agreement with those stated by Metcalfe and Chalk, 1950.

The investigation of phytochemical test of *Coccinia grandis* (L.) Voigt. showed that the alkaloid, glycoside, phenolic compound, α-amino acid, saponin, flavonoid, steroid, terpenoid, reducing sugar and starch. According to this tests, these results were agreement with above the literature of Harborne, 1998; Khandelwal, 2008. Due to the absent of cyanoenic glycoside indicates that the leaves of *Coccinia grandis* (L.) Voigt. were not toxic for human.

Rahman *et al.,* (2016) stated that *Coccinia cordifolia* leaves consist of starch, crude fibers. In the present paper, the nutritional value of the *Coccinia grandis* (L.) Voigt. leaves, the content of ash was higher than the other; crude protein, crude fiber and moisture moderately presence; crude fat and carbohydrate were found as small amount. Some characters were in agreement with those mentioned by Rahman *et al.,* (2016).

According to phytochemical investigation and nutritional contents, *Coccinia grandis* (L.) Voigt. can not only be eaten as vegetable but also used as skin disease in traditional medicine.The present of protein and carbohydrate in this plant, which can used for food in our country. The result of morphological characters, anatomical characters, phytochemical tests and nutritional value on the leaves of *C. grandis* (L.) Voigt. were essentially informative in medicines. So, it is hoped that these results will be useful in medicine and public health care.

Acknowledgements

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Morphological and Histological Characterization of *Clerodendrum serratum* (L.) Moon (Yin-Bya-Net)

Khin Nyo[1] & May Htet Zaw[2]

Abstract

Clerodendrum serratum (L.) Moon (Family Verbenaceae) is an important medicinal plant growing in the tropical and warm temperate regions. This plant is locally known as Yin-bya-net in Myanmar. It was collected from the surrounding area of Government Technological University, Hpa-an in Kayin State. The flowering and fruiting season from May to September. The fresh vegetative and reproductive parts of the plant were used in studying morphological, histological characters and identified with the help of available literatures. The histological characters of the plant were studied, the cell walls of upper and lower surfaces of lamina were deeply wavy, diacytic stomata, uniseriate glandular and non-glandular trichomes were present. Calcium oxalate crystals (druses) were present in mesophyll tissue of lamina and acicular crystals (raphides and styloid) were present in petiole and stem. The vascular bundles were collateral and close type in the midrib, however in the petiole and stem of the vascular bundles were collateral and open type. In transverse section of root, the epidermal cells were disorganized and displayed by periderm. Vascular bundles were arranged in concentric ring. Xylem endarch.

Keywords: *Clerodendrum serratum* (L.) Moon, morphological and histological characters

Introduction

According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compound derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. Plants are the basic source of knowledge of modern medicine. Most of the drugs derived from plants were developed because of their use in traditional medicine (Baker *et. al.,* 1995). The name Verbenaceae was given by Persoon (1806) and has been conserved over older names as Pyrenaceae.

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The genus *Clerodendrum* (L.) Moon [Family Lamiaceae (Verbenaceae)] is very widely distributed in tropical and subtropical regions of the world and is comprised of small trees, shrubs and herbs. The first description of the genus was given by Linnaeus in 1753, with identification of *C. infortunatum.* After a decade later in 1763 Adanson changed the Latin name "*Clerodendron*"; in Greek *Klero* means chance and *Dendrum* means tree (Moldenke, 1985 and Rueda, 1993).

Clerodendrum serratum (L.) Moon is a genus of flowering plants in the Verbenaceae family. The plant *Clerodendrum serratum* (L.) Moon is commonly known as Yin-bya-net in Myanmar and Blue glory in English. According to Ayurvedic Pharmacopoeia, it is also called Bharangi in Hindi. Bharangi is woody medicinal shrub used in ayurvedic system for its various medicinal properties. It is not toxic plant and its flowers and leaves are also edible (Rahman *et.al.*, 2007).

Therefore, the aims of the present study are to identify and confirm the morphological and histological characters of this plant.

Materials and Methods

Botanical Studies

Collection and Identification of Plant Specimens

The specimens used in this research were collected from the surrounding area of Government Technological University, Hpa-an in Kayin State, during flowering period from May to September in 2017. After collection, both the vegetative and reproductive parts of the fresh specimens were identified by using dissecting microscope, available literatures such as Kirtikar and Basu, 1935; Backer and Brink, 1965; Dassanayake, 1983; Hundley and Chit Ko Ko, 1983; Hu Qi-ming and Wu De-Lin, 2009. The habit, leaves, flower as seen, T.S of flower, T.S of ovary, fruits and seeds were presented with photographic record.

Histological study of *Clerodendrum serratum* (L.) Moon

For microscopical studies, free hand sections of fresh specimen from lamina, midrib, petiole, stem and root were made and studied under microscope. Temporary mounts were prepared with glycerin. Powder was also examined to get standardization for traditional medicine. The following chemical and reagents were used to examine for free hand sections and the powdered samples.

(i) Chloral-hydrate solution B.P as clearing reagent.

(ii) Acetic acid and 80% sulphuric acid B.P for calcium oxalate crystals.

The reagents are used in study by the method of Metcalfe and Chalk, 1979 and 1989; Esau, 1965; Pandey, 1978).

Results

Botanical study

Morphological Characters of *Clerodendrum serratum* (L.) Moon

Scientific name	-	*Clerodendrum serratum* (L.) Moon
Myanmar name	-	Yin-bya-net
English name	-	Blue glory, Beetle killer
Family name	-	Lamiaceae / Verbenaceae
Locality	-	Government Technological University, Hpa-an in Kayin State

Habit; perennial shrub, about 6.5-7.2 ft. in high, scarcely woody, not much branched. **Stem;** bluntly quadrangular. **Leaves;** simple, opposite and decussate, apically clustered and variable in size, lamina oblong or elliptic about 8.7-23.8 cm long and 3.6-13.0 cm wide, acute, coarsely and sharply serrate margin, glabrous, base acute, petiolate, about 1.2-1.5 cm long. **Inflorescence;** terminal or axillary, about 17.0-25.0 cm long, green, quadrangular, in lax pubescent, dichotomous cymes, with a pair of acute bracts at each branching and a flower in the fork, each in the axil of a large leafy bract and collectively forming a long lax terminal usually pyramidal erect panicle about 15.0-25.0 cm long. **Flower;** showy, pale blue, about 2.6-3.0 cm long, bracteate, bracteolate, pedicellate, glabrous, complete, bisexual, irregular, zygomorphic, pentamerous, cyclic, hypogynous. **Calyx;** 5, synsepalous, about 0.3-0.5 cm long, puberulous, cup-shaped, connate at the base, enlarge at the middle, apex deeply five-lobe, lobe elliptic, inferior. **Corolla;** (1+4), synpetalous, about 1.6-1.8 cm long, the lower larger lobed, bluish purple, ovate to lanceolate, corolla tube slender, hairy within the tube, inferior. **Stamen;** 4, epipetalous, filament much more curved, densely hair at the base, about 2.2-2.3 cm long, didynamous, white, anther dark purple, dithecous, dorsifixed, longitudinal dehiscence, inferior. **Ovary;** 2, syncarpous, globose, false septum, each with two ovules, axile placentation. **Style;** filiform, about 3.2 cm long, white colour, glabrous, stigma bifid,

purple, disc present, superior. **Fruits;** broadly obovoid, about 0.5-0.9 cm, succulent, dark green in immature, black in matures, glabrous. **Seeds;** normally four lobed with one pyrene in each lobe, about 0.4 cm, dark purple. (Figure 1)

Flowering and fruiting periods	- May-September
Part used	- Leaves
Uses	- Febrifuge, burning sensation, hiccough, cephalalgia, ophthalmic, dyspepsia, bronchitis, asthma.

Habit

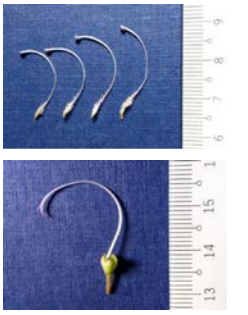
Ventral view of leaves

Dorsal view of leaves

Inflorescence

Flowers

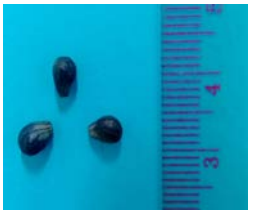
L.S of Flower



Stamens and Pistil

T.S of ovary

Fruits



Seeds

Figure 1. Morphological characters of *Clerodendrum serratum* (L.)

Histological characters of *Clerodendrum serratum* (L.) Moon

Microscopical characters of leaves

Lamina

In surface view, the epidermal cells of upper and lower epidermis are parenchymatous, thin-walled and compactly arranged. Anticlinal walls of their lower surfaces were wavier than the upper one. Diacytic type stomata were present on both surfaces but abundant on the lower surfaces.

In transverse section, the palisade mesophyll cells were made up of 2 to 3 layers of vertically elongated cylindrical cells and the spongy mesophyll layers are composed of 4 to 5 layers of parenchymatous cells. Abundant calcium oxalate crystals (druses) are found in this region.

The vascular bundles were embedded in mesophylls cells, oval in shape. They were collateral type and different in sizes according to their position (Figure 2).

Midrib

In surface view, the epidermal cells are thin-walled parenchymatous and rectangular to polygonal in shape, elongated along the length of the midrib. Anticlinal walls are straight. In transverse section of midrib, the apical portion was convex on both surfaces. The vascular bundles are in the form of ring and collateral type. In apical regions of the leaf only one vascular bundle is left surrounded by prominent bundle sheath. In middle portion, the epidermal cells were one layered, barrel shaped, lower one are similar to the upper epidermal cells, compactly arranged. The cells are thin walled and rounded to polygonal in shape. The vascular bundles were crescent in shaped and embedded in the cortex. The middle portions of the vascular bundles were collateral types. The basal portion of midrib was convex in both surfaces. The vascular bundles are crescent shaped and embedded in the cortex. The basal portions of the vascular bundles were collateral type (Figure 3).

Petiole

In surface view, the epidermal cells are thin walled and rectangular to polygonal in shape with straight wall. In transverse section, the cuticle layer was thin. The epidermal cells were barrel shaped and compactly arranged. The cortex was made up of two different types of tissue. The lamellar collenchymatous cells and parenchymatous cells. Acicular crystals

(raphides and styloid) were scattered in the parenchymatous cells. The vascular bundles were circular in arrangement, bundles were collateral and open type. Patches of phloem fibers surrounded the collateral vascular bundles (Figure 3).

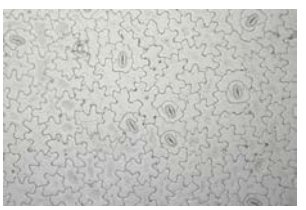
Stem

In surface view, the epidermal cells were rectangular to polygonal-shape parenchymatous cells, thin walled, compactly arranged, anticlinal walls straight. In transverse section, the young was quadrangular in outline. The cortex region was made up of collenchymatous tissue and parenchymatous tissue. The collenchymatous tissues were lamellar types and. The parenchymatous tissue consists of 4 to 5 layers, thin-walled, isodiametric to rounded in shape, the vascular bundles were surrounded the pith region. The vascular bundles were collateral and open type. The patches of phloem fibers were surrounded the collateral vascular bundles (Figure 3).

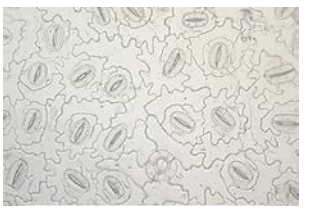
Root

In surface view, the epidermal cells were thin walled, rectangular in shape and compactly arranged. In transverse section, the roots were circular in outline. The epidermal cells were disorganized and displaced by periderm which consists of phloem or cork, the phellogen or cork cambium and phelloderm or secondary cortex. The vascular bundles were arranged in concentric ring. Xylem towards the inner and phloem outside the xylem. The xylem is endarch. The xylem composed of vessels, tracheids, xylem fibres and xylem parenchyma. Protoxylem consists of annular and spiral vessels. The central region of the steel is narrow parenchymatous pith. The thickening of xylem vessels were reticulate and pitted vessels (Figure 4).

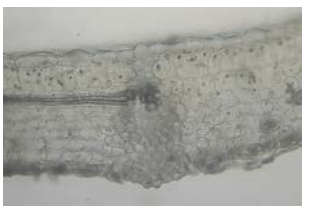
Lamina



Upper epidermis



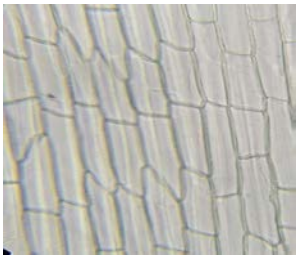
Lower epidermis



T.S of lamina

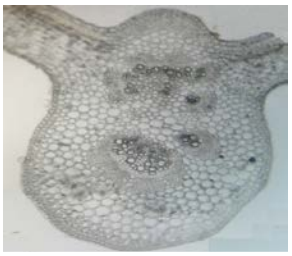
Figure2. Microscopical characters of *Clerodendrum serratum* (L.) Moon

Midrib

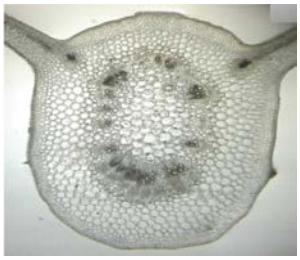


Surface view of midrib

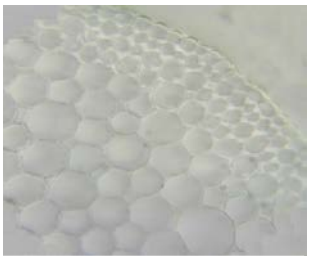
T.S of midrib apex



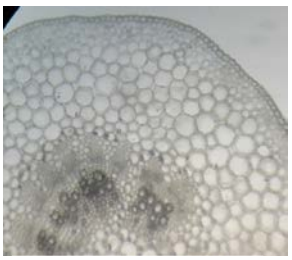
T.S of midrib middle



T.S of midrib basal

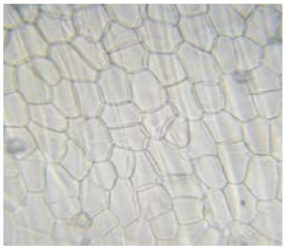


Epidermal and cortical layer

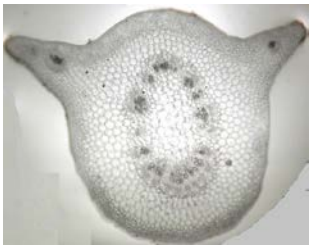


Vascular bundle

Petiole



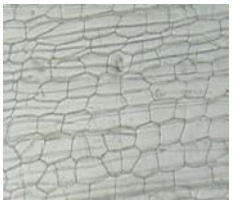
Surface view of petiole



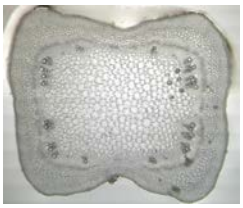
T.S of petiole

Vascular bundles and crystals

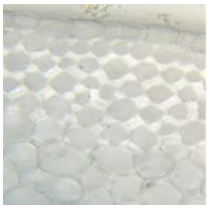
Stem



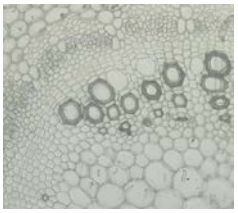
Surface view of stem



T.S of stem



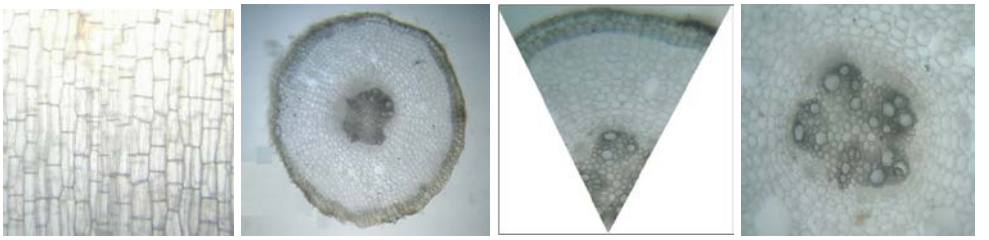
Epidermal and cortical layer



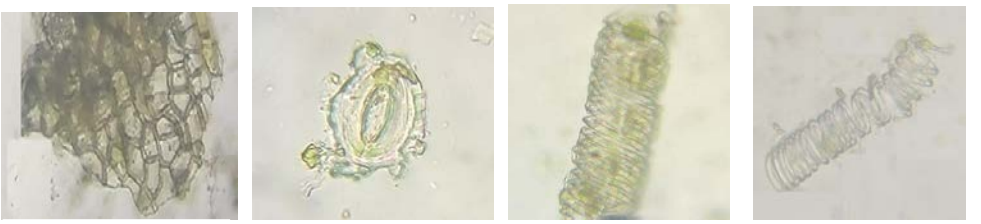
Vascular bundles and crystals

Figure 3. Microscopical characters of *Cleroaenarum serratum* (L.)

Root

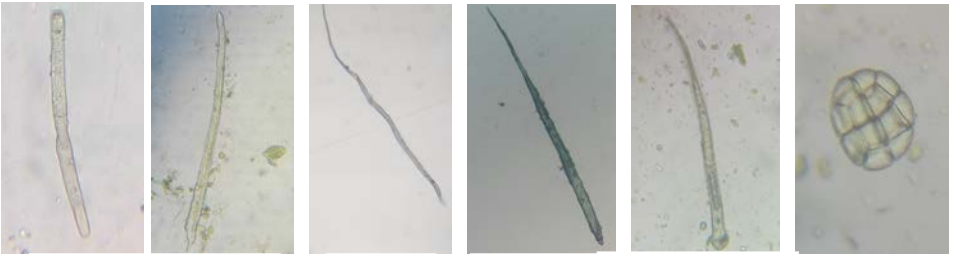


Surface view of root | T.S of root | Cortex region | Vascular bundles



Fragment of epidermal cells | Fragment of stomata | Spiral vessel | Spiral vessel

Spiral vessel | Annular vessel | Scalariform vessel | Pitted vessel



Tracheid | Fibre | Fibre tracheid | Non grandular trichome | Trichome head

Figure 4. Diagnostic characters of powdered leaves of *Clerodendrum serratum* (L.) Moon

Discussion and Conclusion

The study plant *Clerodendrum serratum* (L.) Moon belongs to the Verbenaceae. The family is closely related to the Lamiaceae. The genus is native to tropical and warm temperate regions of the world, with most of the species occurring in tropical Africa and Southern Asia.

In this research, the morphological studies on vegetative and reproductive parts, histological characters of the whole plant have been studied and described. The plant studies are found to be growing wild and cultivated as a pot plant in the tropical.

The plant of *Clerodendrum serratum* (L.) Moon are perennial shrubs, bluntly quadrangular. The leaves are simple, opposite and decussate, apically clustered, sharply serrate margins and glabrous. The inflorescences are both axillary and terminal cymes, solitary flower, in lax pubescent, dichotomous cymes with a pair of acute bracts at each branching and a flower in the fork, pyramidal erect panicle. The flowers of these plants are pale blue, showy, bisexual, irregular, zygomorphic and hypogynous. The calyx is (5), synsepalous, puberulous, cup-shaped and connate at the base. The corolla consists of (1+4) petals, synpetalous, the lower larger lobed, bluish purple, ovate to lanceolate, hairy within the tube, inferior. The ovary is superior, globose, false septum, each with two ovules, axile placentation, stigma bifid, disc present. The fruits are broadly obovoid, succulent, dark green in immature, black in matures, glabrous. The seeds are dark purple. These characters are in agreement with those reported by (Kirtikar and Basu, 1935; Backer and Brink, 1965; Dassanayake, 1983; Hu Qi-ming and WU De- Lin 2009).

In histological studies, the surface views of upper and lower epidermis of the leaves have the epidermal cells with wavy anticlinal walls. The stomata are distributed on both surfaces of the leave and diacytic type. The transverse section of the leaves exhibited the palisade mesophyll cells and spongy mesophyll cells. Calcium oxalate crystals (druses) are found in this region. The vascular bundles were embedded in mesophylls cells. They were collateral type and different in sizes according to their position.

In transverse section of petiole, the epidermal cells were barrel shaped. The cortex was made up of lamellar collenchymatous and polygonal thin walled parenchymatous cells. Acicular crystals (raphides and styloid) were scattered in the parenchymatous cells. In transverse section of

stem, the young stem was quadrangular in outline. The vascular bundles were collateral and open type.

In transverse section of roots were circular in outline. The epidermal cells were disorganized and displaced by periderm. The vascular bundles were arranged in concentric ring. The xylem is endarch. The histological characters of leaves; petiole, stem and root are in agreement with Esau, 1965; Pandey, 1978; Metcalfe and Chalk, 1979 and 1989.

The epidermal characters of *C. serratum* (L.) Moon studied such as size and shape of epidermal cells, distribution of stomata in the surfaces, types of trichome and the presence of druses and acicular crystals were the characteristics of this plant. These characters can be considered as a good diagnostic feature for the identification of species on the basis of epidermal characters of leaf anatomy.

Clerodendrum serratum (L.) Moon has played an important role in Indian system of medicine. In addition to the common local use in respiratory diseases, other ethno medicinal uses include treatment of pain, inflammation, rheumatism and fever especially malarial fever. Therefore, the present research deals to provide a comprehensive overview of the traditional and ethno medicinal uses, phytochemistry of *C. serratum* (L.) Moon. So, this plant should be investigation of effective pharmacological research in the coming future.

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Study on Phytochemical, Physicochemical, Elemental Analysis and Nutritional Values of *Passiflora edulis* Sims

Khin Ohn Myint[*]

Abstract

Passiflora edulis Sims belongs to the family Passifloraceae is known as Pin-hmet in Myanmar. It was collected Insein Township, Yangon Region. This research carried out Phytochemical, physicochemical and elemental analysis of *Passiflora edulis* Sims leaves and nutritional values of its fruits and seeds. Phytochemical analysis of powdered leaves indicated that the presence of alkaloid, glycoside, reducing sugar, saponin, carbohydrate, α-amino acid, phenolic compound, flavonoid, tannin, terpenoid, steroid and starch. As the result of physicochemical properties, the powder of leaves was most soluble in water and moderately soluble in ethanol and methanol. According to the elemental analysis, it was found that potassium (K), chlorine (Cl), calcium (Ca), phosphorous (P), sulphur (S), iron (Fe), strontium (Sr), manganese (Mn), zinc (Zn) and copper (Cu).The nutritional values of powdered fruits such as moisture, ash, protein, fiber, fat, carbohydrate and energy value was conducted. Therefore, it was observed that *Passiflora edulis* Sims is endowed with nutritional value and bioactive constituents that can be provided useful in traditional medicine.

Keywords: phytochemical, physicochemical, elemental analysis and nutrition values of *Passiflora edulis* Sims leaves

Introduction

The World Health Organization (WHO) estimates that up to 80% of the world's populations rely on plants for their primary healthcare. Plants contain chemical constituents such as tannins, flavonoids, steroids, saponins, glycosides, phenolics, terpenes, alkaloids, waxes, essential oils, carbohydrates, amino acids, proteins etc (Stace, 1980).

It is native of Southern Brazil, tropical lowland area and distributed throughout the world of tropical and subtropical area (Verheij and Cornel, 1992). This family consists of 12 genera and about 600 species (Cronquist, 1981). Eight species of genus *Passiflora* were found in Myanmar (Kress, 2003). The plant is used in various medicines and ripe fruit is edible.

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Biochemical constituents of *Passiflora edulis* Sims indicate carbohydrates, proteins, fibres, ash, phosphorous, calciums, irons, enzymaic and non enzymic antioxidants (Devaki and Gopalakrishnan, 2013). Johnson *et al.* (2008) stated that the leaves of *Passiflora edulis* Sims have saponins, steroids, tannins, phenolics, triterpenoids, alkaloids and flavonoids The main constituents of *Passiflora edulis* Sims include terpenoids, flavonoids and alkaloids (Zibadi *et al*., 2004). Alkaloids, glycosides and phenols are major constituents in *Passiflora edulis* Sims (Dhawan *et al.*, 2004).

The aerial parts of *Passiflora* species were used in Europe and America to treat anxiety, insomnia and nervousness (Paris, *et al.*, 2002). Leaves from several *Passiflora* species are widely used in folk medicine as anxiolytics and sedatives (Rebello *et al.*, 2007).

There are six major classes of nutrients: carbohydrates, fats, minerals, protein, vitamins and water. The macronutrients include carbohyrate, fiber, fats, protein and water. The micronutrients are minerals and vitamins. Carbohydrates are a common source of energy in living organisms. Humans are able to obtain 100% of their energy requirement from protein and fats (Maton *et al*., 1993).

Materials and Methods

In this research, the specimens of *Passiflora edulis* Sims were collected from Insein Township, Yangon Region during the flowering and fruiting period. The samples were washed and dried; they were pulverized by grinding machine to get the powder and stored in an air-tight container. The collected specimens were identified with the help of available literature (Hooker, 1879; Brandis, 1907; Bailey, 1939; Lawrence, 1951; Backer, 1963; Kirtikar and Basu, 1975 and Dassanayake, 1996).

(1) Phytochemical investigation of leaves of *Passiflora edulis* Sims

Preliminary phytochemical examination on powdered leaves was carried out in the Department of Botany, University of Yangon according to the method of Marini Bettolo *et al. (*1981), Central Council for Research in Unani Medicine (1987) and Trease and Evans (2002).

(2) Physicochemical properties of leaves of *Passiflora edulis* Sims

Physicochemical characters such as moisture content, content of total ash, acid insoluble ash, water soluble ash and soluble matter content in different solvents of ethanol, methanol, pet-ether, ethyl acetate, chloroform, acetone and water-were determined according to the method of British Pharmacopeia (1965).

(3) Elemental analysis of leaves of *Passiflora edulis* Sims by Energy Dispersive X-ray Fluorescence (EDXRF)

Elemental analysis was performed by Energy Dispersive X-ray Fluorescence (EDXRF) at University Research Center, University of Yangon. The EDX-700 spectrophotometer is used for determination of elements from sodium (Na) to uranium (U). The spectrometer produces the characteristic X-ray spectrum of each sample, consisting of the respective elements.

(4) Nutritional value of fruits pulp and seeds of *Passiflora edulis* Sims

The experimental work for the nutritional value of fruits pulp and seeds of *Passiflora edulis* Sims were carried out at the Food Industries Development Supporting Laboratory (FIDSL). The nutritional value has been undertaken according to the Association of Official Analytical Chemist (AOAC) method.

Results

(1) Phytochemical investigation of leaves of *Passiflora edulis* Sims

The investigation of these test observed that the presence or absence of alkaloid, glycoside, reducing sugar, saponin, carbohydrate, α-amino acid, phenolic compound, flavonoid, tannin, cyanogenic glycoside, terpenoid and steroid, acid or base compound and starch the results were shown in table (1).

Table 1. Preliminary phytochemical test of the leaves of *Passiflora eduls* Sims

No.	Test	Extract	Test reagent	Observation	Results
1.	Alkaloid	1%HCL	(1) Mayer's reagent	White ppts	+
			(2)Wagner's reagent	Brown ppts	+
			(3) Dragendroff's reagent	Orange ppts	+
2.	Glycoside	H_2O	10% lead acetate solution	White ppts	+
3.	Reducing sugar	H_2O	Benedict's solution	Reddish ppts	+
4.	Saponin	H_2O	Distilled water	Frothing	+
5.	Carbohydrate	H_2O	10% α-naphthol + Conc: H_2SO_4	Pink ring	+
6.	α-amino acid	H_2O	Ninhydrin reagent	Violet colour	+
7.	Phenolic compound	H_2O	3 % Ferric chloride	Deep brown ppts	+
8.	Flavonoid	MeOH	HCl / Mg	Pink colour	+
9.	Tannin	H_2O	10 % Ferric chloride	Blue black colour ppts	+
10.	Cyanogenic glycosides	H_2O	Conc: H_2SO_4 Sodium picrate paper	No colour change	-
11.	Terpenoids and steroid	PE	Acetic anhydride + Conc: H_2SO_4	Reddish brown	+
12.	Acid or base compound	H_2O	Bromocresol green Sol:	Green	Neutral
13.	Starch	H_2O	I_2 solution	Bluish black	+

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

According to preliminary phytochemical test of *Passiflora edulis* Sims, the presence of alkaloid, glycoside, reducing sugar, saponin, carbohydrate, α-amino acid, phenolic compound, flavonoid, tannin, terpenoid , steroid and starch were observed in the leaves. However, acid or base and cyanogenic glycosides were absent.

(2) Physicochemical properties of leaves of *Passiflora edulis* Sims

Physicochemical properties were important data for the quality control system for value of medicine. In physicochemical properties, total ash, acid insoluble and water soluble ash and solubility in different solvents of leaves of *Passiflora edulis* Sims were performed. These results were shown in Table (2)

Table 2. Physicochemical examination of leaves of *Passiflora edulis* Sims

No.	Physicochemical characters	Average%
1.	Moisture content	8.51
2.	Total ash content	3.63
3.	Water soluble ash content	29.34
4.	Acid insoluble ash content	10.02
5.	Ethanol soluble matter content	5.10
6.	Methanol soluble matter content	4.13
7.	Pet-ether soluble matter content	0.74
8.	Ethyl-acetate soluble matter content	3.09
9.	Chloroform soluble matter content	1.84
10.	Acetone soluble matter content	2.61
11.	Water soluble matter content	13.37

According to this result, the powders of leaves were soluble in ethanol, methanol, pet-ether, ethyl-acetate, chloroform, acetone and water. Among them, the powder of leaves was most soluble in water and moderately soluble in ethanol and methanol.

(3) Elemental analysis of leaves of *Passiflora edulis* Sims by Energy Dispersive X-ray Fluorescence (EDXRF)

Elemental analysis was used to determine the amount of element in the leaves of *Passiflora edulis* Sims. It was a quantitative indication of the level of nutrients in pant obtained by using Energy Dispersive X-ray Fluorescence (EDXRF) spectrophotometer. It was found that potassium (K), chlorine (Cl), calcium (Ca), phosphorous (P), sulphur (S), iron (Fe),

strontium (Sr), manganese (Mn), zinc (Zn) and copper (Cu) were found. These results were described in Table (3).

Table 3. Elemental analysis of leaves of *Passiflora edulis* Sims by using Energy Dispersive X-ray Fluorescence (EDXRF)

No.	Elements	Percentage present in leaves
1.	Potassium (K)	1.540
2.	Chlorine (Cl)	0.512
3.	Calcium (Ca)	0.500
4.	Phosphorous (P)	0.399
5.	Sulphur (S)	0.086
6.	Iron (Fe)	0.018
7.	Strontium (Sr)	0.006
8.	Manganese (Mn)	0.005
9.	Zinc (Zn)	0.003
10.	Copper (Cu)	0.003

According to this result, concentration of potassium in the powder of leaves were higher than the other elements. chlorine, calcium and phosphorous were moderatly present in the leaves. manganese, zinc and copper were found as trace element in the leaves of *Passiflora edulis* Sims.

(4)Nutritional value of fruits pulp and seeds of *Passiflora edulis* Sims

The experimental work for the nutritional value of fruits pulp and seeds of *Passiflora edulis* Sims was carried out at the Food Industries Development Supporting Laboratory (FIDSL), used Association of Official Analytical Chemist (AOAC) method. According to the experiment, fruits pulp and seeds of *Passiflora edulis* Sims were conducted as moisture, ash, protein, crude fiber, crude fat, carbohydrate, energy values. The results were shown in Table (4) and figure (1).

Table 4. Nutrient contents of fruits pulp and seeds of *Passiflora edulis* Sims

Sr. No.	Test parameter	Results	
		Pulp	Seeds
1.	Moisture	85.09%	55.79%
2.	Ash	0.62%	1.03%
3.	Protein	1.23%	5.42%
4.	Crude fiber	0.10%	12.27%
5.	Crude Fat	0.04%	8.20%
6.	Carbohydrate	12.92%	17.29%
7.	Energy value (Kcal/100g)	56.00	160.00

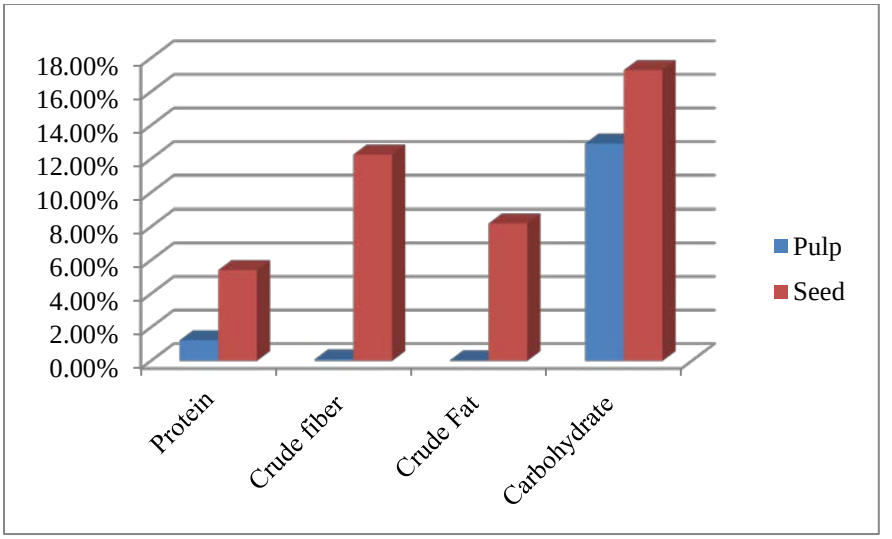

Figure 1. Nutritional contents of fruits pulp and seeds from *Passiflora edulis* Sims

Discussion and Conclusion

In the chemical aspect, preliminary phytochemical test, physicochemical properties and elemental analysis, nutritional value of *Passiflora edulis* Sims have been described. Johnson *et al.* (2008) stated that the leaves of *Passiflora edulis* Sims have saponins, steroids, tannins, phenolics, triterpenoids, alkaloids and flavonoids. In the recent study, the preliminary phytochemical test indicated that the presence of alkaloid, glycoside, reducing sugar, saponin, carbohydrate, α-amino acid, phenolic compound, flavonoid, tannin, terpenoid, steroid and starch. According to this test these results were in agreement with those stated by Johnson *et al.*, (2008).

In physicochemical examination, the powder of leaves was soluble in ethanol, methanol, pet-ether, ethyl-acetate, chloroform, acetone and water. Among them, the powder of leaves was most soluble in water and moderately soluble in ethanol and methanol. Hence the most of the solubility of water, it useful for traditional medicine in our country. The elemental analysis by using Energy Dispersive X-ray Fluorescence Spectroscopy (EDXRF) revealed that potassium (K), chlorine (Cl), calcium (Ca), phosphorous (P), sulphur (S), iron (Fe), strontium (Sr), manganese (Mn), zinc (Zn) and copper (Cu) were present in the leaves of *Passiflora edulis* Sims. The results indicated that the presence of this principal element may be useful for the preparation of medicine (British Pharmacopoeia, 1965). Among them, the concentration of Potassium was higher than other elements in the powdered leaves of *Passiflora edulis* Sims. Potassium helps to reduce the rise in blood pressure. Hence, presence of potassium in this plant might be helping to reduce down the rise in blood pressure.

Nutritional value of fruits and leaves of *Passiflora edulis* Sims was carried out at the Food Industries Development Supporting Laboratory (FIDSL). In this research, protein, crude fiber, crude fat, carbohydrate, energy values were reported. These characters were in agreement with those given by Maton *et al*., (1993).

According to the results, carbohydrate was found to be the highest, it could help to prevent body fat accumulation because carbohydrate is rich in fruit as energy food. Crude fiber was the second highest, which was indigestible portion of the digestive system.

Myanmar is rich in varieties of medicinal plants due to the presence of different climate zones in the country. The medicinal plant *Passiflora*

edulis Sims is widely grown in Myanmar. In conclusion, the plant can be used as herbal medicine due to result of the enrichment of bioactive and nutritional values. Furthermore, the pharmacological actives of *Passiflora edulis* Sims should be undertaken.

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The Distinct Characters of Some *Bulbophyllum* and *Cirrhopetalum* Species Found in Some Western Parts of Southern Shan State

Khin San Win[1], Khin Palè [2] & Yu Yu Tin[3]

Abstract

Bulbophyllum and *Cirrhopetalum* are genera of Orchidaceae. These orchids are epiphytes. They are mostly distributed in hilly and mountainous regions of Myanmar. Shan State is mostly covered by tropical evergreen forests and many wild orchids are found there. The orchids were collected from some western parts of Southern Shan State mainly from Kalaw, Ywa-ngan and Yat-sauk Area. Their morphological characters together with their flowers' distinct characters were recorded during the flowering period. Totally 8 species of *Bulbophyllum* and 2 species of *Cirrhopetalum* orchids were recorded and described their distinct morphological characters with colour plates.

Keywords : *Bulbophyllum, Cirrhopetalum*

Introduction

Bulbophyllum is the largest genus in the orchid family comprising some 2000 species which are widely distributed all over the tropical regions. They are supremely successful epiphytes, invariably found in large colonies. The pseudobulbs carry a single fleshy leaf at its apex or sometime the pseudobulb bearing 2-leaves and the flowers rise from the base of the pesudobulb.

Bulbophyllum the name itself, taken from the Greek words for bulb-leafed or the pseudobulb is surrounded by the leaf.

About 1000 species of *Bulbophyllum* distributed in tropical America and Africa, India and New Zealand.

Cirrhopetalum genus is closely related to *Bulbophyllum* and its members have been until recently considered to be in the genus *Bulbophyllum*. The taxon name comes from Latin *Cirrus* (fringe) and Greek *petalon* (petal), hence meaning fringed-petaled.

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Based on different growing conditions, environments and topographies, orchidaceous plants are classified into 3 categories: terrestrial orchids, saprophytic orchids and epiphytic or lithophytic orchids. It is very interesting that orchids belonging to the same genus may also be terrestrial as well as epiphytic. From the Greek *bolbos* –"bulb" and *phyllon* "leaf", referring to the thick, bulblike leaves or to the short pseudobulb, which bear the leaves.

In the 1830s, Darwin, during his voyages, made an investigation into the tropical jungle in Brazil and marvelled greatly to find orchids growing on trees, calling them "miracle flowers". But like many of his contemporaries, he took the erroneous view that the tree –dwelling orchids were leech-like parasitic plants, only later was it found that they were epiphytic plants, with the trees serving only as their structural base or support. Though they grow on the trunks of trees, they do not assimilate nutriments from them. Some species simply grow on bare rocks or cliffs. Epiphytic orchids have large, thick aerial; roots, which are exposed to the air and absorb moisture there form. They take nutrients from the inorganic salts dissolved in rain water, or from the humus and organic substances around their roots. As epiphytic orchids generally have no reliable water source, most of them have a large, thick fleshy water-keeping organ (the pseudobulb), which guarantees the normal growth of the plant even in dry seasons.

According to the scientists, epiphytic orchids grow high up in the trees in order to avoid the heavy shade and fierce competition for nutrients on the forest floor, and also to let pollen-spreading birds and insects find it easier to spot them; moreover, they can make use of the height to scatter their tiny seeds as far as possible. All this is due to their need to propagate.

The aims the present research is to get a species inventory of the orchids. The objectives are to understand the distribution of various kinds of wild orchids from study area, to provide the knowledge on the different natural resources of study area and to fulfill the compilation of the flora in Myanmar.

Materials and Methods

Bulbophyllum species were collected from some western part of Southern Shan State. The fresh and herbarium specimens were used to identify the species. Identification of plants was made with reference to literature such as Hooker (1885), Java (1965), Ceylon (1987) etc.

Results

In the results section, the data for *Bulbophyllum* and *Cirrhopetalum* are presented.

Bulbophyllum Yhouars, Orch.Illes Fr.tt.92-97. 1882.

Epiphytes with a creeping rootstock and globosely or subglobosely ovoid or ovoid oblong pseudobulbs which are naked or clothed with the remains of old sheaths; leaves one to two on each pseudobulb, coriaceous, oblong, oval or lanceolate, petiolate; flowers very small or large , 1-many in spicate, shortly racemes or umbelled, 2-8-flowered scapes arising at the base of the pseudobulb; sepals sub-equal or the dorsal about half as long as lateral sepals, 5-veined, lateral sepals adnate to the foot of the column forming a short mentum, free or their edges more or less joined; petals much smaller, ovate or lanceolate; lip tongue-shaped, hinged to the end of the column foot, mobile, coriaceous or fleshy, strongly recurved; column short, its base produced into a long upcurved foot, winged or toothed at the top; anther terminal, 2-chambered; pollinia 4, collateral, cohering in pairs by a viscus, ovoid or oblong, the two inner pollinia smaller.

An artificial key to the *Bulbophyllum* species.

1. Pseudobulb bearing 2 leaves---------- 2
1. Pseudobulb bearing only 1 leaf---------- 5
    2. Flowers contemporary with leaves---------- **6**. ***B. shanicum***
    2. Flowers not contemporary with leaves---------- 3
3. Scapes shorter than 2.0 cm ----------**2**. ***B. muscarirubrum***
3. Scapes longer than 3.0 cm----------4
    4. Flowers yellow, very fragrant---------- **7**. ***B. suavissimum***
    4. Flowers reddish-green, pungent foetid---------- **8**. ***B.triste***

5. Margins of sepals and petals with obscurely ciliate, dark violet to blakish violet in color---------- **5**. ***B. secundum***

5. Margins of sepals and petals without ciliate, other colour----------6

6. Flowers pinkish white with purple spots, the tips of petals with tail ---------- **1**. ***B.dixoni***

6. Flowers pale yellow to orange yellow, the tips of petals without tail ---------- 7.

7. Leaves coriaceous, the scape bearing more than16 flowers---------- ---------- **4. *B.rufinum***

7. Leaves membranous, the scape bearing less than 12 flowers---------- ---------- **3**. ***B. polyrhizum***

1. *Bulbophyllum dixoni* Rolfe in Kew Bull.421.1908.

Morphologorum kranzlin in Orchis, 2:89.1908.

The distinct characters of this species are: sympodial epiphytes, one-jointed and fleshy globoid pseudobulbs, single coriaceous, persistent and elliptic-oblong leaf, basally dense racemes with numerous flowers, pinkish-white sepals with purple spots, entire along the petal margin, presence of tails at the apex of petals, absence of lateral lobes in linguiform labellum, horn-like columnar-arms, ovoid compressed pollinia and oblanceolate stigmatic surfaces.

Figure 1. *Bulbophyllum dixoni* Rolfe

2. *Bulbophyllum muscarirubrum,* Seidenf. & Smitinand 419.1961.

The distinct characters of this species are: sympodial epiphytes, one-jointed and globoid pseudobulbs, two deciduous leaves, basally dense short racemes with numerous flowers, scape shorter than 2.0 cm, light reddish purple sepals with many darker dots, acuminate apex of petals, triangular columnar-arms, ovoid compressed pollinia and shining stigmatic surfaces.

Figure 2. *Bulbophyllum muscarirubrum,* Seidenf. & Smitinand

3. *Bulbophyllum polyrhizum* Lindl. , Gen. & Sp.Orch. 53.1830.

The distinct characters of this species are: sympodial epiphytes; one-jointed and ovoid pseudobulbs; single, membranous and deciduous leaf; basally lax racemes with pale yellow flowers, the scape bearing less than 12 flowers; creamy white to pale yellow sepals, short and membranous columnar-arms, ovoid compressed pollinia and very minute stigmatic surfaces.

Figure 3. *Bulbophyllum polyrhizum*

4.*Bulbophyllum rufinum* Rchb.f., Xen. Orch.3:44.219.1881.

The distinct characters of this species are: sympodial epiphytes, one-jointed and ovoid to oblong pseudobulbs, single persistent, coriaceous leaf, basally long racemes with numerous flowers, the scape bearing more than 16 flowers; dark yellow to orange yellow sepals, acuminate apex of petals, short and membranous columnar-arms, ovoid compressed pollinia and shining stigmatic surfaces.

Figure 4. *Bulbophyllum rufinum* Rchb.f., Xen.

5. *Bulbophyllum secundum* Hook.f., Fl. Brit.Ind.5:764; Ic. Pl.2042.1890.

The distinct characters of this species are: sympodial epiphytes, one-jointed and sub-globoid pseudobulbs, single persistent leaf, basally long racemes with secund flowers, dark violet to blakish-violet sepals, ciliate margins of petals, short and membranous columnar-arms, ovoid compressed pollinia and shining stigmatic surfaces.

Figure 5. *Bulbophyllum secundum* Hook.f., Xen.

6. ***Bulbophyllum shanicum*** King & Pantling, J. As.Soc.Beng. 66: 587. 1897.

The distinct characters of this species are: sympodial epiphytes, one-jointed and globoid pseudobulbs, two persistent leaves, basally short lax racemes with scented flowers, pale yellow sepals, narrow triangular petals, short and membranous columnar-arms, ovoid compressed pollinia and shining stigmatic surfaces.

Figure 6. *Bulbophyllum shanicum* King & Pan Xen.

7. ***Bulbophyllum suavissimum*** Rolfe. Gard.Chron.1:297.1889.

The distinct characters of this species are: sympodial epiphytes, one-jointed and ovoi-oblongoid pseudobulbs, two deciduous leaves, basally long racemes with fragrant flowers, scapes longer than 3.0 cm, yellow sepals, ovate and entire or obscurely serrate petals, short and membranous columnar-arms, ovoid compressed pollinia and shining stigmatic surfaces.

Figure 7. *Bulbophyllum suavissimum* Rolfe.

8. ***Bulbophyllum triste***, Reichb.f.in Walp.Ann.6: 253.1861.

The distinct characters of this species are: Sympodial epiphytes, one-jointed and globose pseudobulbs, 2 deciduous oblong leaves, basal long racemes, reddish green or yellowish-green flowers with reddish-purple spots, pungent foetid, scapes longer than 3.0 cm, triangular petals, 4 ovoid pollinia and oblongoid ovary.

Figure 8. *Bulbophyllum triste* Reichb.f Xen.

Cirrhopetalum Lindley, Gen. Sp. Orchid. Pl.45,58. 1830.

Rhizome creeping or sraggling; roots sprouting mainly below pseudobulbs, spreading; new shoots arising from basal node of pseudobulb. Pseudobulbs distinct, apex with one leaf. Leaves persistent, thick. Inflorescences solitary, arising near pseudobulbs, 1-flowered or a ± subumbellate raceme. Pedicel with basal node ± coinciding with attachment of subtending bract. Sepals 3-5-veined; dorsal sepal with margins ciliate, fimbriate, or with paleaceous appendages(with a single apical thread in Bulbophyllum amplifolium); lateral sepals twisted inward near base, with upper margins approaching or meeting and connate, margins glabrous to ciliolate. Petals: margins fimbriate, or with paleaceous appendages, 1-3-veined; lip: margins with or without auricles above base, margins usually ±glabrous, adaxially ± glabrous. Column: stigma protruding at its base, foot distinct; anther: front margin projecting or not, abaxially with or without a low, rounded crest; pollinia 4, inner ca.1/2 as long as outer or longer, all without appendages.

An artificial key to the *Cirrhopetalum* species.

1. Lateral sepals longer than 2.5 cm , the tip of dorsal sepal with tail-------
---------- **2.*C. picturatum***

1. Lateral sepals shorter than 2.0 cm , the tip of dorsal sepal without tail---
----------**1**. ***C. cornutum***

1. *Cirrhopetalum cornutum* Lindl., Bot.Reg.29.Misc.138:75.1838.Seiden 349:1970.

Bulbophyllum helenae (Kze.) J.J.Smith.,Bull.Buitenz.2.s.8:24. 1912.

The diagnostic features of this species are : one-jointed and ovoid pseudobulbs, single oblong leaf, basally umbellate cymes, dark yellow flowers with reddish-brown striations, slightly serrate margins of dorsal sepals, narrowly lanceolate lateral sepals and the margins connate to form a conical tube, ovate petals with serrulate margin, short and thick columnar-arms.

Figure 9. *Cirrhopetalum cornutum* Lindl.

2. *Cirrhopetalum picturatum* G,Loddies in Bot. Reg.Misc.49. 1840.

Bulbophyllum picturatum (Lodd.) Rchb.f.,in Walp.Ann.6:262.1861.

The diagnostic features of this species are : one-jointed and obtusely 4-angled pseudobulbs, single oblong leaf, basally umbellate cymes, greenish-yellow flowers with purple spots, presence of tailed at the tips of dorsal sepals, narrowly lanceolate lateral sepals, ovate petals with serrulate margin, short and thick columnar-arms.

