

Investigation of antimicrobial activities of endophytic fungi from the leaves of *Averrhoa bilimbi* L. and *Averrhoa carambola* L.

Myint Myint Soe¹, Moe Pa Pa²

Abstract

The leaves of the selected plants of *Averrhoa bilimbi* L.(Tayoke -Zaung-Yar) and *Averrhoa carambola* L.(Zaung Yar) were collected from Hinthada District, Ayeyawady Region. Their leaves were used for the isolation of endophytic fungi by surface sterilization method. In this investigation, the five fungi (MMS 01 to MMS 05) were isolated from *Averrhoa bilimbi* and three fungi (MMS 06 to MMS 08) were isolated from *Averrhoa carambola*. The isolated eight fungi were used for antimicrobial activities by paper disc diffusion assay. The seven test organisms were used in this activity. All isolated fungi of inhibited on the *Bacillus subtilis* (35.95 – 15 mm). Except isolated fungi MMS- 08, MMS- 01 to 07 inhibited on *Candida albicans* (33.00-15.00 mm) and *E.coli* (30.00-15.00 mm). Among the isolated eight fungi, MMS 01 was found the best activity of inhibitory zone (35.95 mm) on the *Bacillus subtilis* IFO 90571.

Keywords; *Averrhoa bilimbi* L, *Averrhoa carambola* L, isolation, antimicrobial activities, inhibitory zone

INTRODUCTION

Microorganisms live in all part of the biosphere where there is liquid water, including soil, hot springs, on ocean floor, high in the atmosphere and deep inside rocks within the earth crust. The typical materials for microbial sources are soil living and fallen leaves, leaf litters, dung, insect, fresh water and marine water (Harayama and Isono, 2002).

Fungi is active in decomposing, the major constituents of plant tissues namely cellulose, lignin and pectin. Saprophytic fungi converts dead organic matter into fungal biomass, carbon dioxide and organic acids. These fungi have enzyme that work to “rot” or “digest” the cellulose and lignin found in the organic matter, with the lignin being an important source of carbon for many organisms. Without their digestive activities, organic material would continue to accumulate until the forest became a huge rubbish dump of dead leaves and trees. Fungi due to their competitive saprophytic ability was expressed by the fast mycelia growth, spores production, presence of the efficient and extensive systems of powerful enzymes are able to utilize complex polysaccharides and protein as their carbon and nitrogen sources (Wubah, 1999).

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms, they act primarily against. For example, antibiotics are used against bacteria, antifungals and fungi. They can also be classified according to their functions. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis (Kingston, 2008).

¹ Lecturer, Chemistry Department, Hninthada University

² Associate Professor, Dr, Botany Department, Patheingyi University

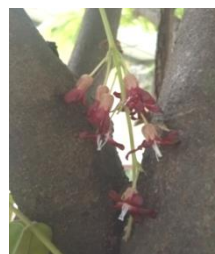
Distinctive Characters of *Averrhoa bilimbi* L.

Scientific Name	- <i>Averrhoa bilimbi</i> L.
Vernacular Name	- Tayoke Zaung Yar
English Name	- Wood sorrel
Family	- Oxalidaceae
Location	- Hinthada University Campus
Plants parts used	- Leaves

Outstanding characters

Small tree, a short-trunk; alternate, quite similar, pinnate compound leaves; flower fragrant, reddish brown; fruit fleshy, ellipsoidal, sometime faintly 5-angle, the juice sour, yellowish-green colored.

Uses- skin eruptions, thrush, cough, treat inflammatory condition, antioxidant, anti-cancer, astringent, diuretic

**Habit****Flower****Fruit****Leaves**Figure 1. Morphological Characters of *Averrhoa bilimbi* L.**Distinctive Characters of *Averrhoa carambola* L.**

Scientific Name	- <i>Averrhoa carambola</i> L.
Vernacular Name	- Zaung Yar
English Name	- Star fruit
Family	- Oxalidaceae
Location	- Hinthada University Campus
Plants parts used	- Leaves

Outstanding characters

Perennial herbs, a short-trunk; the branches are dropping; pinnate compound leaves; cymose inflorescences; flower purplish pink; fruit berry, star fruit and five corner, orange-yellow colored.

Uses- Intestinal worms and fever, Vomiting, Diarrhea, Treat indigestion, Mouth ulcer, Skin rashes, Toothache, Diuretic in kidney



Habit



Flower



Fruit



Leaves

Figure 2. Morphological Characters of *Averrhoa carambola* L.