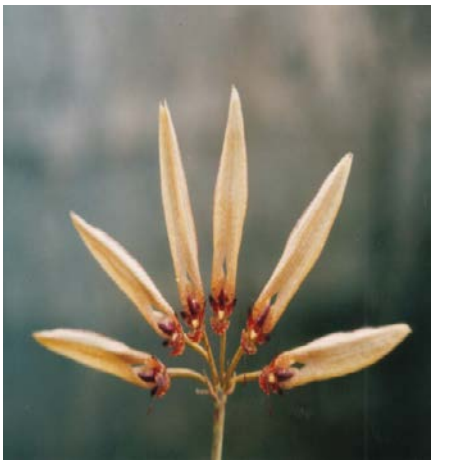

Figure 10. *Cirrhopetalum picturatum* G.Loddies

Discussion and Conclusion

The pseudobulb of *Bulbophyllum shanicum, B. muscarirubrum, B.suavissimum,* and *B.triste* species, bearing two leaves. But, in *B. secundum, B. rufinum, B. polyrhizum, B. doxoni*, the pseudobulb bearing only one leaf.

The main characteristics mentioned by Lindley (1830-1840) stated that the genus *Cirrhopetalum* possess the long oblique lateral sepals that much longer than the dorsal sepal, and the flowers are so dense in the raceme. They are actually radiate from the apex of the scape.

In *Bulbophyllum* species, the pseudobulbs are one-jointed and varied in shape of oblongoid to globoid.

The flowers of *Bulbophyllum suavissimum* are very fragrant. These flowers are called Shan Thazin and very similar to Yakhine Thazin

(*Bulbophyllum auricomum*) but they differ in flower's color and petals shape. But the flowers of *Bulbophyllum triste* are pungently foetid.

Grant(1895) reported that 41 species of *Bulbophyllum* are distributed in Myanmar. 10 species of *Bulbophyllum* from Western part of Southern Shan State have been collected in the present study. *Bulbophyllum picturatum* (Lodd.) Reichenb and *Bulbophyllum helenae*(Kze)J.J.Smith had been transferred to the genus *Cirrhopetalum picturatum* (Lodd.,) and *Cirrhopetalum cornutum* Lindl.

Orchids are not only significant worldwide in the horticulture industry, but in many countries, they have useful their medicinal, nutritional and ornamental qualities.

Most of the orchids can be found in Ywa-ngan township where the elevation and humidity is high. Most of the forest area is strictly defined as a retained area and no one is allowed to cut down the trees of local peoples for various purposes in this area. Therefore, the future of wild orchids may be hopefully in good condition if orchid hunters are not allowed to search and trade orchids illegally in the forests.

Therefore it can be concluded that some of the valuable orchids are still widely thrived in the study area and rare orchid species need to be conserved not to be extinct. In Shan state, orchid plants that are the pride of the state and once thrived abundantly, are now endangered due to over collection and deforestation. The loss of national resources of the country could be prevented by prohibiting collections and sales of orchids without due consideration. Protecting and conserving the forests is a national duty for the state and future generation.

It is sincerely hoped that the present paper can stand up valuable information for the further investigation of students, orchids hunters, orchid crazy, researchers who are facing with some difficulties to know about the species, and anyone who investigate for the diversity of the members of the genus, and so many interest peoples upon the orchids. This study will partially be a compilation of the flora of Myanmar.

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Morphological Characters and Antimicrobial Activity of *Jussiaea repens* L.

Naw Blute Tser[1] & May Thu[2]

Abstract

This study is important and lays down parameters for standardization and authentication of medicinal plants with the help of which adulteration and substitution can be prevented. All the parameters to be evaluated in pharmacognostic study such as morphological study, organoleptic characteristics, anti-microbial activity and uses are listed along with their importance. The present review discusses the need and emphasizes the importance of pharmacognostic study of *Jussiaea repens* L., water primrose in English and Ye-ka-nyut in Myanmar belonging to the family of Onagraceae. The plant sample is collected from Pathein University Campus, Pathein Township during flowering and fruiting period from July to January in the year 2016-2017. The collected plant is classified and identified by its habitat, habit, growth form, leaf characters, flower's parts, colour, period, size, arrangement and fruit. The morphological characteristics are assessed with the help of literatures in Botany Department of Pathein University. The plant is a herbaceous perennial with floating stem. Leaves simple, alternate, stipulate, petiolate, oblanceolate. Inflorescences solitary, axillary and cymose. Flowers white, yellow at the base. Stamens 5+5, biseriate. Ovaries 5 carpels. Fruits capsule. The anti-microbial activity of various crude extracts are tested by using agar-well diffusion method. The antimicrobial activity of ethanol and methanol extracts showed the most effective activity against on *Staphylococcus aureus*.

Keywords: *Jussiaea repens* L., morphological characteristics, anti-microbial activity, medicinal plant

Introduction

Today the value of traditional medicines has been beginning to be recognized. The support of FDA to allow studying traditional medicines in USA is the strong evidence that present society has interest in traditional medicines. About one third of all drugs are plant-based, nearly sixty percent of pharmaceuticals are of plant origin reported by Han Win Shwe *et al.*, 2004.

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In our present study, we have selected a common herb locally known as Ye-Ka-Nyut, scientifically called *Jussiaea repens* L. of Onagraceae family which is a creeping water primrose family or evening primrose family, of flowering plants. It includes about 650 species of herbs, shrubs and trees in 17 genera.

The family is widespread, occurring on every continent from boreal to tropical regions according to Ford and Gottlieb, 2007. The plant can be found in Bangladesh, Cambodia, China, India, Laos, Myanmar, Nepal, Sri Lanka, Thailand and Vietnam mentioned by Ito, *et al*., 2014.

Jussiaea repens L. can pollinate by self and self-fertile. It grows best in areas where annual daytime temperatures are within the range 15-25˙ C but can tolerate 8-30˙C. It prefers a mean annual rainfall in the range 1500-3000 mm but tolerate 1000-4000 mm. It requires sunny position and grows best in a fertile clay soil. It prefers a pH in the range 5.5-7, tolerating 4.5-8.5 mentioned in website 1. It grows in wet places, concentrated around coastal regions, lakes, lagoons, canal, rivers, streams, seas, gutter and water logged areas reported by Oziegbe and Faluyi, 2011.

Plant biologists use morphological characters of plants which can be compared, measured, counted and described to assess the differences or similarities in plant taxa and use these characters for plant identification, classification and description mentioned in website 1.

The goals of antimicrobial testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections reported by Reller and Weinstein, 2009.

Studies of *Jussiaea repens* L. have suggested antioxidant, antibacterial, mollusicidal, and antifertility properties. Decoction of dried material used for colds with fever, intense coughing, inability to urinate and astringent for dysentery. Pounded fresh material applied as poultice to carbuncle, sprains, and snake bites. In the Antilles, used as an emollient. Malays used it for poulticing skin complaints. In Papua New Guinea, stems and leaves of the plant used as contraceptive reported by website 2.

This research work was mainly aiming to identify, separate and evaluate active constituents of *Jussiaea repens* L. from the whole plant. The objectives are to collect and authenticate of selected plant, to describe the characteristic features of a plant parts and to determine antimicrobial activity of crude extract.

Materials and Methods

Botanical studies

Collection and Identification of *Jussiaea repens* L.

The specimens in this study were collected from N 16˚ 48´ 17.273 ˝, E 94˚ 45´10.217˝, Pathein University Campus during flowering and fruiting period from July, 2016 to February, 2017.

After collected, specimens were measured, recorded in detail for taxonomic description, identification by using dissecting microscope and authentication in the department of Botany, Pathein University and were deposited with the help of literature references such as Hooker, 1879; Ridley, 1922; Hutchinson, 1926; Kirtikar and Basu, 1958; Lawrence, 1969; Cronquist, 1981; Hundley and Chit KoKo, 1987; Dassanayake, 1995.

The habits, leaves, inflorescences and flowers of the collected specimens were recorded by using digital camera. The collected plants were prepared for herbarium specimens according to the methods of Lawrence, 1969. The mounted herbarium sheets were prepared by using collected specimens according to the method of Nyo Maung, 2003.

Preparation of powdered samples of *Jussiaea repens* L.

The collected plant sample was thoroughly washed with water to remove impurities. After washing, the samples were air dried for 2 weeks, ground to get powder and stored in air tight container to prevent moisture changes and air-borne contamination for further studies.

Antimicrobial activity

Antimicrobial activity of various solvent extracts of *Jussiaea repens* L.

The whole plant of *Jussiaea repens* L. of various solvent extracts were tested with different types of bacteria and one fungal species such as *Agrobacterium tumefaciens, Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Saccharomyces cerevisiae* by using agar-well diffusion method. The control test was also carried out. The test was conducted at the Biological Resources and Biotechnological Development Center (BRBDC), Pathein University.

Preparation of Antimicrobial activity test

Nutrient agar was prepared according to the method by Cruickshank *et al*., 1975. Nutrient agar was boiled and 20-25 ml of the cultural medium was poured into conical flask and plugged with cotton wool and sterilized at 121°C for 15 minutes in autoclave. After the conical flask were cooled down to 30-35°C and poured into sterilized petridishes and 0.1-0.2 ml of test organisms were added into the dishes. The agar was allowed to set for 2-3 hours, and then 8 mm agar-well was made by the help of sterilized agar-well cutter. After that, about 0.2 ml of samples were introduced into agar-well cutter and incubated at 37°C for 24 hours. The inhibition (clear) zones which appeared around the agar-well were measured. These zones indicated that the presence of antimicrobial activities of the sample. The diameter of the inhibition zones were recorded in millimetre.

Results

Botanical studies

Botanical Name	- *Jussiaea repens* L.
Vernacular Name	- Ye-ka-nyut
English Name	- Water primrose
Family	- Onagraceae
Location	- Pathein University Campus, Pathein Township, Ayeyarwady Region N 16˚ 48´ 17.273 ″, E 94˚ 45´ 10.217″

Morphological characters of *Jussiaea repens* L.

The plant is a herbaceous perennial with floating stem, rooting at the nodes, floating stems are developed into two kinds of roots, absorbing downward growing roots and spongy upward growing roots (pneumatophores) in cluster at nodes of floating stem, much branched, tips ascending, glabrous, pinkish green. Leaves simple, alternate, stipulate, petiolate; petioles 0.2-1.5 cm long, light green to purplish green, glabrous; blades obovate to oblanceolate, narrowed to the petiole, 3.3-5.5 cm long, 0.5-1.5 cm wide, the bases narrowly cuneate, margins entire, the tips sub-obtuse, upper surface green and glossy, the lower slightly green and glabrous. Inflorescences solitary and axillary, cymose, peduncle cylinder,

slightly pubescent, 0.8-1.0 cm long, greenish. Flowers ebracteate, bracteolate, green, brownish at the tip, about 0.1 cm, pedicellate, 1.5-2.5 cm long, glabrous, complete, actinomorphic, white, yellow at the base, 5-merous, epigynous. Calyx 5, fused, calyx tube 0.2-0.3 cm long adnate to the ovary, lobes lanceolate, 0.9-1.0 cm long, 0.2-0.3 cm, light green, pubescent, persistant. Corolla 5, free, petals obovate, glabrous, white with yellow at the base, 1.0-1.4 cm long, 1.0-1.2 cm wide, caducous. Stamens 5+5, biseriate, the outer seriate is shorter than the inner ones, the filaments of outer seriate is about 0.3 cm long and inner ones about 0.3-0.5 cm long, the anthers dithecous, basifixed, extrorse, about 0.2 cm in length on both series, yellowish brown, longitudinal dehiscence, styles simple, slender, 0.5-0.6 cm long, the stigmas capitate, styles and stigmas yellow in colour. Ovaries 5 carpels, fused, ovules many, one ovule in each loculous in transverse section, axile placentation, disk present, epigynous. Fruits capsules, 1.5-2.5 cm long, cylindrical, glabrous, brownish-yellow in colour, calyx persistent. The results were shown in Figures 1-16.

Flowering and fruiting period - throughout the year

Part used - the whole plant

Uses - leaf extract is taken for curing dysentery, whole plant is used as a poultice in ulcers and other skin diseases, extract of leaves and stem exert strong antimicrobial activity, petals possess anti-inflammatory activity

Morphological characters of *Jussiaea repens* L.

Figure 1. Habit with

Figure 2. Habit with

Figure 3. Pneumatophores

Figure 4. Upper surface of leaves

Figure 5. Lower surface of leaves

Figure 6. Stipule

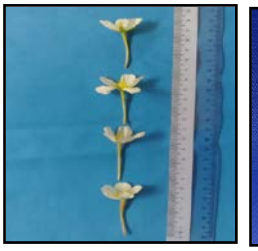


Figure 7. Inflorescence

Figure 8. Flowers

Figure 9. L.S flower

Figure 10. Bracteole

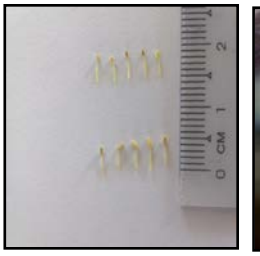


Figure 11. Two seriates of stamens

Figure 12. Pistil

Figure 13. Close up view

Figure 14. L.S

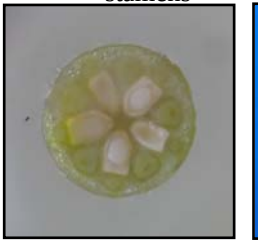


Figure 15. T.S

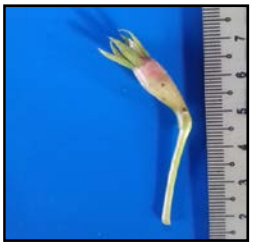


Figure 16. Fruit

Sensory characters of the powdered plant of *Jussiaea repens* L.

The sensory characters of the whole plant powder are brown in colour, undesirable odour, cooling taste and granular texture. The results were shown in Figure 17 and Table 1.

Figure. 17. Po

Table 1. The sensory characters of *Jussiaea repen* L.

Sr. No	Sensory characters	Powdered sample
1	Colour	Brown
2	Odour	Undesirable
3	Taste	Cooling
4	Texture	Granular, fibres

Antimicrobial activity

Antimicrobial activity of various solvent extracts of *Jussiaea repens* L.

In this experiment, the antimicrobial activities of crude extracts were carried out by using various solvent such as distilled water, acetone, chloroform, ethanol, ethyl-acetate, and methanol and petroleum ether. The control test of solvents on test organisms were also carried out.

Distilled water extract did not show activity on *Psedomonas fluorescens* and *Sacchromyces cerevisiae.* Acetone extract showed activity only on *Escherichia coli*. Chloroform extract did not show activity only on *Sacchromyces cerevisiae*. Ethanol extract did not show activity on *Escherichia coli* and *Sacchromyces cerevisiae*. Ethyl acetate extract showed activity on *Agrobacterium tumefaciens* and *Staphylococcus aureus*. Methanol extract did not show activity on *Agrobacterium tumefaciens* and *Sacchromyces cerevisiae*. Petroleum ether extract did not show activity on any tested organisms. Ethanol and methanol extracts showed maximum zone of inhibition for *Staphylococcus aureus*. The results were shown in Figure 18 and Table 2.

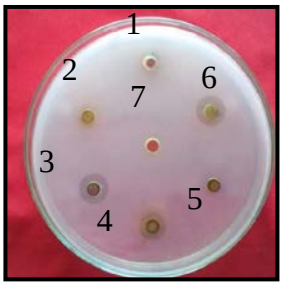
Table 2. Antimicrobial activity of various solvent extracts of *Jussiaea repens* L.

Solvents	Test organisms					
	Control	*Agrobacterium tumefaciens*	*Escherichia coli*	*Pseudomonas fluorescens*	*Staphylococcus aureus*	*Sacchromyces cerevisiae*
Distilled water	-	14 mm	25 mm	-	14 mm	-
Acetone	-	-	22 mm	-	-	-

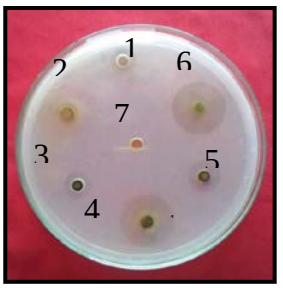
Solvents	Test organisms					
	Control	*Agrobacterium tumefaciens*	*Escherichia coli*	*Pseudomonas fluorescens*	*Staphylococcus aureus*	*Sacchromyces cerevisiae*
Chloroform	-	13 mm	15 mm	17 mm	16 mm	-
Ethanol	-	13 mm	-	15 mm	34 mm	-
Ethyl – acetate	-	19 mm	-	-	20 mm	-
Methanol	-	-	18 mm	17 mm	34 mm	-
Petroleum ether	-	-	-	-	-	-

Agrobacterium tumefaciens

Pseudomonas fluorescens



Escherichia coli



Staphylococcus aureus

Sacchromyces cerevisiae

1. Distilled water,
2. Acetone,
3. Chloroform,
4. Ethanol,
5. Ethyl acetate,
6. Methanol,
7. Petroleum ether

Figure 18. Antimicrobial activity of various solvent extracts of *Jussiaea repens* L.

Discussion and Conclusion

In this research, one of the traditional medicinal plants *Jussiaea repens* L. belongs to the family Onagraceae is presented. It is also known as Ye-kanyut, Kana-baw, Ye-hti-gayon in Myanmar and creeping water

primrose, floating primrose willow in English, recorded by Chakraborty *et al*., 2014; Ito *et al*., 2014 and websites 2, 3, 4.

In this research, morphological characters and antimicrobial activity on *Jussiaea repens* L. had been undertaken.

Jussiaea repens L. is a herbaceous perennial with floating stem, rooting at the nodes, floating stems are developed into absorbing downward growing roots and spongy upward growing roots (pneumatophores) in cluster at nodes of floating stem.

Leaves are simple, alternate, stipulate, petiolate, green, glabrous, blades are obovate to oblanceolate, narrowed to the petiole. Inflorescences are solitary and axillary, peduncle cylinder. Flowers are ebracteate, bracteolate, green, brownish at the tip, pedicellate, complete, actinomorphic, white, yellow at the base, epigynous. Calyx 5, fused, calyx-tube adnate to the ovary, pubescent, persistent. Corolla 5, free, petals obovate, glabrous, caducous.

Stamens 10, biseriate, the outer seriate is shorter than the inner ones, the anthers dithecous, basifixed, longitudinal dehiscence, style simple, slender, the stigmas capitate. Ovaries 5 carpels, fused, ovules many, one ovule in each loculous in transverse section, axile placentation, disk present, epigynous. Fruits capsules. Seeds many, quadrate, corky testa. The above characters are in accordance with those mentioned by Hooker, 1879; Cooke, 1903; Ridley, 1922; Hutchinson, 1926; Swingle, 1934; Gundersen, 1950; Kirtikar and Basu, 1958; Backer and Brink, 1963; Cronquist, 1981; Polunin and Stainton, 1984; Dassanayake, 1995; Truyen and Chan, 1999. Those data are mentioned in and recommended by references.

Cooke, 1903; Hutchinson, 1926; Gundersen, 1950; Kirtikar and Basu, 1958 reported that the stipules are absent. Polunin and Stainton, 1984 metioned that sepals and petals are 4 and free. Kirtikar and Basu, 1958; Polunin and Stainton, 1984 stated that stamens are 8, stigma 4-6 lobed.

From the results of antimicrobial activity of crude extracts were shown to be effective against all tested organisms. It was found that the control tests for antimicrobial activity did not show any effect on tested organisms. The antimicrobial activity of ethanol and methanol extracts showed the most effective activity against on *Staphylococcus aureus* in this study but Ahmed *et al*., 2005 stated that the methanolic extract of *Jussiaea*

repens L. showed a broad spectrum antibacterial activity against all the bacteria tested except *Staphylococcus aureus*.

This plant is used in the treatment of fever, intense coughing, dysentery, carbuncle, sprains, snake bites and skin complaints that occur mostly in Myanmar. The correct identification of this medicinal plant is essential for plant systematic and also useful for valuation of drugs.

To fulfill the requirements partially and share some information about this medicinal plant, this research has been conducted.

Therefore are should be taken when using and investigating medicinal plants by doing proper management, cultivation and analysis. As a result, our country will be developed in herbal medicine industry and many people will be benefited by using medicinal plants as effective drugs.

Acknowledgement

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Online Materials

1. https://en.wikipedia.org/wiki/Plant_morphology
2. http://www.stuartxchange.org/Sigangdagat.html
3. http://aquaplant.tamu.edu/plant-identification/alphabetical-index/water-primrose
4. http://www.missouriplants.com/Yellowalt/Jussiaea_repens_page.html

Phytochemical Investigation of Fruits of *Markhamia stipulata* (Wall.) Roxb. and Its Nutritional Values

Ohn Mar Than[1], Thandar Oo[2] & Phyo Phyo Win[3]

Abstract

The selected plant *Markhamia stipulata* (Wall.) Roxb. belongs to the family Bognoniaceae. It is also called Ma-hlwa. The plant is collected from Bago University campus. Fruits of *Markhamia stipulate* (Wall.) Roxb. exhibits many therapeutic activities. In this paper, phytochemical constituents and nutritional values of fruits of *Markhamia stipulata* (Wall.) Roxb. are presented. The purpose of this paper is to investigate the phytochemical constituents in the fruits of this plant, to study its nutritional values and to ascertain the local medicinal plant resources to be effectively used in health for Myanmar people. In phytochemical investigation, amino acids, saponins, starch, carbohydrate, glycosides and phenolic compounds were found in the parts of fruits. Nutritional values such as fats, proteins, fibres, and carbohydrates contents were detected on fruits of *Markhamia stipulate* (Wall.) Roxb .

Keywords: *Markhamia stipulata* (Wall.) Roxb., phytochemical constituents, nutrition, proteins, carbohydrates

Introduction

Markhamia stipulata (Wall.) Roxb. belongs to the family Bignoniaceae. It is native to South China and Southeast Asia. This species usually grows as a tall tree as in heigh of 5 to15 m. Flowers are pale yellow to dark yellow. Many herbal remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases. Young shoots, Flowers and fruits are used as a vegetable.

Markhamia (Seemann ex K. Schum) is a genus of flowering plants in the family Bignoniaceae with about 100 genera and 800 species. *Markhamia* has been reported among other genera of the family in Nigeria and 10 species are widely distributed in tropical Africa and Asia. Plants of this genus are trees or shrubs with opposite, compound

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imparipinnate leaves and yellow-green flowers grown mostly for social, agrihorticultural, and medicinal purposes.

Markhamia stipulata (Wall.) Roxb. is a usually deciduous, but occasionally evergreen tree growing 15 - 25 meters tall. The upright, cylindrical bole can be 60 - 80cm in diameter. The tree is harvested from the wild for local use as a food and timber; the young shoots are sold as a vegetable in the local markets of Laos. The plant is grown as a pioneer species in reforestation projects in Thailand.

They are mostly found in fringing forests and are drought-resistant. The roots, barks, stems, leaves and fruits of *Markhamia* species have been used by traditional healers for the treatment of miscellaneous disease conditions such as microbial and parasitic diseases, anemia, diarrhea, backache, sore eyes, intercostal pain, pulmonary troubles, gout, scrotal elephantiasis, rheumatoid arthritis, and external skin diseases. The plant has also been used in the treatment of diarrhea, dysentery, pain, and inflammation in veterinary patients.

Phytochemicals are defined as bioactive nutrient plant chemicals in fruits, vegetables, grains, and other plant foods that may provide desirable health benefits beyond basic nutrition to reduce the risk of major chronic diseases. Natural product compounds obtained from medicinal plants have been great contributions in the discovery of numerous clinically useful drugs. Markhamia species have been reportedly used by many cultures in human and veterinary traditional medicines.

Proteins act as enzymes, hormones, and antibodies. They maintain fluid balance and acid and base balance. They also transport substances such as oxygen, vitamins, and minerals to target cells throughout the body. Structural proteins, such as collagen and keratin, are responsible for the formation of bones, teeth, hair, and the outer layer of skin, and they help maintain the structure of blood vessels and other tissues. Leaves of *Paederia foetida* L. are also used as diet by local people so its' vitamin and protein contents were further investigated to record nutritional values of plant materials.

Materials and Methods

Preliminary Phytochemical Test

The fruits of *Markhamia stipulata* (Wall.) Roxb were freshly collected from Bago University Campus at 2019. The fruits were dried in shaded place and samples were washed and cut into small pieces, then air dried in room temperature. After that, dried specimens were crushed with grinding machine to get powder and stored in airtight container.

Powdered fruits of were tested for their chemical constituents such as reducing sugar, alkaloids, flavonoids, terpenoids and steroids, cyanogenetic glycosides, saponins, carbohydrates, tannins, starch, phenolic compounds and amino acids etc. The experimental procedure was prepared by the methods mention as in the methods of Marini-Beltole G.B., et al, (1981); Central Council for Research in Unani medicine, (1989); Trease and Evans, (2002).

Analysis of Nutritional Contents

The nutritional contents in the powdered fruits of *Markhamia stipulata* (Wall.) Roxb. were determined according to the procedure of Willian, 1980. The experiments were detected at the Ministry of Agriculture, Livestock and Irrigation, Small Scale Industries Department, Thudama Main Road, North Okkalapa 137 (A), Yangon, Myanmar. The percentage of moisture and ash contents, protein, fiber, fat and carbohydrate contents were detected.

Results

***Markhamia stipulata* (Wall.) Roxb.**

Scientific Name - *Markhamia stipulata* (Wall.) Roxb.

Family - Bignoniaceae

Myanmar Name - Ma-hlwa

Figure 1. Habit

Figure 2. Flowers

Figure 3 Close up view of flowers

Figure 4. Fruits

Figure 5. Various size of fruits

Phytochemical investigation of fruits of *Markhamia stipulata* (Wall.) Roxb

For preliminary phytochemical screening, powders were subjected to different qualitative chemical tests to determine the presence or absence of various phytochemical constituents. Reducing sugar, amino acids, saponins, starch, carbohydrates, glycosides, flavonoids, phenolic compounds, were observed. The experimental results were shown on the following table.

Table 1. Phytochemical tests of the fruits of *Markhamia stipulata* (Wall.) Roxb.

No	Test	Remark
1.	Alkaloids	-
2.	Amino acids	+
3.	Reducing sugar	+
4.	Saponins	+
5.	Steroid/ Terpenoid	-
6.	Starch	+
7.	Cyanogenic Glycosides	-
8.	Carbohydrates	+
9.	Glycosides	+
10.	Tannin	-
11.	Flavonoids	+
12.	Phenolic compound	+

Analysis of nutritional content

The determinations of percentages of the fats, proteins, fibres, and carbohydrates contents of powdered fruits of *Markhamia stipulata* (Wall.) Roxb. were carried out according to Willam, 1980 and Myanmar Traditional Medicine Formulary, 1989. As a result, it was found that fiber

and carbohydrate were present as major constituents in the samples of fruits.

Table 2. Analysis of nutritional content in fruits of *Markhamia stipulate* (Wall.) Roxb.

No.	Experiment	Nutritional value in fruits (%)
1	Moisture	7.52
2	Ash	4.18
3	Protein	14.29
4	Fiber	17.19
5	Fat	0.72
6	Carbohydrate	56.1

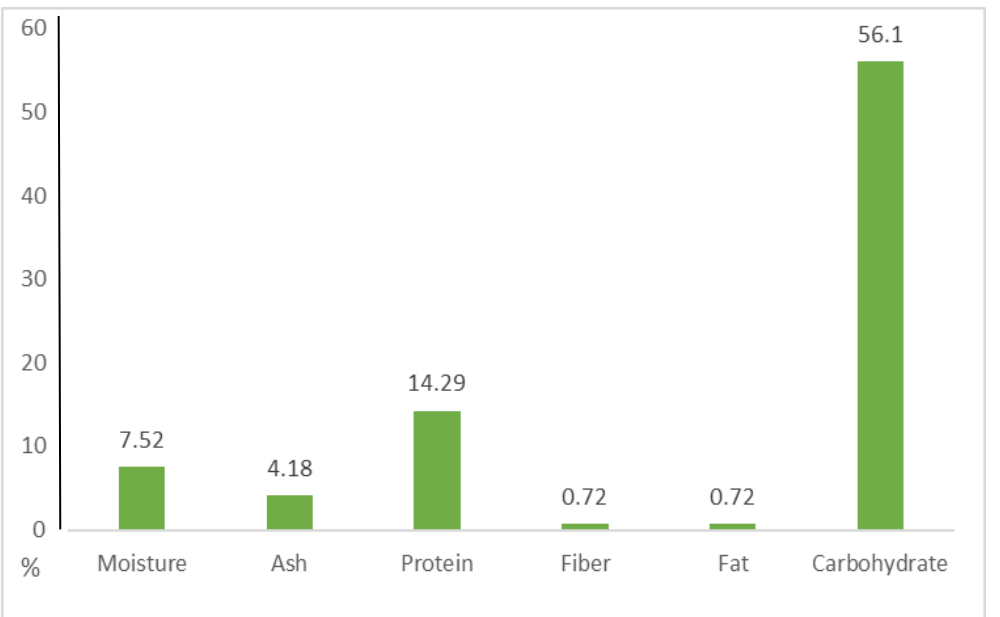

Figure 6. Nutritional values of fruits of *Markhamia stipulata* (Wall.) Roxb

Discussion

In this research, it consists of verified of fruits of *Markhamia stipulata* (Wall.) Roxb. by identification of plant, analysis of phytochemical constituents and nutritional values. Both vegetative and reproductive parts of the specimens were used for identification.

In phytochemical analysis, the preliminary phytochemical screening carried out on the fruits of *Markhamia stipulata* (Wall.) Roxb. The result showed that amino acid, saponin, starch, carbohydrate, glycosides and phenolic compounds were observed. These metabolites have been shown to be responsible for the therapeutic activity of plants.

Amino acids that regulate key metabolic pathways to improve health, survival, growth, development, lactation, and reproduction of organisms. (http://www.globinmed.com) Starch is a type of complex carbohydrate that undergoes several different steps during digestion. Eventually starch is broken down into glucose, which is the main source of fuel for all cells. Since carbohydrates, like starch, play such a big role in providing energy. www.livestron.com.

. Glycosides are found in the plant and they are used in the treatment of heart diseases (http://www. Greenpgarmacy.info). The present of secondary metabolite derived from fruits of *Markhamia stipulata* (Wall.) Roxb. which are used in medicinal purposes for human beings.

In nutritional values of *Markhamia stipulata* (Wall.) Roxb., moisture, ash, protein, fiber, fat and carbohydrate value were significantly found in fruits.

According to the results, carbohydrates are highest amount in the sample. It is principal source of energy for the cell. Carbohydrates are among the first products to arise as a result of photosynthesis. They constitute a large proportion of the plant biomass and are responsible, cellulose for the rigid cellular framework and as starch, for providing an important food reserve (Trease and Evans, 2002).

The content of fibers was also high in samples. Fiber helps regulate the body’s use of sugars, helping to keep hunger and blood sugar in check. Children and adults need at least 20 to 30 grams of fiber per day for good health, but most Americans get only about 15 grams a day. Great sources are whole fruits and vegetables, whole grains and beans. Fiber appears to

reduce the risk of developing various conditions, including heart disease, diabetes, diverticular disease and constipation. (https://britannica.com)

Proteins were present in fruits of *Markhamia stipulata* (Wall.) Roxb. Proteins are building blocks that grow and repair the body. Proteins are needed not only for muscle but also for hair, skin and internal organs. Some proteins travel around the body in the blood as hormones, enzymes and red blood cells. Protein is unique because it is only food sources of nitrogen, which is essential to all plants and animals' life.

Fats were least amounts in the samples. Low-fat diet, in which calories from fat sources are cut dramatically, where once considered the best way to body fat and lower the risk of heart disease and even cancer. Necessary nutritional values were required in diet to maintain good health. Hence, the results confirm the use of the plant in traditional medicine.

Conclusion

In conclusion, the results of the study indicated that *Markhamia stipulata* (Wall.) Roxb. may be used as medicinal plant because of the presence of bioactive secondary metabolites in the plant. The medicinal plant, *Markhamia stipulata* (Wall.) Roxb. possesses many medicinal and nutritional values as protein, fat, fiber and carbohydrate for the human health. Local people use this plant by eating fruits as a vegetable. So, *Markhamia stipulata* (Wall.) Roxb. is saves to be used as both medicinal plants and nutritious vegetable. Therefore, The medicinal plant *Markhamia stipulata* (Wall.) Roxb. can be used as medicine not only for folkloric use but also widely used for traditional medicine.

Acknowledgement

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Investigation of Morphological, Histological Characters and Phytochemical Constituents from the Leaves of *Alpinia galanga* (L.) Willd.

Ohn Mar[1] & Thandar Soe[2]

Abstract

Alpinia galanga (L.) Willd. is locally known as Padegaw-gyi and belongs to the family Zingiberaceae. The plant was collected from Kamyawkin village, Launglon Township, Dawei District, Tanintharyi Region from June to October, 2019. *Alpinia galanga* (L.) Willd. has been chosen in this research because which is an important medicinal plant with diversed pharmacological and phytochemical properties. In this study, the morphological, histological characters and preliminary phytochemical tests from the leaves of *Alpinia galanga* (L.) Willd. were undertaken. Identification of collected plant is carried out by using available literatures. In morphological study, the plant is perennial rhizomatous herb. Leaves are alternate. Inflorescences terminal panicles, flowers are bisexual, zygomorphic, trimerous, epigynous; ovary tricarpellary, syncarpous, axile placentation, inferior. Fruit capsule, globose to ellipsoid. Seed ovoid, black, strongly aromatic. In histological study, tetracytic stomata are present on both surfaces of lamina. In transverse section of lamina, only one layer of hypodermis is present below adaxial surface. In the transverse sections of lamina, midrib and petiole, the main vascular bundles are collateral and closed type. Calcium oxalate crystals are found in the surface view of lower epidermal cells of lamina, midrib and petiole. Oil cells are present in the petiole. The dried powder of leaves has been examined to be used as diagnostic characters in standardization for medicinal purposes. Preliminary phytochemical tests showed the presence of alkaloid, ∝- amino acids, carbohydrate, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, starch, tannin, steroid and terpenoid in leaves of *Alpinia galanga* (L.) Willd.

Keywords: *Alpinia galanga* (L.) Willd., Morphology, Histology, Phytochemical test

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Introduction

Plant and plant products are being used as a source of medicine since long. *Alpinia galanga* (L.) Willd. is used in medication, culinary and cosmetic for centuries. It is widely used in dietary intake as well as in traditional system of medicine, viz. Ayurveda, Unani, Chinese and Thai folk medicine. It is pungent, hot and spicy taste with an aromatic ginger like odour. (Chudiwal *et al*., 2010).

Alpinia galanga (L.) Willd. belongs to the family Zingiberaceae which consisted of 47 genera and 1400 species. The members of this family are distributed throughout the eastern hemisphere particularly in the Indo-Malayan area (Datta, 1970).

Alpinia galanga (L.) Willd. is native to South Asia and Indonesia and cultivated in Malaysia, Laos and Thailand. It is a species of ginger that occur in tropical Asia. *Alpinia galanga* (L.) Willd. is locally known as Padegaw-gyi in Myanmar , Greater galangal in English, Lengkuas in Malay, Hong doukou in Chinese, Arattai in Tamil and Kha in Thai (Subramanian and Nishan, 2015).

Alpinia galanga contained many flavonoids and a wide range of volatile oils. It is used traditionally for the treatment of eczema, bronchitis, coryza, pityriasis, otitis interna, gastritis, ulcers, cholera and to clean the mouth, stimulates the digestive power, appetite and as a purgative. The different parts of the plant possessed many pharmacological effects including antibacterial, antifungal, antiviral, antiprotozoal, anti-oxidant effects, antidiabetic, antiplatelet and many other pharmacological effects (Ali, 2014).

Subash, 2012 stated that phytochemical tests of *Alpinia galanga* were done to detect the presence of secondary metabolites, such as alkaloids, tannins, saponins, resins, flavonoids, steroid, glycosides and terpenoids in the plant under investigation.

In this research, morphological and histological characters of fresh specimens and phytochemical constitutents from leaves of *Alpinia galanga* (L.) Willd. was carried out. The aim and objectives of this research to identify and classify the morphological characters of *Alpinia galanga* (L.) Willd., to examine the histological characters of leaves, to investigate the

diagnostic characters of powdered samples for the standardization of traditional medicine and to determine the preliminary phytochemical constituents from leaves of *Alpinia galanga* (L.) Willd.

Materials and Methods

Morphological characters of *Alpinia galanga* (L.) Willd.

The specimens of *Alpinia galanga* (L.) Willd. were collected from Kamyawkin village, Launglon Township, Dawei District, Tanintharyi Region from June to October, 2019. The collected specimens were made careful notes and recorded by taking photographs to classify and identify it systematically. The morphological study of plant was undertaken with the help of available literatures (Hooker, 1894; Cooke, 1958; Kirtikar and Basu, 1975 and Dassanayake, 1983).

Histological study of *Alpinia galanga* (L.) Willd.

The histological studies, free hand section of lamina, midrib and petiole from the fresh specimens were prepared by using chloral hydrate solution for clearing reagents, safranin solution for testing lignin and concentrated Sulphuric acid for testing calcium oxalate crystals. These characters were determined according to the literatures of Esau, 1953; Wallis, 1967; Tomlinson, 1969; Hussin, *et al*., 1999 and Trease and Evans, 2002.

Preliminary phytochemical investigation of *Alpinia galanga* (L.) Willd

Preliminary phytochemical tests was carried out according to the methods of British Pharmacopoeia, 1968; Marini Bettolo *et al*, 1981; Central Council for Research in Unani Medicine, 1987 and Trease and Evans, 2002.

Results

Morphological characters of *Alpinia galanga* (L.) Willd.

Perennial herbs, underground aromatic rhizome, 2.0-3.0 m high, stem cylindrical, green, stout, glabrous. Leaves alternate, distichous, simple, oblong-lanceolate, green, 16.5-60.0 × 3.8-15.0 cm, the tips acuminate and cristate, the margins entire, the bases cuneate, both surfaces glabrous; petioles cylindrical, sheathing petioles green, glabrous; ligule green;

exstipulate. Inflorescences terminal panicles, erect, many branched, densely pubescent, peduncles cylindrical, pubescent, upper peduncular bracts and lower peduncular bracts enveloped the whole inflorescences in budding time. Flower white, fragrant; bracts ovate; pedicels cylindrical, pubescent; bracteoles white, ovate; calyx of 3-sepals, fused, the calyx tubes tubular, greenish white, the calyx lobes acute, valvate, the inner surfaces glabrous and the outer surfaces pubescent, persistent; corolla of 3-petals, fused, the corolla tubes tubular, greenish white, the corolla lobe unequal, linear-oblong, greenish white, imbricate, posterior lobe is larger than others, the inner surfaces glabrous and the outer surfaces pubescent; stamens one fertile, lateral staminodes absent, other staminodes fused to form a labellum, petalostemonous, the filament of fertile stamen grooved, inserted, pubescent, the anthers dithecous, oblong, incurved, thick, white, basifixed, longitudinal dehiscences, introrse, the labellum spathulate with bifid tips, white, purple stripes present at the base of lateral veins, distinctly clawed, a pair of subulate glands present at the base of the claw, reddish brown; ovary inferior, oblongoid, greenish white, tricarpellary, syncarpous, trilocular, the placentation axile, 2-4 ovules in each locule, pubescent, the style terminal and filiform, inserted within the grooved filament and extending between two anther lobes and protruding out as a stigma, pubescent, the stigma turbinate and ciliate, the stylodes (disc) 2, obtuse. Fruit capsule globose to ellipsoid, green, orange-red when mature, indehiscent, crowned by persistent calyx, glabrous. Seed ovoid, black, strongly aromatic, surrounded by thin arils, glabrous. The results are shown in Figures (1 to 8).

Morphological characters of *Alpinia galanga* (L.) Willd.

Figure 1. Habit

Figure 2. Inflorescence

Figure 3. Flower

Figure 4. L.S of flower

Figure 5. T.S of ovary

Figure 6. L.S of ovary and discs

Figure 7. Fruits

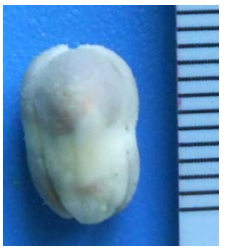

Figure 8. Seeds

Histological characters of leaves of *Alpinia galanga* (L.) Willd.

Lamina

In surface view, the cuticle is thin and smooth. The anticlinal wall in the epidermal cells of both surfaces is straight. The cells are polygonal in shape, thin-walled, parenchymatous. Tetracytic stomata are present on both surfaces but more abundant on lower surface. The stomata are oval in outline with two reniform-shaped guard cells and contain abundant chloroplasts. Prismatic calcium oxalate crystals and silica are present in lower epidermis. (Figures 9 to 12). In transverse section of lamina, cuticle layers are thin and smooth on both surfaces. Epidermal cells are barrel shaped and thin-walled. Only One layer of hypodermis is present below adaxial surface. These cells are isodiametric in shape. Palisade mesophyll cells are one-two layers. These cells are cylindrical vertically narrow and elongated in shape, tightly packed with one another and contained many chloroplasts. The spongy mesophyll cells are 2-5 layers, the cells loosely arranged, irregular in shape, with thin-walled parenchymatous cells and intercellular spaces. Vascular bundles are embedded in the mesophyll cells.

They are collateral and closed type. The phloem tissue composed of sieve tube elements, companion cells, phloem fibre and phloem parenchyma. The xylem composed of vessels, tracheids, fibres and xylem parenchyma. (Figures 13 to 14).

Midrib

In transverse section of the midrib, both upper and lower epidermal cells are barrel shaped, thin-walled parenchymatous cells. Fibre-like cells are present below adaxial epidermis and above abaxial epidermis. Below the upper epidermis, collenchymatous cells are 1-3 layers and above the lower epidermis, collenchymatous cells are 1-2 layers, irregular in shaped. 2-12 layers of parenchymatous cells are found above the main vascular bundles. These cells are irregular and polygonal in shape and thin walled. The main vascular bundles (Main arc I) form a single conspicuous abaxial arc, alternating with air canals and embedded in chlorenchyma. The adaxial conducting system (Centre arc III) consists of 6-8 vascular bundles that are similar in appearance to the main vascular bundles but are smaller in size. The main vascular bundles are furnished with a massive fibrous or sclerenchymatous sheath above the xylem and below the phloem. Vascular bundles are collateral and closed type. Xylem composed of vessels, tracheids, fibres and xylem parenchyma. The phloem tissue composed of sieve tube elements, companion cells, phloem fibre and phloem parenchyma. Prismatic calcium oxalate crystals and silica are scattered in the cortex. (Figures 15 to 17).

Petiole

In transverse section, the cuticle layer is thin-walled. The epidermal cells of adaxial surfaces are barrel shaped and those of abaxial surfaces are rounded in shaped and thin-walled. In adaxial region, the collenchymatous tissue consists of 2-5 layers and 2-9 layers in abaxial region, irregular or polygonal in shaped. 4-20 layers of parenchymatous cells are found above the main vascular bundles. These cells are irregular and polygonal in shape and thin walled. The main vascular bundles (Main arc I) consists of 15-18 vascular bundles that form a single conspicuous abaxial arc, alternating with air canals and embedded in chlorenchyma. The abaxial conducting system (Lower arc II) consists of an arc of vascular bundles that are circular in outline. The adaxial conducting system (Centre arc III) consists of 10-14

vascular bundles that are similar in appearance to the main vascular bundles but are smaller in size. Upper Arc IV closer to the adaxial epidermis and consists of 13-15 vascular bundles, irregularly arranged. Upper arc IV bundles often consisting of few phloem cells completely surrounded by fibres or entirely consisting of fibres only. The main vascular bundles are furnished with a massive fibrous or sclerenchymatous sheath above the xylem and below the phloem. Air lacunae are usually found between Main arc I bundles. Vascular bundles are collateral and closed type. Fibre cells usually form abaxial and adaxial caps, extending to abaxial epidermal cells. Xylem composed of vessels, tracheids, fibres and xylem parenchyma. The phloem tissue composed of sieve tube elements, companion cells, phloem fibre and phloem parenchyma. Prismatic calcium oxalate crystals, silica and oil cells are scattered in the cortex (Figures 18 to 20).

Diagnostic characters of powdered leaves of *Alpinia galanga* (L.) Willd.

The powdered leaves of *Alpinia galanga* (L.) Willd. was green coloured and strong aromatic odor. It was spicy or pungent and fibrous in texture. It consists of fragment of lower epidermal cells with stomata, pitted vessel, tracheids, and fibre as shown in Figures (21 to 24). The sensory characters of powder leaves of *Alpinia galanga* (L.) Willd. as shown in Table (1).

Table 1. Sensory Characters of powder leaves of *Alpinia galanga* (L.) Willd.

Characters	Leaves
Colour	Green
Odour	strong aromatic odour
Taste	spicy or pungent
Texture	Fibrous

Histological characters of leaves of *Alpinia galanga* (L.) Willd.

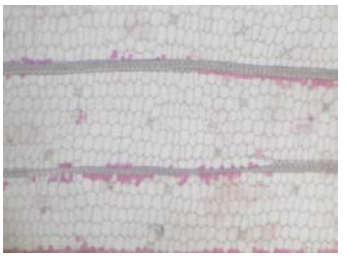

Figure 9. Surface view of upper epidermal cells of lamina (×100)

Figure 10. Close up view of tetracytic stomata (x400)

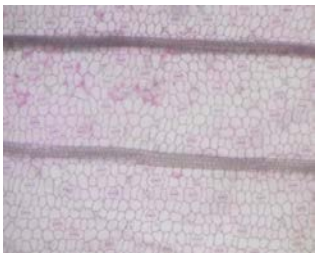

Figure 11. Surface view of lower epidermal cells of lamina (×100)

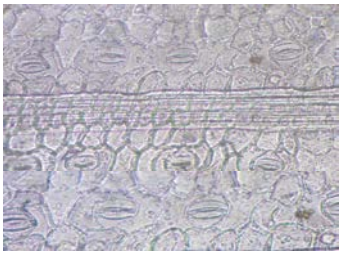

Figure 12. Close up view of tetracytic stomata, prismatic calcium oxalate crystals and silica (x400)

Figure 13. T.S of lamina (×40)

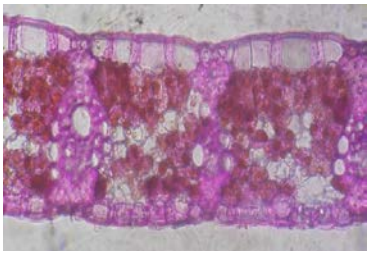

Figure 14. T.S of lamina showing vascular bundles in detail (×400)

Figure 15. T.S of midrib (×40)

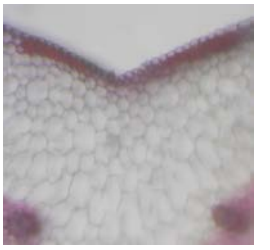

Figure 16. Adaxial portion of midrib showing cortex layer and Centre arc III vascular bundles (x200)

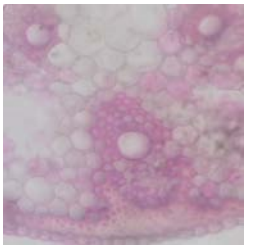

Figure 17. Abaxial portion of midrib showing vascular bundles (Main Arc I) and prismatic calcium oxalate crystals (x200)

Figure 18. T.S of petiole (×40)

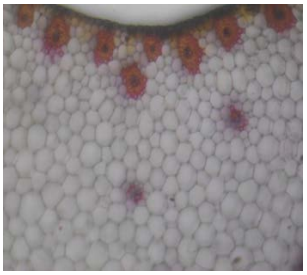

Figure 19. Adaxial portion of petiole showing cortex layer and vascular bundles (Centre arc III and Upper arc IV) (×200)

Figure 20. Abaxial portion of petiole showing vascular bundles (Main arc I, Lower arc II, Centre arc III), oil cell and prismatic

Diagnostic Characters of Powdered Leaves of *Alpinia galanga* (L.) Willd.

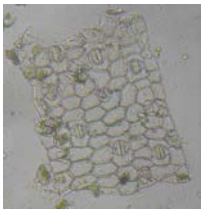

Figure 21. Fragment of epidermal cell with stomata (×400)

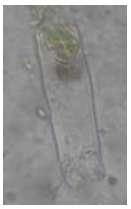

Figure 22. Pitted vessel (×400)

Figure 23. Tracheid (×400)

Figure 24. Fibre (×400)

Phytochemical investigation of the leaves of *Alpinia galanga* (L.) Willd.

Preliminary phytochemical tests indicated the presence of alkaloid, ∝- amino acids, carbohydrate, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, starch, tannin, steroid and terpenoid. The experimental results were shown in Table (2).

Table 2. The results of Phytochemical test from leaves of *Alpinia galanga*

No	Test	Extract	Test reagents	Observation	Results
1.	Alkaloid	1%HCL	(1) Mayer's Reagent (2) Wagner's Reagent (3) Dragendroff's Reagent	White ppt. Reddish brown ppt. Orange ppt.	+ + +
2.	∝- amino acids	H_2O	Ninhydrin reagent	Pinkish purple	+
3.	Carbohydrate	H_2O	10% ∝-napthol + conc: H_2SO_4	Red ring	+
4.	Starch	H_2O	Iodine solution	Blue black	+
5.	Reducing sugar	H_2O	Benedict's solution	Brick red ppt.	+
6	Glycoside	H_2O	10% Lead acetate solution	White ppt.	+
7.	Phenolic compound	HCl /Mg	3% $FeCl_3$	Deep blue or black	+
8.	Saponin	H_2O	Distilled water	Frothing	+
9.	Tannin	H_2O	1% $FeCl_3$ solution	Bluish black	+
10.	Flavonoid	EtOH	HCl / Mg	Pink colour	+
11.	Steroid	P.E	Acetic anhydride + conc: H_2SO_4	Green colour	+
12.	Terpenoid	P.E	Acetic anhydride + conc: H_2SO_4	Pink colour	+

(+) = Present, ppt = Precipitate

Discussion and Conclusion

In this research, the morphological studies on both vegetative and reproductive parts of the plants, microscopical examination and phytochemical invesgation of leaves of *Alpinia galanga* (L.) Willd. have been undertaken. Powder of leaves are also studied.

In morphological study, *Alpinia galanga* (L.) Willd. is perennial rhizomatous herbs. Leaves alternate, oblong-lanceolate, Inflorescences terminal panicles, erect, many branched, densely pubescent. Flower white, fragrant, calyx of 3-sepals, fused, the calyx tubes tubular, greenish white, corolla of 3-petals, fused, the corolla tubes tubular, greenish white, the corolla lobe unequal, linear-oblong, greenish white, stamens one fertile, other staminodes fused to form a labellum, petalostemonous, the labellum spathulate with bifid tips, white, purple stripes present at the base of lateral veins, a pair of subulate glands present at the base of the claw, reddish brown, ovary inferior, oblongoid, tricarpellary, syncarpous, trilocular, the placentation axile, the style terminal and filiform, the stylodes 2, obtuse. Fruit capsule globose to ellipsoid, green, orange-red when mature. Seed 3-5 seeded, ovoid, black, surrounded by thin arils. These characters are in agreement with those described by Hooker, 1894; Cooke, 1938; Kirtikar and Basu, 1975 and Dassanayake, 1983.

In histological study of the surface view of lamina, the epidermal cells of anticlinal walls are straight and cells are polygonal shaped, tetracytic stomata are found in both the surfaces of lamina. Prismatic calcium oxalate crystals are also found in the lower surface of lamina. These characters are agreement with those describe by Hussin, *et al*., 1999. In transverse section of lamina, Only One layer of hypodermis is present below adaxial surface. These characters are agreement with those described by Tomolinson, 1969.

In transverse section of the midrib and petiole, fibre cells usually form abaxial and adaxial caps, extending to abaxial epidermal cells. Fibre cells from both caps are well developed. These characters are agreement with those described by Hussin, *et al*., 1999.

The vascular bundles of midrib consist of Main Arc I and Centre arc III. But those of petiole consist of Main arcs I, Lower arc II, Centre arc III and Upper arc IV. Upper arc IV bundles often consisting of few phloem cells completely surrounded by fibres or entirely consisting of fibres only.

Air lacunae are usually found between Main arc I bundles. Abaxial fibre caps touch abaxial epidermis, continuing as a single layer below it. Prismatic calcium oxalate crystals, silica and oil cells are scattered in the cortex of petiole. These characters are in agreement with those described by Tomolinson (1956) and Hussin, *et al*., (1999).

The powdered leaves of *Alpinia galanga* (L.) Willd. was green coloured and pleasant and strong aromatic odour. It was spicy or pungent and fibrous in texture. These characters are in agreement with those described by Chudiwal *et al*., 2010.

Preliminary phytochemical analysis revealed the presence of various constituents such as alkaloid, ∝- amino acids, carbohydrate, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, starch, tannin, steroid and terpenoid. These characters are in agreement with those described by Subash, 2012.

In conclusion, this plant was observed in morphological and histological characters and phytochemical points of view in this research. The morphological and histological characters for every plants of parts used must be analyzed for the standardization of drugs. It is hope that these finding will be useful in species confirmation. In addition, this research will provide the requirements of valuable taxonomic information on *Alpinia galanga* (L.) Willd. for further researchers. The types of stomata in lamina and arrangement of vascular bundles in midrib and petiole will provide the useful for the diagnostic characters of identification of this plant. For further research programme, pharmacological activities should be carried out concerning *Alpinia galanga* (L.) Willd. possess many medicinal values and other bioactive compounds should be isolated from plants parts.

Acknowledgements

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Study on Some Wild Orchids in Mawlamyine University Campus

Si Si Thein[1], Marlar Aung[2], Sagawa Wynn[3] & Myat Myat Phyo[4]

Abstract

Some members orchidaceae of Mawlamyine University Campus were collected in one season within the year 2018 to 2019. The collection periods covered all seasonal periods. In the present research a study on the comparative morphology of the (6) species, and (6) genera of the orchids have been undertaken. Taxonomic description of genera and species were accompanied by habit, longitudinal sections of the flowers. Easy identification important floral parts such as (labellums, anther caps, stigmatic surface, in some plants) had been respectively. Among them, 3 species of orchids were epiphytes and 3 terrestrial orchids were recorded. All the orchids were presented with color photographs and the detail morphological description.

Keywords: orchids, epiphytes, terrestrial

Introduction

The Orchidaceae are the third or possibly the second largest plant family. Estimates of species numbers vary from 5,000 to 18,000 but 10,000 to 12,000 may be correct. The orchidaceae are a diverse and widespread family of flowering plants, with blooms that are often colourful and fragrant, commonly known as the orchid family. Orchidaceae is one of the largest families of flowering plants, and many of its species are highly valuable as herbal medicines and to the horticultural industry. Common orchids are occurring especially in the southern portions of the continent. Other genera and species occur in the southern United States, and various tropical and subtropical genera and species range into southern Florida (Heywood, 1970).

Orchids attain their greatest development in the tropical rain forest. Much of the jungle has relatively little undergrowth because of the dense shade. The trees standing high above the bare forest floor are covered with scrambling lianas or twining woody vines. Innumerable small plants are

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perched on the upper branches of the trees and in the mass of vines overhead. These epiphytes (plants living upon others plants) include many species of orchids abundant in the humid jungle, and some of the epiphytic orchids and other plants have aerial roots which catch and absorb water from the frequent rain.

Fortunately, Myanmar with a wide range of luxuriant forest vegetations is one of the truely original centers of the world's most interested wild and rare orchids. An attempt has thus been made to study in detail on the morphology of some of the Mon State orchids. Mon State lies between latitude 14° 51' N and 17° 34'N and longitude 96° 53' E and 98° 12' E. It is a coastal region fringed with islands. The more famous islands are balukyun, hinthakyun and kalagokkyun.

In this dissertation, family characters of orchidaceae, comparative morphology of the (6) species, discussion and conclusion are also included. Color photographic plates to show the habit and inflorescence. The aims of the research paper, to study the habit of wild orchids, morphological characters of classification and identification and to know about highly specialized floral structure of orchid flower.

Materials and Methods

Some members of Orchidaceae were collected from Mawlamyine University Campus, the plants are naturally. Field collection was carried out form March 2018 to March 2019. Morphology of collected specimens was studied in the Department of Botany, Mawlamyine University. The family was identified by using taxonomy of Vascular Plants, Lawrence, (1951). Identification of genera and species were carried out by using List of Trees, Shrubs, Herbs and Principal Climbers, ect., Hundley and Chit Ko Ko (1961); Flora and Java, Backer, (1963); Flora of British India, Hooker, (1975); Indian Medicinal Plants, Kirtikar, (1993); Laboratory Manual of Plant Taxonomy, Subrahmanyam (1996), and A checklist of Trees, Shrubs, Herbs, and Climbers of Myanmar, Kress, (2003), Photographs were prepared for each specimen.

Result

***Arundina graminifolia* (Don.)**

Scientific Classification

Kingdom:	Plantae
(Unranked):	Angiosperms
(Unranked):	Monocots
Order:	Asparagales
Family:	Orchidaceae
Subfamily:	Epidendroideae
Tribe:	Arethuseae
Subtribe:	Bletiinae
Genus:	*Arundina*
Species:	*A.graminifolia*

Generic Description

Sympodial terrestrial, erect, evergreen, high, the roots long, cylindrical, thick, fleshy, glabrous, the stems long, teeter, green, nodes as many as leaves, covered with over-lapping leaf sheaths, glabrous. Leaves alternating on opposite side of the stem, simple, 2-ranked, the margins entire, petioles forming sheaths, encircling the internodes, leaving the carious sheaths when old, glabrous. Inflorescences are terminal racemes, many-flowered, peduncle bracts 2-5, lanceolate to ovate, encircling the peduncles, glabrous. Flowers large, resupinate, pale rosy-mauve, bisexual, zygomorphic, trimerous, epigenous, slightly fleshy and glistening, fragrant; floral green, glabrous; pedicels cylindrical, green, glabrous; sepals-3, free, equal, pale rosy-mauve, reserved, usually closely together behind the lip, both surfaces glabrous; petals-3, 2-lateral petals free, equal, broader, the labellum 3-lobed open, trumpet-shaped, terminal lobe emarginated with margin, the labellum usually has a bright rosy-purple end, paler to the throat with purple veins, yellow patch present at the middle, the apex deeply cleft and hairy, with three thin longitudinal keels, the lateral lobes obtuse, reddish purple, convolute, enclosing the column, glabrous, the spur absent; column slender, pale purple, narrowly the top, the anther caps quadrangular

white, yellow, the pairs with their turned stigmatic surface 3-lobed, 2-lateral lobes similar and concave, pale purple, ovary inferiors, 3-carpellate, unilocular, the placentation parietal, numerous ovules in the locule. Fruits capsules, oblongoid, green, dehiscent by 6-longitudinal fissures, glabrous. Seeds are narrowly fusiform, white, glabrous.

Found growing in lowland and mountains, always in open sunny places of the forest and cultivated areas. Flowering and fruiting the from August to September.

Vernacular name : Taung-kyu-pan, wa thitkwa, bamboo orchids.

Area : No.3, Main Road, Mawlamyine University Campus

Habit **Flower** **Inflorescence** **L.S of Flower** **Labellum** **Gynostegium** **Pollen cap**

Figure 1. *Arundina graminifolia* (Don.)

Phaius tankervilleae Blume

Scientific Classification

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Monocots
Order:	Asparagales
Family:	Orchidaceae
Subfamily:	Epidendroideae
Genus:	*Phaius*
Species:	***P. tankervilleae***

Generic Description

Sympodial terrestrials, large, consisting of several internodes, erect, the roots long, cylindrical, glabrous, the stems pseudobulbs, large and thick, oval, enveloped by leaf sheaths, glabrous. Leaves alternate, simple, elliptic-oblong, bright green, borne on a short thick green pseudobulbs, leaves; petioles forming sheaths, encircling the pseudobulb, glabrous. Inflorescences one or more racemes, arising from below the leaves, many-flowered, erect, together, the peduncles erect, peduncular bracts 6 – 10, cymbiform, ovate-oblong, pale green, glabrous. Flowers large, resupinate, pale brownish yellow green, fleshy, fragrant; floral bracts cymbiform, green, the tips acute, the margins entire, pedicels slender, greenish white, glabrous; sepals-3, free, equal, oblong lanceolate, the lower surface white and the upper surface brownish yellow-green, 2-lateral sepals placed under the lip, both surfaces glabrous; petals-3-6, 2-lateral petals free, equal, white on the outside and brownish yellow green on the inside, the labellum 3-lobed open , trumpet-shaped, the edges of the apical part crisped and recurved, the midlobe broadly, blade of lop red inside, mainly whitish outside, base, the upper surfaces with longitudinal-3 ridges, the lateral lobes purple, convolute, enclosed the column, glabrous, the bright yellow, column slender and stout, cream coloured, about long, white hairy; anther 2-celled, the anther caps quadrangular , white, at hairy; yellow, stigmatic surface 3-lobed, 2-lateral lobes similar and concave, cream colored, rostellum small and beak like, ovary inferior 3-carpellate, unilocular, the placenta swollen, glabrous. Fruits are not available.

Found growing in shady primitive forest and cultivated for their showy flowers. Flowering time from March to April.

Vernacular name : Myaysite Thit Kwa, Gamon-zedi gyi.

Area : Tharaphi Hall, Mawlamyine University Campus

Habit **Inflorescence** **L.S of Flower** **Labellum** **Gynostegium** **Pollen cap**

Figure 2. *Phaius tankervilleae* Blume

Spathoglottis plicata Blume

Scientific Classification

Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Monocots
Order:	Asparagales
Family:	Orchidaceae
Subfamily:	Epidendroideae
Tribe:	Epidendreae
Genus:	*Spathoglottis*
Species:	*S.plicata*

Generic Description

Sympodial terrestrials, erect, evergreen, the roots abundant, long, cylindrical, thick, fleshy, the stems pseudobulbs, ovoid, on a creeping rhizome, clustered, glabrous. Leaves alternate, simple, narrowly lanceolate, bright green, the tips finely acuminate, the margins entire, petioles forming sheets, encircling, the pseudobulbs, glabrous. Inflorescences lateral racemes long and many-flowered, erect, showy, arises from the axil of the basal leaves, persistent. Flowers Large, purple, fleshy and fragrant; floral bracts ovate, pale purple, pedicels slender, purple, white hairy; sepals-3, free, equal, ovate-oblong, purple, spreading, 2-lateral sepals usually spreading widely, the petals-3 , 2-lateral petals free, equal, oval-oblong, purple, both surfaces glabrous, the labellum open; the widen at the apex, purple, the tips 2-lobed, the margins entire, larger than the lateral lobes, the claw narrow, with thickening, 2-small ovoid, a yellow with purple spots and white hairy, with the lateral lobes oblong, purple, curved upwards, glabrous; column slender, curved, pale purple, 2- short the ventral margin, broadly ovate tip, anthers 2-celled, the purple, convex at the pollinia location, attached to the process of rostellum, glabrous; in two groups of four, yellow, stigmatic surface 3-lobed, 2-lateral lobes similar and concave, pale purple, glabrous; rostellum small acute, purple, ovary inferior, oblong, 3-carplelate, unilocular, the placentation parietal, numerous ovules in the locule, the

placenta swollen, hairy. Fruits capsules slender, oblongoid, drooping, dehiscent by longitudinal fissures. Seeds are rod-shaped and curved, white, glabrous.

Found growing in semi shady area and cultivated for their showy flowers. Flowering and fruiting time from July to August.

Vernacular name : Mhaysite thitkwa, Guround orchids.

Area : No.1, Staff House, Mawlamyine University Campus

Figure 3. *Spathoglottis Plicata* Blume

Generic Description

Sympodial epiphytes, erect and evergreen, the roots abundant, long, cylindrical, cinereous, in diameter, glabrous, the stems pseudobulbs, many-leaved pseudobulbs, hidden by the leaf-sheaths, leaves alternate, simple, narrowly oblong, bright green, the tips refuse, the petioles forming sheaths encircling the pseudobulbs, persistent. Inflorescences elongate racemes, arises from the base of the pseudobulbs, many-flowered, opening in succession, the peduncles cylindrical. Flowers medium-sized, yellow with reddish brown, fleshy and fragrant; floral bracts ovate, yellow with the bases reddish brown, sepals-3, free, equal, narrow, elliptic-oblong, reddish brown in middle part and yellow along the margins, both glabrous; petals-3, 2-lateral petals free, equal, elliptic-oblong, reddish brown in middle part and yellow along the margins entire, both surfaces glabrous; the labellum 3-lobed open, midlobe obovate downwards, yellow with reddish brown speckles, the tips acute, the margins entire, the spur absent; column very slender, the inner surface reddish brown, the tips 2-lobed, anthers 2-celled, orange, convex at the pollinia location, pollinia-2 , preform on a short stipe, subquadrate gland, yellow, the stipes short, white; stigmatic surface 3-lobed, 2-lateral lobes similar and concave, yellow, rostellum small, obtuse,

ovary inferior, oblong, 3-carpellate, unilocular, the placentation parietal, numerous ovules in the locule. Fruits capsules, oblongoid, brownish green with marron spots, rounded triangular; Seeds minute, subglobose, white, glabrous.

Found growing on the highest trees and semi-shady areas. Flowering and fruiting time from April to July.

Vernacular name : Pan-the she-nyo.

Area : No.7, Main Road, Mawlamyine University Campus

Habit **Inflorescence** **Flower** **L.S of flower** **Labellum** **Gynostegium** **Pollen cap**

Figure 4. *Cymbidium aloifolum* (L)

Dendrobium pulchellum Roxb.

Scientific Classification

Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Monocots
Order:	Asparagales
Family:	Orchidaceae
Subfamily:	Epidendroideae
Tribe:	Podochilaeae
Subtribe:	Dendrobiinae
Genus:	*Dendrobium*
Species:	*D. pulchellum*

Generic Description

Sympodial epiphytes, erect, the roots long, cylindrical, cinereous, thick, fleshy, the stems long and teeter, the old stems purplish, erect, encircling by the leaf sheaths, several internodes glabrous. Leaves alternates on opposite side of the stems, simple, oblong-lanceolate, petioles forming sheaths encircling the internodes, leaf glabrous. Inflorescences lateral raceme, pendulous with several flowers, the cylindrical, penduncular bracts 4 to 6, oblong, purple. Flowers large, pale yellow, fragrant; floral bracts small, ovate, purple, pedicels slender, pale yellow, sepals-3, dorsal pale pinkish yellow, erect, 2-lateral sepals equal, elliptic-oblong, pale pinkish yellow the tips acute, the entire, the labellum one-lobed open, pale pinkish yellow, the tips slightly acute and recurved, the margins fringed, concave, very hairy within, with two large maroon patches at the auricular sides and strap like middle keel purple; funnel-shaped, pinkish pale yellow, column oblong, cream colour, folded, cream colour and inner surface purple, the base 2-lobed, anther 2-celled, white, convex at the pollinia location, pollinia-4, oblong, slightly curved, yellow, waxy, closely nearly uniform; stigmatic surface 3-lobed, 2-lateral lobes similar and concave, folded, forming white, ovary inferior, 3-carpellate, unilocular, the placentation parietal, numerous ovules in the locule, glabrous. Fruits not available.

Found growing in full sunny and on tall trees. Flowering time from December to January.

Vernacular name : Sinma- myet-kwin.

Area : Tharaphi Hall, Mawlamyine University Campus

Habit **Inflorescence** **Flower** **L.S of Flower** **Labellum** **Gynostegium** **Pollen cap**

Figure 5. *Dendrobium pulchellum* Roxb

Rhynchostylis retusa Blume.

Scientific Classification

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Monocots
Order:	Asparagales
Family:	Orchidaceae
Subfamily:	Epidendroideae
Genus:	*Rhynchostylis*
Species:	*R. retusa*

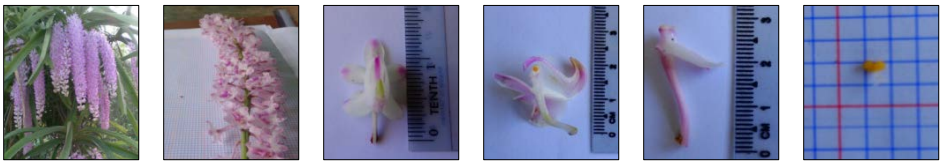
Generic Description

Monopodial epiphytes, erect, evergreen, roots long, cylindrical, abundant aerial roots present, glabrous, the stems stout, green, long simple, creeping in trees, the nodes as many as the leaves present, covered with overlapping bases, the internodes several, glabrous. Leaves alternating on opposite side of the stems, simple, linear-oblong, persistent petioles forming sheaths, enclosed the internodes, glabrous. Inflorescences axillary racemes, many-flowered, drooping, stout, dense, showy, opening in succession, placed peduncular bracts 4-8, ovate-oblong, glabrous, persistent. Flowers medium-sized, resupinate, bisexual zygomorphic, trimerous, epigynous, fleshy and waxy, fragrant; floral bracts ovate, green, pedicels slender, pale purple, glabrous; sepals-3, the tips acute and recurved, the margins entire, 2-lateral sepals, free, equal, both surfaces glabrous; petals-3, 2-lateral petals, free, equal, white with purple speckles, the tips obtuse, both surfaces glabrous, the labellum one-lobed, obtuse, white, much hairy on the inner side, column short , pale purple with purple speckles, the tips 2-lobed, anthers 2-celled, purple, convex at the pollinia location, pollinia-2, subglobose, stigmatic surface 2-lobed, abovate, purple, ovary inferior, oblongoid, 3-carpellate, unilocular, the placentation parietal, numerous ovules in the locale. Fruits were capsule, ovate, purplish green, glabrous. Seeds were not available.

Found growing in wild forest and semi-shady areas. Flowering and fruiting time from May to July.

Vernacular name : Kyaung-myi-tu, Kyet-tu-ywe.

Area : No.4, Main Road, Mawlamyine University Campus



Habit **Inflorescence** **Flower** **L.S of Flower** **Gynostegium** **Pollen cap**

Figure 6. *Rhynchostylis retusa* Blume

Discussion and Conclusion

In this paper, six species and six genera belong to the family orchidaceae of Mawlamyine University Campus area has been undertaken. The specimens collected and identified in this paper are to accordance with the specific characteristics mentioned by Lawrence (1951), Backer & Bakhuizew Van Den Brink (1963), Hooker (1975), Dassanayake, (1981) and Subrahmanyam, (1996). In this study, there are one species of monopodial and five species of sympodial type.

Dassanayake (1981), state the *Arundina graminifolia* is a terrestrial perennial orchid with reedy stems. Leaves are linear or oblong lanceolate. The tips acuminate. Inflorescence is terminal racemes with rosy and purple flowers. These characters are agreed with project paper.

According to Dassanayake (1981), state the *Phaius tankervilleae* is terrestrial plants and large leaves arise from bulbs in the ground. The flowers have brown on the inside and white on the outside. These characters are agreed with project paper.

Hooker (1885), State the *Spathoglottis plicata* is an evergreen, terrestrial herb which forms tall clumps. The petals are about the same length as the sepals but significantly wider. At the tip of the column there is a cap, under which masses of yellow pollen grains can be seen. These characters are agreed with project paper.

Subrahmanyam (1996), state the *Cymbidium aloifolium*, sympodial epiphytes, many-leaved pseudobulbs, hidden by the leaf-sheaths, leaves alternate, simple, narrowly oblong, bright green, fleshy and coriaceous, glabrous, the petioles forming sheaths encircling the pseudobulbs, glabrous, persistent. These charcters are agreed with project paper.

Hooker (1885), State the *Dendrobium pulchellum* sympodial epiphytes, erect, leaves alternates on opposite side of the stems, simple, oblong-lanceolate, inflorescences lateral raceme, pendulous with several flowers. These characters are agreed with project paper.

According to Dassanayake (1981), the flower of *Rhynchostylis retusa* is white with purple speckles, labellum is one, lobed, adnate to the column, mauve, white, the anther caps obovate, pollinia 2, yellow, rostellum is purple.

Myanmar is an endangered, epiphytic and deciduous orchid. It flowers once a year and the blooms have admirable fragrance and has high horticultural value is local market. It is valued and worn by Myanmar women.

Therefore, the conservation of orchids and their host trees are important and necessary more than commercial value and medicinal value. Conservation of wild orchids is now a matter of universal concern. Conservation measures have to be strengthened based on traditional knowledge and value systems with which is local communities could identify the revival of the sacred grove concept to protect the forests which help to conserve the orchid diversity present in this area as nature's gift. Understanding of traditional knowledge on conservation of orchids of the local people of will be helpful for sustainable orchid resource management of this region.

Acknowledgement

I would like to express my deepest gratitude to Dr. Aung Myat Kyaw Sein, Rector, Dr San San Aye, Pro-rector of Mawlamyine University for their allowing me to do this research paper. I am special thanks to Dr. Malar Aung, Professor and Head, Dr Moe Moe Shwe, Professor, Department of Botany Mawlamyine University for their encouragement. I would like to thank my beloved parents, U Soe Thein and Daw San Myint, for their physical, moral and financial supports throughout this study.

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Physicochemical Properties and Phytochemical Analysis of *Ficus lacor* Buch.-Ham.

Thaw Maw Moe*

Abstract

Health is the most important things for human being. Plants have been used as medicine, diet and other useful. *Ficus lacor* Buch.-Ham. is belong to family Moraceae of Ficus about 73 genera and over 1000 species. *Ficus lacor* Buch-Hum. is synonym of *Ficus infectoria* Roxb. It is locally known as Nyaung Chin Phu and is large deciduous, rapidly growing closely foliaceous free near about 20 meter height with fine shaped crown. It was collected from Kyaing Tong University Campus. In the present study, the morphology, organoleptic characters of powdered of leaves, phytochemical constituents, physicochemical properties, elemental analysis and medicinal uses and other useful of this plant were mentioned. Preliminary phytochemical in the powdered of leaves include alkaloid, flavonoid, saponin, glycoside, α amino acid, reducing sugar, phenolic compound, carbohydrate, steroids, terpenoids, starch are present while tannin is absent. The physicochemical properties showed that the solubility of the powdered samples were more soluble in D/W, methanol and chloroform than other solvents. The mineral contents were analyzed by using Energy Dispersive X-Ray Florescence (EDXRF) Spectrophotometer in West Yangon University. Finally the medicinal and other useful were described from local people.

Keywords: phytochemical, physicochemical, elemental analysis, medicinal value

Introduction

Genus *Ficus* belonging to Moraceae family is one of the largest genera of flowering plants. *Ficus* trees are native to Indo Australian region, Central and South America and Africa. *Ficus* is a genus of about 800 species and 2000 varieties and of woody trees, shrubs and veins in the family Moraceae according in most tropical and sub-tropical forests worldwide (Hamed, 2011) and some species of woody plants occurring in most tropical and subtropical forests throughout the world. Moraceae is a family of flowering plants commonly known as the mulberry or fig family. It comprises about 73 genera and over 1,000 species of plants widespread in

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tropical and subtropical regions, less common in temperate climates (Siva, 2016).

Siva, 2016, stated that the word *ficolin*, which appears similar to *Ficus* and refers to a lectin like compound combining the first parts of the words for *fibrinogen* and *collagen.*

Ficus lacor Buch.-Ham. is distributed worldwide in Australia, South East Asia, Burma, India, Bhutan, Indochina, Myanmar and Nepal. It is native to a wide area of Asia from India through Myanmar (Burma), Thailand, Southeast Asia, Southern China and Malaysia. Useful parts include aerial root, bark, leaves, buds, fruits, and latex. Different parts of the plants are used for various medicinal purposes. Chaudhary *et al*, 2012).

Plants are a rich source of secondary metabolites with interesting biological activities. In general these secondary metabolites are an important source with a variety of structural arrangement and properties. The plant derived compounds have a long history of clinical use, better patient tolerance and acceptance.

Ficus lacor Buch.-Ham. leaves are also used for various skin problems.The leaves have been reported the presence of several compounds including α-amyrin, β-amyrin, lupeol, stigmasterol, and compesterol. *Ficus lacor* leaves have been reported the presence of several compounds including α-amyrin, β-amyrin, lupeol, stigmasterol, and compesterol. The other compounds such as infectorin, scutellarein, glucoside, sorbifolin, and bergapten, bergaptol were isolated from the whole plant. The other compounds such as infectorin, scutellarein, glucoside, sorbifolin, and bergapten, bergaptol were isolated from the whole plant (Gamble, 1922).

The aim and objectives are the morphological and organoleptic characters, described the medicinal uses, physicochemical properties, phytochemical analysis and determine the elemental analysis of leaves of *Ficus lacor* Buch.-Ham.

Materials and Methods

Collection of Plant Material

In the present study, specimen is collected during the field exploration. Plant was collected from Kyaing Tong University Campus Area, Kyaing Tong Township during the February to April, 2017. The plant

was identified at the Department of Botany, Kyaing Tong University, with the help of available literatures (Hundley and Chit Ko Ko, 1961; Lawrence, 1964: Hooker, 1885; Burmmitt, 1992; Kirtikar and Basu, 1993; Kress, 2003). The collected leaves were detached and washed with tap water and dried under shady place with good ventilation for 15 days. Then the dried samples were powdered using kitchen blender and stored in air tight containers for further study. Physicochemical investigation of leaves of *Ficus lacor* Buch.-Ham. was made according to The British Pharmacopoeia 1968. Phytochemical investigation on leaves was tested for the presence of active secondary metabolites by using the methods expressed in (Trease and Evans, 2002).

Qualitative elemental analysis was performed by EDXRF (Energy Dispersive X-ray Fluorescence) at West Yangon University. The EDX 720 spectrometer is used for determination of the elements comprising in samples.

Results

Taxonomic classification of *Ficus lacor* Buch.-Ham.

Family	:	Moraceae
Scientific Name	:	*Ficus lacor* Buch.-Ham.
Vernacular Name	:	Nyaung chin
Local Name	:	Nyaung chin phu
English Name	:	Java fig

Large deciduous tree with milky latex juice. Leaves simple, spirally arrange; stipule broadly ovate-lanceolate; petiole long; leaves blade ovate-elliptic; 8-14 x 3-8 cm, 5-10 veins, margin entire. Receptacle axillary in pairs, globose, whitish, basal bract 3, ovate - rounded, small and closed by 3 flat apical bracts; internal bristle white. Male flower few ostiolar, near the mouth of receptacle, 2-3 ring; tepal 2-3. Gall flower sessile; tepal reddish, spathulate. Female flower sessile; tepal 3-4, style short in gall flower, long in pistillate, stigma thicken.

Medicinal and other uses

Traditionally used in treatment of several diseases is diabetes, menstrual disorders, washing ulcers, leucorrhoea.

The fleshy leaves 3-5 washed thoroughly with water and eaten to dysentery. The leaves washed thoroughly with water and boiled in water, the decoction about ⅓ left when drink for dysentery. The roasted leaves are put into hot water and drink such is taken especially for diabetes. Decoction of leaves is used for gargling to treat mouth ulcer. Leaves paste is applied herpes and wounds.The leaves macerated and made into paste with and smeared on skin at the place where the scorpion stings, an antidotes for poison. The leaves are used in dyspepsia, cough, and wound treatment. The leaves are decoction for the treatment of diarrhoea, obesity and heavy bleeding during menstruation and analgesic problem and skin diseases. Leaves juice used for asthma, ear-ache and toothache. Young shoot and sour leaves eaten raw or cooked. The leaves with water to boiled and mixed with onion, garlic, soil bean powder are eaten either as salad or cooked with pork by local people.

Figure 1. Habit of *Ficus lacor* Buch.-Ham.

Figure 2. Leaves of *Ficus lacor* Buch.-Ham.

Organoleptic characters of leaves of *Ficus lacor* Buch.-Ham.

In the present study, the powders of leaves were yellowish green in color with pungent and unpleasant odor and fibrous texture, sour and astringent in taste.

Physicochemical properties of leaves of *Ficus lacor* Buch.-Ham.

The result pointed out the highest yield was obtained from distilled water, chloroform and methnol of the leaves. (Table 1)

Table 1. Physicochemical properties of leaves of *Ficus lacor* Buch.-Ham.

No	Physicochemical properties	Leaves Average%
1	Petroleum ether soluble content	5.7
2	Ethyl Acetate soluble content	5.3
3	Acetone soluble content	6.0
4	Methanol soluble content	6.2
5	Ethanol soluble content	4.8
6	Distilled water soluble content	10.5
7	Chloroform soluble content	7.0

Phytochemical analysis of leaves of *Ficus lacor* Buch.-Ham.

Preliminary phytochemical in the powdered of leaves include alkaloids, flavonoid, saponin, glycoside, α amino acid, reducing sugar, phenolic compound, carbohydrate, steroid, terpenoid, protein, starch are present while tannin is absent.(Table. 2)

Table 2. Preliminary phytochemical of leaves of *Ficus lacor* Buch.-Ham.

No	Test	Extract	Test reagent	Observation	Results
1	Alkaloids	1%HCL	Mayer's reagent Wagner's reagent	cream ppt yellow ppt	+ +
2	α amino acid	H_2O	Ninhydrin	violet ppt	+
3	Carbohydrate	H_2O	Fehling A Fehling B	reddish brown ppt	+
4	Flavonoid	EtOH	HCL Magnesium tannin	pink ppt	+
5	Glycoside	H_2O	Sodium hydroxide	yellow ppt	+
6	Phenol	H_2O	10% $feCl_3$	blue or green ppt	+
7	Protein	H_2O	Million's reagent	white ppt Reddish ppt	+
8	Reducing sugar	H_2O	Benedicts solution	reddish brown ppt	+
9	Saponin	H_2O	Distilled water	frothing	+
10	Starch	H_2O	Iodine	blue black ppt	+
11	Steroid	P.E	$CHCL_3$ + Conc: H_2SO_4	blue green ppt	+
12	Terpenoid	P.E	$CHCL_3$ + Conc: H_2SO_4	reddish brown ppt	+
13	Tannin	H_2O	$FeCl_3$	violet ppt	-

+ present

- absent

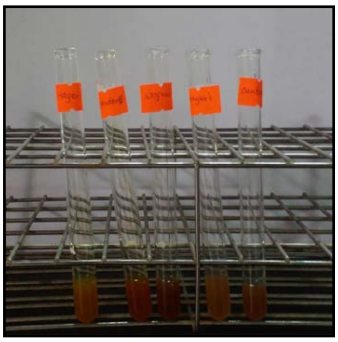

Figure 3. Alkaloids test

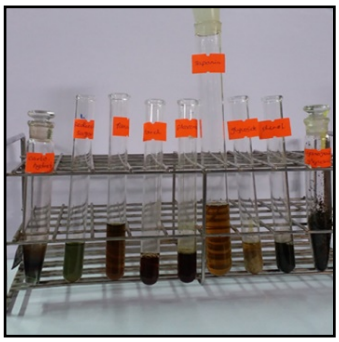

Figure 4. Other nine test

Figure 5. α Amino acid test

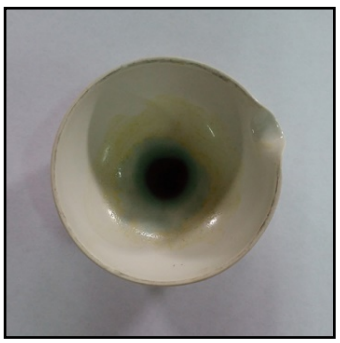

Figure 6. Steroid and terpenoid test

Elemental analysis of leaves of *Ficus lacor* Buch.-Ham. by using Energy Dispersive X-Ray Fluorescence (EDXRF)

The content element in powdered of leaves was quantitatively determined by using EDXRF. It was found that Potassium (K), Phosphorous (P), Sulphur (S) and Calcium (C) are found to be as macronutrients. Iron (Fe), Rubidium (Rb), Manganese (Mn), zinc (Zn) and Copper (Cu) are found to be as micronutrients. Percentage of potassium and calcium are higher than other elements in powdered leaves. (Table.3)

Table 3. Elemental analysis by EDXRF

No	Elements	Quantitative Result
1	K	2.940**
2	P	0.566**
3	S	0.287**
4	Ca	0.251**
5	Fe	0.009*
6	Rb	0.007*
7	Mn	0.005*
8	Zn	0.005*
9	Cu	0.002*

** = macronutrients

* = micronutrients

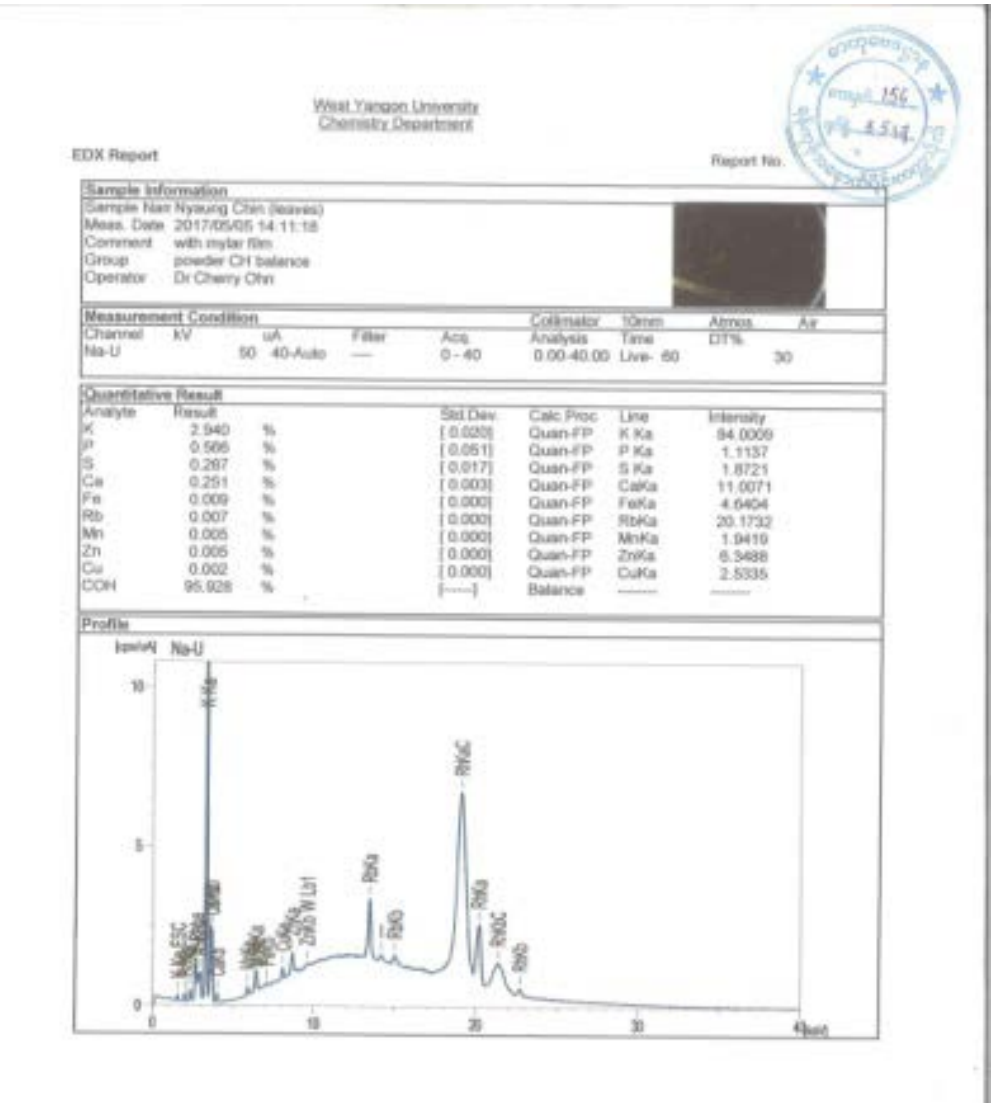

Figure 7. EDXRF Spectrum of leaves of *Ficus Lacor* Duch.- Ham.

Discussion and conclusion

In this study, the morphological and organoleptic characters, physicochemical properties, phytochemical analysis, elemental analysis of *Ficus lacor* Duch.- Hum. and described the medicinal and other uses.

Large deciduous tree with milky latex juice. Leaves simple, spirally arrange; stipule broadly ovate-lanceolate; petiole long; leaves blade ovate-elliptic; 5-10 veins, margin entire. Receptacle axillary in pairs, globose. Male flower few ostiolar, near the mouth of receptacle. Gall flower sessile; tepal reddish, spathulate. Female flower sessile; tepal 3-4, style short in gall flower, long in pistillate, stigma thicken. These characters agreed with (Lawrence, 1969, Hooker, 1885, Burmmitt, 1992, Kirtikar & Basu, 1993).

Herbal medicine is the study and use of medicinal properties of plants. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases (Mahalingam, 2018).

The roasted leaves are put into hot water and drink such is taken especially for diabetes. Decoction of leaves is used for gargling to treat mouth ulcer. Leaves paste is applied herpes and wounds. . The leaves are decoction for the treatment of diarrhoea, obesity and heavy bleeding during menstruation and analgesic problem and skin diseases. Decoction of aerial roots is given in ⅓ to treat diarrhoea and control blood sugar level in diabetics. Find powder of aerial roots is treating to sprinkled over wounds.

Sangita, 2017, stated that the usage of these plant leaves resulted in a better decreased blood sugar level in both the cases. This species then combined with Oat flour and Amla powder lowered the blood sugar level in the human diabetic subjects. Plants are natural antioxidants and effective herbal medicines, in part due to their anti-diabetic compounds, such as flavonoids, tannins, phenolic, and alkaloids that improve the performance of pancreatic tissues by increasing the insulin secretion or decreasing the intestinal absorption of glucose.

The physicochemical properties showed that the solubility of the powdered samples were more soluble in distilled water, chloroform and methanol than other solvents. These extracted showed are used for further chemical study and preparation of extracts for pharmacological study.

The various phytochemical analysis demonstrated the presence of alkaloids, α amino acid, carbohydrates, glycoside, phenol, starch, steroids,

terpenoids, reducing sugar, flavonoids, saponins and protein are present while tannin is absent.

Many phytochemical compound detected are known to have beneficial importance in medicinal science (Aliyu *et al*, 2009).

Alkaloids have been used to treat diseases like malaria and glycosides serve as defense mechanism against many microorganisms. Carbohydrate is a high energy rich food substance.

Saponin is used in the human diet for controlling cholesterol and for weight loss. Saponin protects the plant against microbes and fungi (Aiyelagbe, 2009).

Terpenoids possess anti-inflammatory, anticancer and antioxidant properties (Kuo-Reen-Yen - 2009).

All over the world, there is increasing interest in the importance of dietary minerals in the prevention of several diseases. Minerals are of critical importance in the diet, major minerals are those required in amounts greater than 150 mg per day and they represent 1% or less of body weight (Brook and Caldwell, 1954).

These include calcium, potassium, phosphorous, sulphur, chloride and magnesium. Essential trace elements are zinc, iron, copper, floride, iodine, chromium. The body required these minerals and vitamins for vital processing. (Brooks and Caldwell, 1954).

In this study plant leaves in Phosphorous (P), Potassium (K) and Sulphur (S) were found abundantly. Potassium is a mineral that is crucial for life. It is necessary for the heart, kidneys and other organs to work normally. Low potassium is associated with a risk of high blood pressure, heart disease, stroke, cancer digestive disorder and infertility (Kilander, 1951). Phosphorous is a mineral that makes up 1% of a person's total body weight. It is the second most abundant mineral in the body. Phosphorous is needed for the body to make protein for the growth, maintained and repair of cells and tissues (Allsopp, 2012). Sulphur is an essential element for all life. All living things need sulphur. It is especially important for humans because it is part of the amino acid (Hammond, 2000).

Numerous plants synthesize substances that are useful in the maintenance of health in humans and animals with a view that to increase the wide range of medicinal usages, the present day entails new drugs with

more potent and desired activity with less or no side effects. According to (FAO) more than 50000 plant species are being used in the traditional folk medicine throughout the world.

For further research programme, pharmacological activities should be carried out. As *Ficus lacor* Duch.- Hum. possess many medicinal values other bioactive compounds should be isolated from other plant parts.

Acknowledgements

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Pollen Morphology and Antimicrobial Activities of Leaves of Coleus Amboinicus Lour.

Nwe Ni Tin[1], Nang Moon Sar[2] & Aung Zin Oo[3]

Abstract

Pollen morphology and antimicrobial activity of leaves from Coleus amboinicus Lour. from Family Lamiaceae (Mint family) was presented in this paper. In taxonomy, this plant is perennial, strongly aromatic, succulent; stems obtusely 4- angular, fistular. Leaves simple, opposite and decussate, exstipulate; blades broadly ovate, hirsute above. Verticillasters terminal, spike-like. Flowers pale purple; bracts ovate with acute apex. Stamens 4, exserted, anthers dithecous, subreniform. Ovary 4-lobed, ovoid, bilocular with 2 ovules in each locule on the axile placentae, stigma bifid. Nutlets globoid and coloured photograph of this species have been presented. For pollen morphology, shape, size, aperture type and exine sculpture of pollen grains were studied. The results showed that pollen grains were 6- zonocolpate; the exines were reticulate. Antimicrobial activities constituents from the leaf extract were studied by using different kinds of solvents. Then, it's of crude leave extracts were evaluated by agar-well diffusion method. The experiment revealed that the hexane, methanol and pet-ether extract had the most efficient impact against all the test organism of the microbes under study especially against Candida albicans. Ethanol extracts proved to be the rather limited in antimicrobial activity against Bacillus pumalis.

Keyword: Pollen morphology, antimicro bial activities of Coleus amboinicus Lour.

Introduction

Family Lamiaceae (Mint family) is spread over 200 genera and 3200 species. Willis (1966) mentioned 180 genera and 3500 species, cosmopolitan in distribution. Especially abundant in the Mediterranean region, the old world and the mountains of subtropics. In India, the family is represented by about 69 genera and 421 species. Approximately 261 species are endemic (Priti Shukla and Shital P. Misra, 1979).

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Predominantly annual or perennial herbs, sometimes shrubs, rarely trees, or erect, perennial marsh plant. Stem herbaceous or woody as in tree members, glandular – pubescent, erect sometimes prostrate and with suckers as in *Mentha viridi*s, quadrangular. Leaves simple opposite - decussade, estipulate, petiolate, with varied type of margins, surface glandular hairy. These glands contain volatile oils which makes the leaves aromatic. Inflorescence is typical of the family. It is a spike or raceme of pairs of dichasial cymes at each node, rarely solitary, axillary as in *Scutellaria*. Flowers hermaphrodite, bracteolate, zygomorphic. Fruit typically a group of 4 nutlets, enclosed by the persistent (Bhattacharyya & Johri, 1998).

Pollen grains embody the male partners in sexual reproduction. They are generally shed in a desiccated condition and their moisture level is less than 20%. At the time of shedding, pollen grains are either two celled - a large vegetative cell enclosing a generative cell, or three cell – a vegetative cell and two sperm cells formed by the division of the generative cell. There is considerable variation in the size and shape of pollen grains (Shivanna & Sawhney, 1997).

The wall of the pollen grain is made up of two layers: an outer, acetolysis – resistant exine composed of sporopollenin and an inner pectocellulosic intine. One of the conspicuous structural features of pollen grains is the ornamentation of the wall form by the outer part of the exine (Shivanna & Sawhney, 1997). When both pores and furrows occurs together (*colporate*), the pores are always located in the furrows, one pore per furrow (Erdtman, 1952).

Much research has given evidence of the importance of pollen as a valuable morphological component in Angiosperm. Characters of pollen are shape, size, polarity, apertures type, number, position and exine structure. The shapes varied widely from one species to another. Even in the same species, it may vary according to the sub-species, varieties or cultivars. Pollen morphology may be of significant value sharing in solving problems in the classification of Lamiaceae members. (Wodehouse, 1935)

Uses the leaves of *Coleus amboinicus* Lour. are strongly flavoured and used for stuffings of meats and poultry, beef, lamb and game. The herb is used as a substitute for oregano to mask the strong odors and flavours of fish, mutto and goat. Fresh leaves are used to scent laundry and hair. It is also grown as an ornamental plant. It is widely used in folk medicine to

treat conditions like cold, asthma, constipation, headache, cough, fever and skin diseases. (Chatterjee A & Pakrashi SC. 1997)

Essential oil or volatile oils of plants have been variously reported in medicinal applications. The volatile oil extracts of *Coleus amboinicus* showed significant antibacterial activity against human pathogens such as *Bacillus, Pseudomonas* and *Staphylococcus sp*. In this, *Coleus amboinicus* showed greater effect against all species. (Baslas R.K & Kumar, 1981)

Therefore the aim and objectives of the present study is to collect and identify the *Coleus amboinicus* Lour., to give the record of knowledge and information of pollen morphological characteristics from the taxonomic point of view and to continue the future studies with the systematic research works of Family Lamiaceae, to promote an intensive application in Myanmar traditional medicine scientifically, to evaluate the antimicrobial activity of crude extracts from eight different kinds of solvents were prepare for healthy of human being.

Materials and Methods

Plant collection and plant identification

The plants of *Coleus amboinicus* Lour. were cultivated in Hwe Khar village, Mine - Zin tract, Kyaing Tong District. The specimens were collected during January to May, 2020 at the period of anthesis and fructification. Natural habit and flowers had been photographed. The plant material was identified and specimens are deposited in our Department Herbarium, Kyaing Tong University.

Collection of pollen samples

A few stamens of *Coleus amboinicus* Lour. were taken from buds (than in full bloom flowers). Collected pollens was stored in small glass vials with 1 cc of glacial acetic acid and labelled. The sample bottle was warmed in a water bath and a drop of polllliniferous jelly was taken out with a pair of forceps and placed on the glass slide, and then covered with a cover slip. Size measurements for the pollen grains were taken according to Erdtman (1970). The shape is basically, determined by dividing the equatorial width into the polar length of the pollen grain (P / E) Erdtman (1952). The pollen sample was examined by using electric Novex

Trinocular Microscope with 40 X and imaged by taking Oppo Digital Camera. A micrometer was used to measure the size of the grain.