MATERIALS AND METHODS

Plant materials of two *Averrhoa* species were used for the isolation of endophytic fungi and these isolated endophytic fungi were used for the study of the antimicrobial activity.

Collection and Identification of Plant Samples

Plant samples were collected from Hinthada District, Ayeyarwaddy Region. These plant samples were collected on the basis of medicinal properties of plants. The collected plants were identified by Botany Department, Patheingyi University. This study was conducted from May to October, 2021.

Surface Sterilization Method of Endophytic Fungi (Ando, 2004)

The endophytic fungi isolated from various parts of selected plant with following procedures.

1. Healthy fresh part of selected plants were washed in running tap-water for 30 mins.
2. The leaf blade, leaf sheath and rhizome were cut into about 1 cm piece.
3. The pieces were sterilized by soaking in 70% ethanol for about one mins.
4. Then, the leaves were next sterilized by immersing in 5% sodium hypochloride for five mins.
5. After that pieces were sterilized by soaking in 75% ethanol for 30 seconds.
6. The pieces were then rinsed several times in the sterile distilled water.

7. Then these pieces were placed in the sterilized tissue paper and dried about one hours.
8. Dried pieces were cut into smaller pieces and incubated on LCA medium. After three to five days, the microorganisms were picked and purified by subculturing. The pure strains were maintained in the test tubes.

Low Carbon Agar medium

(Ando, 2004)

(composition per liter)

Glucose	2.0 g
Sucrose	2.0 g
K ₂ HPO ₄	1.0 g
MgSO ₄ .7H ₂ O	0.5 g
KNO ₃	1.0 g
KCL	0.5 g
Agar	18.0 g
pH	6.5

Potato Glucose Agar Medium (PGA)

(composition per liter)

Potato	200.0 g
Glucose	20.0 g
Agar	18.0 g
pH	6.5

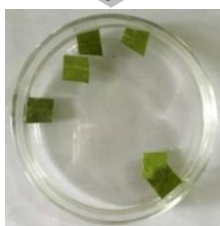
(After autoclaving chloramphenicol was added to the medium)



under running tap-water (30 mins)



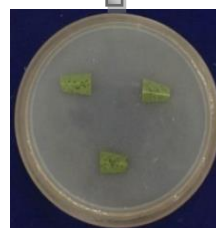
cut into about 1 cm pieces



soaking in 70% ethanol (about one mins)



subculturing on PGA medium



incubated on LCA medium



placed in sterilized tissue paper and dried (about one hours)

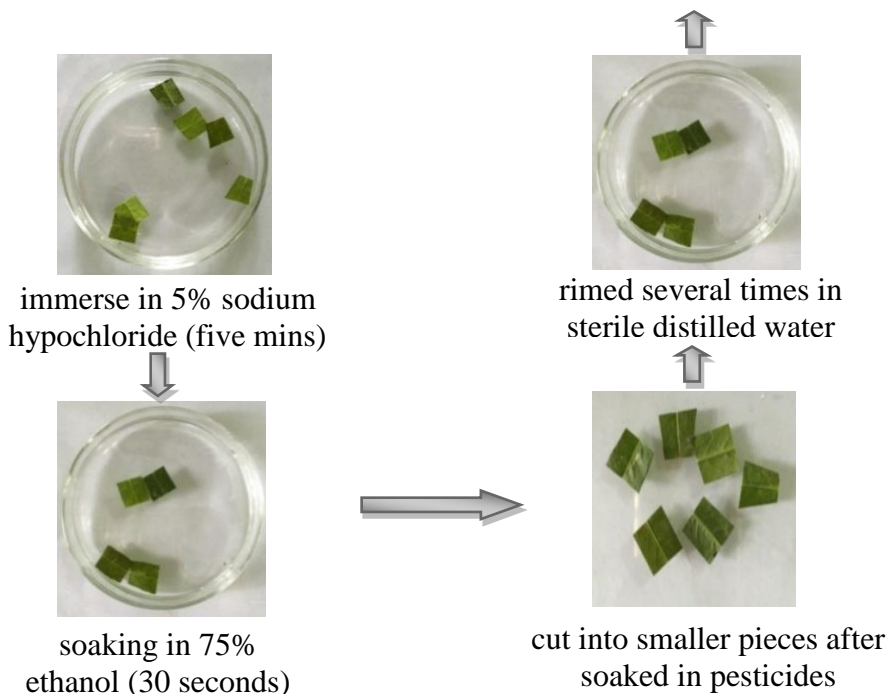


Figure 3. Surface sterilization method of endophytic microbes
(Ando, 2004)