Antimicrobial activities

The samples were dried and powdered. The leaf powder was subjected to examine antimicrobial activities by Ager-well diffusion method after (Cruickshank, 1975). The crude sample was subjected to antibacterial screening against some pathogenic organisms. These organisms *Agrobacterium tumefaciens*, *Bacillus pumalis*, *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Xanthomonas oryzae.* These investigations were conducted at the Department of Botany, University of Yangon, Yangon Division.

Antimicrobial activities are conducted by using available organisms. This microorganism and the diseases that they cause are as follow:

Table 1. Type of test organisms and diseases that they cause

No	Test Organism	Source	Diseases
1.	*Agrobacterium tumefaciens*	-	Plant tumor cell
2.	*Bacillus Pumalis*	IFO-1202	Eye infection, soft tissue and cutaneous infections
3.	*Candida albicans*	IFO-1060 -	Skin and cardiac infection, oral and vaginal infection, candida and alimentary tract infection
4.	*Pseudomonas aeruginosa*	IFO-3080 -	Pneumonia, septic shock, urinary tract infection, respiratory system, skin and soft tissue, bone and joint, gastro intestinal, chronic lung, ear, burn, septicaemia and ocular infections.
5.	*Staphylococcus aureus*	ATCC-12277	Skin disease, food poison, boils wound infection

No	Test Organism	Source	Diseases
6.	*Xanthomonas oryzae*	-	Bacteria for leaf blight

(Cruickshank 1975)

Results

***Coleus amboinicus* Lour., Fl. Cochinch.2: 372.1790. (Fig.1 A-E)**

Coleus aromaticus Benth. in Wall., Pl. As. Rar.2:15.1831.

English name : Country borage

Myanmar name : Ziya-ywet

Shan name : Ziya

Lahu name : Khaw-pute-thai

Ahka name : Ky-harr-nan

Family : Lamiaceae

Flowering period : May to October

Perennial, erect herbs to under shrub, strongly aromatic, succulent; stems obtusely 4-angular, fistular, dotted with sesile oil glands in between hairs. Leaves simple, opposite and decussate, exstipulate; blades broadly ovate, rounded to truncate at the base, crenulate along the margin, rounded at the apex, fleshy, hirsute above, gland-dotted and tomentose beneath. Verticillasters terminal, spike-like; verticils distinct, dense, 8 to 16 flowered. Flowers pale purple; bracts ovate with acute apex. Calyx campanulate; upper lip erect, ovate-oblong, ciliate; lateral lobes of lower lip short, median ones lanceolate, acuminate. Corolla boat-shaped, pink or lilac; tube about 5 mm long, infundibular above, geniculate below; upper lip erect, suborbicular; lower lip declinate, suborbicular, entire sparsely hirtellous without. Stamens 4, exserted, hiding in the lower lip; filaments connate below into short tube around the style, the posterior two, the anterior two white; anthers dithecous, subreniform. Disk unequal-sided, produced anteriorly, minute, pale green. Ovary 4-lobed, ovoid, glabrous,

bilocular, with 2 ovules in each locule on the axile placentae; style filiform, bent; stigma bifid. Nutlets globoid, brown, glabrous.

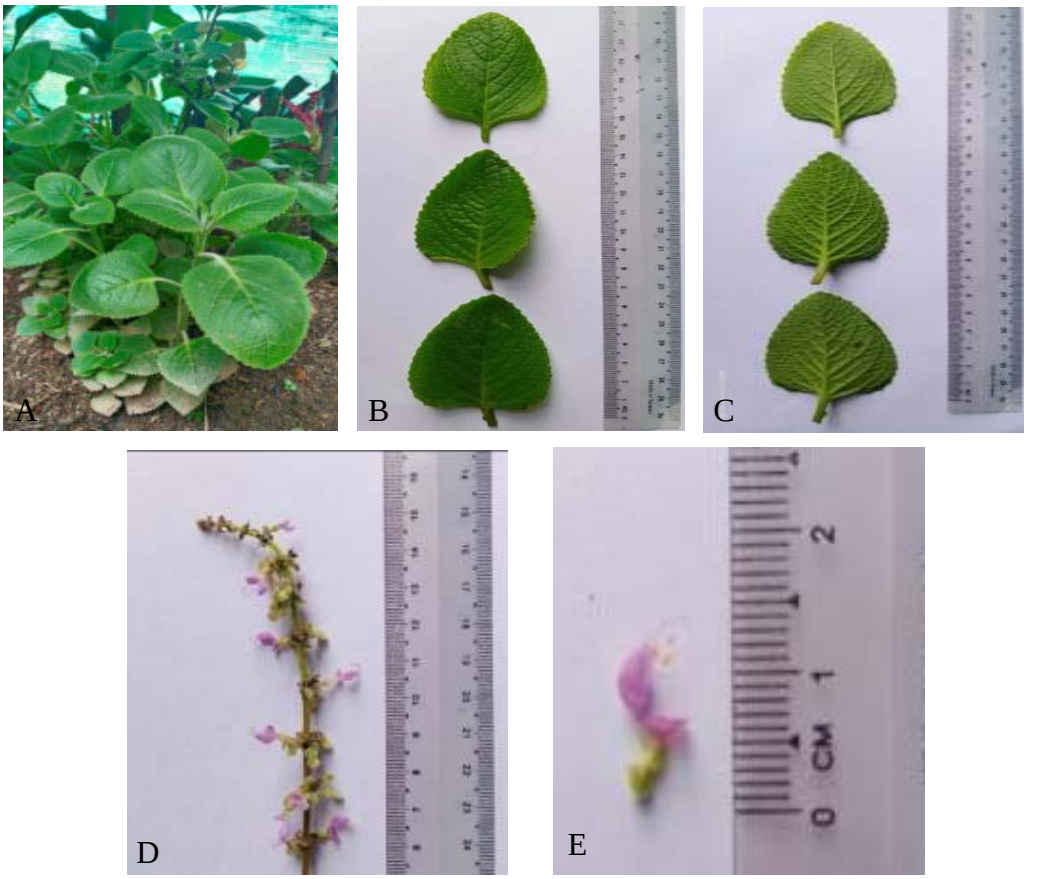


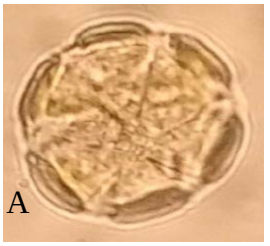
Figure 1. Morphological characters of *Coleus amboinicus* Lour.

A. Habit B**.** Upper surface of leaves C. Lower surface of leaves D. Inflorescence E. Flower

Pollen morphology of *Coleus amboinicus* Lour. (Fig. 2 A-B)

The present research concerns the palynology of *Coleus amboinicus* Lour. of Family Lamiaceae (Mint family). The palynological data is shown with Fig. 2.

In this *Coleus amboinicus* Lour**.,** Aperture condition hexa - colpate (6 - zonocolpate), small size, pollen shape oblate - spheroidal, 17.5-18.8 X 18.3-19.5 µm in length and breadth; amb spheroidal; colpi longicolpate, about 16.3-17.5 x 1.3-2.0 µm in length and breadth; exine thicker than nexine; sculpturing reticulate.



A B

Figure 2. Pollen Morphological characters of *Coleus amboinicus* Lour.

A. Polar view B. Equatorial view

Antimicrobial activities of leaves of *Coleus amboinicus* Lour.

Antimicrobial activities were conducted at the Department of Botany, University of Yangon, Yangon Division. The results showed that the hexane, methanol and pet - ether extract had the most efficient impact against all the test organism of the microbes under study especially against *Candida albicans* with the maximum inhibition zone of 16 mm in area. The water extract also showed the impact upon all the test organism but with the inhibitory zone rare limited in area. The acetone and ethanoic acid extract had the most efficient impact against all the test organism of the microbes under study especially against *Agrobacterium tumefaciens* and *Staphylococcus aureus* with the maximum inhibition zone of 14 mm. The chloroform extract also showed the impact upon the entire test organism but especially against *Staphylococcus aureus* with the inhibitory zone14 mm in

area. The ethanol extract showed to possess the inhibitory effect upon the all the test organism of the microbes under study especially against *Bacillus pumalis.* The Antimicrobial activities data is shown with Table (2) and Fig. 3 as follow -

Table2. Test for Antimicrobial activities of leaves of *Coleus amboinicus* Lour.

No	Test organism	Solvents							
		Aceto-ne	Chlor-oform	Ethan-oic acid	Etha-nol	Hexa-ne	Metha-nol	Pet - ether	Water
1	*Agrobacterium tumefaciens*	14mm (+++)	12mm (++)	14mm (+++)	8mm (+)	14mm (+++)	10mm (+)	10mm (+)	12mm (++)
2	*Bacillus pumalis*	8mm (+)	10mm (+)	10mm (+)	14mm (+++)	12mm (++)	12mm (++)	8mm (+)	10mm (+)
3	*Candida albicans*	12mm (++)	12mm (++)	12mm (++)	12mm (++)	16mm (+++)	16mm (+++)	16mm (+++)	12mm (++)
4	*Pseudomonas aeruginasa*	14mm (+++)	12mm (++)	12mm (++)	10mm (+)	14mm (+++)	14mm (+++)	12mm (++)	12mm (++)
5	*Staphylococc-us aureus*	14mm (+++)	14mm (+++)	14mm (+++)	12mm (++)	12mm (++)	12mm (++)	12mm (++)	12mm (++)
6	*Xanthomonas oryzae*	10mm (+)	10mm (+)	12mm (++)	10mm (+)	8mm (+)	10mm (+)	8mm (+)	8mm (+)

Agar well size = 6 mm

8 mm ~ 10 mm (+), (inactive),

11mm ~ 13 mm (++), (partially active)

14 mm above (+++), (active)

Agrobacterium tumefaciens *Bacillus pumalis* *Candida albicans*

Pseudomonas aeruginasa *Staphylococcus aureus* *Xanthomonas oryzae*

Figure 3. Antimicrobial treatment of different solvent extracts of leaves of *Coleus amboinicus* Lour.

Discussion and Conclusion

Coleus amboinicus Lour. were cultivated in Hwe Khar village, Mine - Zin tract, Kyaing Tong District. The specimens were collected during January to May, 2020. Pollen morphology and antimicrobial activity of leaves from *Coleus amboinicus* Lour. from Family Lamiaceae (Mint family) was presented in this paper.

In taxonomy point of view this plant is perennial, strongly aromatic, succulent; stems obtusely 4- angular, fistular. Leaves simple, opposite and decussate, exstipulate; blades broadly ovate, hirsute above. Verticillasters

terminal, spike-like; verticils distinct, dense. Flowers pale purple; bracts ovate with acute apex. Calyx campanulate; upper lip erect, ovate-oblong. Corolla boat-shaped, pink or lilac. Stamens 4, exserted, anthers dithecous, subreniform. Ovary 4-lobed, ovoid, bilocular with 2 ovules in each locule on the axile placentae, stigma bifid. Nutlets globoid found in study area.

Shivanna & Rangaswamy, 1992, In plant systematic, pollen are especially used to determine the pollen size, pollen shape, pollen type, structure of the pollen wall, pollen architecture, number of aperture, aperture position and aperture shape. In pollen morphology of *Coleus amboinicus* Lour**.** aperture condition hexa - colpate (6 - zonocolpate), small size, pollen shape oblate - spheroidal, 17.5-18.8 X 18.3-19.5 µm in length and breadth; amb spheroidal; colpi longicolpate, about 16.3-17.5 x 1.3-2.0 µm in length and breadth; exine thicker than nexine; sculpturing reticulate evaluated in study area.

The antimicrobial activities of the studied plant *Coleus amboinicus* Lour. inhibited microorganisms such as *Agrobacterium tumefaciens*, *Bacillus pumalis*, *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Xanthomonas oryzae*. According to the Hoadley and Dutka, 1977 to examined antimicrobial activities, leaf extract in eight different kinds of solvent namely acetone, chloroform, ethanoic acid, hexane, petroleum ether, methanol, ethanol and water were used by agar well diffusion method. It exhibited antimicrobial activities against all the organisms. The experiment revealed that the hexane, methanol and pet-ether extract had the most efficient impact against all the six species of the microbes under study especially against *Candida albicans* which causes skin and cardiac infection, oral and vaginal infection, candida and alimentary tract infection with the maximum record inhibition zone of 16 mm in area.

According to the Cruickshank 1975 and Hoadley and Dutka, 1977 Acetone, chloroform and ethanoic acid extracts proved to be the inhibitory zone rather limited in antimicrobial activity against *Agrobacterium tumefaciens*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which cause plant tumor cell; skin disease, food poison, boils, wound infection and pneumonia, septic shock, urinary tract infection, respiratory system, skin and soft tissue, bone and joint, gastrointestinal, chronic lung, ear, burn, septicaemia and ocular infection respectively with the maximum record inhibition zone of 14 mm.

According to the Soltys, 1963 Ethanol extracts proved to be the rather limited in antimicrobial activity against *Bacillus pumalis* which cause eye infection, soft tissue and cutaneous infection. Water extract proved to be the rare antimicrobial activity against the entire organism.

*Coleu*s is a plant that has been used since ancient times to treat heart disordres such as high blood pressure and chest pain (angina), as well as respiratory disorders such as asthma, constipation, headache, cough, fever and skin diseases. Forskolin is a chemical found in the roots of the *Coleus* plant. The herb *Coleus* is household herb in Asian countries. It can be grown in kitchen gardens and used for culinary purpose. The antimicrobial, antioxidant and flavouring potentials are continuously being explored and validated by researchers in different parts of the world. (Dadasaheb D,Wadikar and Prakash E. Patki, 2016)

Coleus amboinicus Lour**.** is a commonly available medicinal herb in India. The antimicrobial activity of this herb is tested as a cure for reproductive tract infections (RTI) among women. Fresh leaf has been used as a disc in different diameters to test the antimicrobial activity, against RTI causing microbes. (Warrier PK, Nambiar VP, Ramankutty C, 1995)

Based on the result of antimicrobial activities *C. amboinicus* Lour.

could be applied for the treatment on the diseases resulting from *Candida albicans*. Therefore, hexane, methanol, pet-ether extract of the leaves of *Candida albicans* Lour. could be useful as herbal medicine for the treatment of skin and cardiac infection, oral and vaginal infection, candida and alimentary tract infection.

In this result, palynology is usefulness and applicability of pollen investigation in plant systematic for Family Lamiaceae. Antimicrobial activities had been an important aid in various fields of science for promoting the Myanmar traditional medicine scientifically.

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We would like to express my heartfelt sincere gratitude to Dr. Ni Lar Myint, Acting Rector and Dr. Min Lwin and Dr Khin Lay Yee Pro-Rectors, Panglong University for their permission to carry out this paper and encouragement. We also want to show our deepest appreciation to Dr Khin Cho Cho Oo, Professor and Head and Dr Khin Lay Phyu, Professor, Department of Botany, University of Panglong for their guidance and encouragement.

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Terminology

Angulaperturate	:	those apertures situated at the angles of the amb (the sides of the amb may be convex, straight or concave).
Aperture	:	apertures are specially delimited, generally thin Walled areas in the outer pollen wall or exine through which the pollen tube
Colpate	:	pollen grain with an elongate aperture or furrow.
Colporate	:	with a composite aperture, consisting of both a colpous and a pore.
Exine	:	the main outer usually resistant layer of a sporoderm.
Heterobronchate	:	with brochi of ± distinctly different sizes.
Lumina	:	the spaces between the muri of a reticulation (sing. lumen)
Muri	:	ridges separating the lumina of an ordinary reticulum.
Polar axis	:	a perpendicular line connecting the poles of a spore.
Porate	:	with one or more pori.
Reticulate	:	with a reticulum.
Retipilate	:	with a reticuloid pattern with pila instead of muri.
Sexine	:	the outer sculpture part of the exine.
Simplibaculate	:	walls of the reticulum with a single row of bacular or beads.
Reticulum	:	network formed by muri and luminae. (Pl. reticula)

Effect of Different Growth Hormones on Seed Germination Rates and Survival Rates of *Momordica charantia* L.

San Win[1] & Sann Sann Oo[2]

Abstract

The paper presents the results of studies on seed germination and the influence of different media of plant growth on seeds germination and seedling growth experiment of *Momordica charantia* L. were conducted at Department of Botany, Loikaw University from 22nd July to 5th August, 2020. *Momordica charantia* L. (kyet-hin-ga) including the comparative growth patterns of the seedlings. The germination percentage, survival percentage and plant height of seedlings were computed. The seeds were collected from Mar-lar-myaing fruits and flower garden, Kamayut Township, Yangon Division. The seeds were soaked in water, gibberellic acid (GA_3), B_1 and Indoe-acetic acid (IAA) solution. In this experiment, determinations the testing of germination rates for kyet-hin-ga seeds are used in five different treatments T_1 (control), T_2 (soaked in water), T_3 (soaked in GA_3), T_4 (soaked in B1) and T_5 (soaked in IAA) by method of germination in sand with three replicates. In the treatments, the result showed the highest rate of the germination (100%) in the treatment T_3 (seed soak in GA_3), T_4 (seed soak in B_1) and T_5 (seed soak in IAA) second highest rate (93%) in T_2 (seed soak in water) and the lowest rate in T_1 (control) (90%). The experiment indicates the treatment T_3 (seed soak in GA_3) is the best for germination rate and survival rate of *Momordica charantia* L. seed in field.

Keywords: *Momordica charantia* L.; gibberellic acid (GA_3); B_1; Indoe-acetic acid (IAA)

Introduction

Momordica charantia L. belongs to the family Curcurbitaceae including about 110 genera and about 640 species (Trease and Evans, 2002).

The vernacular names were mentioned as kyet-hin-ga in Myanmar (Hundely and Chit Ko Ko, 1987).

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In Thailand, it was known as Bitter cucumber. In the Philippines, it was called Amargoso. The names of this plant in English are Carilla fruit and Bitter gourd. These plants are used as vegetables. The local people used the leaves and fruits in the treatment of diabetes. Bitter gourd is one of the most popular vegetables in South-East Asia. It is a member of the cucurbit family along with cucumber, squash, watermelon and muskmelon. Native to China or India, the fast growing vine is grown throughout Asia and is becoming popular worldwide. Depending on location, bitter gourd is also known as bitter melon or balsam pear. Pre-sowing seed treatments with growth substances such as gibberellic acid have been found to improve the seedling growth of many species (Shanmungavelu 1970, Singh *et al.* 1989).

The present study is concerned with the investigation of germination and survival rate. The main aims are to know about the viability of *Momordica charantia* L. seeds from market, to know about the comparison of plant growth hormone and water treatments for germination seeds and to record the germination and survival rate of *Momordica charantia* L.

Materials and Methods

Collection of Planting Materials

The seeds of F_1 *Momordica charantia* L. seeds were collected from Mar Lar Myaing Market, Kamaryut Township, Yangon Division. The seeds of F_1 *Momordica charantia* L. seeds were used as the planting materials in this experiment.

Seed Germination

The seeds were soaked in different media GA_3, B_1 and IAA. Germination studies were conducted with Okra seeds treated with different treatments (control, 6 hours soak in water, 6 hours soak in GA_3, 6 hours soak in B_1 and 6 hours soak in IAA, first count and final count were on 3^{th} and 14^{th} day. The germination was expressed as the percentage of seed which produced normal seedlings (ISTA, 1996).

The seeds of *Momordica charantia* L. (F_1) were germinated into the plastic trays containing 2500g of prepared soil medium. Firstly, the soil was made the holes of 1 cm depth. Ten holes were made in plastic trays. One seed was sown in a hole. There were three treatments for germination test and the total applied seeds were 150.

The treatments were carried out as follows:

T_1 = control;

T_2 (seed soak in water);

T_3 (seed soak in $GA_{3)}$;

T_4 (seed soak in $B_{1)}$;

T_5 (seed soak in IAA)

Germination experiments

Germination experiments were tested using three replications of 30 seeds per each treatment. Germinated seeds and rotted seeds were counted every other day and removed. The germination test was recorded every other day for 14 days.

Germination rate

The germination rate was calculated using the following formula developed by Soupe (2009).

$$\text{Rate of seed germination} = \frac{\text{No. of germinated seeds}}{\text{Total number of sown seeds}} \times 100$$

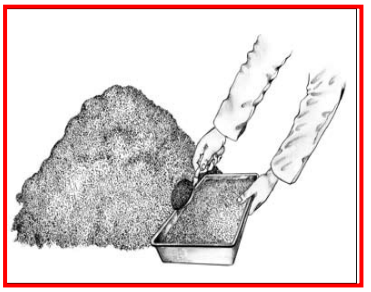
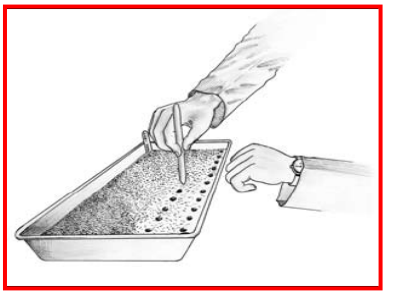
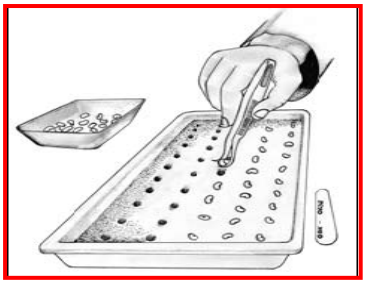
Survival rate

$$\text{Survival rate} = \frac{\text{No. of survival seeds}}{\text{Total number of sown seeds}} \times 100$$

Data collection and statistical analysis

All the observation on germination percentage, growth parameter of seedling length and root length were collected at the time of germination. The data were analyzed using the IRRISTAT software, version 4, developed by International Rice Research Institute (IRRI), Philippines. The mean separation was calculated by Least Significant Different (LSD) (Gomez and Gomez, 1984).


Figure 1. *Momordica charantia* L. Seeds from East-West Seed International Co. Ltd

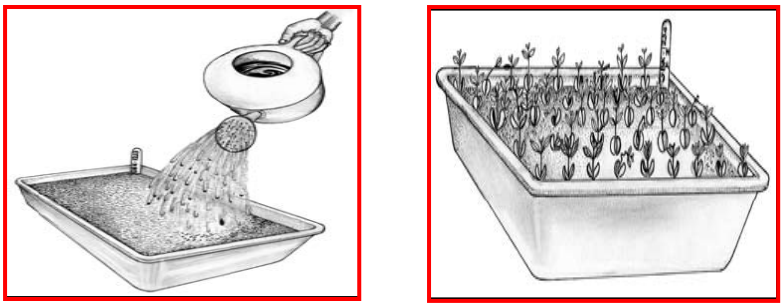



Figure 2. Seed germination testing in sand.

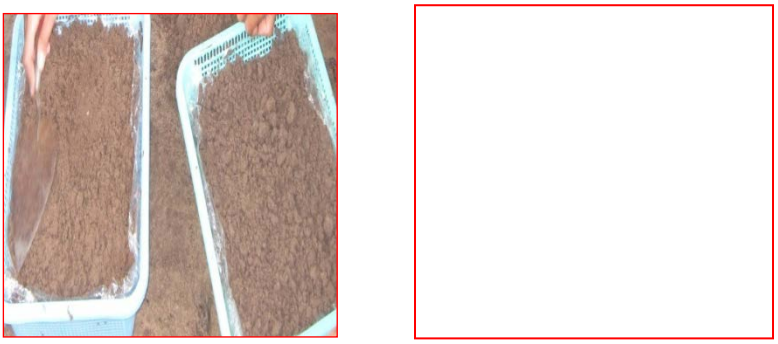


Figure 3. Soil preparation for germination of *Momordica charantia* L.

Results

Morphological Characters of *Momordica charantia* L.

Scientific Name - *Momordica charantia* L.

Myanmar name - Kyet- hin- ga

English name - Carilla fruit, Bitter gourd

Family - Cucurbitaceae

Duration - Annual

The plants are monoecious annual vine plant; Stems slender, 5-angles with tendrils, green, hairy; leaves are light green, hairy, alternate, simple. Fruit a berry, ellipsoid, green when young, bright orange when ripe; seeds obovoid, compressed, aril red, corrugate on the margin, sculptured a both surfaces.

Habit with flowers

Habit with a fruit

Female flower

Male flower

Figure 4. Morphological Characters of *Momordica charantia* L.

Effect of different hormones treatment on germination percentage of *Momordica charantia* L.

In this study, the germination of *Momordica charantia* L. seeds was started at 3days after sowing (DAS) and it was continued to 7 DAS.

The result indicated that the germination from T_3 (seed soak in GA_3), T_4 (seed soak in B_1) and T_5 (seed soak in IAA) was the maximum rate 100 %, followed by T_2 (seed soak in water) 93 %, and T_1 (control) 90 % respectively (Table 1, Figure 5).

Table 1. Effect of different hormones treatment on germination percentage of *Momordica charantia* L.

Treatment	Number of Germinated Seedlings					
	3 DAS	4 DAS	5 DAS	6 DAS	7 DAS	Germination %
T_1control	0	0	10	15	27	90 %
T_2(seed soak in water)	0	5	18	20	28	93 %
T_3(seed soak in GA_3)	0	10	22	27	30	100%
T_4(seed soak in B_1)	0	7	20	27	30	100%
T_5 (seed soak in IAA)	0	5	22	25	30	100 %

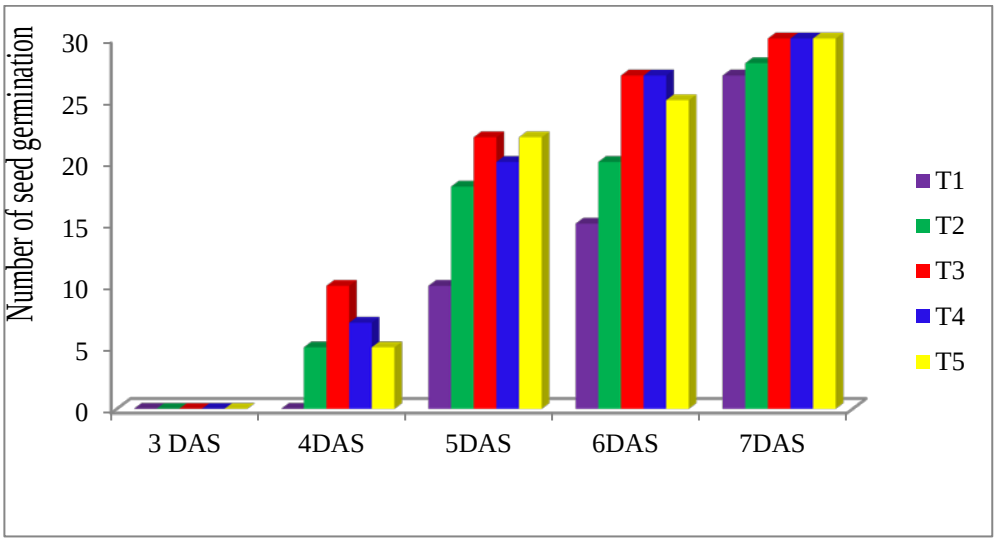


Figure 5. Effect of different hormones treatment on germination percentage of *Momordica charantia* L.

Effect of different hormones treatment on survival rate of *Momordica charantia* L.

In this study, the survival rate of *Momordica charantia* L. seeds was started at 8 days after sowing (DAS) and it was continued to 14 DAS.

The result indicated that the survival rate from T_3 (seed soak in GA_3) was the maximum rate 100 %, followed by T_5 (seed soak in IAA) 96.67%, T_4 (seed soak in B_1) 93.33%, T_2 (seed soak in water) 86.67 %, and T_1 (control) 80 % respectively (Table 2, Figure 6).

Table 2.Model data sheet to record survival rate of *Momordica charantia* L.

Treatments	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
8 DAS	27	28	30	30	30
9 DAS	27	27	30	29	30
10 DAS	27	27	30	29	30
11 DAS	26	27	30	29	30
12 DAS	25	27	30	29	30
13 DAS	25	27	30	29	29
14 DAS	24	26	30	28	29
Total Survival	24	26	30	28	29
Survival (%)	80%	86.67%	100%	93.33%	96.67%

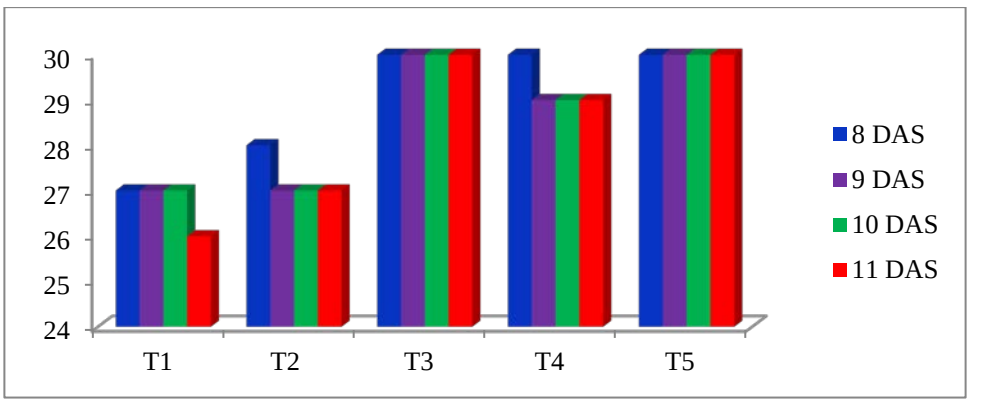


Figure 6. Effect of different hormones treatment on survival rate of *Momordica charantia* L.

Effect of different hormones treatment on plant height of *Momordica charantia* L.

The result indicated that the plant height from T_3 (seed soak in GA_3) were the maximum 18.62 cm, followed by T_4 (seed soak in B_1) 15.48 cm, T_5 (seed soak in IAA) 14.83 cm, T_2 (seed soak in water) 11.88 cm and T_1 (control) 11.27 respectively. When compared plant height, T_3 (seed soak in GA_3) hormone was more efficient germination than other media (Table 3, Figure 7).

Table 3. Effect of different hormones treatment on plant height of *Momordica charantia* L.

Treatment	Plant height (cm)				
	7DAS	9DAS	12DAS	14DAS	Mean
T_1control	7.51	10.97	12.55	14.08	11.27
T_2(seed soak in water)	7.84	12.05	13.74	13.92	11.88
T_3(seed soak in GA_3)	13.78	18.81	20.73	21.18	18.62
T_4(seed soak in B_1)	10.93	18.25	19.63	13.11	15.48
T_5 (seed soak in IAA)	13.66	12.99	14.43	18.25	14.83
F-test	**	**	**	**	
5% LSD	1.93	1.88	1.88	2.32	
CV%	35.2	25.2	22.7	28.2	

Each value represented the mean from 3 replications. Mean differences in each column was determined by LSD. **= Highly significant at 1%.

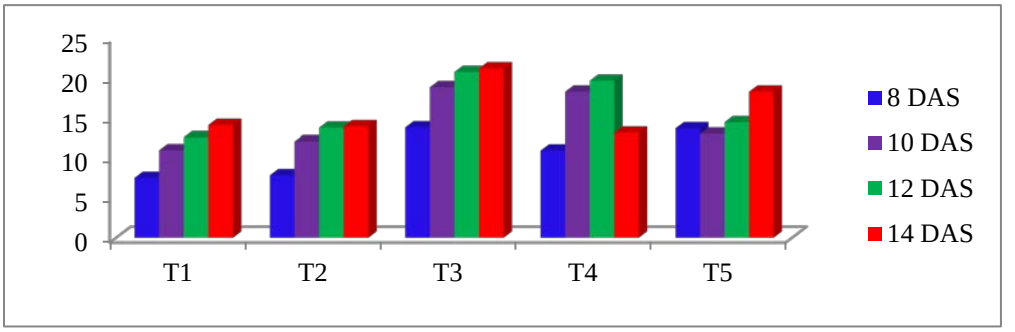


Figure 7. Effect of different hormones treatment on plant height of *Momordica charantia* L.

Figure 8. Seed germination testing in sand for (7) days

Figure 9. Seed germination testing in sand for (14) days

Discussion and Conclusion

Seed germination of *Momordica charantia* L. (kyet-hin-ga) using different growth hormones treatments was studied at the Botany Department, Loikaw University, for the period from 22nd July to 5th August, 2020. The percentages of germination rate and survival rate were calculated according to the rules for seed testing (ISTA, 1996). Germination characteristics were recorded daily. After two weeks, ten representative seedlings from each replication of a treatment (30 seedlings for each treatment) were selected for measuring growth parameters.

The seed germination results show that T_3 (seed soak in GA_3), T_4 (seed soak in B_1) and T_5 (seed soak in IAA) were the maximum 100 % followed by T_2 (seed soak in water) 93 %, and T_1 (control) 90 % respectively. The results show germination rate and seedling length of GA_3 media was more efficient germination than other hormones. GA_3 application influenced the rate and the final percentage of germinated seeds. The present results are in agreement with the findings of Dzayi (2010).

From the study, it is concluded that the soak of GA_3 hormone promoted the growth of *Momordica charantia* L. (kyet-hin-ga). Plant height is a very important factor for good plant growth and ultimate yield. Along with plant height pod length, number of pods, number of leaves and other vegetative and reproductive attributes were also affected. This study helps to find concentrations of GA_3 which has the highest effect on the

components of growth which are also economic enough to be suggested to the farmers.

Acknowledgements

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In particularly, we are greatly indebted to Dr. Sann Sann Oo, Professor and Head and Dr. Khin Myo Myint, Professor, Department of Botany, Loikaw University, for her guidance, moral support, and cooperation throughout study.

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Antibacterial Activity of Endophytic Fungi Isolated from the Leaves of *Naringi crenulata* (Roxb.) Nicolson.

Tin Tin Mar[1] & Khin Mar Kyu[2]

Abstract

In the present study, ten kinds of endophytic fungi species were isolated from the leaves of *Naringi crenulata* (Roxb.) Nicolson. belonging to the family Rutaceae. In investigation of their antibacterial activities, one test organism was used and supported by NITE (National Institute of Technology and Evalution, Japan). Four isolated fungi (F-02, F-05, F-06, and F-10) showed the activities against the test organism, *Staphylococcus aureus*. Then, the macroscopical characters of endophytic fungi and the microscopical characters of antibacterial activities of endophytic fungi were studied to identify.

Keywords: *Naringi crenulata*, Rutaceae, antibacterial activity, *Staphylococcus aureus*.

Introduction

Endophytes are microbes which colonize living, internal tissues of plants without causing any harm to their host. Endophytic fungi are extremely common and highly diverse microorganisms that live within plant tissues, but don't cause any disease symptoms. They are found in all plant species (Azevedo *et al.*, 2000).

Endophytes can promote the growth of host plants, enhance resistance to biotic and abiotic stresses and the endophytic fungi play important physiological and ecological roles in their host life (Carroll & Carroll, 1978).

Endophytic fungi produce varieties of secondary metabolites, such as antibiotics, antimycotics, immunosuppressant and so on to name a few. Secondary metabolites from endophytic fungi show important biological activities such as antioxidant, anticancer, immunomodulatory, antivirus, antituberculosis, anti-parasite and insecticides (Agusta, 2009).

The endophytic fungi are considered to be rich source of novel compounds (Strobel, 2003).

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The medicinal plants of endophytic fungi protect their hosts from infectious agents and adverse conditions by secreting bioactive secondary metabolites. The medicinal plants are known as harbour endophytic fungi that are believed to be associated with the production of pharmaceutical products (Zhang *et al.*, 2006).

Medicinal plants are used medicinally in different countries and are the source of potential and powerful drugs. According to World health organization (WHO) more than 80% of the world population rely on traditional medicine for their primary health care needs (Sudhakar *et al*, 2006).

Naringi crenulata (Roxb.) Nicolson. is the best known member of the family Rutaceae. In the ayurvedic system of medicine, *Naringi crenulata* (Roxb.) Nicolson.is practiced as a traditional medicinal plant in the Indian sub continent , China and Southeast Asia including Thailand .In Thai traditional medicine, the leaves of *Naringi crenulata* (Roxb.) Nicolson.have been used for the treatment of fungal infection, skin diseases, allergies, cancers, and inflammation (Hussain *et al*., 2014).

Naringi crenulata (Roxb.) Nicolson. is an endangered medicinal plant. It is used as skin diseases, ringworm, and snake bite. This plant has been utilized in folk remedies to cure various diseases such as diabetes, hepatitis, eczema, pulmonary tuberculosis, hypertension and cancers (Siripong *et al*, 2006).

The aims and objectives of this research are to investigate the isolated endophytic fungi from *Naringi crenulata* (Roxb.) Nicolson. and to find out macroscopical characters and to identify the microscopical characters of isolated endophytic fungi and to study their anibacterial activities.

Materials and Methods

Collection of plant samples

The plant samples were collected from Aung Tha village in Monywa Township. The collected plants were identified according to the outstanding characters shown in the literatures of Backer (1968), Hooker (1885), Hundley and Chit Ko Ko (1987), Kress W.J and Daw Yin Yin Kyi (2003).

Isolation of Endophytic Fungi

The isolation of endophytic fungi was referred by the method of Ando and Inaba (2004) and the following in Fig.1. The leaves of the plant of *Naringi crenulata* (Roxb.) Nicolson. were washed in running tap water for 5 minutes. They were separated and cut into small pieces 2 cm in length. The pieces were surface sterilized by immersing in a plate containing 70% ethanol for 2 minutes. The pieces were rinsed in the sterile distilled water. These pieces were placed on sterilized tissue paper and dried for one hour. Dried pieces were cut into smaller pieces and transferred to LCA plates. The cultures were incubated at room temperature for 3 to 7 days (Fig.1).

After fungal colonies were obtained, the observed colonies were cultured separately in PGA medium. Then subcultures of isolated fungi were done 3-5 times with PGA medium until the pure cultures were obtained. The pure strains are maintained as culture in test tubes.

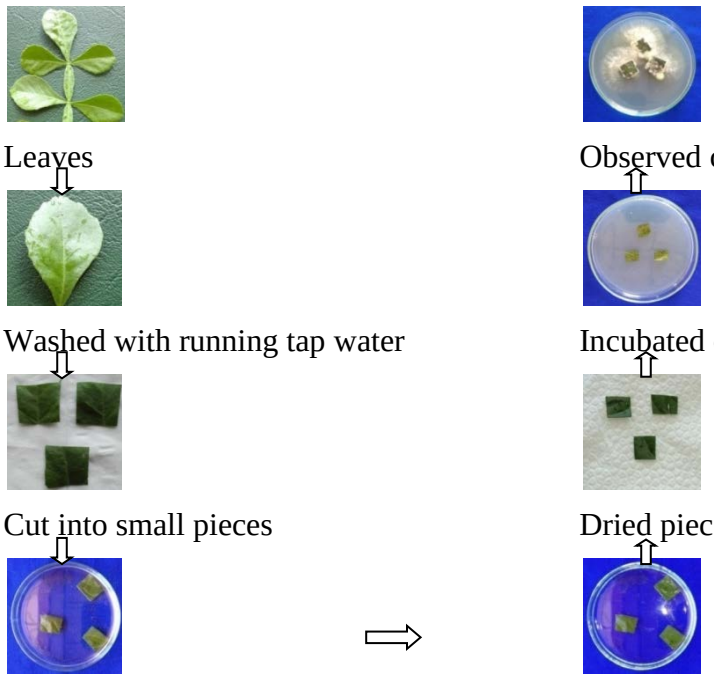


Figure 1. The isolation procedure of the endophytic fungi.

Medium used for the Isolation Fungi

Low Carbon Agar Medium (Ando, 2004)

Glucose	0.2 g
Sucrose	0.2 g
K_2HPO_4	0.1 g
$MgSO_4$. $7H_2O$	0.05g
KNO_3	0.1 g
KCl	0.05g
Agar	1.8 g
Distilled water	100 mL
pH	6.5

(after autoclaving Chloramphenicol 0.2g was added to the medium)

PGA medium (Ando, 2004)

Potato Glucose Agar Medium	
Potato	20g
Glucose	2g
Agar	1.8g
Distilled water	100 mL
pH	6.5

WGA (Water Glucose Agar) Medium

Glucose	1.8g
Agar	1.8g
Distilled Water	100 mL
pH	6.5

(after autoclaving Chloramphenicol 0.2g was added to the medium)

Preliminary Study on Antibacterial Activity of Isolated Fungi by Paper Disc Diffusion Assay (Tomita, 1988)

The isolated fungi were grown at 25ºC for 7 days on PGA medium. These fungi were inoculated on seed medium and incubated 25ºC for 3 days. Ten mL of seed culture was transferred into the fermentation medium (40 mL) and incubated at 25ºC for 7 days. The fermented broth (20 µL) was used to check the antibacterial activity against test organisms by paper disc diffusion assay (Figure.2). Paper disc having eight milimeter diameter (Advantec, Toyo Roshi Kaisha Co; Ltd; Japan) was utilized for antibacterial assay.

The assay medium (Glucose 1%, Polypetone 0.3%, KNO_3 0.1%, Agar 1.8%, Distilled water 100 mL, pH 6.5-7.0) was used for the antibacterial activity test. One percent (1.5×10^8/ mL of spore suspension) of test organism was added to assay medium, then poured into plates. After solidification, paper discs impregnated with samples (fermented broth) were applied on the agar plates and the plates were incubated for 24-36 hours at 28ºC to 30ºC. Clear zones (inhibitory zones) surrounding the test disc indicate the presence of bioactive metabolites which inhibit the growth of test organism.

Seed Medium (Ando, 2004)		**Fermentation Medium (Ando, 2004)**	
Glucose	2.0g	Glucose	2.0g
Polypeptone	0.3g	Yeast extract	1.0g
KNO_3	0.1g	K_2HPO_4	0.001g
K_2HPO_4	0.2g	$MgSO_4$	0.0001g
D.W	100 mL	D.W	100 mL

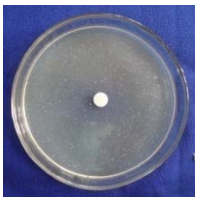
7 days old culture

1. The isolated were growing 27˚C for 7 days on PGA medium.

100 mL conical flask containing 50 seed medium

2. The isolated fungi were inoculated on seed medium and incubated at 27˚C for 7 days.

3. Ten mL seed culture was transferred into the fermentation medium and in incubated at 27˚C for 7 days.



4. 20 μL of fermented broth was put on paper disc and placed on assay plate containing test organisms.

Figure 2. Procedure for antibacterial activity test.

Table 1. Test organisms used in antibacterial activities (NITE, 2005)

No.	Test organism	Infections
1	*Staphylococcus aureus* AHU 8465	Boil, food poisoning and skin disease

Results

Collection of plant samples

Outstanding characters of *Naringi crenulata* (Roxb.) Nicolson.

Scientific name - *Naringi crenulata* (Roxb.) Nicolson. In saldanha & Nicolson, Fl. Hassan Distr. 387.1976.

Local name - Thanat-kha

English name - Chinese Box Tree

Family - Rutaceae

Flowering period - March to May

Deciduous small trees. Leaves unipinnately imparipinnate compound, alternate; petioles narrowly winged; leaflets 5 -7, terminal leaflet largest, ovate-rhomboid, cuneate at the base, crenulate along the margin. Inflorescences axillary or terminal racemes. Flowers bisexual, actinomorphic, hypogynous, tetramerous, creamy-coloured. Calyx 4, connate, campanulate, pubescent. Petals 4, free, elliptically oblong. Stamens 8, equal, around an annular disk; filaments linear; anthers dithecous, basifixed, introse. Ovary superior, globose, 1 - to 4- loculed, with solitary

ovule in each locule on the axile placentae; style terminal, stout; stigma globose. Fruits small, globose, 1- to 4- seeded. Seeds oblongoid.

Habit

Inflorescence

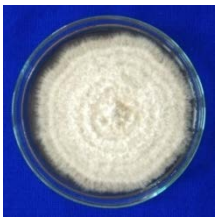
Figure 3. *Naringi crenulata* (Roxb.) Nicolson.

Isolation of endophytic fungi

Table.2. Isolated endophytic fungi from *Naringi crenulata* (Roxb.) Nicolson.

No	Part used	Species of Rutaceae	Isolated endophytic fungi
1	Leaves	*Naringi crenulata* (Roxb.) Nicolson.	F-01, F-02, F-03, F-04, F-05, F-06, F-07, F-08, F-09, F-10

Macroscopical characters of endophytic fungi



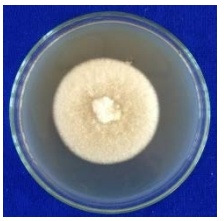
Macroscopical character of F-01

Macroscopical character of F-02

Macroscopical character of F-03

Macroscopical character of F-04

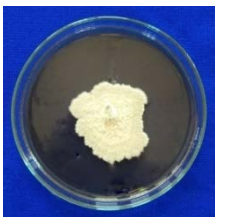
Figure .4. Macroscopical characters of F-01, F-02, F-03 and F-04.



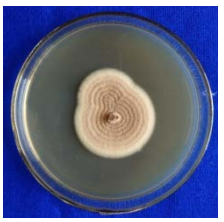
Macroscopical character of F-05

Macroscopical character of F-06

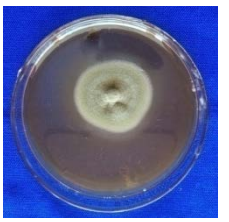
Macroscopical character of F-07



Macroscopical character of F-08



Macroscopical character of F-09



Macroscopical character of F-10

Figure 5. Macroscopical characters of F-05, F-06, F-07, F-08, F-09, and F-10.

Preliminary study on the antibacterial activities of isolated endophytic fungi

All endophytic fungi isolated from the leaves of *Naringi crenulata* (Roxb.) Nicolson. were shown antibacterial activities on *Staplylococcus aureus.* The data of antibacterial activities of four fungal isolated against test organisms were indicated in Table (3). Among them, the isolated fungi F-02, F-05, F-06, and F-10 were shown the antibacterial activities against *Staphylococcus aureus* at 5 days fermentation period.

Table 3. Antibacterial activities of isolated fungi

Isolated fungi	*Staphylococcus aureus*	Isolated fungi	*Staphylococcus aureus*
F-01	No activity	**F-06**	**21.28 mm**
F-02	**21.25 mm**	F-07	No activity
F-03	No activity	F-08	No activity
F-04	No activity	F-09	No activity
F-05	**29.02 mm**	**F-10**	**22.59 mm**

Figure 6. The activity of endophytic fungus F-02 against on *Staphylococcus aureus.*

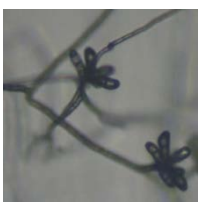


Figure 7. Microscopical character of endophytic fungus F-02.

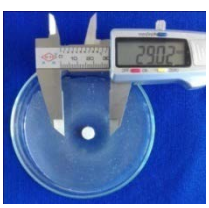


Figure 8. The activity of endophytic fungus F-05 against on *Staphylococcus aureus.*

Figure 9. Microscopical character of endophytic fungus F-05.

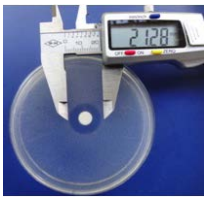


Figure 10. The activity of endophytic fungus F-06 against on *Staphylococcus aureus.*

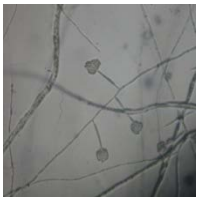


Figure 11. Microscopical character of endophytic fungus F-06.

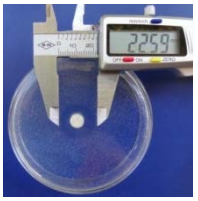


Figure 12. The activity of endophytic fungus F-10 against on *Staphylococcus aureus.*

Figure 13. Microscopical character of endophytic fungus F-10.

Discussion and Conclusion

In this investigation, a total of ten endophytic fungi were isolated from the leaves of *Naringi crenulata* (Roxb.) Nicolson. All ten endophytic fungi were studied on antibacterial activity with one test organism. This test organism was supported by NITE (National Institute of Technology and Evaluation, Japan) for the cooperation researches. The antibacterial activity of ten endophytic fungi against one test organism was studied by paper disc diffusion assay method (Tomita,1988). In the antibacterial activities of these endophytic fungi, F-02, F-05, F-06 and F-10 showed the antibacterial activity against on *Staphylococcus aureus.*

Special types of endophytic fungi of medicinal plants may be associated with the production of specific bioactive compounds needed by human. This study revealed the presence of bioactive secondary metabolites produced by endophytic fungi from medicinal plant of *Naringi crenulata* (Roxb.) Nicolson. with antibacterial activity. Pharmacological exploration of *Naringi crenulata* (Roxb.) Nicolson. is essential (Chopra *et al*., 1995).

These results indicated that the endophytic fungi can be isolated and they possess high antibacterial activity. The endophytic fungus F-05 was selected for the production of highest antibacterial compound against the *Staphylococcus aureus*.

The natural ingredients can be contained from the plant which has an antibacterial activity. *Naringi crenulata* (Roxb.) Nicolson. leaf has an antibacterial activity against *Staphylococcus aureus* bacteria (Jayapriya and Shoba, 2015). *Staphylococcus aureus* is one of the bacterial floras on human skin which can be a pathogen and causes diseases by the environment change (Kobayashi *et al*., 2015).

The present investigation aims to carry out antibacterial activities of *Naringi crenulata* (Roxb.) Nicolson. leaves of antibacterial potential against gram positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* and gram-negative bacteria such as *Salmonella thyphi, Pseudomonas fluorescens and Escherichia coli* (Sattar *et al*., 2004).

These endophytic fungal strains are associated with medicinal plants. Therefore, endophytic fungi are one of the best sources of bioactive compound. Their compound is active against a number of human pathogenic bacteria.

Acknowledgement

First of all, I would like to express my gratitude to Dr Htay Aung, Pro-rector, Loikaw University for his permission to undertake this research and for his valuable instruction and guidance.

I would like to express thanks to Dr Sann Sann Oo, Professor and Head of Botany Department, Loikaw University for her kind guidance, helpful advice, comments, numerous invaluable suggestions and permitting me to carry out the research in the Department. I am also indebated to all my colleagues and my pupils for their help in collection of the specimens.

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Elemental Analysis and Antimicrobial Activity on Leaves of *Polygonum hydropiper* L. (Smartweed)

Tin Tin Maw*

Abstract

The study was carried out to assess the elemental analysis and antimicrobial activity on leaves of smartweed. *Polygonum hydropiper* L. is also called smartweed which is belonging to family Polygonaceae. It is native to entire northern hemisphere (Europe, Asia and North America). The specimens were collected from Lashio area and identified with the help of available literatures. The energy dispersive X-ray fluorescence spectrometer (EDX 720, Shimadzu) used to determine the elemental analysis of leaves powder, showed that Potassium (K), Calcium (Ca), Sulfur (S) and Phosphorus (P) are major elements, and then Chlorine (Cl), Manganese (Mn), Iron (Fe), Zinc (Zn) and Copper (Cu) are trace elements. In antimicrobial activities of leaves extract is performed by agar well diffusion method, ethanol and methanol extracts of leaves are effective on seven tested organisms and ethanol extract is found to be significant against *Agrobacterium tumefaciens*. Therefore smartweed has provided in improving human health care system and further investigation.

Keywords: Smartweed, elemental analysis, antimicrobial activity

Introduction

Medicinal plants play a very important role in promoting the likelihood of human beings. Many new drugs from discoveries have actually exhibited vital impacts on health of human beings and many new drugs from plants are yet to be discovered. One of them from these important plant is smartweed, as its name suggests this plant is actually smart because it has various important medicinal uses (Website 4). *Polygonum hydropiper* L. is also called as smartweed, marshpepper, knoteweed. It is a plant of Polygonaceae family. All plant parts have been commonly used in traditional system of medicine (Moyeenul Haq *et al*., 2014). The plant grows in shallow water and in damp places and it is mainly found in New Zealand, Australia, Temperate Asia, North America (Pooja

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Arora, Lamba & Pankaj Sharma, 2018). In Myanmar, this plant is distributed in Shan State, Kachin State, Sagaing Division and Yangon Division (Kress & Yin Yin Kyi, 2003).

In recognition of the important role that major and trace elements play in health and disease of human body, in the building up and restoration phenomenon, it was observed that during the last 20 years remarkable progress has occurred in this area of health and sciences. Elements research has definitely been part of this explosion of scientific knowledge (Said, Saeed, Silva, Zubairy & Bano 1996). Impressive developments in the field of mineral elements have taken place in the chemical, biochemical and immunological areas of research. Deficiency of trace elements in human subjects can occur under the most practical dietary conditions and in many diseased statuses. In recent year, scientists and nutritionalists have started believing in the therapeutic role of metals in human health (Udayakumar & Begum, 2004).

Trace elements play both curative and preventive role in combating diseases. These is a vast scope to exploit the preventive medicinal aspects of various trace elements such as Cu, Cr, etc. Medicinal plants play the most important role in the traditional medicines (Kaneez, Qadirruddin, Kalhoro, Khaula & Bader, 1998). Mineral elements though usually form a small portion of total composition of most plant materials and of total body weight; they are nevertheless of great physiological importance particularly in the body metabolism. Their effects are related to concentration and recorded observation range from a deficiency state, to role as biological essential component to imbalance created when excess of one interferes with the function of another, to pharmacological activities (Bamiro, 1995)

Medicinal plants are potential sources of potent antimicrobial drugs and are used in many countries to treat infectious diseases (Bhaskarwar, Itankar & Fuluke, 2008). Over the years, traditional phytotherapy is in practice for the treatment of microbial origin diseases (WHO, 1978). World Health Organization (WHO) estimates that approximately 80% population of underdeveloped countries rely on medicinal plants for their primary health care (Farnsworth, Akerele, Bingel, Soejarto & Guo, 1985). Domestically the plant is used as anti-inflammatory, carminative, astringent, diuretic, CNS stimulant, diaphoretic, stomachic, emmenagogue, anthelmintic, in bleeding disorders and in diarrhea (Sharma, 2003). Conventionally, the whole plant decoction is used to treat different diseases

like dyspepsia, menorrhagia, hemorrhoids and skin itching (Chevallier, 1996).

The aims of the current study was to evaluate the elemental analysis and antimicrobial activity on leaves of smartweed.

Materials and Methods

Samples collection, identification and preparation

The specimens were collected from Lashio area, Northern State of Myanmar in December 2019. The collected specimens were identified according to Hooker, 1885; Backer, 1946; Lawrence, 1951; Dassanayake, 1997. The leaves of smartweed were washed with tap water and then air dried in shade at room temperature for two weeks. After completely dried, the samples were ground by grinding machine to get powders and stored in air tight containers for further studies.

Elemental analysis of sample by using EDXRF

The analysis of elemental concentration of powered leaves was carried out by using EDXRF spectrometer (EDX 720, Shimadzu) at University Research Centre (URC).

Antimicrobial activities of samples

The powdered samples were used to measurement of antimicrobial activities. The samples were extracted with ethyl acetate, ethanol, methanol and water. In this experiment, antimicrobial activities of four solvent extract samples were tested on seven microorganisms such as *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Bacillus pumilus*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Agar well diffusion method was used (Cruickshank, Duguid, Marmior & Swaim, 1975). These investigations were carried out in Department of Botany, Dagon University, Yangon Division.

Results

Morphological characters

Family name : Polygonaceae

Myanmar name : Phet-phe

English name : Smartweed, marshpepper, knoteweed

Flowering period : October to December

Polygonum hydropiper L. , Sp. Pl. ed 1, 361.1753.

Annual, sub-erect herbs, 40-870 cm high; stems and branches slender, glabrous. Leaves simple, alternate; stipules ochreate, tubular 1-1.5 cm long, membranous, sparsely to densely strigose; petioles 4-8 mm long, sheathing at the base, minutely brownish yellow or black glandular punctate throughout, strigose ciliate; blades lanceolate, 4-8 cm by 1-2.5 cm, obtuse at the base, entire along the margin, acuminate at the apex, glabrous on both surfaces. Inflorescences terminal spikes-like racemes, at ends of branches, with 3-5 subsessile flowers in axils of most leaves, raceme very slender, filiform, flexuous, 3-8 cm long, axis minutely glandular. Flowers pink or white, 4-5 mm in diameter, sessile; bracts ochreola, funnel-shaped, 2-3 mm long, glabrous, ciliate, densely glandular. Tepals 5, free, elliptic, 1.5-3 mm by 1-1.5 mm, densely glandular. Stamens 6, included; filaments filiform; anthers dithecous, minute. Ovary unilocular with one ovule on basal placenta; style 2, filiform; stigma simple. Achenes trigonous, 2-3 mm in diameter, dull-brown, minutely punctulate. [Figure 1].

Specimen examined : Northern Shan State, Lashio, Dr. Tin Tin Maw, December 15, 2019.

A. Inflorescence B. Fresh leaves C. Dry leaves D. Powdered leaves

Figure 1**.** Mophological characters of Smartweed

Elemental analysis of smartweed leaves by using EDXRF

The analysis of elemental concentration of powderd leaves was carried out by using EDXRF spectrometer at URC. The experimental data was shown in Table 1 and Figure 2.

Table 1. The relative concentration elements in the leaves of smartweed by using EDXRF

No.	Elements	Concentration value (%)
1	Potassium (K)	1.090
2	Chlorine (Cl)	0.883
3	Calcium (Ca)	0.645
4	Sulphur (S)	0.288
5	Phosphorus (P)	0.161
6	Manganese (Mn)	0.037
7	Iron (Fe)	0.018
8	Rubidium (Rb)	0.002
9	Zinc(Zn)	0.002
10	Copper (Cu)	0.001
11	Strontium (Sr)	0.001

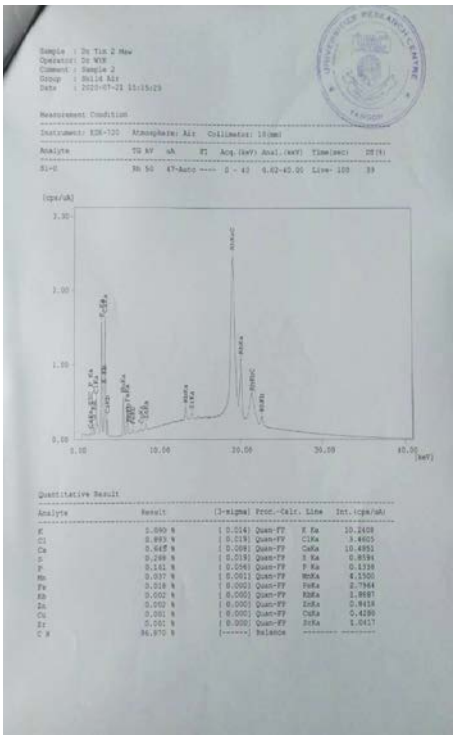

Figure 2. The relative concentration elements in the leaves of smartweed by using EDXRF

According to this results, eleven elements were found in leaves of smartweed. Among them, Potassium (K), Calcium (Ca), Sulphur (S) and Phosphorus (P) were found as major elements in smartweed leaves. And then, Chlorine (Cl), Manganese (Mn), Iron (Fe), Rubidium (Rb), Zinc (Zn), Copper (Cu) and Strontium (Sr) are trace elements in sample. Potassium (K) is found to be higher percentage in leaves.

Antimicrobial activities of smartweed leaves

The antimicrobial activities of ethyl acetate extract, ethanol extract, methanol extract, and water extract of smartweed leaves were conducted by Agar well diffusion method against various microorganisms.

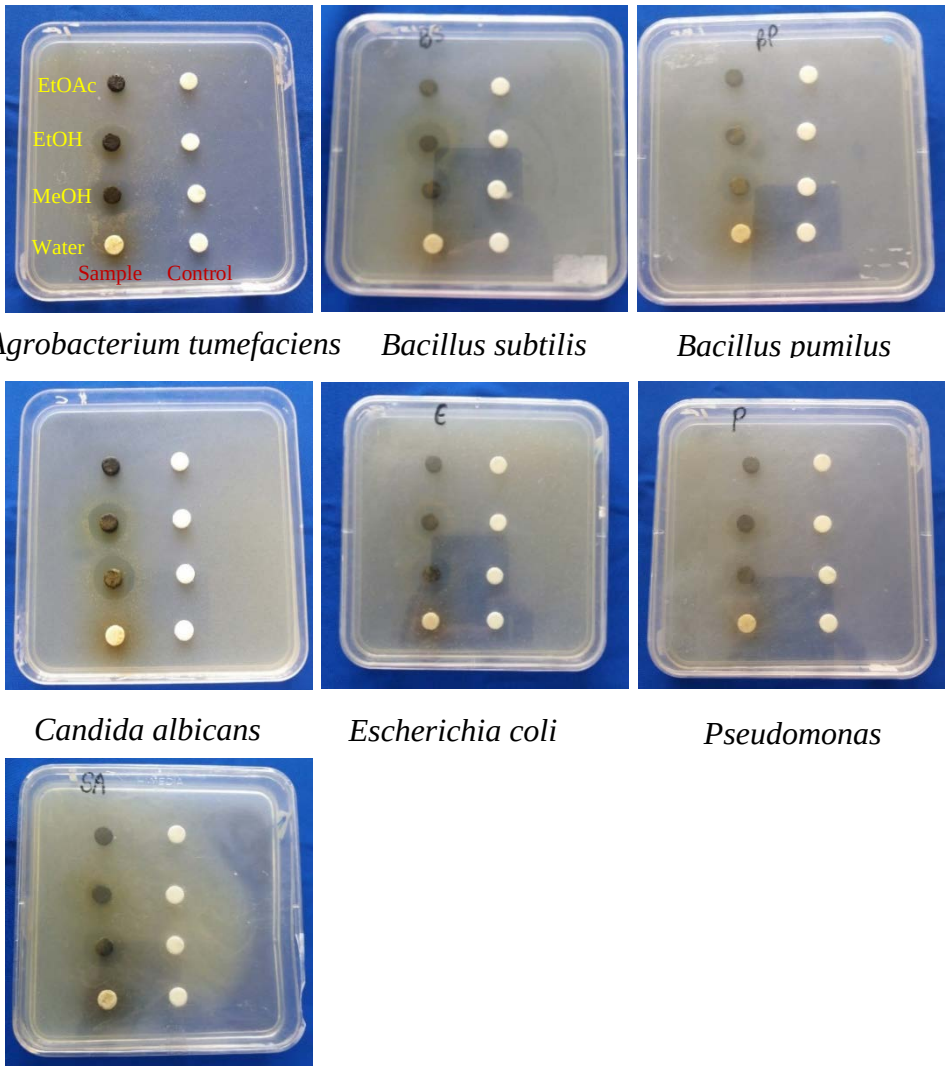

Figure 3. Antimicrobial activies of different solvent extracts of smartweed leaves

Table 2. Antimicrobial activities of different solvent extracts of smartweed leaves

Test organisms / **Solvents**	1	2	3	4	5	6	7
Ethyl acetate	-	-	-	-	-	-	-
Ethanol	23 (+++)	18 (++)	18 (++)	20 (+++)	19 (++)	19 (++)	18 (++)
Methanol	20 (+++)	21 (+++)	21 (+++)	18 (++)	16 (++)	17 (++)	12 (+)
Water	-	-	-	-	-	-	-

1. *Agrobacterium tumefaciens*
2. *Bacillus subtilis*
3. *Bacillus pumilus*
4. *Candida albicans*
5. *Escherichia coli*
6. *Pseudomonas aeruginosa*
7. *Staphylococcus aureus*

Agar well - 8 mm

10 mm ~ 14 mm (+) = minimum inhibitory concentration

15 mm ~ 19 mm (++) = medium inhibitory concentration

20 mm above (+++) = maximum inhibitory concentration

In antimicrobial activities, ethanol and methanol extracts of leaves are effective on seven tested organisms. But ethyl acetate and water extracts of leaves are no effective on all tested organisms. Among them, ethanol extract of leaves is more sensitive against on *Agrobacterium tumefaciens* and *Candida albicans*, but responds medium activity on *Bacillus subtilis*, *Bacillus pumilus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Methanol extract of leaves is more sensitive against on *Agrobacterium tumefaciens*, *Bacillus subtilis* and *Bacillus pumilus*, but responds medium activity on *Candida albicans*, *Escherichia coli* and *Pseudomonas aeruginosa*, and gave low activity on *Staphylococcus aureus* (Figure 3, Table 2).

Discussion and Conclusion

In the present study, elemental analysis and antimicrobial activities on leaves of smartweed were carried out.

According to elemental analysis, Potassium (K), Calcium (Ca), Sulphur (S) and Phosphorus (P) were found as macronutrient elements whereas Chlorine (Cl), Manganese (Mn), Iron (Fe), Rubidium (Rb), Zinc (Zn), Copper (Cu) and Strontium (Sr) were found as micronutrient elements. (Table 1, Figure 2)

Potassium is one of the seven essential macrominerals. A high potassium intake reduces the risk of overall mortality by 20 percent. It also decreases the risk of stroke, lowers blood pressure, protects against loss of muscle mass, preserves bone mineral density, and reduces the formation of kidney stones (Website 6). Calcium is a mineral that is necessary for life. In addition to building bones and keeping them healthy, calcium enables our blood to clot, our muscles to contract, and our heart to beat. About 99% of the calcium in our bodies is in our bones and teeth (Website 11). Sulfur is one of the basic building blocks of vibrant health, essential for maintaining everything from youthful skin and joints to a healthy digestive system (Website 5). Phosphorus offers numerous health benefits because it affects many different systems in the body. Some of the benefits of phosphorus include keeping the bones and teeth strong, healping the muscles contract, aiding muscle recovery after exercise, filtering and removing water from the kidneys, promoting healthy nerve conduction throughout the body, making DNA and RNA, and managing the body' s energy usage and storage (Website 9).

Chlorine chemistry is critical to manufacturing medicines we depend on, including some that help lower cholesterol, control arthritis pain and relieve allergy symptoms (Website 2). Manganese is a trace mineral. It is vital for the human body, but people only need it in small amounts. Manganese contributes to many bodily functions, including the metabolism of amino acids, cholesterol, glucose, and carbohydrates. It also plays a role in bone formation, blood clotting, and reducing inflammation (Website 10). Iron helps to preserve many vital functions in the body, including general energy, and focus, gastrointestinal processes, the immune system, and the regulation of body temperature (Website 7). Zinc is needed for healthy growth. It plays a vital role in immune function, wound healing, blood clotting, thyroid function, and much more. Consequently, a potential

medical role of zinc for the treatment of Covid-19 has been highlighted. Currently, however, there is no evidence that taking zinc will protect against Covid-19 or make the disease milder. Nevertheless, epitomizing the role of zinc for the human body is certainly worthwhile (Website 3). Copper is an essential nutrient for the body. Together with iron, it enables the body to form red blood cells. It helps maintain healthy bones, blood vessels, nerves, and immune function, and it contributes to iron absorption. Sufficient copper in the diet may help prevent cardiovascular disease and osteoporosis, too (Website 8)

Rubidium is very similar to potassium. Normal human adults contain about 300 mg in all tissues, more than most of the other ultra trace elements. It also acts as nutritional substitute for potassium. The metabolism of rubidium are closely related to potassium, and they show interchangeability with potassium in a variety of biological systems (Website 1). Strontium resembles calcium element in it properties; like calcium, it is taken up and is preferably implanted into the bone. Strontium may have both beneficial and deleterious effects on human, depending on the amount received. (Hollriegl and Munchen, 2011).

In antimicrobial activities of ethanol and methanol extracts of leaves showed active against seven microorganisms. But ethyl acetate and water extracts of leaves displayed no active on all tested organisms. From these experimental results, ethanol extract indicated the most large inhibition zone on *Agrobacterium tumefaciens* (23 mm). The ethanol and methanol extracts of smartweed leaves played antibacterial activity against *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Bacillus pumilus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and antifungal activity against *Candida albicans* (Figure 3, Table 2).

Therefore leaves of smartweed can be used for mysterious skin disease, illness, food poisoning, sore throat, toxic shock, eye infection syndrome, respiratory tract infection, dermatitis, soft tissue infection, joint infection, gastrointestinal infection, cancer, AIDS, diarrhea, pneumonia, urinary tract infection, and genital yeast infection.

The present study aims to evaluate the elemental analysis and antimicrobial activity on leaves of smartweed. Based on the result it can concluded that the macronutrient elements and micronutrient elements play a vital role in human body. Smartweed has antimicrobial activity and can be

used to treat in many diseases. Therefore, the current research has provided in improving human health care system and further investigation.

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Survey on Ten Benthic Algae from Ngwe Taung Dam in Demoso Township, Kayah State

Khin Khin Phyu[1], Thant Sin Nwe[2], Htay Htay Myint[3] & Myat Myat Moe[4]

Abstract

The benthic algae of the Ngwe Taung Dam were taken from different habitats epipelic, epiphytic, epilithic and epizoic. These samples were collected from different localities along the abutment of the dam in May, 2018. Total of 10 taxa were identified. Among them, 5 species belong to division Chlorophyta, 2 species belong to Division Chrysophyta and 3 species belong to Division Cyanophyta. The results of this survey give the information about habitat of existing benthic algae species of Ngwe Taung Dam and their microscopical characters.

Keywords: Benthic algae, Cyanophyta, Chrysophyta, Chlorophyta

Introduction

Ngwe Taung Dam was set in 1965 which is located in the Demoso Township of Kayah State, eastern part of Myanmar. It has a geographical area of over 6000 sq ft and the height of dam is 35 ft. It possesses beautiful freshwater bodies.

Algae are considered the important biological organisms. They are the source of oxygen and the first ring of the food chains in aquatic ecosystems (Round,1973). Algae are primarily oxygen releasing photosynthetic organisms which have been shown to have a higher photosynthetic efficiency than terrestrial plants (2.8% compared to 1.2%) resulting in a solar to chemical energy conversion (Taylor, 2013).

The simplest form is the single, self-sufficient cells. Numerous one-celled algae may clump together to form a colony. Other algae are multicellular organisms. In the simplest multicellular algae, the cells are joined end to end and forming filaments, both branched and unbranched. The most complex algae have a variety of specialized tissues, including a

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rootlike holdfast, a stipe (Chandrakant, 2013).Many species are capable of self-locomotion by twisting, bending, gliding, swimming, motile spores and gametes develop in many species (Pooja, 2011).

Reproduction in algae can be vegetative, asexual or sexual. Vegetative reproduction occurs through formation of spores and binary fission whereas sexual reproduction take place by fuse of two haploid gametes. For the exception of the Cyanobacteria, algae are eukaryotes. Most algae have rigid cell walls composed largely of cellulose like plants. An exception is Diatom which cell wall is composed of silica. The division of algae is distinguished from each other based on a combination of characteristics, including photosynthetic pigments starch-like reserve products, cell covering and other aspects of cellular organization (Chandrakant, 2013).

Algae are influenced by the temperature, sunlight, nutrients and other biotic and abiotic factor. The food chain of animals is numerous and varied. Some fishes feed directly on the algae, while others obtain the energy bound up in the plants by eating animals that feed on algae. Algae are economically important both directly and indirectly. Commercial transactions involving the collection, processing and sale of algae amount annually to several millions dollars. When exposed to light, some of them are green and synthesize both sugar and amino acid, others remain colorless and obtain food only from external sources (Pooja 2011).

Benthic algae are present in the bottom sediments of almost all aquatic systems, where they require adequate light to carry out photosynthesis and growth. Particular taxonomic groups, such as filamentous green algae and pennate Diatoms are typical of benthic conditions, while Volvocales and centric Diatoms are characteristic of the pelagic environment. Benthic algae in freshwater habitats are mainly dominated by Cyanobacteria, green algae, Diatoms and red algae. In benthic algae, epiphytic, epilithic, epizoic, and epipelic algae are classified as their habitat (Sigee, 2004).

The aim and objectives of this study were to describe some benthic algae flora found in Ngwe Taung Dam and to study the microscopical characters and habitats in water bodies.

Figure 1. Location Map of Ngwe Taung Dam

Figure 2. View of Ngwe Taung Dam

Materials and Methods

Specimens Collection

A total of 10 samples from different localities of Ngwe Taung Dam at latitudes 19º 32' N and longitudes 97º 10' E comprising of various habitats, e.g., aquatic plants, shells of snails, rocks, dead leaves under water and sediments during May 2018. All of the samples were placed under favorable conditions to get good light and air.

Observation the Specimens

Epiphytic algae from aquatic plants, epizoic algae from shells of snails; epilithic algae from rocks, epipelic algae from dead leaves and sediments were observed using the electronic microscope for presence of

algal form at the Department of Botany, Dagon University. The measurements of cell length and width were calculated by micrometry.

Identification of the Specimens

The samples were identified on the thallus shapes, sizes, color, chloroplasts, pyrenoids and sinus structures by following standard monographs of Smith (1950), Prescott (1962) and John *et al.* (2008). According to John *et al*. (2008), the identified algae were classified.

Results and Discussion

In this survey, algae were randomly collected from the abutment of the Ngwe Taung Dam and analyzed under electronic microscope. 10 species, 9 genera, 6 families, 5 orders, 3 classes and 3 divisions were recorded as benthic algae. According to their habitats, the collected benthic algae were epiphytic, epipelic, epilithic and epizoic (Table 1). The micrograph of collected algae were showed in Figure 3.1 – 3.3.

Division - Cyanophyta

Class - Cyanophyceae

Order - Oscillatoriales

Oscillatoria limosa (Roth) C. Agardh, 1812

Trichomes usually forming a very dark blue-green attached to a submerged object, straight, tapering little or not at all toward the apex; apical cell rounded, the outer membrane thickened but without calyptra; not constricted at the cross walls which are granular. Cells 10 µm in diameter, 2.5 µm long.

Phormidium tenue (Menegh) Gomont, 1892

Trichomes forming a blue-green; sheaths diffluent, mucilaginous and indistinct; straight except at apices, where they are bent and attenuated; apical cell conical, smooth; constricted at the cross walls, which are not granulate; cell contents homogeneous. Cells 2.5 µm in diameter, 5 µm long.

Lyngbya taylorii Drouet & Strickland, 1940

Trichomes floating free, solitary, straight and not tapering toward the apices; sheaths thin and colorless; apical cell broadly convex; very little

constricted at the cross walls; cell contents granular. Cells quadrate, 7.5 µm in diameter, 5 µm long.

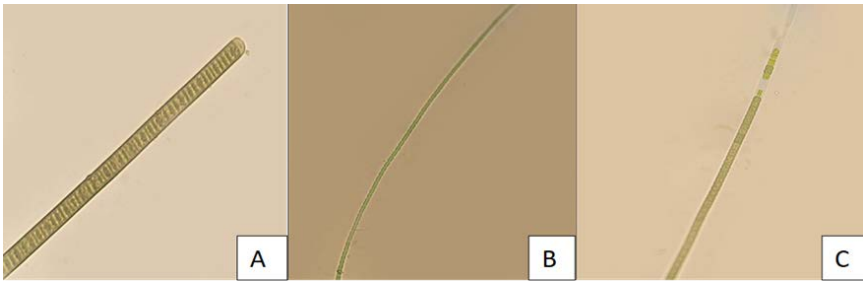

Figure 3.1. A. *Oscillatoria limosa* (Roth) C. Agardh
B. *Phormidium tenue* (Menegh) Gomont
C. *Lyngbya taylorii* Drouet & Strickland

Division - Chrysophyta

Class - Bacillariophyceae

Order - Pennales

Gomphonema truncatum Ehrenberg, 1832

Frustules colony, epiphytic on gelatinous peduncles; cuneiform in girdle view; rectangular in apical end, with attenuated base; raphe present; cell 12.5 µm in diameter, 37.5 µm long.

Amphora proteus W. Gregory, 1857

Frustules broadly elliptic with truncate end in girdle view, reniform in valve view; apical axis 12.5 µm; cell 30 µm in diameter, 50µm long.

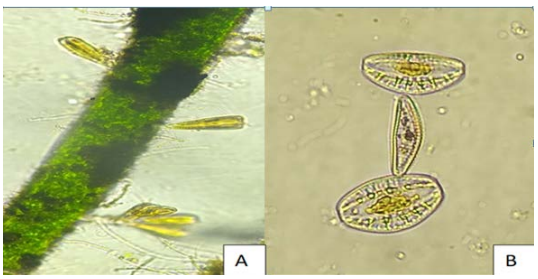

Figure 3.2. A. *Gomphonema truncatum* Ehrenberg

B. *Amphora proteus* W. Gregory

Division-Chlorophyta

Class -Chlorophyceae

Order -Ulotrichales

Ulothrix tenuissima (Weber & Mohr) Kutzing, 1833

Filament long; cell cylindrical that are shorter than wide, without constrictions at the crosswall; chloroplast folded, parietal plate, about 2/3 the length of the cell, with several pyrenoids; cell 20 μm in diameter, 15 μm long.

Ulothrix zonata (Weber & Mohr) Kutzing, 1833

Filament attached, long; cell short with constriction at the cross wall; chloroplast a complete circular band, with several pyrenoids; cell 27.5 μm in diameter, 12.5 μm long.

Division-Chlorophyta

Class -Chlorophyceae

Order - Cladophorales

Cladophora profunda Brand, 1902

Thallus composed of attached, irregularly and much branched filaments growing from a prostrate. Basal branches ending in colorless rhizoid-like cells, upper branches irregular in arrangement; cell 40 μm in diameter, 250 μm long.

Division-Chlorophyta

Class -Chlorophyceae

Order -Oedogoniales

Bulbochete C. Agardh, 1817

Filaments richly branched, each cell with a bulbous base and two such hairs on a terminal cell; chloroplast parietal and net-like, usually with several pyrenoids; vegetative cells 17 μm in diameter, 2 times long as wide.

Division-Chlorophyta

Class -Chlorophyceae

Order -Chloroccocales

Characium A. Braun in Kutzing, 1849

Cell solitary, symmetrical, saccate, stipe short; chloroplast parietal; cell 15 µm in diameter, 30 µm long.

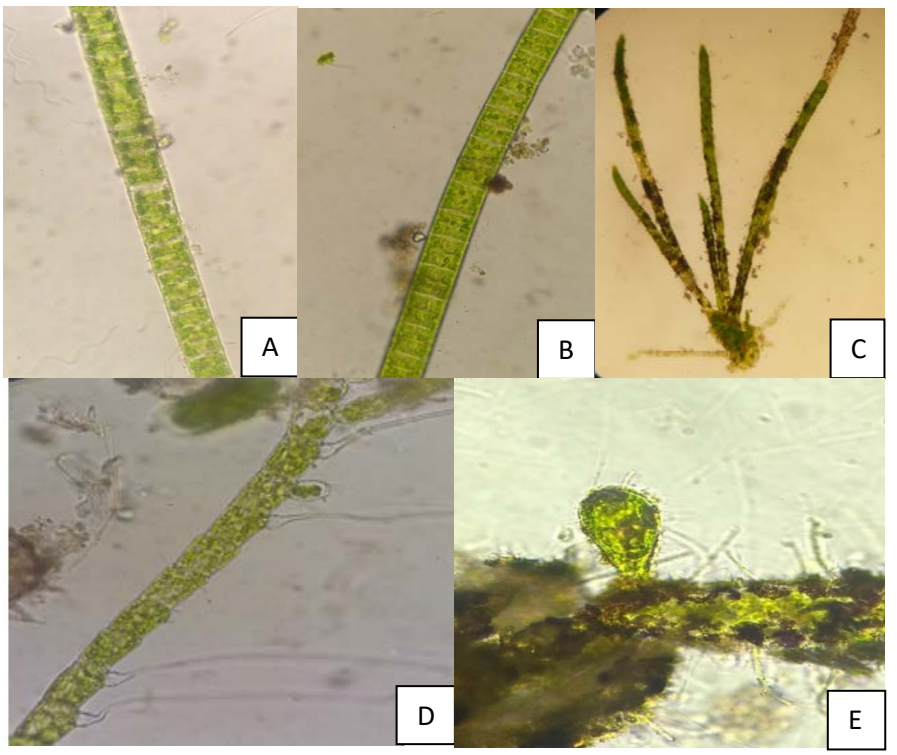

Figure 3.3. A. *Ulothrix tenuissima* (Weber & Mohr) Kutzing
B. *Ulothrix zonata* (Weber & Mohr) Kutzing
C. *Cladophora profunda* Brand
D. *Bulbochete* C. Agardh
E. *Characium* A. Braun in Kutzing

Table 1. Different habitats of benthic algae found in Ngwe Taung Dam

No.	Scientific Name	Epiphytic	Epilithic	Epipelic	Epizoic
1	*Oscillatoria limosa*			+	
2	*Phormidium tenue*			+	
3	*Lyngbya taylorii*		+	+	
4	*Amphora proteus*		+	+	
5	*Gomphonema truncatum*	+			
6	*Ulothrix tenuissima*				+
7	*Ulothric zonata*	+	+		
8	*Cladophora profunda*				+
9	*Bulbochete*	+	+		
10	*Characium*	+			

The present data were collected from Ngwe Taung Dam in May, 2018. Total of 10 species, 9 genera,6 families, 5 orders, 3 classes and 3 divisions were recorded as the benthic algae. In Division Chlorophyta, the collected genera were *Ulothrix*, *Cladophora*, *Bulbulchete* and *Characium*. Among them, *Ulothrix* has multicellular and unbranded filaments. The other *Bulbochete* and *Cladophora* are also multicellular but their thallus is branched. The genus *Characium* is unicellular. In *Ulothrix*, 2 species were surveyed. These were *Ulothrix tenuissima* and *U.zonata.* The cell of both species are short and cylindrical but *U.zonata* has constrictions at the cross walls. Chloroplasts of the genus *Ulothrix* is parietal bands. In genus *Cladophora*, *C.profunda* is an attached thallus which composed irregularly and much-branched filaments growing from a prostrate. The genus *Characium* is also attached and unbranched, their stalks are short and stout. Cell is club-shaped. All the characters of above algae agreed with Smith (1983), Presscott (1962) and John *et al* (2008).

Some Diatoms belong to the Division Chrysophyta are also surveyed. *Gomphonema truncatum* is not only the colonial form but also solitary and epiphytic on gelatinous peduncles cuneiform in girdle views.

The genus *Amphora* of diatoms belongs to Chrysophyta is broadly elliptic in girdle views, strongly and convexly curved in valve views. These characters agreed with Smith (1950).

According to present data, *Cladophora profunda* and *Ulothrix tenuissima were found* on shells of snails. *Ulothrix zonata and Bulbochete* found as epiphytic on aquatic plants and rocks. *Characium* attached on the genus *Cladophora. Gomphonema truncatum* attached with gelatinous stalk on the genus *Cladophora*. *Oscillatoria limosa, Phormidium tenue, Lyngbya taylorii* and *Amphora proteus* were found in sediment over the dead leaves. *Lyngbya taylorii* and *Amphora proteus* also were also found on rocks. These habitat characters of the surveyed algae were in agreement with Smith (1950), Prescott (1962) and John *et al* (2008).

Sigee, (2004) reported that filamentous green algae and pennate Diatoms are typical of benthic conditions, while Volvocales and centric Diatoms are characteristic of the pelagic environment and benthic algae in freshwater habitats are mainly dominated by Cyanobacteria, green algae, Diatoms and red algae. Exception of the presence of red algae, the other conditions agreed with present study.

According to Poulickova *et al.,* (2008), epiphytic algae are the primary producers that fix carbon and uptake essential nutrients from the water column, thereby making these accessible at higher trophic levels. Epipelic algae perform a range of ecosystem functions including biostabilisation of sediments, regulation of benthic-pelagic nutrient cycling, and primary production. There is a growing need to understand their ecological role in the light of current and future alterations in sediment loading resulting from land-use change and land management practices. Although the majority of recent work on benthic algal species has been conducted within freshwater, significant advances have also been made in both freshwater and estuarine ecosystems.

Conclusion

This present survey was taken as a preliminary study it is difficult to describe the definite information about existing algae in the surveyed area. This present survey can give little basic information for further researchers in the study of the freshwater algae in Ngwe Taung Dam.

Acknowledgments

We would like to express our thanks to Dr. Myat Myat Moe, Professor, and Head of Botany Department, Dagon University, Myanmar for providing the departmental facilities during study periods and we would like to especially thank Dr. Thar Tun Maung, Rector, Dagon University for allowing us to undertake this survey.

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Phytochemical Properties of *Capsicum minimum* Roxb. and It's Antibacterial Activity

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Abstract

Capsicum minimum Roxb. was cultivated throughout in Myanmar. A characterization study on morphology of the plant was done according to available literatures and assumed to be coherent with those authors citation. In phytochemical tests indicated the presence of alkaloid, glycoside, phenolic compound, steroids, tannin, saponin, reducing sugar, ∞ amino acids and carbohydrates but no cyanogenic glycosides. In physicochemical characterization, the aqueous extract of these species was found to be most soluble in water and less soluble in pet-ether and ethylacetate. The antibacterial activity of the fruits extracts of ethylacetate, acetone, pet-ether, methanol and ethanol showed the prominent antibacterial activity against the *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albican* and *E.coli.*

Keywords: Morphology, phytochemical, antibacterial activities

Introduction

The fruits of *Capsicum* are known as chillies and belong to the family Solanaceae, which are commercially used as daily diet, spices, dyes and medicinal purposes. Chilli peppers are indigenous to the tropical regions of America and West Indies, but are cultivated now throughout the tropics. They are popular in West African soups. Peppers were not known in Europe before the discovery of America. Most of the species are domestically important as appetizer and used daily in Myanmar curries. The fruits of *Capsicum* species are widely known to be important in medicine as counter-irritant and carminative action (Wealth of India, 1950). It can be used to treat sore-throat, dyspepsia and diarrhoea. It also has rube facient, laxative and antiseptic effects (Indian Medical Plants, 1975). It is powerful stimulant and useful to treat cholera, diarrhoea. Externally can be used as an antiseptic gargle to relax sore-throat and chronic hoarseness (Hand book of natural food's by Jiva daya U San Hla).

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Materials and Methods

Botanical studies

Collection, Identification and Preparation of *Capsicum minimum* Roxb.

Specimens of *Capsicum minimum* Roxb. were collected from Mingaladone Townships, Yangon Division. The vegetative and floral parts of fresh specimens were used for identification according to (Backer, 1965, Dassanayoke, 1983, Hundley & Chit Ko Ko ,H.G, 1978, Hooker .1885, Kress, 2003, Kirtikar, K.R & Basu, 1975). The fruits were dried in shade for several days. When completely dried, these were pulverized by grinding machine to get the powdered and stored in an airtight container for the chemical and microbiological studies.

Chemical Studies

Phytochemical investigation of *Capsicum minimum* Roxb.

Preliminary phytochemical examination for the fruits were carried out to detect the organic compounds qualitatively (British Pharmacopoeia, 1965; Harbone, 1973; Trease and Evans, 1980). These results were shown in Table (1).

Physicochemical characterization of *Capsicum minimum* Roxb.

The quantitative analyses, namely determination of moisture content, determination of total ash, acid insoluble ash, water soluble ash and extractive values for the various solvents were made according to (British Pharmacopoeia, 1980). These results were shown in Table (2).

Microbiological studies

Antimicrobial activities tests

The powdered samples were extracted by using pet-ether, acetone, methanol, ethyl acetate, ethanol and water. The various solvent extracts were tested against six pathogenic microorganisms *(Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albican and Escherichia coli)* by using agar-well diffusion method (Cruickshank R., 1975). This test was conducted at the

Development Centre for Pharmaceutical Food Technology (DCPFT).

Preparation of antimicrobial activities tests

Nutrient agar was prepared according to agar-well diffusion method described by Cruickshank R. in 1975. 20 - 25 ml of boiled Nutrient agar was poured into each test-tube, plugged with cotton wool and autoclaved at 121° C for 15 minutes. The tube were cooled down to about 30 - 35° C and poured into sterilized petri dishes. Then 0.1 - 0.2 ml of test organisms were also seeded respectively into each petri dishes. The agar was then allowed to gel for 2-3 hours. After 10 mm agar-well were made with the help of sterilized agar well cutter. After that, about 0.2 ml of each sample plant extract was introduced into the agar-well and incubated at 37° C for 24 hours. The appearance of inhibition zone around agar-well indicates the presence of antimicrobial activity. This results were shown in Table (3).

Results

Botanical studies

Morphological characters of *Capsicum minimum* Roxb.

Perennial erect small shrubs, the stems angular with shallowly longitudinal 4-5 ridges at the apex, terete at the base. Leaves alternate, simple, sometimes whorled near the apex, exstipulate, petioles slender, long, glabrous on both surfaces, oblique at the base, entire at the margin , acuminate at the apex. Inflorescences terminal and axillary solitary cyme. Flowers ebracteate, pedicellate, ebracteolate, bisexual, regular, actinomorphic, pentamerous, hypogynous. Sepals (5), cup-shaped, valvate, sepaloid, persistent. Petals (5), campanulate, valvate in bud, acute at the tip, petaloid (white).Stamens 5, epipetalous inserted within the lobes near the throat; the filaments filiform, glabrous, purple; the anther dithecous, basifixed, introrse, longitudinal dehiscence, purple. Ovary(2), bicarpellary, syncarpous, bilocular, obliquely placed, numerous ovules in each locule, axile placentation; the style slender, the stigma simple; the ovary superior. Fruits berries, linear-cylindrical, slightly curved and tapering to the apex, board at the base, green when young, red when ripe with persistent calyx, hot in taste; seeds reniform, compressed, somewhat wrinkled, glabrous, cream - coloured. (Fig.1)


Habit


Leaves


Inflorescence

Flower

Fruits


Seeds

Figure 1. Morphological characters of *Capsicum minimum* Roxb.

Chemical Studies

Phytochemical Investigation

In phytochemical investigation indicated the presence of alkaloid, glycoside, phenolic compound, steroids, tannin, saponin, reducing sugar, ∞ amino acids and carbohydrates but no cyanogenic glycosides.

Table 1. Preliminary phytochemical test on the fruits of *Capsicum minimum* Roxb.

No	Type of Product	Test reagent	Observation	Results
1.	Alkaloid	Mayer's reagent Dragendorff's reagent Sodium picrate solution	White ppt Orange ppt Yellow ppt	+ + -
2.	Glycoside	10% lead acetate	White ppt	+
3.	Phenolic compound	$FeCl_3$ solution	Blue colour	+
4.	Steroids	Benzene, Acetic anhydride, and conc: H_2SO_4	Green colour	+
5.	Tannin	(a)Ferric chloride test solution (b) lead subacetate solution	Deep Blue colour Brown ppt	+ +
6.	Saponin	Distilled water	Frothing	+
7.	Cyanogenic Glycoside	Sodium picrate solution , conc: H_2SO_4	No colouration	-
8.	Reducing sugar	Benedict's solution	Brick-red ppt	+
9.	α-amino acids	Ninhydrin reagent	Pink colour	+
10.	Carbohydrates	10% α-Naphthol, conc: H_2SO_4	Red ring	+

+ = Present - = Absent

Physicochemical characterization of *Capsicum minimum* Roxb.

In physicochemical characterization, the percentage of moisture content, total ash, acid insoluble ash, water soluble ash, water soluble matter, ethanol soluble matter, methanol soluble matter, ethyl acetate soluble matter, acetone soluble matter and pet-ether soluble matter.

Table 2. Physicochemical properties of *Capsicum minimum* Roxb.

No.	Physicochemical characters	Quality determined percentage
1.	oisture content	5.09
2.	Total ash	7.59
3.	Acid insoluble ash	1.82
4.	Water soluble ash	11.05
5.	Water soluble matter	70.09
6.	Ethanol soluble matter	26.31
7.	Methanol soluble matter	32.35
8.	Ethyl acetate soluble matter	1.45
9.	Acetone soluble matter	19.91
10.	Pet-ether soluble matter	5.76

Microbiological studies

Antimicrobial activities of different solvents extracts of *Capsicum minimum* Roxb.

The antimicrobial activities of *Capsicum minimum* Roxb. fruits extracts by different solvents were estimated by agar well diffusion method. In this test, ethanol extracts revealed the inhibitory zone of 19 mm against *Escherichia coli*, 18 mm against *Pseudomonas aeruginosa*, 17 mm against *Bacillus pumalis, Candida albican* and 16 mm against *Bacillus subtilis, Staphylococcus aureus*. Methanol extracts revealed the inhibitory zone of 17 mm against *Staphylococcus aureus*, *Escherichia coli* and 15 mm against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albican*. Ethyl acetate extracts revealed the inhibitory zone of 15 mm against *Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa*, *Bacillus pumalis, Candida albican* and *Escherichia coli*. Acetone extracts showed the inhibitory zone of 17 mm against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, 16 mm against *Candida albican*, *Escherichia coli* and 15 mm against *Bacillus subtilis*. Pet-ether extracts showed the inhibitory zone of

15 mm against *Bacillus subtilis* and 13 mm against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albican* and *Escherichia coli*. Water extracts showed the inhibitory zone of 12 mm against *Escherichia coli*, 11 mm against *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albican* and did not show the inhibitory zone of *Bacillus subtilis* and *Staphylococcus aureus*.

Table 3. Antimicrobial activities of different solvent extracts of *Capsicum minimum* Roxb.

Organisms						
Solvent	***Bacillus subtilis***	***Staphylococ-cus aureus***	***Pseudomonas aeruginosa***	***Bacillus pumalis***	***Candida albican***	***E - coli***
Pet-ether	15 mm	12 mm	12 mm	12 mm	12 mm	12 mm
Methanol	15 mm	17 mm	15 mm	15 mm	15 mm	17 mm
Acetone	15 mm	17 mm	17 mm	17 mm	16 mm	16 mm
Ethyl acetate	15 mm	15 mm	15 mm	15 mm	15 mm	15 mm
Ethanol	16 mm	16 mm	18 mm	17 mm	17 mm	19 mm
Water	-	-	11 mm	11 mm	11 mm	12 mm

Agar well - 10 mm

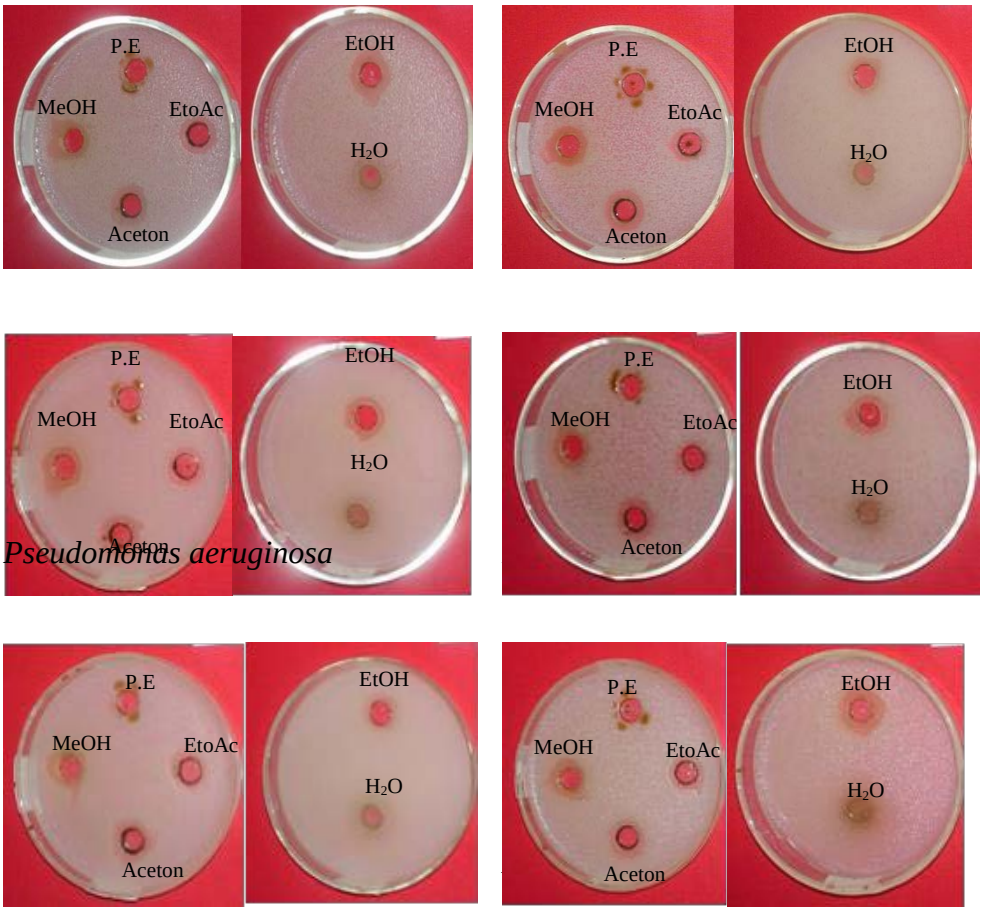

Figure 2. Antimicrobial activities of *Capsicum minimum* Roxb.

Discussion

The plants of *Capsicum minimum* Roxb. was cultivated throughout the Myanmar. The morphological characters of this plants are agreement with available literatures.

In phytochemical tests indicated the presence of alkaloid, glycoside, phenolic compound, steroids, tannin, saponin, reducing sugar, ∞ amino acids and carbohydrates but no cyanogenic glycosides. In physicochemical characterization, the aqueous extracts of *Capsicum minimum* Roxb. was found to be most soluble in water and less soluble in pet-ether and ethyl acetate.

In antimicrobial activities, the fruit extracts of *Capsicum minimum* Roxb. of ethanol, methanol, ethylacetate, acetone and pet-ether against the six pathogenic organisms and water extracts showed the antibacterial activity against the *Pseudomonas aeruginosa, Bacillus pumalis, Candida albican* and *E.coli.*

Conclusion

Capsicum minimum Roxb. is widely cultivated in Myanmar. The fruits are commercially valuable. The morphological study of the plant *Capsicum minimum* Roxb. belonging to the family Solanaceae.