Preliminary study for antimicrobial activities by paper disc diffusion assay of microorganisms (Hokkaido Uni., 1992)

The isolated fungi were grown at 25°C for 5 days on PGA medium for sporulation. The isolated fungi were inoculated on seed medium and incubated at room temperature for 3 days. Twenty mL of seed culture was transferred into the fermentation medium and incubated at 25°C for 5 days. Twenty µL of fermented broth was put on paper disc (8 mm) and placed on assay plate containing test organisms. Paper disc having eight millimeter diameter (Advantec, Toyo Roshi Kaisha Co. Ltd., Japan) that were utilized for antimicrobial assays.

The assay medium (Glucose 20 g, Polypepton 3 g, KNO₃ 10 g, Agar 18 g, Distilled water 1000 mL) was used for the antimicrobial activity test. One percent of test organism was added to assay medium and then poured into plates. After solidification, paper disc impregnated with fermented broth that were applied on the agar plates and the plates were incubated for 24-36 hours at 25°C to examine the inhibitory zones.

The test organisms were used in paper disc diffusion assay for the antimicrobial activities such as *Agrobacterium tumefaciens* NITE 09678, *Bacillus subtilis* IFO 90571, *Candida albicans* NITE09542, *E. coli* AHU5436, *Micrococcus luteus* NITE83297, *Pseudomonas fluorescens* IFO94307, and *Salmonella typhi* AHU 7943. These test organisms were obtained from NITE (National Institute of Technology and Evaluation, Kisarazu, Japan).

Seed medium		Fermentation medium	
Glucose	20.0 g	Glucose	20.0 g
Polypeptone	3.0 g	Yeast extract	8.0 g
KNO ₃	1.0 g	K ₂ HPO ₄	0.01 g
K ₂ HPO ₄	1.0 g	MgSO ₄	0.01 g
DW	1000 mL	CaCO ₃	1.0 g
pH	6.5	DW	1000 mL
* BR-BDC- Screening Media (2004)		pH	6.5

RESULTS AND DISCUSSION

The plants of *Averrhoa carambola* L. and *Averrhoa bilimbi* L. were grown in Hinthada University campus, Hinthada Township, Ayeyawady Region. The isolation of microorganisms was carried out by Surface Sterilization Method. Low Carbon Agar medium (LCA) and Potato Glucose Agar (PGA) were used for the isolation of soil fungi in this study. In this investigation, the endophytic three fungi can be isolated from the leaf blades of *Averrhoa carambola* L.. The leaf blades of *Averrhoa bilimbi* L. were isolated the endophytic five fungi. So, eight fungi were collected from the leaves of these plants. The endophytic eight fungi were used for the antimicrobial activities by using paper disc diffusion assay method. Among them, the fungus MMS-01 was selected and continued to study the based on the results of the antimicrobial activity especially against *Bacillus subtilis*. Therefore, this endophytic fungus (MMS-01) was found to be better than others.

Isolation of endophytic fungi from two selected plants

Table 1. Endophytic fungi isolated from two selected plant

Collected Plant	Plant parts	Collected places	Isolated fungi
<i>Averrhoa bilimbi</i> L. (Tayoke Zaung Yar)	Leaf blade	N 17° 63' 97.57" E 95° 43' 86.65" Hinthada District	MMS-01to 05
<i>Averrhoa carambola</i> L. (Zaung Yar)			MMS-06to 08



MMS-01 (Front View)



MMS-02 (Front View)



MMS-03 (Front View)



MMS-01 (Reverse View)



MMS-02 (Reverse View)



MMS-03 (Reverse View)

Figure 4. The morphological characters of MMS-01 to MMS-03 on PGA medium (5 days old culture)