The antibacterial activity of the fruits extracts of ethyl acetate, acetone, pet-ether, methanol and ethanol showed the prominent antibacterial activity against *the Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albican* and *E.coli.*

Thus, further investigation is needed for the application in medicinal uses to upgrade through systematic evaluation to confirm its bioactive constituents.

Acknowledgements

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Study on Pollen Morphology of Twelve Herbaceous Plants Grown in Khin Gyi Village, Wundwin Township

May Thu Khin[1], Thida Myint[2] & Swe Swe Linn[3]

Abstract

In this research, the description of pollen morphology being 7 families of 11 genera of 12 species of Angiosperm species were identified, classified and described. All the specimens were collected from Khin Gyi Village, Wundwin Township. The collected species were prepared with the standard method by Erdtman (1960). Pollen of all the species have been recorded with their aperture number, position, shape, size, and sculpture of the exine; all investigated species were monads varing from colpate, polycolpate, polyporate, colporate, and inaperturate. The sculpture pattern of the above ranged from reticulate, coarsely reticulate, obscursely reticulate and foveolate, echinate, retipilate and croton-pattern. The pollen key to the species of Angiosperms has also been constructed on the basis of the acquired palynological data. Photo-micrographs of all species have included both equatorial and polar views.

Keywords: Pollen morphology, monads, key, Khin Gyi Village, Wundwin Township

Introduction

Palynology is the study of the pollen grains and spores. In a wider sense, it also comprises the study of microfossils other than pollen grains and spores Hyde and Williams, 1944).

Pollen is a source and transport unit for the male gametes. The unicellular pollen grains represent the microspore of seed plants, the multicellular pollen grain represent the male gametophytic generation. The development of a pollen grain includes microsporogenesis and microgametogenesis (Gomez *et al*., 2015: Keijzer and Willemse, 1988).

Pollen grains and spores can be divided into groups on the basis of the number, position and character of their apertures. The classification is

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basically simple and consistent. The number of aperture of apertures is indicated attaching the prefixes mono-, di-, tri-, tetra-, penta- and hexa- before the terms colpate, porate and colporate. More than six apertures are indicated by the use of the prefix poly-. In most cases the pori and/ or colpi are arranged equidistantly around the equator of the grain; This is indicated by the prefix zono-. If the apertures are scattered all over the surface of the grain the prefix panto- is used (Moore, Webb, Collinson 1991).

With the polar axis comprising the major axis of the ellipse, the pollen may be termed spherical, prolate-spheroidal, subprolate, prolate, and perprolate, while those-(where the polar axis comprises the minor axis of the ellipse) are termed spherical, oblate-spheroidal, suboblate, oblate and peroblate (Erdtman 1954).

The pollen wall of seed plants is formed by two main layers; the outer exine and the inner intine. The exine consists mainly of sporopollinin, which is an acetiolysis and decay-resistant biopolymers. The intine is mainly composed of cellulose and pectin.

Pollen wall patterns are so diverse and so characteristic of each species that they have long been used for taxonomic classification and even for forensic identification (Szibor *et al*., 1998).

Pollen morphology supports the taxonomic suggestion to separate the species in a special section or genus (Keri and Zetter, 1992). Also it supports a phylogenetic study of molecular and morphological data (Bradford and Barnes, 2001).

The study area is lied in Khin Gyi Village on the north eastern border of Wundwin Township. The village is about 4.5 miles (11.7) distance form Wundwin Township. It is located at the interestion of 31° 51′ 54.3″ N latitude and 30° 19′ 04.0″ E longitude.

The aim of this study is to document of pollen morphology of (12) species belonging to genera, families found in Khin Gyi Village, Wundwin Township, to determine pollen variation was significant within and between (12) species of various plants and to support for further studying in morphological characters.

Materials and Method

The specimens were collect from Khin Gyi Village, Wundwin Township during the flowering and fruiting period. The collected plants were photographed to record their habit and flower characters. Identification was carried out by using floristic literature Hooker (1874), Nair (1962), Kress *et al.* (2003), Hundley and Chit Ko Ko (1978) and Dassanayake (1988). All pollen samples were freshly collected from the anthers of open flowers. The collected specimens of each species were stored in small glass vials with 1 cc of glacial acetic acid and labeled. The collected pollen samples in glacial acetic acid were acetolysed by the standard acetolysis method (Erdtman 1960). The acetolysis solution was prepared using a measuring cylinder; 9 parts of acetic acid and 1 part of sulphuric acid were added. Acetolysis mixture 1 cc was poured into the test tube containing the pollen samples and stirred with a glass rod. The test tube was heated by a water-bath with 80°C for approximately 4 minutes (up to 10 min). The test tube was allowed to cool and the samples were diluted with distilled water and centrifuged for 30 minutes at 3000 rpm. After centrifuging and decanting, the samples were washed in acetic acid and 3 times with water. A few diluted glycerine jelly was then added to the samples and this was then transferred to the storage bottles and labeled. The mounted slides were observed under light microscope to study pollen morphology.

Results

Pollen descriptions are provided in alphabetical order by families, genus and species. Pollen morphology of 12 species belonging to 8 families of Angiosperms. The resulting palynological data are provided in Table 1. and illustrated in figures 1 – 12 respectively.

Table 1. Plant List of Studied Species from Khin gyi Village, Wundwin Township

No.	Family	No.	Scientific Name	Myanmar Name
1.	Acanthaceae	1.	*Aechmanthera tomentosa* Nees.	Unknown
		2.	*Andrographis paniculata*	Sa ga gyi

No.	Family	No.	Scientific Name	Myanmar Name
			(Burm. f.) Wall.	
		3.	*Dicliptera paniculata* (Forssk.) I. Darbysh.	Unknown
		4.	*Lepidagathis cuspidata* Nees.	Unknown
2.	Asteraceae	5.	*Tridax procumbens* L.	Hmwe zoke ne gya
3.	Euphorbiaceae	6.	*Croton calococcus* Kurz.	Kanako gale
4.	Malvaceae	7.	*Pavonia odorata* Wild.	Ba la pin
		8.	*Sida acuta* Burm.	Ta byet si pin, shwe ta daing
		9.	*Sida spinosa* L.	Unknown
5.	Nyctaginaceae	10.	*Boerhavia diffusa* L.	Pa ran nawa
6.	Papaveraceae	11.	*Argemone mexicana* L.	Kha ya, Khone kha ya
7.	Portulacaceae	12.	*Portulaca umbraticola* Kunth.	Unknown

Description of pollen Morphology

1. *Aechmanthera tomentosa* Nees in Wall., Pl. As. Rar. 3. .87. 1837.

(Figure 1, A, B)

Monad, tricolporate, zonocolporate, prolate, large, 50 - 65 x 32 – 47 µ in length and breadth; amb rounded; pori lalongate, about 1.00 x 3.75 – 5.00 µ in length and breadth; colpi longicolpate, 44.0 – 59.0 x 5.0 – 12.5 µ in length and breadth, distinctly pseudocolpi present, the number of pseudocolpi 16; exine about 2.5 µ thick, sexine thicker than nexine; sculpturing reticulate, the lumina heterobrochate, 1.25 – 5.00 µ in width, the muri simplibaculate, about 0.75 µ wide.

2. *Andrographis paniculata* (Burm. f.) Wall. Pl. Asiat. Rar. 3 (12): 116. 1832. (Figure 2, A)

Monad, tricolporate, zonocolporate, prolate, medium, 32.50 – 40.00 x 30.00 x 37.50 µm in length and breadth; amb rounded triangular, angulaperturate; pori lolongate, 8.75 – 10.00 x 7.50 – 8.75 µm in length and breadth; colpi longicolpate, 27.50 – 32.50 x 7.50 – 10.00 µm in length and breadth; exine about 2.00 µm thick, sexine thicker than nexine; sculpturing reticulate, the lumena heterobrochate, 1.00 – 2.50 µm in width, the muri simplibaculate, 0.50 µm wide.

3. *Dicliptera paniculata* (Forssk.) I. Darbysh. Kew Bull. 62 (1): 122-123. 2007. (Figure 3, A, B)

Monad, tricolporate, zonocolporate, prolate, medium, 27.00 – 37.50 x 17.50 – 22.00 µm in length and breadth; amb rounded triangular, angulaperaturate; pori circular, 2.50 – 5.00 µm in diameter; colpi longicolpate, 25.00 – 32.00 x 3.00 – 4.50 µm in length and breadth; pseudocolpi distinctly present, the number of puseudocolpi 6; exine about 2.50 µm thick, sexine thicker than nexine; sculpturing reticulate, the lumina heterobrochate, 1.25 – 2.50 µm in width, the muri simplibaculate, 1.25 µm wide.

4. *Lepidagathis cuspidata* Nees in Wall., Pl. As. Rar. 97. 1832.

(Figure 4, A, B)

Monad, tricolporate, zonocolporate, prolate, medium, 25.0 – 35.0 x 17.5 – 25.0 µ in length and breadth; amb rounded triangular, angulaperturate; pori circular, 3.75 – 5.00 µ in diameter; colpi longicolpate, 21.00 – 31.00 x 2.50 - 3.75 µ in length and breadth; exine 2.5 µ thick, sexine as thick as nexine; sculpturing coarsely reticulate, the lumina heterobrochate, 1 - 5 µ in width, the muri curvimurate, simplibaculate, about 0.5 µ wide.

5. *Tridax procumbens* L. Sp. Pl. 2:900. 1753. (Figure 5, A, B)

Monad, tetracolporate, zonocolporate, spheroidal, medium, 22.50 – 37.50 µm in diameter; amb rounded, angulaperturate; pori circular, 0.75 – 1.25 µm in diameter; colpi brevicolpate; in length and breadth; exine about 3.75 µm thick, sexine thicker than nexine; sculpturing echinate, spines 5.00

– 6.25 µm in length, poined, the basal cushion present, 3.75 – 5.00 µm in width, 2.50 – 3.75 µm in height, interspinal space 3.75 – 4.50 µm wide.

6. *Croton calococcus* Kurz, Journ. As. Soc. Bentg. 42 (2):42. 1873.

(Figure 6, A, B)

Monad, inaperturate, spheroidal, medium, 37.50 – 45.00 µm in diameter; amb rounded; exine about 3.25 µm thick, sexine thicker than nexine; sculturing croton pattern, the lower part of the muroid ridges separated by rounded faveoloid areas, the upper part of well defined regular pegs or warts situated on top of the ridges; each faveoloid area encircled by 5 - 6 roundly triangular pegs, each peg about 2.00 µm in diameter, situated between 3 faveoloid areas.

7. *Palvonia odorata* Wild. S1p. Pl. 3:837, 1822. (Figure 7, A, B)

Monad, polyporate, pantoporate (about 60), spheroidal, very large, 120.50 – 180.50 µm in diameter; amb rouonded; pori circular, 1.75 – 2.25 µm in diameter, interporal space 12.00 – 21.50 µm in width; exine about 2.50 µm thick, sexine thicker than nexine; sculpturing echinate, spine 12.50 – 21.50 µm in height, pointed, slender, basal cushion absent, interspinal space 12.75 – 15.50 µm wide.

8. *Sida acuta* Burm. f., Fl. Ind. 147. 1768. (Figure 8, A, B)

Monad, polyporate, pantoporate (about 40), spheroidal, large, 75.00 – 87.50 µm in diameter; amb rounded, pori circular, 1.50 -2.00 µm in diameter; interporal space 7.50 – 10.00 µm; exine about 2.50 µm thick, sexine thicker than nexine; sculpturing echinat, spine 5.00 – 7.50 µm in length, pointed, basal cushiong distinctly present, 7.50 – 8.75 in width, 2.50 – 3.75 µm in height, interspinal space 5.00 – 7.50 µm wide.

9. *Sida spinosa* L., Sp. Pl. 683. 1753. (Figure 9, A, B)

Monad, polyporate, pantoporate (about 26), spheroidal, large, 45.00 – 75.00 µm in diameter; amb rounded; pori circular, 2.50 – 5.00 µm in diameter; interporal space 12.50 – 20.00 µm in width; exine about 2.75 µm thick, sexine thicker than nexine; sculpturing echinate, spines 5.00 – 7.50 µm in length, slender, tips pointed, basal cushion distinctly present, 6.25 – 7.50 µm in width; 2.50 – 3.75 µm in height, interspinal space 10.00 – 12.50 µm wide.

10. *Boerhavia diffusa* L. Sp. Pl. 1:3. 1753. (Figure 10, A, B)

Monad, polyporate, pantoporate (about 20), spheroidal, large, 55.00 – 75.00 µm in diameter; amb rounded; pori circular, 5.00 – 7.50 µm in diameter; interporal space 12.50 – 15.50 µm in width; exine about 2.00 µm thick, sexine thicker than nexine; sculpturing microechinate and foveolate, spines short, 2.50 – 3.75 µm in length, pointed, basal cushion present, 1.25 – 2.00 µm in width, 1.00 -1.50 µm in height, interspinal space 7.50 – 10.00 µm wide; the foveola 2.50- 3.00 µm in diameter.

11. *Argemone mexicana* L. Sp. Pl. 1:508 – 509, 1753. (Figure 11, A, B)

Monad, tri to tetracolpate, zonocolpate, prolate, medium, 30.00 – 37.50 x 25.00 – 30.00 µm in length and bredth; amb rounded triangular to quadencular; colpi logicolpate, 5.25.00 – 32.50 – 2.50 - .75 µm in length and breadth; exine about 1.50 µm thick, nexine slightly thicker than sexine; sculpturing reticulate, the lumina heterobrochate, 0.75 – 1.25 µm in width, the muri simplibaculate, about 0.5 µm wide.

12. *Portulaca umbraticola* Kunth Nov. Gen. Sp. (quar. ed) 6:72, 1823. (Figure 12, A, B)

Monads, polycolpate, pantocolpate (about 30), spheroidal, large, 70.00 – 90.00 µm in diameter; amb rounded; colpi pentagonal surface pattern, 12.50 – 18.50 µm x 2.50 – 5.50 µm in length and breadth, exine about 3.50 µm thick, sexine thicker than nexine; sculpturing obscursely reticulate and perforate, 0.75 – 1.00 µm in diameter.

Pollen key to the studied species by pollen morpholgy

1. Pollen grain aperturate ---------- 2

1. Pollen grain inaperturate ---------- (6) *Croton calococcus*

2. Pollen grain colpate or porate ---------- 3

2. Pollen grain colporate ---------- 8

3. Polycolpate or tri to tetracolpate ---------- 4

3. Polyporate ---------- 5

4. Polycolpate ---------- (12) *Portulaca umbraticola*

4. Tri to tetracolpate ---------- (11) *Argemone mexicana*

5. Sculpturing microechinate and perforate ---------- (10) *Boerhavia diffusa*

5. Sculpturing echinate ---------- 6

6. Basal cushion absent ---------- (7) *Pavonia odorata*

6. Basal cushion present ---------- 7

7. Pori size about 1.50 μm in diameter ---------- (8) *Sida acuta*

7. Pori size 2.50 – 5.00 μm in diameter ---------- (9) *Sida spinosa*

8. Pollen grain tetracolporate ---------- (5) *Tridax procumbens*

8. Pollen grain tricolporate ---------- 9

9. Pseudocolpi absent ---------- 11

9. Pseudocolpi present ---------- 10

10. Number of pseudocolpi 16 --------- (1) *Aechmanthera tomentosa*

10. Number of pseudocolpi 6 ---------- (3) *Dicliptera paniculata*

11. Sculpturing reticulate ---------- (2) *Andrographis paniculata*

12. Sculpturing coarsely reticulate ---------- (4) *Lepidagathis cuspidate*

Discussion and Conclusion

The present study deals with the pollen morphology of 7 families which comprised 11 genera belonging 12 species distributed in Khin Gyi Village, Wundwin Township.

The classification of pollen morphology was studied and described on shape, size, type of pollen grains, aperture type and exine sculpture. The thick exine was proposed generally with detailed measurement.

The pollen grains found in study area were differently occurred in various pollen sizes ranging from medium, large and very large. Six species medium, five large species and only two species of very large were found. Among all species, small size was not found.

In this study all species examined was showed aperturate grains except that of the genus *Croton calococcus* Kurz. The aperturate types pollen grains were recorded polyporate, polycolpate, tri to tetracolpate, tricolporate and tetracolporate.

Polyporate and tricolparate pollen grains were the most abundant in this study. *Palvonia odorata* Wild. Nees, *Sida acuta* Burm. *S. spinosa* L. Nees and *Boerhavia diffusa* L. (Nyctaginaceae) were polyprate species.

All this studied species of Acanthaceae were tricolporate aperturate type, *Aechmanthera tomentosa* Nees., *Andrographic paniculata* (Burm.f) Wall, *Dicliptera paniculata* (Forssk.) I.Darbysh. and *Lepidagathis cuspidata* Nees

Polycolpate (*Portulaca umbraticola* Kunth. Portulacaceae), tri to tetracolpate (Argemone mexicana L. Pappveraceae) and tetracolpae (Tridax procumbens L. Asteraceae) type were observed in this study.

Pollen grains were mostly spheroidal in this research. Polyporate, polycolpate and inaperturate, tetracolporate granis were spheroidal and tricolporate, tri to tetracolpate species were prolate.

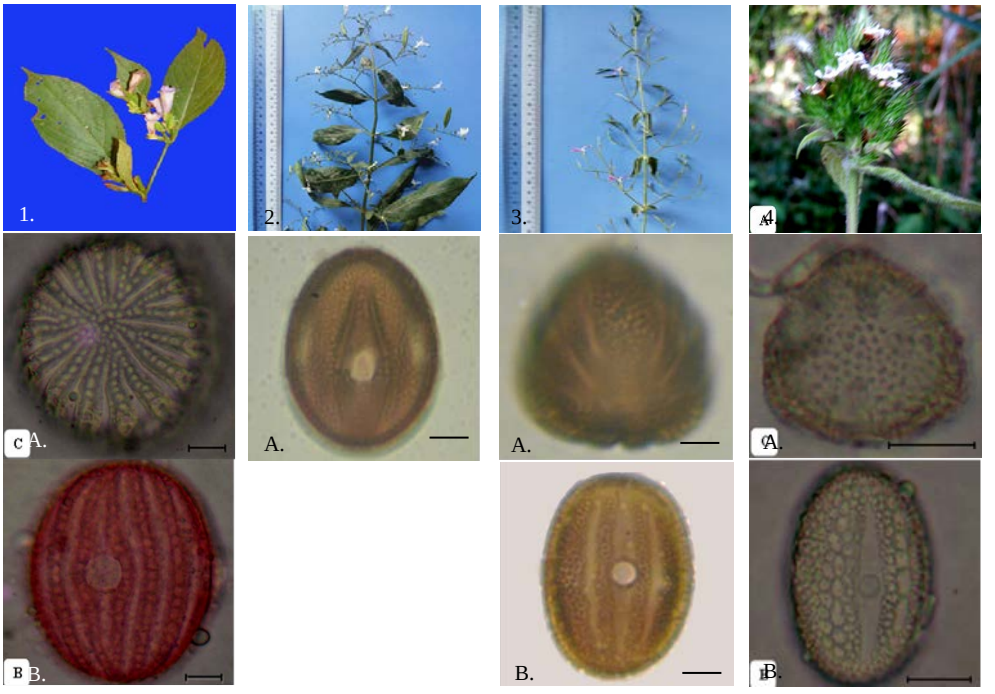
Pseduocolpi was found in two species, *Aechmanthera tomentosa* Nees., *Andrographic paniculata* (Burm.f) Wall. and the number of pseudocolpi was 16 and 6.

The exine sculpturing was differently showed in this research. Reticulate sculpturing was observed in four species, coarsely reticulate, obscursely reticulate and perforate, microchinate and foveolate in one species, echinate in four species, and croton-pattern in one species.

Minor changes in size may however occur due to nutrition of the plants and preparation of the materials. Processing and mounting pollen material may cause changes in size and form. Acetolysis has been reported to cause swelling of materials (Reitsma 1969; Martin 1973). This procedure does influence the final form of colpi and pori; glycerine jelly cause swelling of grains. Silicone oils have been suggested as an better alternative (Andersen 1960). For these reasons an attempt has been made as far as possible for uniformity in the preparation of all specimens.

Pollen morphology supports the taxonomic suggestion to separate the species in a special section or genus. Also it supports a phylogenetic study of molecular and morphological data.

So, it was concluded that all these above pollen morphological findings of the study are expected for helping in the study of morphological characters and identification of taxonomy in further investigation.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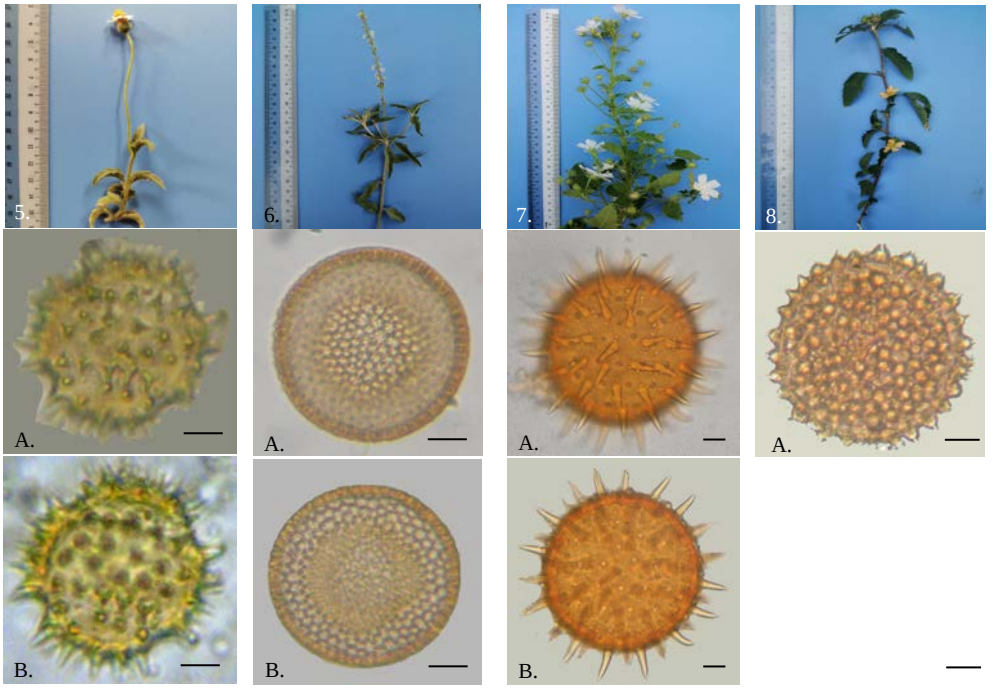
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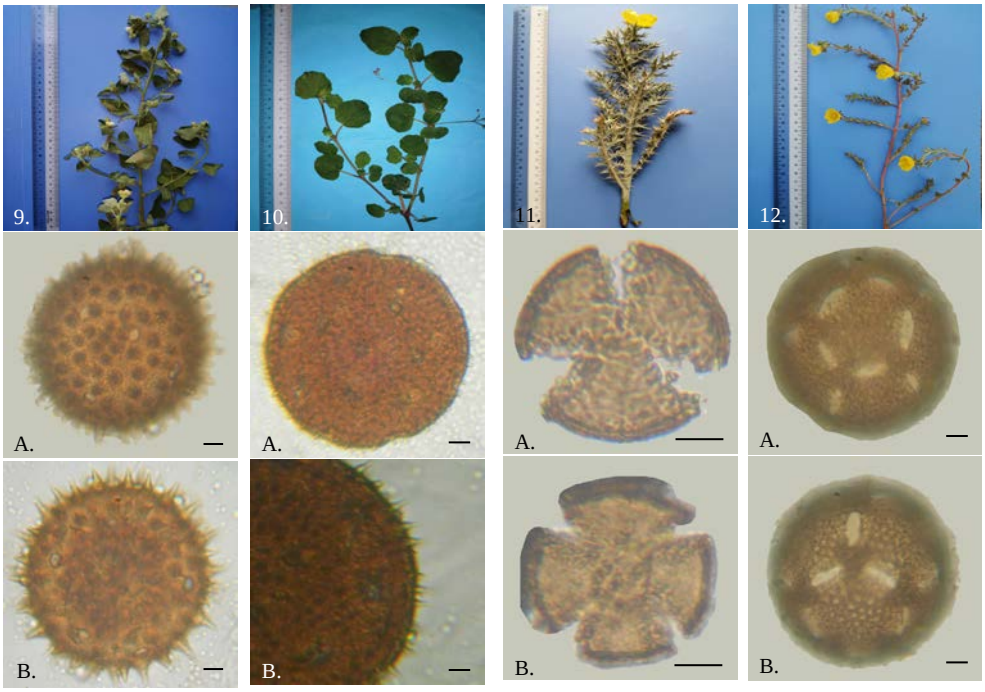

Figure 1. Inflorescence, A. Polar View, B. Equatorial View of *Aechmanthera tomentosa* Nees.

2. Inflorescence, A. Equatorial View of *Andrographis paniculata* (Burm.f.)Wall.
3. Inflorescnece, A. Polar View, B. Equatorial View of *Dicliptera paniculata* (Forssk.) I. Darbysh.
4. Inflorescence, Polar View, B. Equatorial View of *Lepidagathis cuspidate* Nees.
5. Inflorescence, Polar View, B. Equatorial View of *Tridax procumbens* L.
6. Inflorescence, A. Surface View, B. Exine sculpture of *Croton calococcus* Kurz.
7. Inflorescence, A. Surface View, B. Exine sculpture of *Pavonia odorata* Wild.

8. Inflorescence, A. Surface View, B. Exine sculpture of *Sida acuta* Burm.
9. Inflorescence, A. Surface View, B. Exine sculpture of *Sida spinosa* L.
10. Inflorescence, A. Surface View, B. Exine sculpture of *Boerhavia diffusa* L.
11. Inflorescence, A. Polar View, B. Polar of *Argemone mexicana* L.
12. Inflorescence, A. Surface View, B. Surface View of *Portulaca umbraticola* Kunth. Scale bar = 10μm

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Effect of Seed Sizes on Germination and Seedling Growth of *Vigna radiata* (L.) R. Wilczek

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Abstract

The green gram, *Vigna radiata* (L.) R. Wilczek (Family Fabaceae) is one of the important pulse crops. In the present study, the effect of seed sizes on germination and seedling growth of green gram was carried out by sieving to obtain different seed sizes such as large, medium and small seed sizes designated as Treatment 1, Treatment 2 and Treatment 3, respectively. Control treatment (Treatment 0) was laid out without sieving. The growth parameters such as plant height, number of leaves and fresh and dry weight at 23 days after sowing were recorded. Treatment 1 (large seed size) gave the best germination percentage (94.0%) and growth parameters such as plant height (14.32 cm), number of leaves (1.72) and fresh and dry weight (103.41 gm and 12.33 gm) at 23 days after sowing. Thus, large seeds should be chosen in the cultivation of green gram because they possess higher percentage of germination and have a profound effect on the plant growth.

Keywords: Green gram, *Vigna radiata* (L.) R. Wilczek, seed size, germination, seedling growth

Introduction

The central dry zone of Myanmar is usually defined to include the majority of three regions (Magway, Mandalay and Sagaing) occupying the centre of Myanmar and accounting for approximately 17 per cent of national territory. It is strongly influenced by climate: although average annual rainfall levels (960 mm) are lower than in other areas of the country, they are nevertheless moderate. The zone is the principal production area for the dryland crops such as pulses, oilseeds and sorghum in Myanmar. In 2012, Myanmar exported US $ 804 million of pulses, making it the fifth largest exporter of pulses and representing 8.9 % of world exports. Pulse exports rely heavily upon black gram and green gram (National Export Strategy, 2015-2019).

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The green gram, *Vigna radiata* (L.) R. Wilczek (Family Fabaceae) is one of the important pulse crops. This is consumed in the form of split pulse as well as the whole pulse which is an essential supplement of cereal-based diet. In addition to being an important source of human food and animal feed, green gram also plays an important role in sustaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen (Ramesh *et al.*, 2011).

Seed size is an important parameter which influences the germination, growth and biomass of the seedlings (Wood *et al*., 1977). Large-sized seeds germinate more quickly, it is stronger in the developmental stages and higher yield than small ones (Snyder and Filban, 1970; Benati *et al*., 1988, Ambika *et al*., 2014). Seed size in *Senna occidentalis* (L.) Link exhibited a significant effect on seed germination and emergence percentage with large seeds showing greater germination as well as emergence percentages (Saeed and Shaukat, 2000). Hojjat (2011) reported that the germination parameters were significantly related by seed weight and large seeds germinated early and showed better germination than small seeds of lentil. Pollack and Roos (1972) reported that larger seeds possessed more vigour than smaller seeds due to the presence of more food material. Seed size is positively correlated with seed vigour, that is, larger seeds tend to produce more vigorous seedlings in wheat (Ries *et al*., 1976).

In the present study, the main objectives were; to obtain the best seed size for the maximum germination percentage, to examine the effect of seed sizes on growth of seedling and to inform the role of seed sizes in green gram cultivation.

Materials and Methods

Experimental Study

The experiment was conducted at the Pakokku University Campus from July, 2018 to August, 2018. The seeds of *V. radiata* (L.) R. Wilczek were collected from the local farmers cultivated green gram in the vicinity of Pakokku Township. The collected seeds were confirmed by Department of Agriculture, Pakokku Township.

For the germination test, about 750 seeds of green gram were used. Firstly, all the seeds were sieved twice with two ordinary household sieves

to grade large, medium and small seed sizes. Different seed sizes were soaked in separate beakers containing purified water for about 10 hours. Large seed size was designated as Treatment 1; medium seed size as Treatment 2 and small seed size as Treatment 3. No sieving seeds were used as control (Treatment 0). Trays for germination test were prepared with top soil from the Campus. Tray size was 30 cm × 22 cm. In each tray, 50 seeds were sown for one treatment and each treatment had three replications. Thus, there were 12 trays for all treatments in this experiment. The emergence of the radicle was regarded as the criterion for seed germination. The number of germinated seeds was counted daily and final germination percentage of each cultivar was calculated by using following formula.

$$\text{Germination percentage} = \frac{\text{Total No. of Germinated Plants}}{\text{Total No. of Cultivated Seeds}} \times 100$$

Moreover, growth parameters at 23 DAS (days after sowing) such as plant height, number of leaves, fresh weight and dry weight were recorded. Mean values with standard deviation were presented in every measurable data.

Results

Germination Test

Some of the seeds of *V. radiata* (L.) R. Wilczek used in this investigation were started to germinate at three days after sowing. Data for germination percentage were recorded daily after germination. The results of the germination percentage were presented in Table 1 and Figure 1.

Table 1. Germination Percentage of *V. radiata* (L.) R. Wilczek

Days After Sowing	Germination Percentage			
	T0 (Control)	T1	T2	T3
Three days	48.6	63.2	61.2	33.2
Four days	66.6	88.0	75.3	59.3
Five days	79.2	94.0	81.2	72.6
Six days	79.2	94.0	81.2	72.6

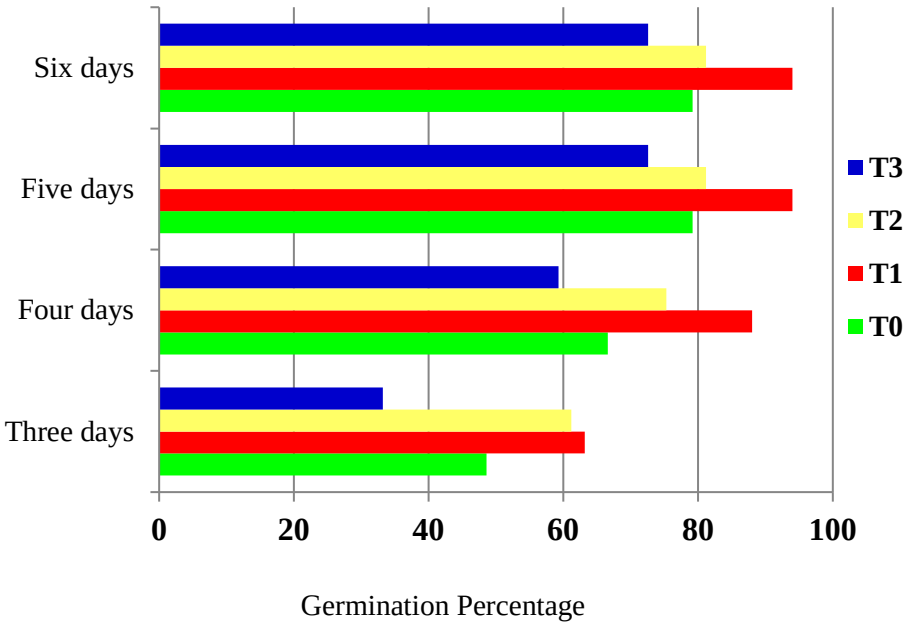

Figure 1. Germination Percentage of *V. radiata* (L.) R. Wilczek

Effect of Seed Sizes on Plant Height of *V. radiata* (L.) R. Wilczek

At 14 days after sowing, the plant height of Treatment 0 was 10.07 cm; Treatment 1, 13.11 cm; Treatment 2, 12.40 cm and Treatment 3, 9.15 cm, respectively. Among these treatments, Treatment 1 gave the maximum plant height. After 23 days cultivation, the highest plant height (14.32 cm) was obtained from Treatment 1 followed by Treatment 2 (12.81 cm), Treatment 0 (10.95 cm) and Treatment 3 (10.80 cm). Table 2 and Figure 2

Table 2. Mean Values of Plant Height (cm) of *V. radiata* (L.) R. Wilczek

Treatment	14DAS*	17DAS	20DAS	23DAS
T0	10.07 ± 1.19	10.25 ± 1.20	10.67 ± 1.49	10.95 ± 1.20
T1	13.11 ± 0.90	13.82 ± 0.83	14.27 ± 0.54	14.32 ± 0.82
T2	12.40 ± 1.31	12.41 ± 1.49	12.74 ± 1.58	12.81 ± 1.49
T3	9.15 ± 0.91	9.80 ± 0.94	10.45 ± 1.08	10.80 ± 0.94

*DAS Days after sowing

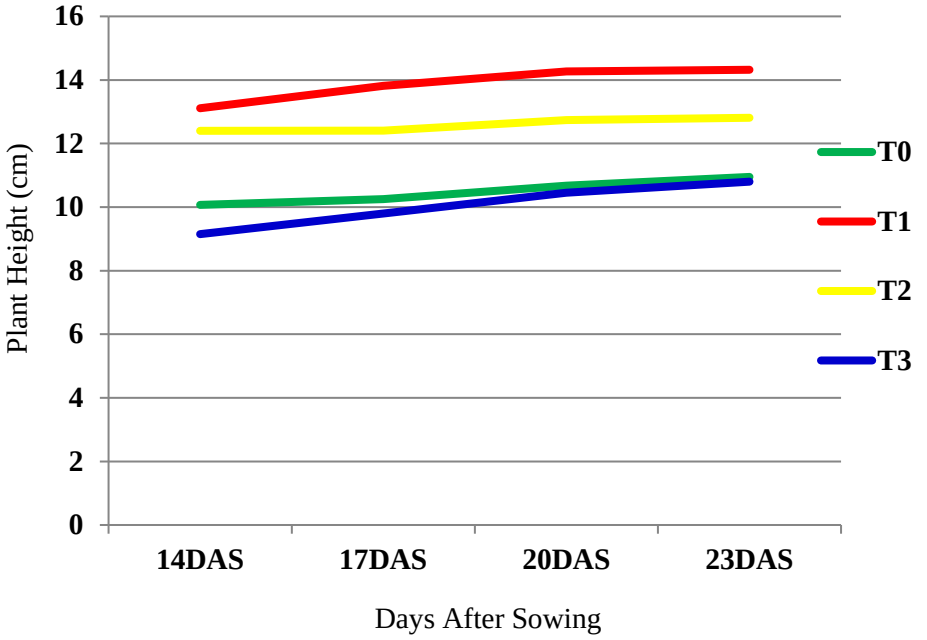

Figure 2. Effect of Seed Sizes on Plant Height

Effect of Seed Sizes on Number of Leaves of *V. radiata* (L.) R. Wilczek

After 14 days sowing, the highest number of leaves was recorded from Treatment 1 (1.10 leaves per plant) while Treatment 3 provided the least number of leaves (0.73 leaves per plant). Treatment 0 had 1.12 leaves; Treatment 1, 1.72 leaves; Treatment 2, 1.50 leaves and Treatment 3, 1.13 leaves at 23 days after sowing. In all treatments, Treatment 1 showed the top ranking. Table 3 and Figure 3

Table 3. Mean Values of Leaves per Plant *V. radiata* (L.) R. Wilczek

Treatment	14DAS*	17DAS	20DAS	23DAS
T0	0.84 ± 0.03	0.93 ± 0.44	1.10 ± 0.17	1.12 ± 0.62
T1	1.10 ± 0.01	1.22 ± 0.13	1.55 ± 0.03	1.72 ± 0.21
T2	0.74 ± 0.21	1.05 ± 0.12	1.23 ± 0.03	1.50 ± 0.22
T3	0.73 ± 0.06	0.88 ± 0.27	1.01 ± 0.09	1.13 ± 0.07

*DAS Days after sowing

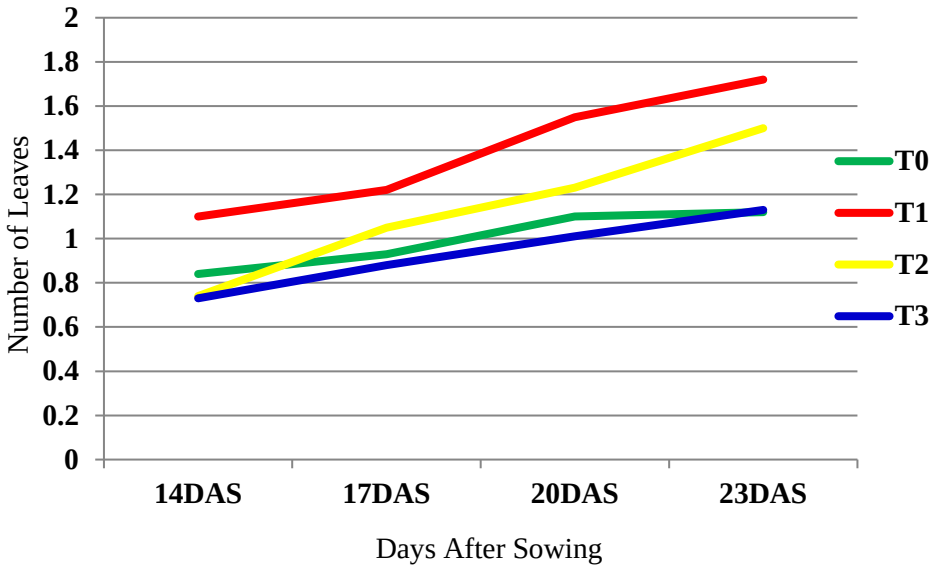

Figure 3. Effect of Seed Sizes on Number of Leaves

Effect of Seed Sizes on Fresh and Dry Weight of *V. radiata* (L.) R. Wilczek

Fresh weight in 23 days after sowing showed that Treatment 0 had 94.67 grams; Treatment 1, 103.41 grams; Treatment 2, 100.73 grams and Treatment 3, 90.15 grams, respectively. Among these treatments, Treatment 1 showed the top ranking in fresh weight followed by Treatment 2, Treatment 0 and Treatment 3. Dry weight in 23 days after cultivation recorded that Treatment 1 gave the optimal weight (10.01) while Treatment 3 showed the minimal one (9.79 grams). Treatment 2 had 11.51 grams and Treatment 0, 10.01 grams. Table 4 and Figure 4

Table 4. Mean Values of Fresh and Dry Weight (in gram)

Treatment	Fresh Weight (gram)	Dry Weight (gram)
T0	94.67 ± 3.21	10.01 ± 2.81
T1	103.41 ±1.40	12.33 ± 2.01
T2	100.73 ± 6.63	11.51 ± 3.25
T3	90.15 ±0.84	9.79 ± 2.44

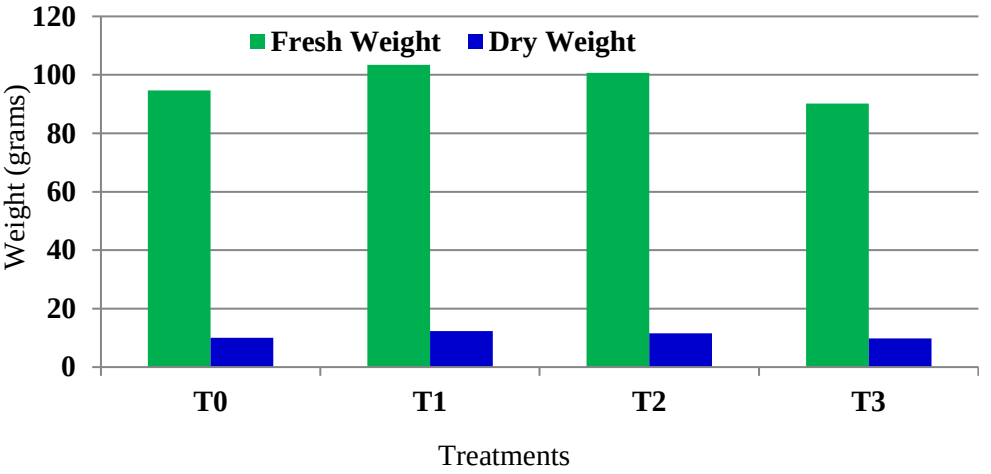

Figure 4. Effect of Seed Sizes on Fresh and Dry Weight

Discussion and Conclusion

In the present investigation, the effect of seed sizes on germination and seedling growth of green gram was carried out by sieving to obtain different seed sizes such as large seed size, medium seed size and small seed size designated as Treatment 1, Treatment 2 and Treatment 3, respectively. Control treatment (Treatment 0) was made without sieving. Furthermore, growth parameters of *Vigna radiata* (L.) R. Wilczek, such as plant height, number of leaves and fresh and dry weight at 23 days after sowing were recorded in this study.

Seed size is an important component in plant fitness. It is thought commonly to be an important focus of selection on the life histories of plants (Janzen, 1977), because the likelihood of dispersal (Howe and Kerckhove, 1980), germination (Putievsky, 1980) and survival (Schaal, 1980) can all depend on seed size. Large seeds produced the highest germination per cent, coleoptiles fresh weight, coleoptiles dry weight, radicle fresh weight and 1000 seed weights compared to other seed size in safflower (Sadeghi *et al*., 2011). Seed size can show considerable variation within population and this variability is often associated with variability in seedling size (Schaal, 1980).

Vadivelu and Ramakrishnan (1983) reported that the large size and medium size seeds showed significantly higher seed recovery, 1000 seed weight, germination, seedling dry matter, vigour index and field emergence

compared to small size and ungraded seeds in bengal gram. Bhor *et al*. (1988) recorded higher number of pods per plant, seed yield with higher seed size compared to small and medium seed size in gram. But, the maximum seed yield per plant produced from the larger seeds than small seeds in groundnut (Borale *et al*., 1993).

According to the results analyzed from the collected data, Treatment 1 (large seed size) gave the best germination percentage and growth parameters such as plant height, number of leaves and fresh and dry weight at 23 days after sowing. Thus, large seeds should be chosen in the cultivation of green gram because they possess higher percentage of germination and have a profound effect on the plant growth and yield.

It may be concluded from this experiment that positive relation was found between large seed grade and germination and seedling growth in all the test parameters. The research has revealed that large size seeds have high seedling survival and growth. However, standard germination values can't be directly used to predict field emergence because the experiment was used to small-scale trial.

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Isolation and Identification of Airborne Fungi Around the Pineapple Yards of Two Plantation Sites (Myaung Mya and Pyin Oo Lwin)

Nu Yin Aye[1] & Thuzar Tin[2]

Abstract

The samples of airborne fungi were captured from three regions (Yangon, Ayeyarwady and Mandalay) of pineapple yards and then isolation from Potato Dextrose Agar medium. According to the present study, the isolation of seven fungi, six fungi and four fungi were isolated from Myaung Mya and Pyin Oo Lwin respectively. The isolation of seventeen fungi were belong to ten genera were identified by their color, structure of mycelium and spores formation according to the Larone (1995), Cruickshank (1968), Barnett (1969) and Roberts (1984).

Keywords: Pineapple, fungi

Introduction

Microorganisms are found almost everywhere, and their presence in the air was demonstrated by the work of Lazzaro Splallanzani in 1768 and of Louis Pasteur at the end of the 19th century. However, air is not a natural medium for growth and reproduction of microorganisms, any organism, that airborne contain must have originated from a living or non living source (humans, animals, plants, food, water or soil). Every day people are exposed to millions of bioaerosols, including whole microorganisms, which can have both beneficial and detrimental effects. The airborne microbiome of the built environment in characterizing the various sources of airborne microorganisms and the relative contribution of each (Prussin *et al*., 2015).

Exposure to these airborne particles can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions. In addition, long-term contact of people with bioaerosols can influence a person's mental power and learning ability. Different environmental conditions such as temperature, UV light, dryness and humidity, play a role in controlling the growth of airborne particles. Nevertheless the microbes manage to reach

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new hosts through the air for its survival. The importance of bio-aerosols has been emphasized in recent decades due to their effect on human health (Muhammad *et al*., 2017).

The main objectives of present research work are to discover what types of microbes preexisting in the aerosphere in the pineapple yard, to study solation, purification and identification of fungi.

Materials and Methods

Study Area

Plantation site of Thinlargatsu village, Myaung Mya Township, Ayeyarwady Division; and Lepangon village, Anesakan, Pyin Oo Lwin Township, Mandalay Division were selected for this study. The township located 5m above sea level. Myaung Mya Township located in eastern district of Ayeyarwady Division between North 16°12′ and East 94°40′. The township located 4m above sea level. Pyin Oo Lwin Township located in eastern district of Mandalay Division between North 22º31′ and East 96º28′. The township located 1078m above sea level.

Collection of Air sampling, isolation and identification of airborne microbes

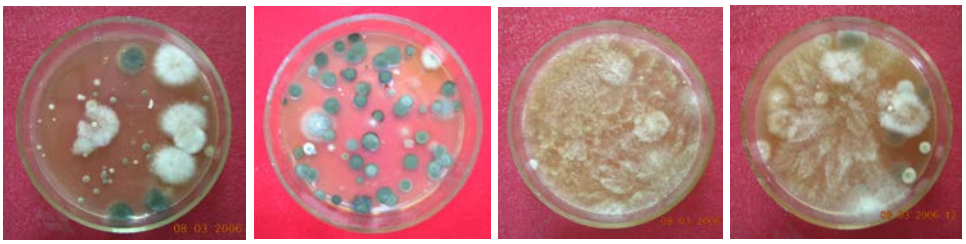
Airborne fungi were captured above pineapple plants with exposed petridishes which is Potato Dextrose Agar medium (Atlas, 1993). . Leaved for 20 minutes and then covered it according to Muhammad *et al*., 2017. Petridishes were placed at room temperature for 3 to 7 days. When fungi were grown on surface of the agar medium each of pure fungi can be obtained by direct transfer to a growth medium. Subculture of fungal isolates was carried out 5-7 times successfully with starch yeast agar slants medium, respectively, until the final pure culture was obtained. Pure culture was maintained at room temperature and subculture was prepared every weeks. Each of pure fungi and spores formation was examined and identified after 5-7 days (Jacob *et al*., 2015). The identification of isolated fungi was performed according to Larone (1995), Cruickshank (1968), Barnett (1969) and Roberts (1984).

Results

The characters of pure colony, morphology and spores formation of isolated fungi are described in following Figures.

Characteristics of isolated fungi in Myaung Mya Township

Characters of mycelium, spore formation and identification of each fungi were presented in Fig. (1 & 2).



M1 M2 M3 M4

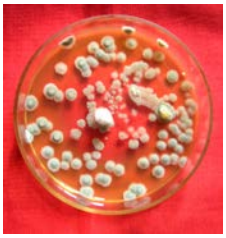
Figure 1. Capturing of airborne Fungi sample from Myaung Mya Township.

Scientific classification

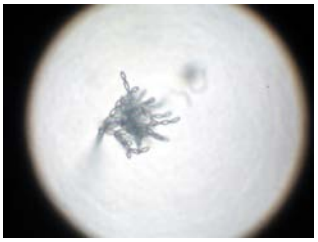
Division	- Amastigomycota
Subdivision	- Deuteromycotina
Form-Class	- Deuteromycetes (imperfect fungi)
Form-Subclass	- Coelomycetidae
Form-Order	- Moniliales
Genus	- Cladosporium
Species	- *Cladosporium* sp.

Characters of mycelium and spore formation

Conidiophores dark, branched variously near the apex or middle portion, clustered or single; conidia dark, one- or two- celled, variable in shape and size, ovoid to sylindrical and irregular, some typically lemon-shaped. Hyphae are septate and dark; conidiophores are dark and branched, vary in length, and usually produce two or more conidial chains (Fig. 2-a).