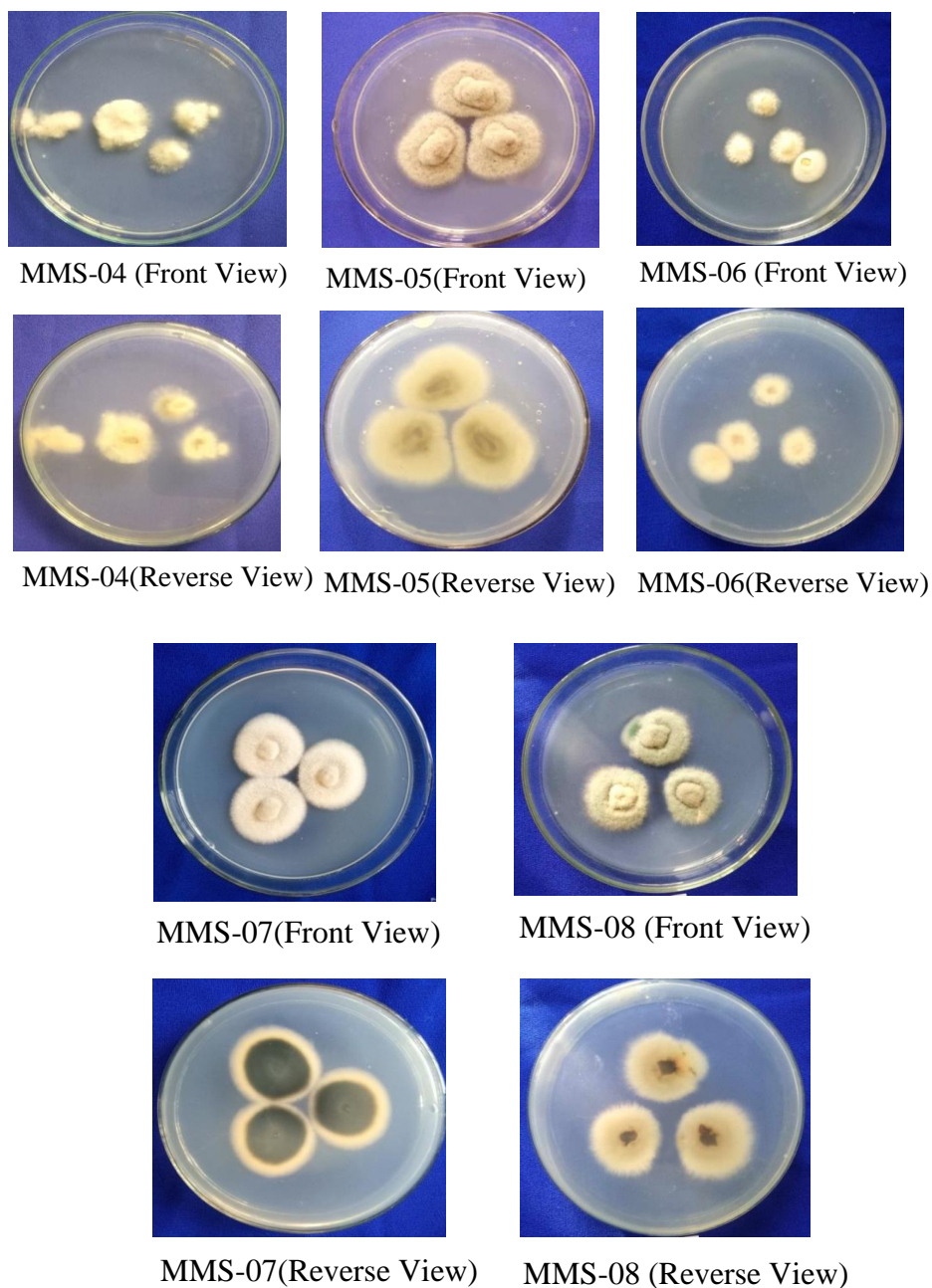


Figure 5. The morphological characters of MMS-04 to MMS-07 on PGA medium (5 days old culture)

Preliminary study for antimicrobial activities by paper disc diffusion assay of microorganisms

In the isolation, the leaf blade of *Averrhoa bilimbi* L. can isolate the endophytic five fungi. Leaf blade of *Averrhoa carambola* L. was used for the isolation of three fungi. These endophytic fungi were used for the antimicrobial activities by using paper disc diffusion assay. Among them, the endophytic fungus MMS-01 was selected for further investigations based on the results of the antimicrobial activities especially against *Bacillus subtilis*. So, this fungus was continually studied to be more precise. According to the principle of continuance studied it was clear that the good thing was high performance.

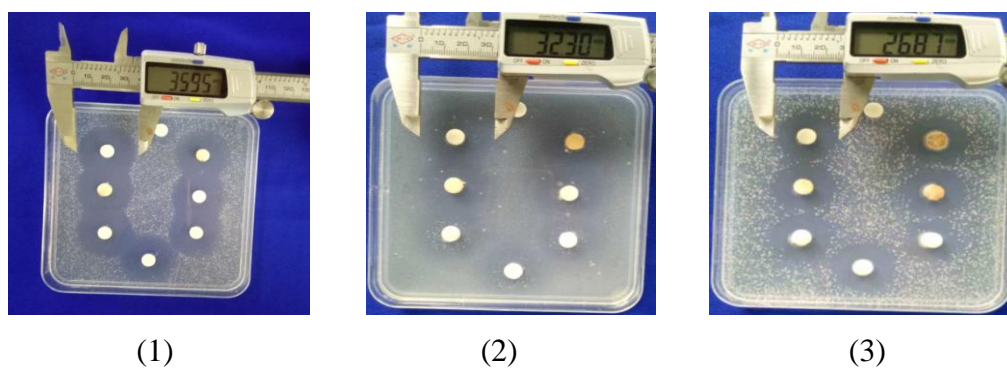


Figure 6. Antibacterial activities of endophytic fungi on
(1) *Bacillus subtilis* (2) *Candida albicans* (3) *E. coli*

Table 2. Antimicrobial activities of isolated fungi from two selected plant (1st time)

Test organisms	MMS-01	MMS-02	MMS-03	MMS-04	MMS-05	MMS-06	MMS-07	MMS-08
<i>Agrobacterium tumefaciens</i> (mm)	-	-	-	-	-	-	-	-
<i>Bacillus subtilis</i> (mm)	35.95	35.83	35.67	35.63	34.58	34.48	34.63	15.00
<i>Candida albicans</i> (mm)	32.30	32.00	25.00	20.00	15.00	22.00	33.00	-
<i>E. coli</i> (mm)	26.87	26.45	26.55	30.00	15.00	25.00	23.00	-
<i>Micrococcus luteus</i> (mm)	-	-	-	-	-	-	-	-
<i>Pseudomonas fluorescens</i> (mm)	-	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i> (mm)	-	-	-	-	-	-	-	-

Table 3. Antibacterial activities of isolated fungus especially against *Bacillus subtilis* (2nd time)

Selected Fungus	Fermentation Periods				
MMS-01	3 days	4 days	5 days	6 days	7 days
	30.77 mm	32.83 mm	31.03 mm	24.59 mm	22.25 mm