Pure fungal colony (Light green color) (showing 5-7 days old culture)



Micrograph of conidia and conidiophore (40x)

Figure 2.(a) Isolated fungi of M1

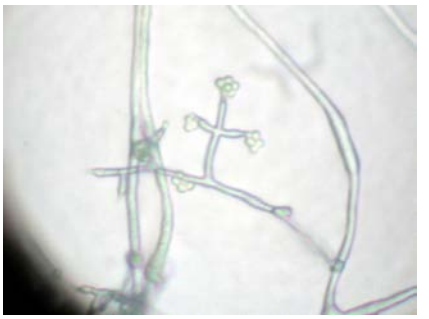
Scientific classification

Subdivision	- Deuteromycotina
Form-Class	- Deuteromycetes (imperfect fungi)
Form-Subclass	- Coelomycetidae
Form-Order	- Moniliales
Genus	- Trichoderma
Species	- *Trichoderma* sp.

Characters of mycelium and spore formation

Conidiophores hyaline, upright, much branched, not verticillate, phialides single or in groups; conidia hyaline, one-celled, ovoid, borne in small terminal cluster; usually easily recognized by its rapid growth and green patches or cushion of conidia (Fig. 2-b).

Pure fungal colony (Green color) (showing 5-7 days old culture)



Micrograph of conidia and conidiophore (40x)

Figure 2.(b) Isolated fungi of M2

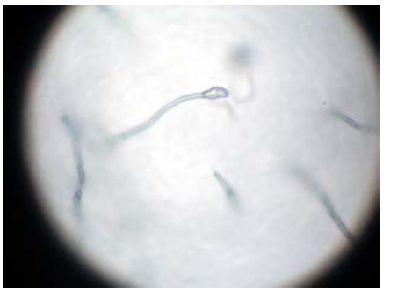
Scientific classification

Division	- Mastigomycota
Subdivision	- Diplomastigomycotina
Form-Class	- Oomycete (imperfect fungi)
Form-Order	- Peronosporales
Genus	- Pythiaceae
Species	- *Phytophthora* sp.

Characters of mycelium and spore formation

The production of distinct sporangiophores, which are easily distinguishable from the somatic hypha. Sporangia are lemon-shaped with a distinct papilla. Many species attack aerial parts of plants and cause fruit rot, canker and leaf-blight disease (Fig. 2-c).

Pure fungal colony (Green color) (showing 5-7 days old culture)



Micrograph of conidia and conidiophore (40x)

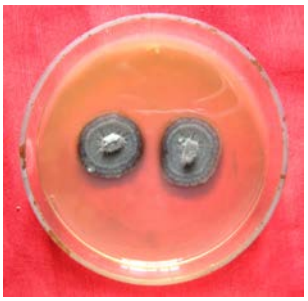
Figure 2.(c) Isolated fungi of M3

Scientific classification

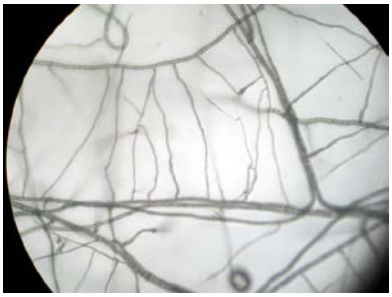
Division	- Amastigomycota
Subdivision	- Deuteromycotina
Form-Class	- Agonomycetes (imperfect fungi)
Form-Subclass	- Hyphomycetiadae
Form-Order	- Mycelia sterilia
Genus	- Rhizoctonia
Species	- *Rhizoctonia* sp.

Characters of mycelium and spore formation

A sexual fruit bodies and spores lacking; sclerotia brown or black, variable in form, frequently small and loosely formed among and connected by mycelial threads; hyphae of mycelium brown with long cells, septa of branch set off from main hypha (Fig. 2-d)



Pure fungal colony (Black green color) (showing 5-7 days old culture)



Micrograph of mycelium (40x)

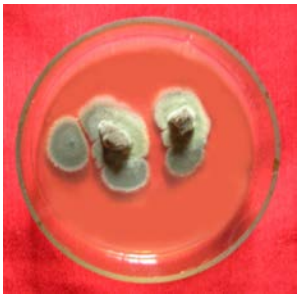
Figure 2.(d) Isolated fungi of M4

Scientific classification

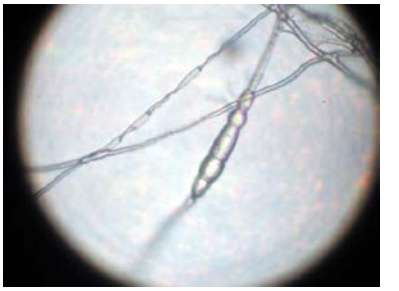
Division	- Amastigomycota
Subdivision	- Deuteromycotina
Form-Class	- Deuteromycetes (imperfect fungi)
Form-Subclass	- Coeloycetidae
Form-Order	- Moniliales
Genus	- Trichophyton
Species	- *Trichophyton* sp.

Characters of mycelium and spore formation

Mycelium cottony, in culture; microconidia hyaline, small, one-celled, subspherical or ovoid, borne on sides of hyphae, single or in clusters; macroconidia large, several celled, thin-walled, hylialine, clavate (Fig. 2-e).



Pure fungal colony (Green color) (showing 5-7 days old culture)



Micrograph of conidia and conidiophore (40x)

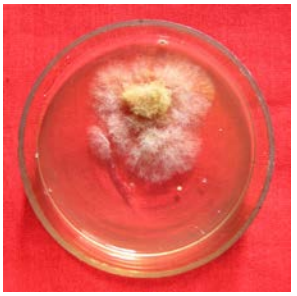
Figure 2.(e) Isolated fungi of M5

Scientific classification

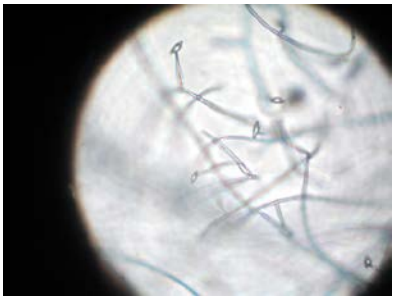
Division	- Amastigomycota
Subdivision	- Deuteromycotina
Form-Class	- Deuteromycetes (imperfect fungi)
Form-Subclass	- Coelomycetidae
Form-Order	- Moniliales
Genus	- Verticillium
Species	- *Verticillium* sp.

Characters of mycelium and spore formation

Conidiophores cylinder, branched, at least some of the branches verticillate (in whorls); conidia ovoid to ellipsoid, hyaline, one celled, borne singly (Fig. 2-f).



Pure fungal colony (White color)
(showing 5-7 days old culture)

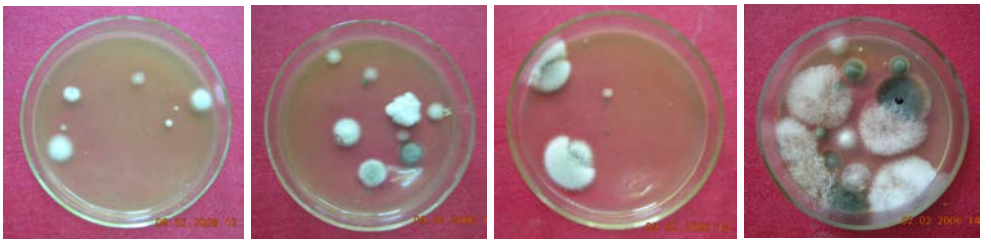


Micrograph of conidia and
conidiophore (40x)

Figure 2.(f) Isolated fungi of M6

Characteristics of isolated fungi in Pyin Oo Lwin Township

Characters of mycelium, spore formation and identification of each fungi were presented in Fig (3 & 4).



P1 P2 P3 P4

Figure 3. Capturing of airborne fungi sample from Pyin Oo Lwin Township

Scientific classification

Class – Conidia phycomycetes

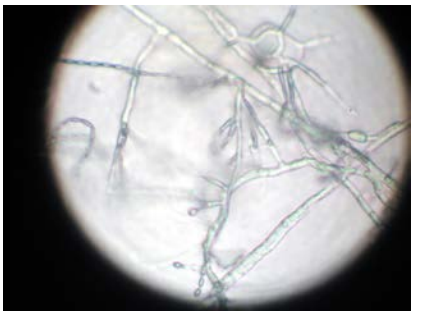
Genus – Cochlonema

Species – *Cochlonema* sp.

Characters of mycelium and spore formation

Aerial mycelium, slender, haustoria coiled, conidia one-celled, hyaline, catenulate (Fig. 4-a).

Pure fungal colony (White color) (showing 5-7 days old culture)



Micrograph of conidia and conidiophore (40x)

Figure 4.(a) Isolated fungi of P1

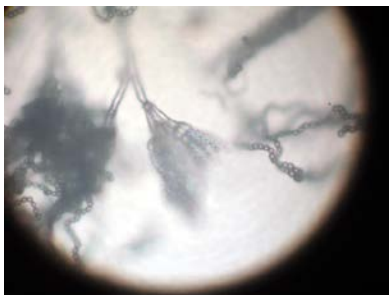
Scientific classification

Division	- Amastigomycota
Subdivision	- Deuteromycotina
Form-Class	- Deuteromycetes (imperfect fungi)
Form-Subclass	- Coelomycetidae
Form-Order	- Moniliales
Genus	- Paecilomyces
Species	- *Paecilomyces* sp.

Characters of mycelium and spore formation

Conidiophores mostly arising from aerial hyphae; phialides in lose verticillate group on the conidiophore; basal portion of phialide nearly cylindrical, tapering gradually to a long slender tube conidia produced successively (basipetally) and helps together in chains, one-celled, hyaline. The conidia are elliptical or oblong and occur in long, unbranched chains (Fig. 4-b).

Pure fungal colony (White color)
(showing 5-7 days old culture)



Micrograph of conidia and
conidiophore (40x)

Figure 4.(b) Isolated fungi of P2

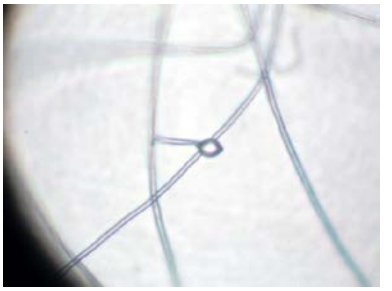
Scientific classification

Division	- Mastigomycota
Subdivision	- Diplomastigomycotina
Form-Class	- Oomycete (imperfect fungi)
Form-Order	- Peronosporales
Genus	- Phytophthora
Species	- *Phytophthora* sp.

Characters of mycelium and spore formation

The production of distinct sporangiophores, which are easily distinguishable from the somatic hypha. Sporangia are lemon –shaped with a distinct papilla. Many species attack aerial parts of plants and cause fruit rot, canker and leaf-blight disease (Fig. 4-c).

Pure fungal colony (Green color)
(showing 5-7 days old culture)



Micrograph of conidia and
conidiophore (40x)

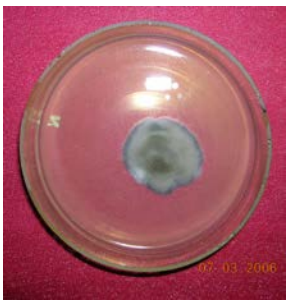
Figure 4.(c) Isolated Fungi of P3

Scientific classification

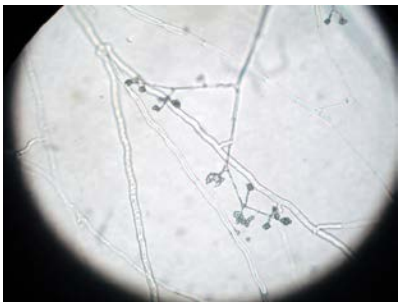
Subdivision	- Deuteromycotina
Form-Class	- Deuteromycetes (imperfect fungi)
Form-Subclass	- Coelomycetidae
Form-Order	- Moniliales
Genus	- Trichoderma
Species	- *Trichoderma* sp.

Characters of mycelium and spore formation

Conidiophores hyaline, upright, much branched, not verticillate, phialides single or in groups; conidia hyaline, one-celled, ovoid, borne in small terminal cluster; usually easily recognized by its rapid growth and green patches or cushion of conidia (Fig. 4-d).



Pure fungal colony (Green color)
(showing 5-7 days old culture)



Micrograph of conidia and
conidiophore (40x)

Figure 4.(d) Isolated Fungi of P4

Discussion and Conclusion

In the present study the screening of fungi in the aerosphere and has been performed by the standard methods of microbiology in the two regions (Myaung Mya and Pyin Oo Lwin). A total of seventeen isolated fungi were collected during the cause of this study out of which six, four fungi were collected from Myaung Mya and Pyin Oo Lwin respectively. The isolated fungi were examined macroscopically and microscopically, and compared to the morphology of published fungal species. It was found that eight species of fungi are observed in the pineapple yards of two regions as shown in Table (1). The present results have been agreed with the literature of Muhammad *et al*., 2017.

Table 4. Different types of isolated fungi were observed in Two Divisions

No.	Species	Isolated fungi From Myaung Mya	Isolated fungi from Pyin Oo Lwin
1	*Blastomyces* sp.	–	–
2	*Cladosporium* sp.	Figure – 4a	–
3	*Cochlonema* sp.	Figure – 4g	Figure – 6a
4	*Curvularium* sp.	–	–
5	*Paecilomyces* sp.	–	Figure – 6b, 6f

No.	Species	Isolated fungi From Myaung Mya	Isolated fungi from Pyin Oo Lwin
6	*Phytophthora* sp.	Figure – 4c	Figure – 6c
7	*Rhizoctonia* sp.	Figure – 4d	–
8	*Trichophyton* sp.	Figure – 4e	–
9	*Tricoderma* sp.	Figure – 4b	Figure – 6d
10	*Verticillium* sp.	Figure – 4f	Figure – 6e

It is also noted that out of two different area the least number at fungi was detected on the pineapple plants grown in Pyin Oo Lwin Township (24%) compared with Myaung Mya (35%) Township. Because the environment of Myaung Mya is more humid and high temperature than Pyin Oo Lwin and the environment of Pyin Oo Lwin is higher elevation. Although different strains of fungi were captured in the aerosphere, no severe damage of pineapple leaves and fruits had been recorded in the present study.

Acknowledgements

I would like to thank to Dr. Aye Pe, Professor and Head, Dr Myint Aung, Dr Bay Dar and Dr Thandar Aye, Professors, Department of Botany, University of Yangon, for their valuable advices. I would like to express my deep appreciation to Dr U Win, Reactor (Retired), Department of Botany, Hinthada University, for his valuable suggestion and helpful advices.

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Qualitative and Quantitative Analysis of Leaves of *Nymphoides indica* (L.) Kuntze (Kya-Lin-Ban)

Pyae Sandi Win[1] & Ohnmar Ye Win[2]

Abstract

Nymphoides indica (L.) Kuntze belongs to the family Menyanthaceae. It is known as Ka-Lin-Ban in Myanmar and Water Snowflake in English. The plant was collected from Loikaw Township. In this paper, phytochemical analysis of various extracts of leaves showed the presence of alkaloids, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, terpenes, steroids and the absence of tannins. The dried leaves powder was subjected to extraction with different solvents like pet-ether, chloroform, methanol, acetone, ethyl acetate, ethanol and water. Standardization of crude drug plays a very important role in identifying the purity and quality of crude drugs. The physicochemical investigation revealed standardization which includes moisture content, total ash, acid insoluble ash, water soluble ash, water soluble matter, pet-ether, chloroform, methanol, acetone, ethyl acetate, and ethanol soluble matter.

Keywords: *Nymphoides indica* (L.) Kuntze., phytochemical and physicochemical

Introduction

The World Health Organization (WHO) (1998) estimates 4 billion people (80%) of the world's populaion presently use herbal medicine for one form of primary health care or another. Medicinal herb is considered to be a chemical factory as it contains a multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpenes (Ahmad *et al*. 2013). *Nymphoides*, one of the most diverse and widespread floating leaved groups, approximately consists of 50 species worldwide.

The flowers are held upright above the leaves. Some have rather broad distributions but most are restricted to a single continent or even to a local geographic region. According to WHO, more than 80% people in developing countries depend on traditional medicines for their primary health needs *N. indica* (L.) Kuntze is antiscorbutic and febrifuge.Whole plants are used as hepatoprotectie and promote pregnancy, reduce fever, invigorate carminative and antiscorbutic (Chowdhury 2012).

[1] Demonstrator, Department of Botany, Loikaw University
[2] Associate Professor, Dr., Department of Botany, Taunggyi University

The purpose of this research was to study the phytochemical and physicochemical tests of the leaves powder investigated for its constituents and solubility for further research.

Materials and Methods

The specimens used in this paper were collected from Loikaw Township during September 2017 to February 2018.

Qualitative Analysis

Preliminary phytochemical investigation was carried out in the Department of botany, Loikaw University, according to the methods of British Pharmacopoeia, 1968; Trease and Evans, 1978; Harbone, 1993.

Quantitative Analysis

Physicochemical investigation was determined according to WHO, 1998; Central Council for Research and Unani Medicine, 2014.

The elemental analysis of the leaves on *Nymphoides indica* (L.) Kuntze was carried out by EDXRF spectrometer (RIGAKU Corporation, ME65-1220120301) at Department of Physics, Taunggyi University.

Results

Scientific Name : *Nymphoides indica* (L.) Kuntze (Fig 1. A and B)
Family : Menyanthaceae
Myanmar name : Kya-Lin-Ban
Part Used : Leaves

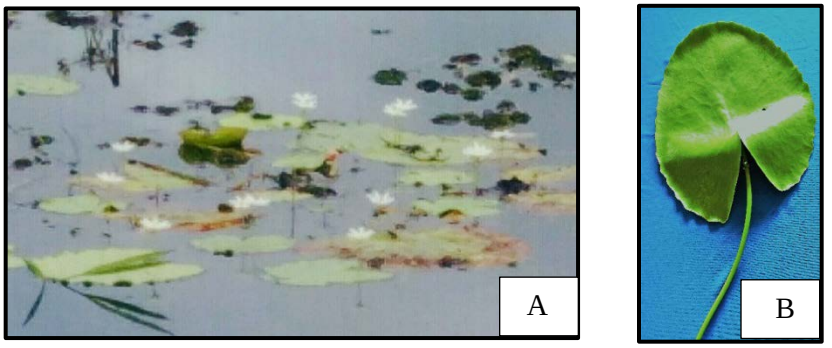

Figure 1. *Nymphoides indica* (L.) Kuntze
(A) Habit (B) Leaf

Qualitative Analysis

In preliminary phytochemical investigation, the leaves of *Nymphoides indica* (L.) Kuntze indicated that alkaloid, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, terpene, steroid was detected and tannin was not detected. The results of preliminary phytochemical investigation were shown in Table 1 and Fig. 2.

Table 1. Result of preliminary phytochemical investigation of the leaves of *Nymphoides indica* (L.) Kuntze

No.	Tests	Solvent Extract	Test Reagents	Observation	Remark
1.	Alkaloid	1% HCl	Dragendorff's reagent	Brown ppt.	+
			Wagner's reagent	Reddish ppt.	+
2.	Flavonoid	95% EtOH	Mg truning, con: HCl	Greenish brown ppt.	+
3.	Terpene	95% EtOH	Acetic anhydride, $CHCl_3$, con:H_2SO_4	Pink colour	+
4.	Steroid	95%EtOH	Acetic anhydride, con: H_2SO_4	Deep green colour	+

No.	Tests	Solvent Extract	Test Reagents	Observation	Remark
5.	Reducing sugar	Distilled water	Benedict's solution	Brown ppt.	+
6.	Saponin	Distilled water	Distilled water	Frothing	+
7.	Phenolic compound	Distilled water	10% $FeCl_3$	Deep green ppt.	+
8.	Glycoside	Distilled water	10% Lead acetate	White ppt.	+
9.	Tannin	Distilled water	10% $FeCl_3$, dil.H_2SO_4solution	ND	-

(+)= detected, (-)= not detected

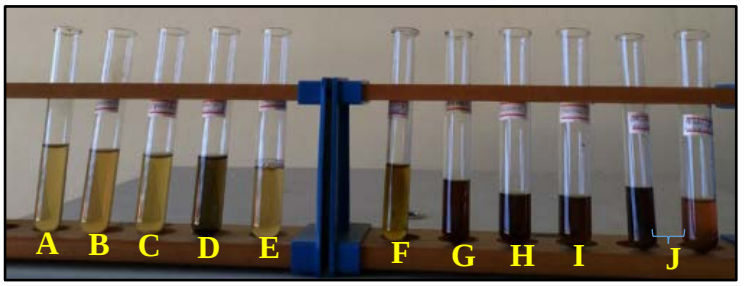

Figure 2. Result of Preliminary phytochemical investigation
(A) Control (B) Tannin (C) Glycoside
(D) Phenolic compound (E) Saponin (F) Reducing sugar
(G) Steroid (H) Terpene (I) Flavonoid
(J) Alkaloid (Dragendorff's and Wagner's reagent)

Quantitative Analysis

In physicochemical studies, seven different solvents are used. It is found that the solubility of powdered sample is the highest in ethanol. The least soluble in pet-ether is observed. These findings were shown in Table 2.

Table 2. Physicochemical properties

No.	Physicochemical characters	Quantity determination in leaf (%)
1.	Moisture content	88.6
2.	Total ash	3.33
3.	Acid insoluble ash	33.33
4.	Water soluble ash	83.33
5.	Water soluble matter content	15.2
6.	Methanol soluble matter content	13.6
7.	Ethanol soluble matter content	16.6
8.	Ethyl-acetate soluble matter content	13
9.	Acetone soluble matter content	8.2
10.	Pet-ether soluble matter content	7
11.	Chloroform soluble matter content	8.2

According to elemental analysis, K, Ca, Mg, P, S are found as macronutrient elements whereas Cl, Fe, Mn, Zn, Cu are found as micronutrient elements. These results were shown in Table 3 and Fig. 3.

Table 3. Relative concentration of elements in the leaves powdered sample by using EDXRF

No.	Component	Result (%)
1.	Mg	0.860
2.	P	0.168
3.	S	0.197
4.	Cl	0.537
5.	K	0.629
6.	Ca	1.45
7.	Mn	0.0795
8.	Fe	0.0971
9.	Cu	0.0009
10.	Zn	0.0040

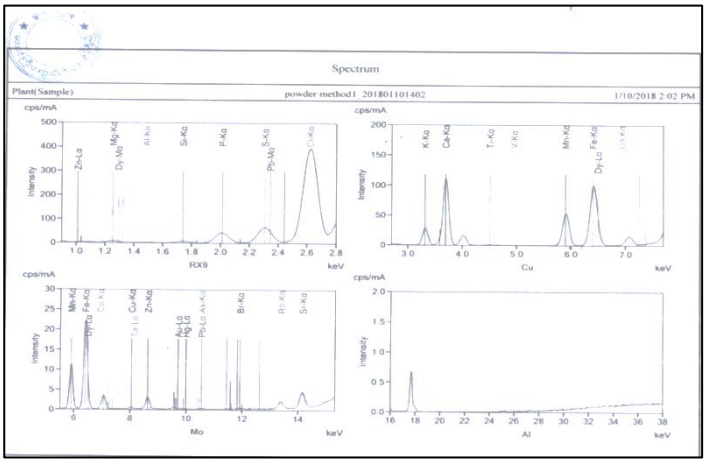

Figure 3. EDXRF spectrum of leaves of *Nymphoides indica* (L.) Kuntze

Discussion and Conclusion

In this research, the aquatic medicinal plant *Nymphoides indica* (L.) Kuntze belonging to the family Menyanthaceae has been studied. This plant is collected as wild which is abundantly found in Htay Ngar Lyar pond, Loikaw Township.

In preliminary phytochemical investigation, the leaves of *Nymphoides indica* (L.) Kuntze contain alkaloid, flavonoid, terpene, steroid, reducing sugar, saponin, phenolic compound and glycoside. Tannin is not detected in the present study. Harbone *et al*. (2013) described that tannin is present in the leaves of *Nymphoides indica* (L.) Kuntze.

Alkaloids can be used as life-saving drugs in some serious disorders like heart-failure, cancer, blood pressure. Flavonoids are highly diversified plant pigments that are present in a wide range of fruits, vegetables, nuts and beverages. Terpenes are aromatic compounds that are found in thousands of plant species, and are responsible for the various flavors and fragrances of cannabis. Steroids are effective for asthma and reduce inflammation. This is important in conditions like arthritis. Reducing sugar intake can lead to improve mental health such as depression or anxiety. Reducing sugar can help minimize symptoms of mental health conditions, boost energy and improve resilience to stress. Saponins are foaming characteristics. Saponins have many health benefits: blood cholesterol

levels, cancer, bone health. Phenolic compounds have strong antiseptic- and antibacterial properties and act as nerve stimulants and immunostimulants. Glycosides can be used for atrial flutter, atrial fibrillation, paroxysmal tachycardia and congestive heart failure. These findings are consistent with previous reports by Amin *et al.* (2016).

In physicochemical studies, seven different solvents are used. It is found that the solubility of powdered sample is the highest in ethanol. The least soluble in pet-ether is observed.

According to elemental analysis, K, Ca, Mg, P, S are found as macronutrient elements whereas Cl, Fe, Mn, Zn, Cu are found as micronutrient elements. In this study, O is found to be the highest percentage in leaves. Ca, Mg, K, and Cl are also found the moderate percentage in leaves. These findings are consistent with previous reports by Chowdhury (2012) and Amin *et al.* (2016).

In conclusion, *Nymphoides indica* (L.) Kuntze is one of the Myanmar indigenous aquatic herbs. Preliminary phytochemical and physicochemical tests are also useful in pharmacognostic study.

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I would like to express my special thanks of Dr. Htay Aung, Rector, Loikaw University, for permitting me to write this paper.

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Spore Morphology of Ferns from Lashio Township

Swe Swe Win[1] & Phue[2]

Abstract

This study deals with the spore morphology of ferns which are commonly found in the Sin Taung, Ye Kan Taung and Sarsana 2500 hill of Lashio Township. The 13 species of 10 genera belonging to 6 families were collected. The ferns were found as terrestrial or epilithic in 9 species and epiphytic or epilithic in 4 species. Most members of fern were erect and rarely climbing found in *Lygodium flexuosum* (L.) Sw. The fronds were mostly monomorphic and dimorphic in *Platycerium wallichii* Hook. The sori types are indusiate in 3 species, false indusiate in 4 species and exindusiate in 6 species. The morphological characters of spores were examined under a light microscope by the method of Erdtman (1957). Two types of spores; monolete and trilete were observed. The various spores colour were found as reddish brown, yellow, pale brown and greenish brown. The spore sizes were ranged from 35µm to 50µm. The spore surface patterns vary as ornate, granulate, tuberculate, rugulate, echinate and verrucate. This study would provide as the taxonomic evidences for the classification of ferns.

Keywords: Spore Morphology, ferns, Lashio, taxonomic.

Introduction

A fern is a group of about 20,000 species of plants classified in the Division Filicophyta, and also known as Filicinophyta or Pteridophtyta. Fern is a common name for the cryptogamous or spore producing plants. Within the vascular plants, the Pteridophytes constitute the third major group besides the angiosperms and the gymnosperms. A fern is a member of vascular plants that reproduce spores and have neither seeds nor flowers. Fern stems are often called rhizomes. Many ferns from tropical rain forests are epiphytes which mean they only grown on other plant species, their water comes from the damp air or from rainfall running down branches and tree trunks. There are also some purely aquatic ferns such as water velvet (*Salvinia molesta*) and mosquito ferns (*Azolla* sp.) (Sundas *et al.*, 2006).

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[2] Demonstrator, Department of Botany, Lashio University

In ferns, leaf is often referred to as a frond. New leaves typically expand by the unrolling of a tight spiral called a crozier or fiddlehead. This incurling of the leaf is termed as circinate vernation. There are two types of leaves in ferns. A leaf that does not produce spores is called trophophyll. A leaf that produces spores is called sporophyll. Under surface of leaves one can find sometimes cluster of spores (sorus, plural sori) sometimes covered by a protective scale called indusium. Ball-shaped sporangia are formed that are covered by a thick wall called annulus. It serves to eject the spores. Meiosis occurs in these sporangia, leading to haploid spores (Smith, 1955).

The study of pollen and spore is called palynology. Spores are involving in distribution of the plants. Very small spore travel to long distance in the pteridophytes species. The spore characters were useful to differentiate some genera and compared to other spore-bearing vascular plant (Gamal, 2012). The palynology of Pteridophyte was done by Erdtman (1957). Therefore, spores are also taxonomical tools in determining phylogenetic relationship amongst fern species. According to Vijayakanth & Sathish (2016), the most advanced structure of spores were monolete and the primitive structure of spores were trilete. In literature survey, there are some valuable morphological studies of living and fossil fern species Zenkteler (2012). The spore morphology of the ferns was studied by many authors: Makgomol (2006), Zenkteler (2012), Vijayakanth and Sathish (2016) and Vijayakanth *et al*. (2017).

The study area, Lashio Township, is situated in Northern Shan State of Myanmar. It lies between 22º 39' 53" and 23º 04' 27" N latitude and 97º 30' 10" and 97º 47' 40" E longitude. Generally, the elevation of this part is roundly 855m above the sea level. The total area of Lashio is 4832 square kilometers. It is divided into 12 quarters (Mya Min, 2010).

Due to the mountainous region, there are many hills around the Lashio area. These hills are rich in the diversity of plants including the angiosperm to the lower group such as Pteridophytes, Bryophytes, lichens, fungi and algae. Among them, distribution of ferns is widely and abundantly found almost anywhere in this area. However, the fern researches concerning with the ferns species and fern data have been limited in Myanmar. The spore morphology of fern species from Lashio had also not been recorded by other researchers. Therefore, the present study attempts to describe the spore morphology of ferns from the Lashio Township.

The aim of this study was to study the morphology and spore characters of ferns found in the Lashio Township. The objectives were to identify the collected fern species and to record the morphological variation in the sori of ferns for the taxonomic information of ferns.

Materials and Methods

The plant specimens were collected from the Sin Taung, Ye Kan Taung and Sarsana 2500 hill of Lashio Township. The field works were carried out from May 2017 to November 2018. The mature fertile fronds were collected and recorded. For spore morphology, the sori were crushed with a glass rod on the glass slide. Spores were rinsed with 95% alcohol and mounted in glycerin jelly and prepared by method of Erdtman (1957). The spore characters were studied under the light monocular microscope and observations were made with a camera (Cannon A 3500 IS) under 40X using a 10X eye piece. At least 10 spores per species were measured the length of equatorial diameters and polar diameters. The microscopic characters of spore morphology of all species were presented by plant systematics order. Identification of fern species have been carried out by referring to Hennipman (1982), Shing (1983) and Zhengyi *et al*. (2013). The spore terminology used in this research was in accordance with Erdtman (1957) and Wrinter *et al*. (2003).

Results

The spore morphology of 13 species belonging to 10 genera of ferns were studied. The results were showed in Figure 1 and 2. The spore descriptions of fern species were presented and arranged in the systematic order.

Microscopic characters of Spores

Lygodium flexuosum (L.) Sw.

Family	: Lygodiaceae
Common name	: Climbing fern
Spore producing period	: July to September

Spores trilete, radial symmetry, tetrahedral in outline, triangular with straight sides in polar views, convex in proximal views, hemispheric in distal views, equatorial diameters about 42.5µm, polar diameters about 40.5µm, leasurae narrow, surface patterns spheroidal-tuberculate.

Adiantum capillus-veneris L.

Family : Pteridaceae

Common name : Venus hair fern

Spore producing period : July to October

Spores trilete, radial symmetry, tetrahedral in outline, triangular with straight sides in polar views, convex in proximal views, hemispheric in distal views, equatorial diameters about 37.5µm, polar diameters about 45.5 µm, reddish brown, leasurae narrow, surface patterns irregular granulate.

Adiantum malesianum J. Ghatak,

Family : Pteridaceae

Common name : Maidenhair fern

Spore producing period : August to November

Spores trilete, radial symmetry, tetrahedral in outline, triangular with straight sides in polar views, convex in proximal views, hemispheric in distal views, equatorial diameters about 37µm, polar diameters about 35µm, reddish brown, leasurae narrow, surface patterns irregular granulate.

Adiantum philippense L.

Family : Pteridaceae

Common name : Walking fern

Spore producing period : July to September

Spores trilete, radial symmetry, tetrahedral in outline, triangular with straight sides in polar views, convex in proximal views, hemispheric in distal views, equatorial diameters about 37.5µm, polar diameters about 45.5µm, reddish brown, leasurae narrow, surface patterns irregular granulate.

Pteris vittata L.

Family : Pteridaceae

Common name : Ladder fern

Spore producing period : May to October

Spores trilete, radial symmetry, tetrahedral in outline, triangular with straight sides in polar views, convex in proximal views, hemispheric in distal views, equatorial diameters about 50µm, polar diameters about 37.5µm, greenish brown, leasurae narrow, surface patterns rugulate.

Cyclogramma omeiensis (Bak.) Tagawa,

Family : Thelypteridacae

Common name : Marsh fern

Spore producing period : September to January

Spores monolete, lateral symmetry, ellipsoidal in outline, oblongoid in equatorial views, oblongoid in proximal views and distal views, equatorial diameters about 45µm, polar diameters about 32.5µm, yellow, leasurae wide, surface patterns vermiculate.

Athyrium filix-femina (L.) Roth.

Family : Athyriaceae

Common name : Lady fern

Spore producing period : August to October

Spores monolete, lateral symmetry, ellipsoidal in outline, oblongoid in equatorial views, oblongoid in proximal views and distal views, equatorial diameters about 45µm, polar diameters about 32.5µm, pale brown, leasurae wide, surface patterns rugulate.

Nephrolepsis exaltata (L.) Schoot,

Family : Nephrolepidaceae

Common name : Boston fern

Spore producing period : August to November

Spores monolete, radial symmetry, ellipsoid in outline, reniform in equatorial views, oblongoid in polar and distal views, equatorial diameters

about 50µm, polar diameters about 32.5µm, brown, leasurae wide, surface patterns ornate.

Arthromeris mairei (Brause) Ching,

Family : Polypodiaceae

Common name : Maire joint-pinna fern

Spore producing period : July to October

Spores monolete, radial symmetry, orbicular in outline, reniform in equatorial views, circular in polar and distal views, equatorial diameters and polar diameters about 25µm, brown, leasurae narrow, surface patterns granulate.

Microsorum scolopendria (Burm.f.) Copel.

Family : Polypodiaceae

Common name : Musk fern

Spore producing period : June to September

Spores monolete, lateral symmetry, ellipsoidal in outline, ellipsoid in equatorial views, oblongoid in proximal views and distal views equatorial diameters about 35µm, polar diameters about 25µm, yellow, leasurae wide, surface patterns granulate.

Platycerium wallichii Hook.

Family : Polypodiaceae

Common name : Staghorn fern

Spore producing period : July to November

Spores monolete, lateral symmetry, ellipsoidal in outline, reniform in equatorial views, oblongoid in proximal views and distal views, equatorial diameters about 45µm, polar diameters about 32.5µm, yellow, leasurae wide, surface patterns minutely echinate.

Pyrrosia lingua **(**Thunb.) Farw.

Family : Polypodiaceae

Common name : Tongue fern

Spore producing period : May to November

Spores monolete, lateral symmetry, ellipsoidal in outline, reniform in equatorial views, proximal views and distal views, equatorial diameters about 45µm, polar diameters about 32.5µm, yellow, leasurae wide, surface patterns verrucate.

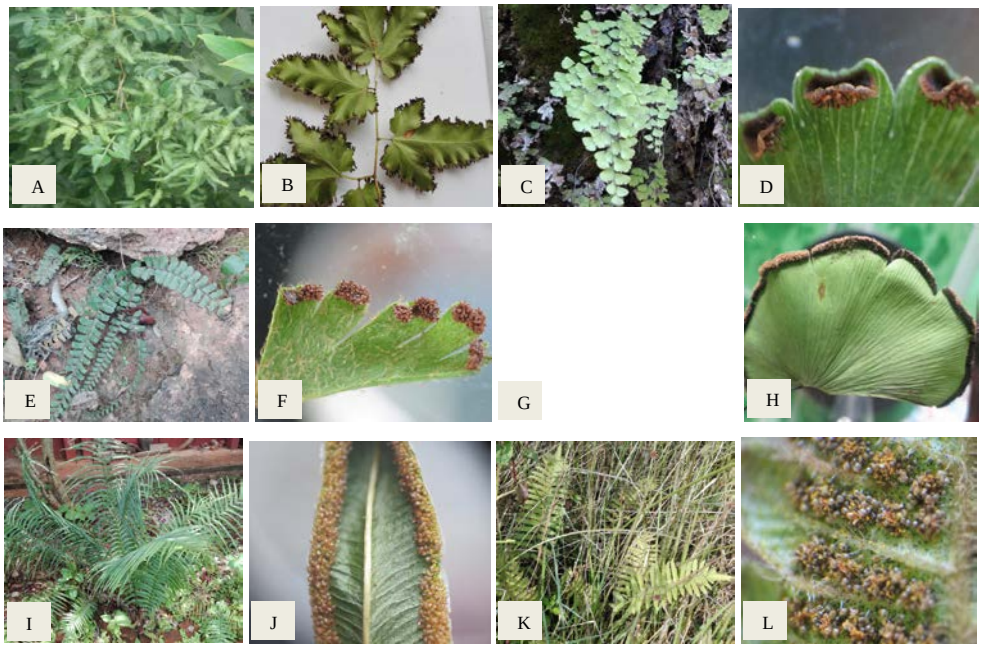
Pyrrosia stigmosa (Sw.) Ching,

Family : Polypodiaceae

Common name : Felt fern

Spore producing period : October to January

Spores monolete, lateral symmetry, ellipsoidal in outline, oblongoid in equatorial views, proximal views and distal views, equatorial diameters about 45µm, polar diameters about 32.5µm; yellow, leasurae wide, surface patterns verrucate.



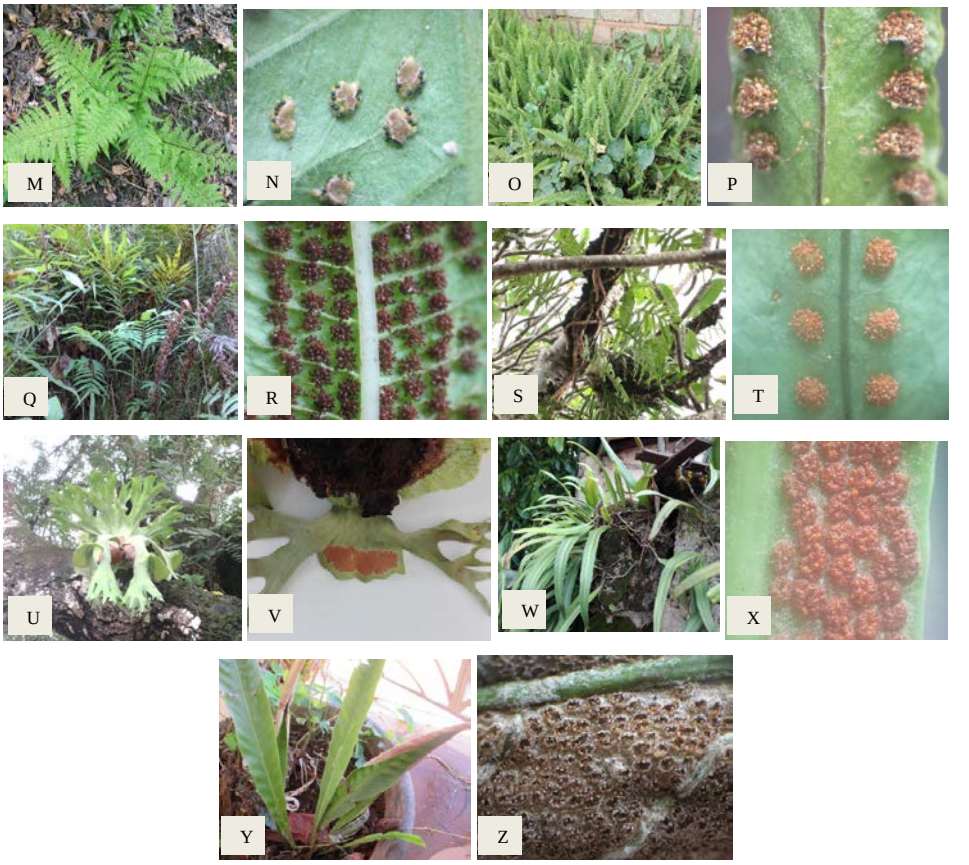
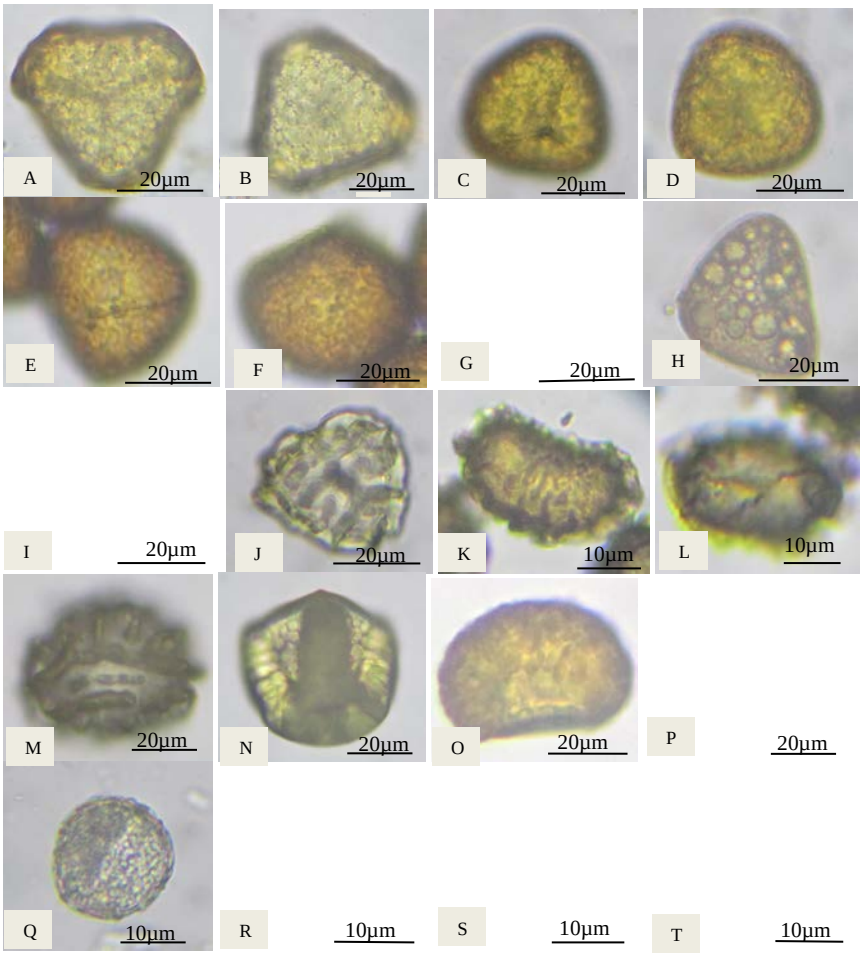


Figure 1. Habits and fertile fronds of collected species.
A,B. *Lygodium flexuosum* (L.) Sw. C,D. *Adiantum capillus-veneris* L.,E,F. *Adiantum malesianum* J. Ghatak, G,H. *Adiantum philippense* L., I,J. *Pteris vittata* L.,K,L. *Cyclogramma omeiensis* (Bak.) Tagawa,M,N. *Athyrium filix-femina* (L.) Roth,O,P. *Nephrolepsis exaltata* (L.) Shoot Q,R. *Arthromeris mairei* (Brause) Ching, S,T. *Microsorum scolpendria* (Burm.f.) Copel, U,V. *Platycerium wallichi* Hook, W,X. *Pyrrosia lingua* (Thunb.) Farw.,Y,Z. *Pyrrosia stigmosa* (Sw.) Ching,

Discussion and Conclusion

All of the studied species in this research were fern plants under the Class Pteridopsida and Division Pteridophyta. In this study, 13 species belonging to 10 genera of 6 families were collected from Sin Taung, Ye Kan Taung and Sarsana 2500 hill of Lashio Township. As a result, *Microsorum scolpendria*, *Platycerium wallichi*, *Pyrrosia lingua* and *Pyrrosia stigmosa* were found as epiphytic or epilithic ferns the rest were terrestrial or epilithic ferns. Ferns favor the sheltered areas under the forest canopy, along creeks and streams, and other sources of permanent moisture contents. They cannot grow readily in hot dry seasons like the flowering plants.



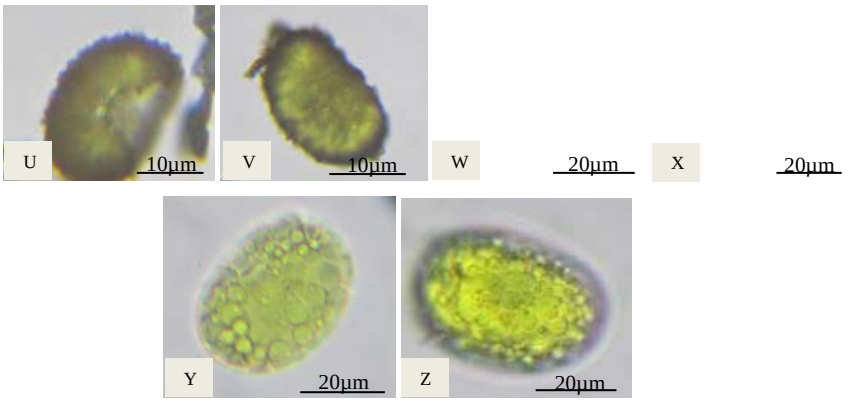


Figure 2. Proximal view and equatorial view of spore.
A,B. *Lygodium flexuosum* (L.) Sw. C,D. *Adiantum capillus-veneris* L.,E,F. *Adiantum malesianum* J. Ghatak, G,H. *Adiantum philippense* L., I,J. *Pteris vittata* L.,K,L. *Cyclogramma omeiensis* (Bak.) Tagawa,M,N. *Athyrium filix-femina* (L.) Roth,O,P. *Nephrolepsis exaltata* (L.) Shoot Q,R. *Arthromeris mairei* (Brause) Ching, S,T. *Microsorum scolpendria* (Burm.f.) Copel, U,V. *Platycerium wallichi* Hook, W,X. *Pyrrosia lingua* (Thunb.) Farw.,Y, Z. *Pyrrosia stigmosa* (Sw.) Ching,

Ferns are not as important economically as seed plants throughout the world. In the study area, only one species was found as an ornamental plant of *Nephrolepis exaltata* (L.) and the rest species were wild plants. Smith (1955) reported that some of the ornamental fern genera are *Adiantum*, *Lygodium*, *Nephrolepis* and *Pteris*. These findings were confirmed to the present study.

The tender leaf blades called fronds (croziers) are very palatable when well cooked. But vegetable ferns were not presented in this study. Some ferns protect their sporangia with thin semi-transparent membranes called indudia (Smith, 1955). In this study, indusiae were found in 3 species, false indusiae in 4 species and exindusiae in 6 species. The shape of sporangia is globose in all species. It was provided by short or long stalk but sessile in *Lygodium flexuosum* (L.) Sw. They produce a large number of spores. Annulus was found on the outer rim of the sporangium. They serve as the spore dispersal. It consists of a ring of dead water-filled cells with

differentially thickened cell walls that stretches about two-third around each sporangium (Smith, 1955).

This study described not only the visual taxonomic characters of ferns but also the microscopic spore characters of collected species. The types of spores, shapes, sizes, color and surface patterns were studied.

In ferns, the spores are generally monolete (a single leasura) and trilete (three laesura). The trilete spores have radial symmetry and were flat or convex at the distal face, while monolete spores, ellipsoidal or reniform spores have bilateral symmetry. In this study, trilete spores were observed in 9 species belonging to 4 genera. Monolete spores were found in 4 species belonging to 4 genera. The color of spores was yellow, reddish brown, greenish brown and pale brown. Most of the trilete spores are tetrahedral. Monolete spores are ellipsoidal and that of one species is globose. Most of the spores were varied in the size class that classified by Erdtman (1957). In this study, medium size and large size of spores were observed. Spore sizes generally ranges between 35µm and 50µm and the largest spore size of 50 µm was found in *Pteris vitata* L.