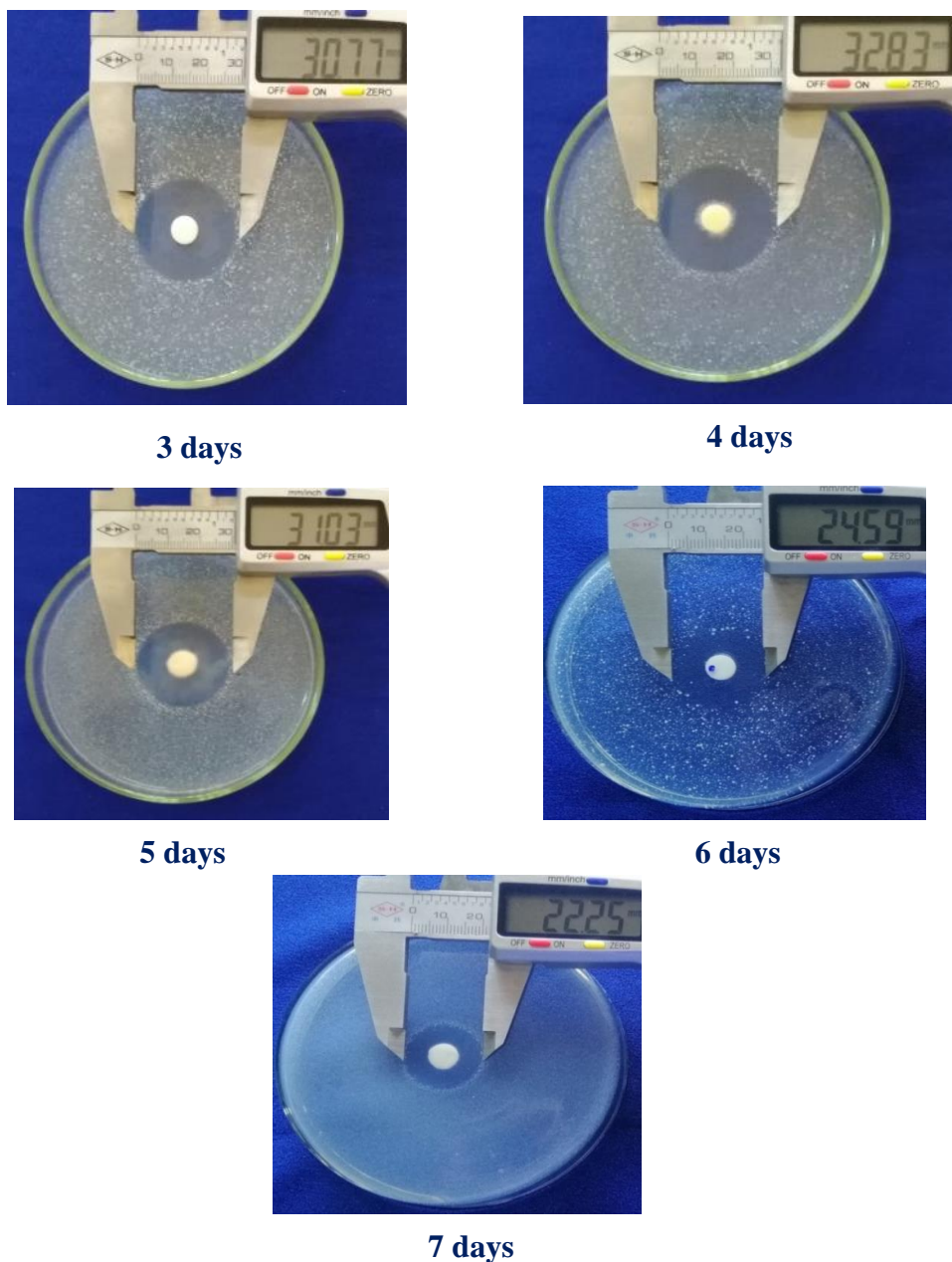


Figure 7. Antibacterial activity of endophytic fungus MMS-01 on *Bacillus subtilis*

CONCLUSION

In this research paper, eight endophytic fungi were isolated from *Averrhoa bilimbi* L.(Tayoke-Zaung-Yar) and *Averrhoa carambola* L. (Zaung Yar) by using surface sterilization method. These fungi were used for the antimicrobial activities by paper disc diffusion assay. In antimicrobial activities of isolated fungi, all isolated fungi did not inhibit on *Agrobacterium tumefaciens*, *Micrococcus luteus*, *Pseudomonas fluorescens* and *Staphylococcus aureus*. Isolated fungi (MMS-08) inhibited on *Bacillus subtilis* among the tested organisms. Isolated fungi (MMS-01 to MMS-07) were the best activity of inhibitory zone(35.95-34.48 mm) on *Bacillus subtilis*. Among the isolated eight fungi (MMS-01 to MMS-08), MMS-01 was the best activity of inhibitory zone (35.95 mm) on *Bacillus subtilis* IFO 90571. Therefore, the endophytic fungi from the leaves of *Averrhoa bilimbi* and *Averrhoa carambola* may be

remedy for some diseases such as intestinal worms and fever, diarrhea, skin rashes, skin eruption, etc., .

Acknowledgements

First of all, I am also truthful thanks to Dr Tin Htwe Mu, Professor and Head, Chemistry Department, Hinthada University, for her invaluable supports and instructions.

Furthermore, may I acknowledge my sincere gratitude to Dr Moe Moe Aye, Professor and Head, Botany Department, Patheingyi University, for her exemplary suggestions. Special thanks are due to my supervisor Dr Moe Pa Pa, Associate Professor, Department of Botany, Patheingyi University, for her heartfelt encouragement and moral support to do my research.

Finally and especially, I appreciate my parents, for their supporting and understanding.

References

- Crueger, W., and Crueger, A. 1989. Methods of fermentation, *in* Biotechnology, A Textbook of Industrial Microbiology, Internal Student Edition; 64-74
- Harayama, T. and K. Isono, 2002. "Sources of microorganisms". *J. Microbiology*, **48**:46-50.
- Heywood, V. H., 1978., "Flowering Plants of the World Oxford London Melbourne".
- Hooker, J. D., 1885., "Flora of British India". Vol. IV REEVE and Co. Ltd. London.
- Kingston W., 2008. "Irish contributions to the origins of antibiotics". *Irish Journal of Medical Science*. **177** (2): 87–92. doi:10.1007/s11845-008-0139-x. PMID 18347757.
- Lab. of Applied Microbiology, 1992. "Screening of soil microorganism", Faculty of Agriculture, Hokkaido University, Japan.
- NITE; 2004-2005. "Medium for fermentation to produce the metabolites," National Institute of Technology and Evaluation, Kisarazu, Japan.
- Wubah, A.D. 1999. An Excerpt on Fungal Nutrition.