Lygodium flexusoum (L.) Sw., climbing fern, can easily be distinguished from other fern by its climbing habit. On the Sarsana 2500 hill, the ground of the vegetation is dominant and abundantly covered by this species. The spores of this species are trilete, tetrahedral and surface patterns were spheroidal-tuberculate. Those finding agreed with the statements of Makgomol (2006). Three species of *Adiantum* were collected from Chinese temple near Yae-kan-taung, Sin-taung and Sarsana 2500 hill. *Adiantum* is distinctive in appearance with dark, often black stipes and rachises and bright green often delicately flabellate leaf or frond. The fronds are unipinate in *A. philippense* L.and *A. maleasianum* J. Ghatak. and bi- to tri-pinnate in *A. capillus-veneris* L. The sori were borne submarginally and were covered by reflexed flaps of leaf tissue which resembled growing indusial. The spores of *Adiantum* were uniformity in their morphological characters. Nayar & Santha Devi (1968) and Vijayakanth *et al.* (2017) recorded that the spores of *Adiantum* are trilete, tetrahederal, reddish brown and irregular granulate. These findings were confirmed to the present study.

Pteris vittata L. can easily be distinguished by the sori arrangement on the fronds. In this species, the sori form linear along the edge of each pinna. The dark green fronds of this species are cut and often used as additions to flower arrangement. The spore characters of this species were

in accordance with those described by Vijayakanth and Sathish (2016). *Athyrium filix-femina* (L.) Roth, was widely grown in Sarsana 2500 hill area. Zenkteler (2012) reported that the spore of this taxa was monolete, ellipsoid, yellow and rugulate-papillate. The results of this study were confirmed with Zenkteler (2012). *Nephrolepis exaltata* (L.) Schoot, the sword fern can be found as an ornamental plant in the study area. It is also a very popular house plant for its beautiful fishbone like leaf structure. This fern possesses small potatoes like balls, and bulbs from which the new fern plants grow. The results of spore characters of this species corroborate with those described by Vijayakanth and Sathish (2016) in relation to the spore being monolete, ellipsoidal, brown and ornate.

The distinct characters of *Microsorum scolopendria* (Burm.f.) Copel, are the presence of long creeping rhizomes with scales, pinnately lobed leaves, winged rachis and wort-like sori on the frond undersides. Makgomol (2006) indicated that the spores of this species was monolete, ellipsoid, brown and tuberculate; Vijayakanth *et al.* (2017) described it as shallow verricate surface pattern. The result of this study agreed with Makgomol (2006). In *Platycerium wallichii* Hook., the fronds were extreme dimorphic which means the fertile and sterile fronds were different in shapes, sizes and structure. The spores of this species were reported by Pal and Pal (1970) and Makgomol (2006) as having monolete, ellipsoid, brown, scattered globules. Therefore, the spore surface patterns of those plant were considered as echinate.

In this study, the fronds were found as simple in *Pyrrosia lingua* (Thunb.) Farw., and pinnate or pinnatified in other fern species. The spores of *Pyrrosia* was studied by Vijayakanth *et al.* (2017). Those finding agreed with the present study of monolete, ellipsoid, yellow and verrucate surface patterns. In the literature survey, it was noted that the spore morphology of *Cyclogramma omeiensis* (Baker) Tawaga and *Arthromeris mairei* (Brause) Ching are not reported by other authors. In the present study, the spores of these species were found as monolete type, ellipsoid in *Cyclogramma omeiensis* and globoid in *Arthromeris mairei*. The surface patterns were vermiculate in *Cyclogramma omeiensis* species and granulate in *Arthromeris mairei* species.

In conclusion, it can be concluded that ferns are easily recognizable by their fronds with circinate vernation, presences of sori on underside of the fronds and often graceful of fronds. According to this study, the

different morphological characters of fronds, sori, and sporangia would be provided the taxonomic information for other fern researches. The spore characters together with morphological features of the sporophytes are considered the useful complementary taxonomic tools leading to the classification of fern group in the genus level. Therefore, the study of spore morphology would be useful in solving the problems of taxonomy and phylogeny in Pteridophytes.

Acknowledgements

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Morphological, Microscopical Studies and Phytochemical Investigation on Leaves of *Diospyros malabarica* (Descr.) Kostel.

Thet Su Hlaing*

Abstract

Diospyros malabarica (Descr.) Kostel. is evergreen tree, belongs to the family Ebenaceae. It is locally known as Bok-pyin (Yengan-bok). The specimens were collected from East Yangon University Campus, during the months of April to July, in the year 2019. The vegetative and reproductive parts of the fresh specimens were identified and studied by the literatures. In morphological study, the plant is dioecious tree. The leaves are simple and alternate. The inflorescences are axillary fascicles. The flowers are pale yellow and fragrant. The fruits are globose, berry and orange-colour. The seeds are 4-8, elliptical-wedge-shaped. In microscopical study, the upper and lower epidermal cells of leaves were irregular in shape. Stomata were present anomocytic type on the lower surfaces. The vascular bundles were collateral and closed types in midrib and petiole. The preliminary phytochemical tests were also performed from the powdered leaves of *Diospyros malabarica* (Descr.) Kostel. The prescence of alkaloid, glycoside, phenolic compound, flavonoid, steroid, terpenoid, tannin, saponin, reducing sugar starch and carbohydrate were found in phytochemical investigation. *Diospyros malabarica* (Descr.) Kostel. contain many valuable phytochemicals which might contribute to medicinally useful plant.

Keywords: Morphological, microscopical and phytochemical tests

Introduction

Diospyros malabarica (Descr.) Kostel. (Synonyms: *Diospyros peregrina* (Gaertn.) Gurke) belonging to the family Ebenaceae is a medium size evergreen plant. It is distributed throughout the tropics and has many medicinal values.

Different phytochemicals have been isolated from leaf and bark which include β-sitosterol, betulin, betulinic acid, oleanolic acid, lupenol and gallic acid. The plant is traditionally used for the treatment of dysentery and menstrual problems. Timber moderately hard and heavy, used for boat building and construction (Dassanayake, 1981).

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The leaves are used to headaches, as a poultice for boils and as a cure for rheumatism. The leaves and bark are used in dyspepsia, leprosy, diarrhoea, dysentery, haemorrhages, skin burning, diabetes, wounds, flatulence, scabies, carminative, laxative and tonic (Warrier, 1996).

Aim of the present research work is to examine the medicinal plant scientifically which have effective medicinal values. The objectives are to identify and study the morphological and microscopical characters and to perform the preliminary phytochemical test of the leaves of *Diospyros malabarica* (Descr.) Kostel.

Materials and Methods

Collection and Identification

The plant materials *Diospyros malabarica* (Descr.) Kostel. were collected from East Yangon University Campus during flowering and fruiting periods from April to July in 2019. Fresh specimens were used to identify by literature such as Backer (1965) and Dassanayake (1981).

Microscopical Studies

The fresh specimens lamina, midrib and petiole were done by free hand sections according to the methods by Metcalfe and Chalk (1950), Esau (1965), Trease and Evans (2002) and Pandey (2004). The reagents were used to examine the cut sections and the powdered samples and observed under the compound microscope. Chloralhydrate solution as clearing agents. Iodine solution B.P.C for testing starch and aleurone grains. Solution of phloroglucinol B.P. followed by concentrated hydrochloric acid for testing lignin. Acetic acid B.P. for testing calcium oxalate. Ferric chloride solution B.P.C for testing tannin. Sudan III B.P.C for testing essential oil.

Preliminary phytochemical investigation on the leaves of *Diospyros malabarica* (Descr.) Kostel.

The preliminary phytochemical investigation on the leaves of *Diospyros malabarica* (Descr.) Kostel. were carried out to determine the presence or absence of alkaloid, α-amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, starch, terpenoid and tannin. The experimental procedure was prepared by the methods of described in Vogel (1956), British Pharmacopoeia (1968), Marini Bettalo *et*

al., (1981), Robison (1983), Harbone (1993), Trease and Evans (2002) and Kokate (2009).

Extraction

Five gm of dried powdered leaves of *Diospyros malabarica* (Descr.) Kostel. was extracted with 100 ml of distilled water.

Test for alkaloid

The leaves extracts were stirred with 2ml of dilute hydrochloric acid separately and filtered. Each filtrate was divided into three portions and tested with Mayer's reagent, Wagner's reagent and Dragendroff's reagent. The precipitate formed on addition the reagent indicates the presence of alkaloid (Robison, 1983).

Test for α- amino acid

Extract 2 ml was added with two drops of ninhydrin reagent. The formation of violet color appears due to ·the presence of α-amino acid (Marini Bettolo, *et. al.,* 1981).

Test for carbohydrate

Extract 2 ml was introduced into a test tube and a few drops of 10% α- napthol was added and shaken. The test tube was then inclined at an angle of 45° and concentrated sulphuric acid was added slowly along the side of the tube. A red ring or violet ring was formed between the two layers, showing the presence of carbohydrate (Marini Bettolo, *et. al.,* 1981).

Test for flavonoid

Extract 2 ml was added with a few fragments of magnesium ribbon and 2 drops of concentrate hydrochloric acid and pink scarlet, crimsom red or occasionally green to blue color appears after few minutes indicating that the presence of flavonoid (Robison, 1983).

Test for glycoside

Extract 2 ml was added with 10% lead acetate solution in a test tube separately. White precipitate or pale yellow precipitate was observed which showed that the presence of glycoside (Marini Bettolo, *et. al.,* 1981).

Test for phenolic compound

Extract 2 ml was added 2 drops of 4 % ferric chloride solution in a test tube separately. If formation of green or blue color which may indicate that the presence of phenolic compound (Marini Bettolo, *et. al.,* 1981).

Test for reducing sugar

Extract 2 ml was added a few drops of Benedict's solution in a test tube separately solution appear green, yellow or red precipitate, which indicated that the presence of reducing sugar (Vogel, 1956).

Test for saponin

Extract 2ml was added a few drops of distilled water. Then the mixture was vigorously shaken for a few minutes. Observation was made to see if foaming took place, indicating that the presence of saponin (Marini Bettolo, *et. al.,* 1981).

Test for starch

Extract 2ml was added two drops of iodine solution in a test tube separately. The formation of bluish black precipitate indicated that the presence of starch (Central council for Research in Unani Medicine, 1987).

Test for steroid and terpenoid

Extract 2 ml was added two drops of concentrate H_2SO_4. This formation of blue or green color which shows the presence of steroid and formation of deep red color greenish color or blue color indicate that the presence of terpenoid (Central council for Research in Unani Medicine, 1987).

Test for tannin

Extract 2 ml was added with a few drops of 1% ferric chloride solution in a test tube separately. If yellowish brown precipitate or blue green color was resulted indicating that the presence of tannins (Kokate, 2009).

Results

Morphological characters of *Diospyros malabarica* (Descr.) Kostel.

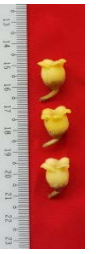
Evergreen dioecious tree, up to 14 m high and 70 cm diam. Leaves are simple, alternate, petiolate, exstipulate, oblong-lanceolate, coriaceous (9−22 × 3 – 5 cm). Inflorescences are axillary cyme, fascicle. Flowers are bracteate, bracteolate, pedicellate (very short), incomplete, unisexual, regular, actinomorphic, 4−5 merous, hypogynous. Sepals (4), synsepalous, valvate, sepaloid, inferior, persistent, cup-shaped (10 mm long) in male flower and lobes ovate tapered (15 mm long) in female flower. Petals (4−5) merous, synpetalous, imbricate, petaloid (pale yellow), campanulate shape (8−13 mm long) in male flower and campanulate shaped tip recurved (18−25 mm long) in female flower. Stamens numerous, apostamenous, filament short, pilose, dithecous, introrse, basifixed, longitudinal dehiscence, inferior. Staminodes 4−5, adnate to corolla, short, carpel (8), syncarpous, unilocular, free central placentation, many ovules in the locule, style 4, short, stigma forked and tips lobed, superior. Fruits are berry, short pedicel, globose, orange colour, up to 6 cm diam., calyx patent-reflexed, pulp sweet and astringent. The seeds are 4−8 seeds (25 mm long), elliptical−wedge shaped, soft and endosperm hard.

Habit

Leaves

Male Inflorescence

Female Inflorescence



Male flowers

Female flower | L.S male flower | L.S female flower | T.S ovary | Fruit | Seeds

Figure 1. Morphological characters of *Diospyros malabarica* (Descr.) Kostel.

Microscopical characters of *Diospyros malabarica* (Descr.) Kostel.

Lamina

In surface view, the epidermal cells of both surfaces were thin-walled parenchymatous cells and irregular in shape. The anticlinal walls were thick and straight. Anomocytic type stomata were found only in the lower epidermis. Long unicellular and two arms unicellular trichomes were present on lower surfaces.

In transverse section, the lamina was dorsiventral, thin cuticle present on both surfaces. The epidermal cells were thin-walled and barrel shaped parenchymatous cells on both sides. The mesophyll was composed of palisade and spongy parenchymatous cells. The single layered thick palisade cells were vertically elongated and compactly arranged with numerous chloroplasts. The spongy mesophyll composed of 8−12 layers of cells, irregular to rounded shaped and loosely arranged.

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. The xylem composed of vessels, tracheids, fibers and xylem parenchymatous cells. The vessels have simple perforations and simple pitted. The fiber cells were long with pointed ends.

Midrib

In surface view, the epidermal cells were thin walled, compactly arranged and rectangular and irregular shaped parenchymatous cells. Unicellular with two arms trichomes and brown tanniferous contents were present.

In transverse section, convex at both sides covered with thick cuticle. Both epidermals cells were barrel shape. The lower epidermals cells were similar to those the upper epidermal cells. The collenchymatous cells were present 1−2 layers below the epidermis. The parenchymatous cells were 6 to 8 layers above the vascular bundle and 10 to 12 layers below the vascular bundle. They were thin-walled and irregularly rounded or oval shaped. The vascular bundle was crescent shaped, collateral and closed type. The bundle was surrounded by a sheath of sclerenchymatous cells.

The phloem cells were thin-walled and mainly composed of sieve tubes and companion cells. The xylem consisted of vessles, tracheids, fibers and xylem parenchymatous cells. The vessels have simple perforations and simple pitted. The fiber cells were long with tapering pointed.

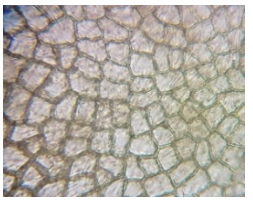
Petiole

In surface view, the epidermal cells were thick-walled and irregular shaped parenchymatous cells. Unicellular trichomes and unicellular with two arms trichomes were present in the cells.

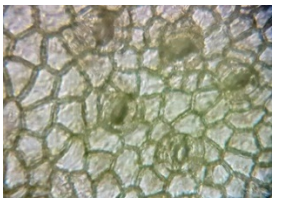
In transverse section, the upper was flat and the lower was convex and covered with thick cuticle. Both epidermal cells were rounded shaped parenchymatous cells.

The cortex was composed of 16−20 layered parenchymatous cells. They were thin-walled and oval to rounded cells. The intercellular spaces were absent. Stone cells and starch grains were distributed throughout the cortex.

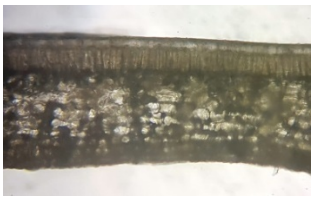
The vascular bundles were crescent shaped and collateral type. The pericyclic fibers formed as a ring around the bundle. Phloem was composed of sieve tube and companion cells. Xylem cells were lignified, thick-walled and polygonal to isodiametric in shape. Rays were sclerenchymatous cells, radiating in between xylem vessels, 6−10 cells in each row, the cells lignified.



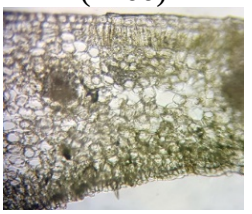
Surface view of upper epidermis (×400)



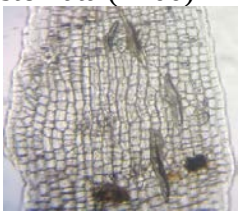
Surface view of lower epidermis showing stomata (×400)



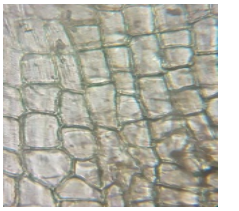
Transverse section of lamina (×100)



T.S of lamina showing mesophyll cells and trichomes (×100)



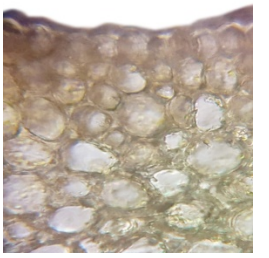
Surface view of midrib showing tannin and trichomes (×40)



Surface view of midrib showing epidermal cells(×400)

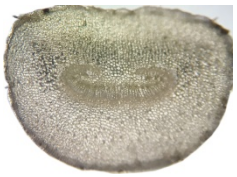
Figure 2.(a) Microscopical characters of leaves of *Diospyros malabarica* (Descr.) Kostel.

T.S of midrib showing epidermal cells, cortex and vascular bundle(×40)

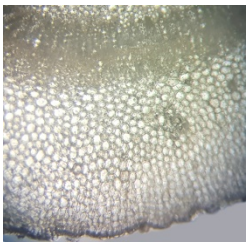


T.S of midrib close up view of epidermis and cortex (×400)

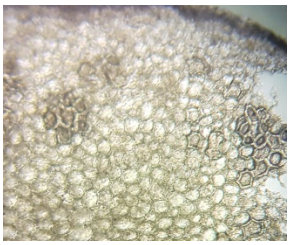
Surface view of petiole showing epidermal cells with trichome(×400)



T.S of petiole showing starch grains and trichomes(×40)

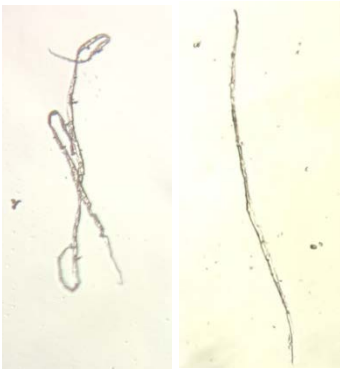


T.S of petiole showing cortex and vascular bundle (×100)

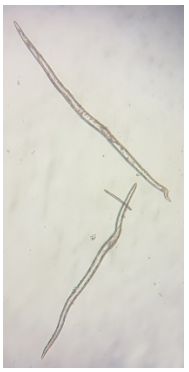


T.S of petiole showing cortex with stone cells (×100)

Figure 2.(b) Microscopical characters of leaves of *Diospyros malabarica* (Descr.) Kostel.



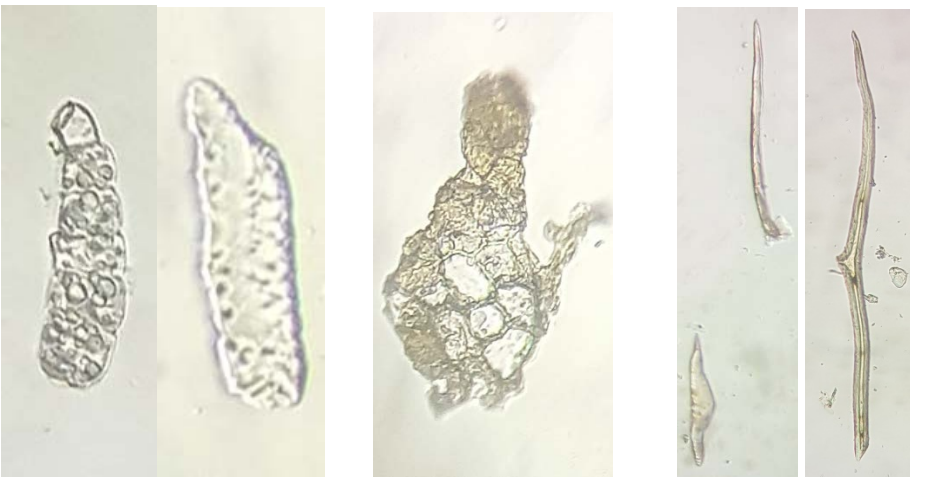
Fibers (×100)



Fiber-tracheid (×100)

Tracheid (×400)

Figure 3.(a) Diagnostic characters of powdered leaves of *Diospyros malabarica* (Descr.) Kostel.



Pitted vessels(×400) Epidermal cells(×400) Trichomes(×100)

Figure 3.(b)Diagnostic characters of powdered leaves of *Diospyros malabarica* (Descr.) Kostel.

Preliminary phytochemical test of *Diospyros malabarica* (Descr.) Kostel.

The preliminary phytochemical investigation was carried out on the powdered leaves. The results were shown in Table (1) and Figure (4).

Table 1(a). Preliminary phytochemical test of leaves from *Diospyros malabarica* (Descr.) Kostel.

No.	Chemical constituents	Reagent	Extract	Observation	Results
1.	Alkaloid	Mayer's reagent	H_2O + 1% HCl	Pale brown ppt	+
		Wagner's reagent	H_2O + 1% HCl	Dark brown ppt	+
		Dragendroff's reagent	H_2O + 1% HCl	Pale brown ppt	+
2.	α-amino acid	Ninhydrin reagent	Distilled water	Brown	−

No.	Chemical constituents	Reagent	Extract	Observation	Results
3.	Carbohydrate	10% α-napthol+ H_2So_4 (Conc:)	Distilled water	Red ring White ppt	+

Table 1(b). Preliminary phytochemical test of leaves from *Diospyros malabarica* (Descr.) Kostel.

No.	Chemical constituents	Reagent	Extract	Observation	Results
4.	Flavonoid	Mg/HCl (Conc:)	Distilled water	Brown	+
5.	Glycoside	10% lead acetate solution	Distilled water	White ppt	+
6.	Phenolic Compound	4% $FeCl_3$ solution	Distilled water	Blue	+
7.	Reducing sugar	Benedict's solution	Distilled water	Yellow	+
8.	Saponin	Distilled water	Distilled water	Frothing	+
9.	Starch	I_2 solution	Distilled water	Black ppt	+
10.	Terpenoid/ steroid	H_2SO_4 (Conc:)	Distilled water	Blue green	+
11.	Tannin	1% $FeCl_3$ solution	Distilled water	Dark brown	+

+ = present, − = absent

Figure 4. Preliminary phytochemical investigation on powdered leaves of *Diospyros malabarica* (Descr.) Kostel.

Discussion and Conclusion

In this research, the morphological studies on both vegetative and reproductive parts of the plant, the microscopical studies and the preliminary phytochemical studies of leaves of *Diospyros malabarica* (Descr.) Kostel. were present.

In the morphological study, leaves are elliptic-oblong, coriaceous. Inflorescence are 1−5 flowers. Flower very shortly stalked, pale yellow, fragrant, calyx 4 lobed, corolla campanulate, staminodes 4, adnate to tube, short, style 4, short, each forked and the tips lobed. Fruit short pedicel and globose, berry, orange-coloured or tawny lobes of fruiting calyx patent-reflexed. Seeds 4−8, elliptical wedge-shaped. The morphological characters in this study were agreed with those mentioned by Backer (1965) and Dassanayake (1981).

In the microscopical study, Metcalfe and Chalk (1950) reported that ranunculaceous stomata were present on the lower surfaces and epidermis composed of cells having straight anticlinal walls, with very much thickened in certain species of *Diospyros*. Long unicellular trichomes, brown tanniferous contents, starch grain and oil were also present.

Metcalfe and Chalk (1950) also stated that unicellular with two arms trichomes to the leaf surface and vascular bundle of the vein accompanied by well-developed sclerenchyma. Petiole of *Diospyros* exhibiting a solitary, crescentic vascular strand, open and slightly concave to deep with strongly

incurved ends and stone cells present in the parenchymatous. The findings in this study were confirmed with those described by Metcalfe and Chalk (1950), Pandey (2004) and Trease & Evans (2002).

In the preliminary phytochemical tests from the leaves of *Diospyros malabarica* (Descr.) Kostel. contained alkaloid, glycoside, phenolic compound, flavonoid, steroid, terpenoid, tannin, saponin, reducing sugar, starch and carbohydrate. These qualitative analysis characters were similar to those described by Satish (2016).

From the above mentioned studies it can be concluded that the morphological and microscopical characters can be used as valuable resource for confirmation and identification of the plant. *Diospyros malabarica* (Descr.) Kostel. contain many valuable phytochemicals which might contribute to medicinally useful plant. Therefore, the present study may support to application of medicinal purposes.

Acknowledgements

I would like to express grateful to Dr. Daw San Khaing, Professor and Head, Department of Botany, East Yangon University, for her invaluable advices and kind suggestion. I am also thankful to Dr. Thidar Htoo, Professor, Department of Botany, East Yangon University, for her beneficial advices and constant encouragement.

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Investigation on Isolation and Identification of Isolated Compounds from fruit's Methanol Extract of *Trapa bispinosa* Roxb.

Khin Thawtar Win*

Abstract

Trapa bispinosa Roxb. was one of the flowering plants belonging to the family Trapaceae. In the present investigation, isolated compounds were examined by extraction, isolation and identification on methanol extract. The isolated compounds were identified by melting point, thin layer chromatography and FTIR spectroscopy. According to thin layer chromatographic analysis, isolated compound A using petroleum ether: ethyl acetate (9:1v/v) solvent system, showed a single spot with R_f value 0.72 which was similar with R_f value of oleanolic acid, thin layer chromatography of isolated compounds B and C using petroleum ether: ethyl acetate (5:1v/v) solvent system, showed a single spot and R_f value 0.50 and 0.31, which was similar with R_f value of β-sitosterol and β-amyrin and thin layer chromatography of isolated compound D using petroleum ether : ethyl acetate (3:1v/v) showed a single spot and R_f value 0.43 which was similar with R_f value of gallic acid were observed. In addition, the results of Infra- red spectral data from isolated compounds were also presented.

Keywords: Isolation, Identification, Thin Layer Chromatographic, R_f values

Introduction

Plants consist of macro and micro elements, kinds of elements contained in plants are more or less similar to each other. But compound of such plants are quite different in structure.

All plants produce chemical compounds as part of their normal metabolic activities. Sterols, terpenes, flavonoids, saponins, alkaloids, phenols, tannins are known as secondary or special metabolites. *Trapa bispinosa* Roxb. is not only for treatment of medicine, but also having food value. Edible part of the plant consists of minerals and organic compounds that are needed for human.

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Trapa bispinosa Roxb. was afforded a mixture of polysaccharides which on hydrolysis gave mainly D- galactose, L-arabinose, D- galacturonic acid and D- xylose together with a very small amount of glucose and rhamnose. Fractionation of the polysaccharides gave an acidic arabinogalactan and a xylan. The arabinoglactan was shown to contain linear chains of 1, 5 linked L-arabinofuranose residue and 1, 4 linked D-galactopyranose and D-galacturonic acid residues. The xylose units in xylan are linked by 1, 4 bonds with branching in 3- positions (Chemical Abstracts, 1981).

So, in this research the edible part of *Trapa bispinosa* Roxb. were chosen to carry out the qualitative and quantitative analysis of chemical constituents; extraction, isolation and identification of isolated compounds.

Materials and Methods

Extraction, Isolation and Identification of isolated compounds from fruits of *Trapa bispinosa* Roxb.

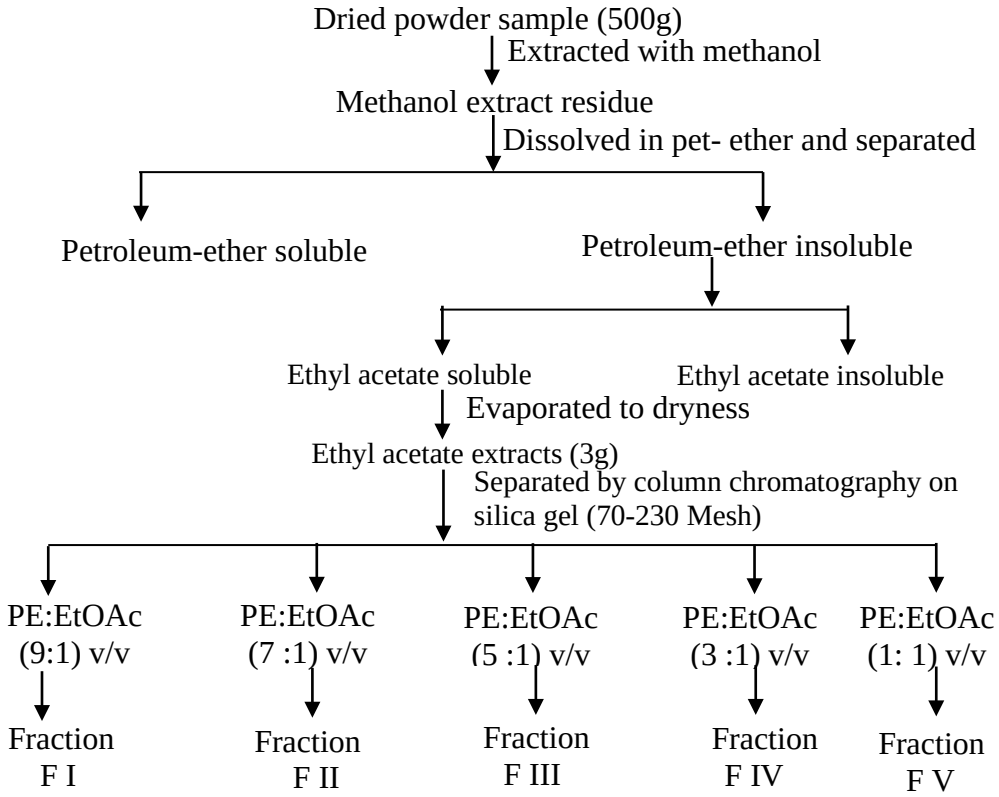
Extraction

The dried powdered sample (500g) was soaked with methanol in the percolator for 1 week and then drained off. The percolation was carried out another 2 times with fresh solvent. This methanol extract was concentrated and the residue was defatted with petroleum ether. The insoluble residue was extracted with ethyl acetate and filtered. The filtrate was concentrated on water bath and finally they were evaporated to dryness. Among them, 5 g of dried ethyl acetate extract was mixed with 15 g of silica gel. The moisture was continuous agitation in room temperature. So that free-flowing dry silica gel on which the sample was uniformly absorbed.

Isolation of Compound A, B, C and D

The glass column (2.5 × 23) cm with a stop cock attached was clamped, so that it was perfectly vertical. The column was filled with solvent system and plugged by pushing small pieces of cotton through the solvent with the aid of a glass rod. Silica gel 100 g was mixed with solvent system and the suspension was the roughly stirred in a beaker. A portion of the slurry was poured into the column and at the same time, the stop cock was opened so that the solvent flowed down slowly with constant rate. The slurry was continuously poured into the column and the column material was slowly settled to the bottom, the side of the column was tapped with

rubber tubes to free air bubble and attain uniform packing. When the stable silica gel column was formed, a uniform layer absorbed gel was placed on top of the column. The solvent above the top was allowed to enter the gel by opening the stopcock. The column was completely filled with solvent system and fraction was started. Flow rate was adjusted about 1 drop per second. Elution was successively performed with petroleum: ethyl acetate (9 : 1, 7: 1, 5 : 1, 3 : 1, 1 : 1, 1 : 2, 1 : 4, 1 : 1, ethyl acetate only) respectively. These fractions were checked by TLC. The fractions gave the similar appearance on TLC was combined and finally five main fractions (FI-FV) were collected. Fraction FI, FII, FIII, and FV showed only one spot with different R_f values. Compound A from fractions FI, compound B from fractions FII, compound C from fractions FIII and compound D from fractions FV were obtained. The flow diagram of extraction, isolation of compounds A, B, C and D were shown in Figure (1).



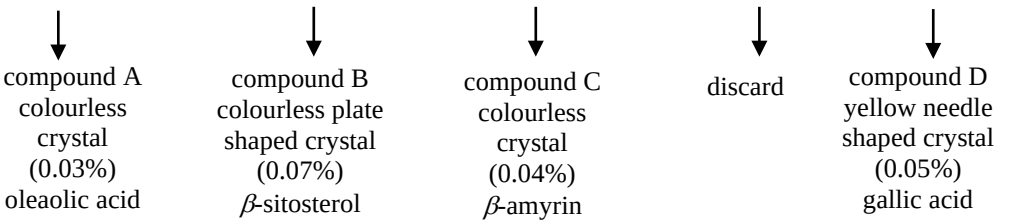


Figure 1. Chromatographic separation of EtOAc fraction of defatted MeOH extract from fruits of *Trapa bispinosa* Roxb.

Identification of compound A, B, C and D by thin layer chromatography

The isolated compound A, B, C and D were characterized by thin layer chromatography using the precoated silica gel plates (GF_{254} aluminum plates, Merck). The isolated compounds were dissolved in minimum amount of solvent and applied on silica gel plate by using capillary tube. The spots were allowed to dry and developed with solvents systems. The solvent systems were petroleum ether: ethyl acetate (9:1v/v) for compound A, petroleum ether: ethyl acetate (5:1v/v) for compound B and C, petroleum ether: ethyl acetate (3:1v/v) for compound D respectively.

When mobile phase solvent was reached near the upper edge, the plate was taken out from the glass chamber and dried at room temperature. The spots were visualized by spraying with 5% sulphuric acid for the compound A, iodine vapor for the compounds B, C and D respectively. When spraying with 5% sulphuric acid which needed to be heated in oven at 110°C. R_f value were calculated by the following equation. The results were shown in Figure (6–9).

$$\text{Relative flow } (R_f) = \frac{\text{distance travelled by the solute from the point of origin}}{\text{distance travelled by the solvent from the point of origin}}$$

Identification by Infra-red spectroscopy

The Infra-red spectrum of isolated compound A, B, C and D were recorded by using Shimadzu, Perkin Elmer spectrum GXFTIR spectrometer at the Universities' Research Centre, Mandalay and Department of Medical Research, Yangon. The isolated

compounds A, B, C and D were sampled with 1% KBr pellet. The FTIR spectrums were shown in Figures (10- 13).

Fig. 3. water-bath and extract

Fig. 4. Stuart scientific melting point apparatus

Fig. 5. Partition Chromatogra - phic Column

Fig. 2. Habit and fruit of *Trapa bispinosa* Roxb.

Results and Discussion

Extraction, Isolation and Identification of compound A, B, C and D from fruits of *Trapa bispinosa* Roxb.

Colorless needle shaped crystal 0.03% of compound A, 0.07% and 0.04% of colorless plate shaped crystal of compounds B and C and 0.05% of fawn-colored needle shaped crystal of compound D were isolated from ethyl acetate extract of powdered fruits using column chromatographic method. Isolated compounds A, B, C and D were identified by TLC, melting point and FTIR spectroscopic methods.

Identification of isolated compounds A, B, C and D by using TLC, melting point and FTIR spectroscopic method

Identification by thin layer chromatography

Isolated compound A was dissolved in alcohol and spotted on silica gel GF_{254} plate, pet ether : ethyl acetate (9:1v/v) was used as solvent system. After spraying with 5% H_2SO_4, purple spot was observed. R_f value of compound A was 0.72, which was similar with R_f value of oleanolic acid. R_f value of compounds B and C were found to be 0.53 and 0.31 by using pet ether: ethyl acetate (5:1v/v) as solvent system and after visualization with iodine vapor and spraying reagent 5% sulphuric acid and anisaldehyde. The R_f value of compound D was found to be 0.43 by using pet ether: ethyl acetate (3:1v/v) as solvent system and after visualization with iodine vapor and spraying reagent anisaldehyde. The R_f value of compound B, C and D

were identical with that of *β*-sitosterol, *β*-amyrin and gallic acid. The results were shown in Table (2- 5) and Figures (6- 9).

Table 1. Melting point of isolated compounds

Isolated Compounds	Melting point		References
	Isolated	Literature	
A (oleanolic acid)	307-310°C	310°C	Stecher, 1968
B (*β*-sitosterol)	139°C	140°C	Grasselli, 1975
C (*β*-amyrin)	197°C	197°C	Grasselli, 1975
D (gallic acid)	233-238°C	235-240°C	Stecher,1968,Harbone, 1993

Identification by melting point

The melting point of the isolated compound such as oleanolic acid, *β*-sitosterol, *β*-amyrin and gallic acid were determined by using a capillary with a Stuart Scientific melting point apparatus were described in Table (1).

Identification by FTIR spectroscopic study of Compound A

In the FTIR spectrum, the characteristic of isolated compound A was observed: OH-stretching group at 3414 cm^{-1}, CH-stretching group at 2932, 2870cm^{-1}, C=O stretching group at 1712 cm^{-1}, C=C stretching group at 1643, 1555, 1454 cm^{-1}, CH_3-bending group at 1377 cm^{-1}, C–O–C stretching for acid group at 1250cm^{-1}, C–O–C stretching for alcohol group at 1185 cm^{-1}, OH bending group at 1076 cm^{-1}, CH out of plane bending group at 756 cm^{-1}, FTIR spectrum of isolated compound A was shown in Figure(10). On the basis of melting point, thin layer chromatography and FTIR spectrum data of isolated compound A may be oleanolic acid.

Identification by FTIR spectroscopic study of Compound B

In the FTIR spectrum, the characteristic of isolated compound B was observed: OH-stretching for alcohol group at 3409, 3306 cm^{-1}, CH stretching of olefin at 3095 cm^{-1}, CH-stretching for CH_2andCH_3 group at 2939, 2870 cm^{-1}, C=C stretching group at 1639 cm^{-1}, asymmetric CH_3 bending at 1462 cm^{-1}, symmetric CH_3 bending in gem dimethyl group at

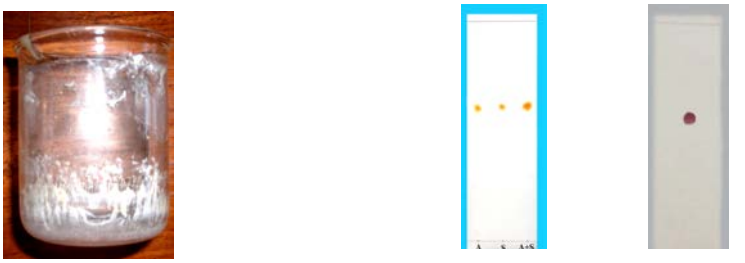
1373 cm^{-1} and 1321 cm^{-1}, C–O–C stretching group at 1053 cm^{-1}, FTIR spectrum of isolated compound B was shown in Figure (11). On the basis of melting point, thin layer chromatography and FTIR spectrum data isolated of compound B were the same as those given in the literature. So, isolated compound B may be β-sitosterol.

Identification by FTIR spectroscopic study of Compound C

In the FTIR spectrum, the characteristic of isolated compound C was observed: OH stretching for alcohol group at 3425 cm^{-1}, CH stretching for $>CH_2$ and $-CH_3$ group at 2932 cm^{-1}, C=C stretching group at 1636 cm^{-1}, C–H bending of $>CH_2$ and $-CH_3$ group at 1450,symmertic CH_3 bending at 1375 cm^{-1}, C–O stretching group at 1080 cm^{-1}, CH out of plane bending group at 795 cm^{-1}, FTIR spectrum of isolated compound C was shown in Figure (12). On the basis of melting point, thin layer chromatography and IR spectrum data of compound C were the same as those given in the literature for (Koji Nakanishi, 1962). So, isolated compound C may be β-amyrin.

Identification by FTIR spectroscopic study of Compound D

In the FTIR spectrum, the characteristic bands of isolated compound D was observed: OH-stretching for alcohol group at 3418 cm^{-1} C–H stretching for $>CH_2$ and $-CH_3$ group at 2993 cm^{-1}, C=O stretching for ketone group at 1697 cm^{-1}, C=C stretching group at 1620, 1550 and 1450cm^{-1}, C–O–C stretching for acid group at 1334 cm^{-1} , C–O–C stretching for alcohol group at 1242 cm^{-1}, OH bending at 1026 cm^{-1} , FTIR spectrum of isolated compound D was shown in Figure (13). On the basis of melting point, thin layer chromatography and FTIR spectrum data of isolated compound D were the same as those given in the literature. So, isolated compound D may be gallic acid.

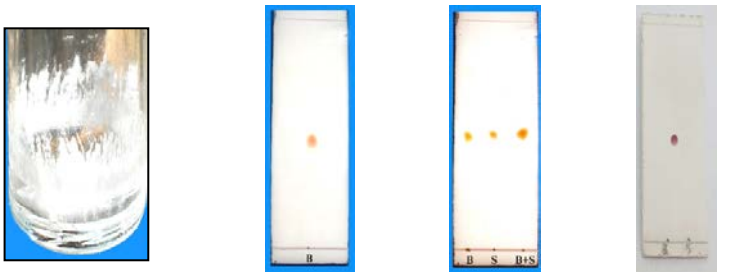


A= Isolated compound A (Oleanolic acid) S= Standard

Figure 6. Thin layer chromatogram of isolated compound 'A'

Table 2. Physicochemical properties of isolated compound 'A'

Experiment	Observation	Remark
R_f	0.72	PE : EtOAc (9:1)v/v
UV (254 nm & 356 nm)	Inactive	Inactive
Liebermann-Burchard Test	Purple	Terpenoid
Vanillin-H_2SO_4, Δ	Pink purple	On TLC
Anisaldehyde-H_2SO_4, Δ	Violet blue	On TLC
Iodine vapour	Brown yellow	On TLC

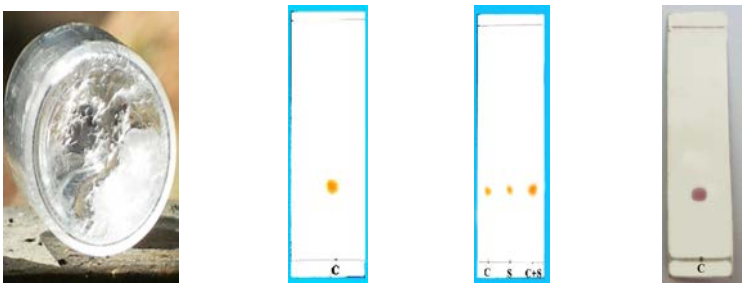


B= Isolated compound 'B' (β-sitosterol) S= Standard

Figure 7. Thin layer chromatogram of isolated compound 'B'

Table 3. Physicochemical properties of isolated compound B

Experiment	Observation	Remark
R_f	0.50	PE:EtOAc (5 :1) v/v
UV (254 nm & 356 nm)	Active	Active
Liebermann-Burchard Test	Colouration	Steroid/terpenoid
Vanillin-H_2SO_4, Δ	Purple	On TLC
Anisaldehyde-H_2SO_4, Δ	Purple	On TLC
Iodine vapour	Yellow	On TLC

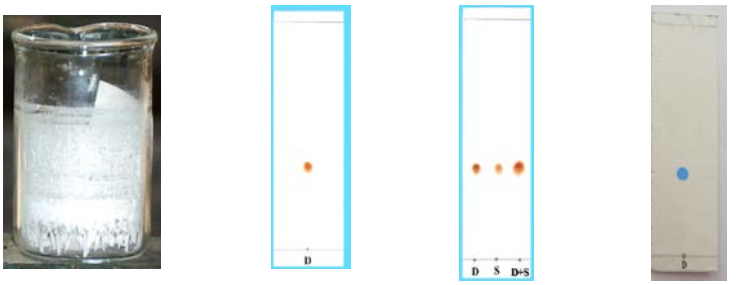


C= Isolated compound 'C' (β-amyrin) S= Standard

Figure 8. Thin layer chromatogram of isolated compound 'C'

Table 4. Physicochemical properties of isolated compound C

Experiment	Observation	Remark
R_f	0.31	PE:EtOAc (5:1) v/v
UV (254 nm & 356 nm)	Inactive	Inactive
Liebermann-Burchard Test	Deep gray	Terpenoid
Vanillin-H_2SO_4, Δ	Pale purple	On TLC
Anisaldehyde-H_2SO_4, Δ	Purple	On TLC
Iodine vapour	Yellow	On TLC



D= Isolated compound 'D' (Gallic acid) S= Standard

Figure 9. Thin layer chromatogram of isolated compound 'D'

Table 5. Physicochemical properties of isolated compound D

Experiment	Observation	Remark
R_f	0.43	PE : EtOAc (3:1) v/v
UV (254 nm & 356 nm)	Active	Active
1% $FeCl_3$ solution Test	Deep blue	Phenolic OH present
Anisaldehyde-H_2SO_4, Δ	gray	On TLC
Iodine vapour	brown	On TLC

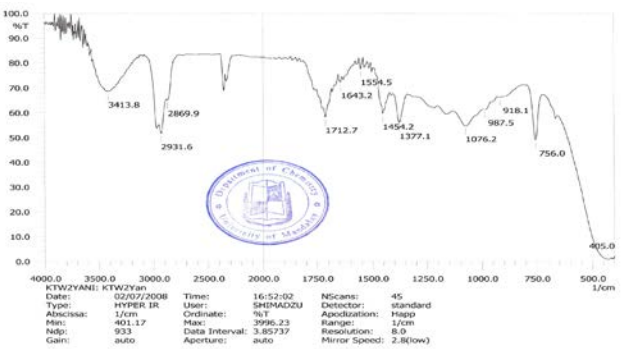


Figure 10. Infra-red spectral data of isolated compound 'A' as oleanolic acid

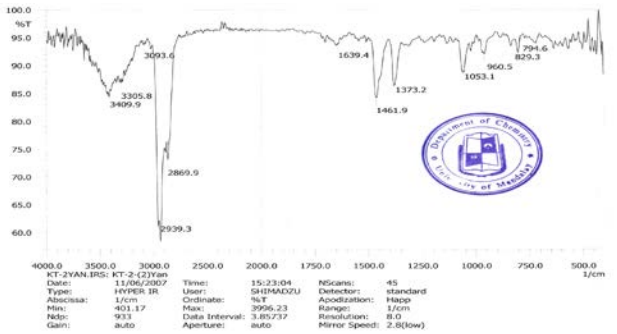


Fig. 11. Infra-red spectral data of isolated compound 'B' as *β-sitosterol*

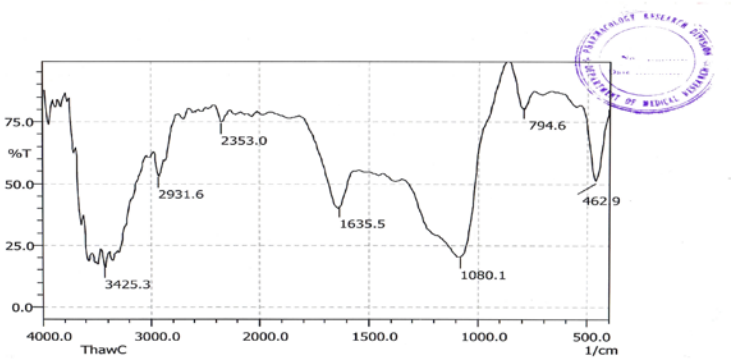


Figure 12. Infra-red spectral data of isolated compound 'C' as *β- amyrin*

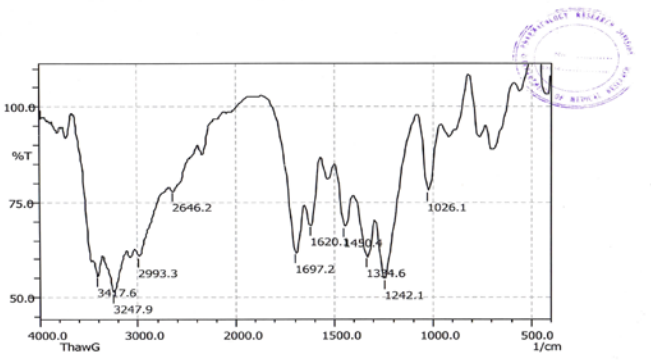


Figure 13. Infra-red spectral data of isolated compound 'D' as gallic acid

Conclusion

In the present study, extraction, isolation and identification of isolated compounds were examined. According to thin layer chromatographic analysis, isolated compound A using petroleum ether: ethyl acetate (9:1v/v) solvent system. They gave a single spot with R_f value 0.72 which was similar with R_f value of oleanolic acid mentioned in (Stahl, 1969). Melting point of isolated compound A was (307-310°C), which was agreed with those of oleanolic acid described in Stecher (1968). FTIR spectral bands of this compound indicated the functional group of oleanolic acid. Thus, isolated compound A may be oleanolic acid.

Thin layer chromatography of isolated compounds B and C using petrolreum ether: ethyl acetate (5:1v/v) solvent system, showed a single spot and R_f value 0.50 and 0.31, which was similar with R_f value of β-sitosterol and β-amyrin mentioned in (Stahl, 1969 & Stecher, 1968). Melting point of β-sitosterol and β-amyrin described in (Grasselli, 1975). FTIR spectral data of isolated compounds B and C indicated the spectral characteristics of β-sitosterol and β-amyrin described in (Grasselli, 1975 & Stecher, 1968). Thus, isolated compounds B and C may be β-sitosterol and β-amyrin.

Thin layer chromatography of isolated compound D using petroleum ether: ethyl acetate (3:1v/v) showed a single spot and R_f value 0.43. Melting point of isolated compound D was (235-238°C), which was similar with melting point of gallic acid described in (Stecher, 1968). FTIR spectral data of isolated compound D indicated that the spectral characteristics of gallic acid described in (Stecher, 1968). According to the above factors, isolated compound D may be gallic acid.

It is concluded that the finding of the present study provide considerable contribution to improvement of Myanmar traditional medicine.

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