# PHYTOCHEMICAL AND ANTIMICROBIAL INVESTIGATION ON LEAVES OF *AVERRHOA BILIMBI* L.

Thet Su Hlaing[1]

## Abstract

*Averrhoa bilimbi* L. is small tree, belongs to the family Oxalidaceae. It is locally known as Tayoke-zaung-yar. The plant was collected from Hinthada University Campus. The vegetative and reproductive parts of the fresh specimens were identified with the help of available literatures. In morphological study, the plant is small tree. The leaves are unipinnately compound and imparipinnate. The inflorescences are cauliflorous, arising in fascicles from main stem and thicker branches. The flowers are dark red-purple and hypogynous. Stamens 5+5, free and unequal. Ovary (5), axile placentation and stigma 5 fid. The fruits are berry fleshy with 5 blunt longitudinal ridges. The seeds are flattened disc like, smooth and white. The presence of alkaloid, glycoside, phenolic compound, flavonoid, steroid, terpenoid, tannin, saponin, α-amino acid, protein, reducing sugar, starch and carbohydrate were found in phytochemical investigation. According to physicochemical properties, the powdered leaves were most soluble in methanol and least soluble in chloroform. The nutrient contents of powdered leaves were examined by using David Pearson and Kjeldahl method. The presence of protein, moisture, ash, fat, fiber and carbohydrate were found in the examination. Antimicrobial activities of various crude extracts were carried out by using paper disc diffusion assay with six test organisms. Chloroform and ethyl acetate extracts indicated more effective against the test organisms. Methanol and ethanol extracts showed moderate antimicrobial activity. These results showed that *Averrhoa bilimbi* L. leaves are rich in many active constituents, nutrients and antimicrobial properties and has potential to be used as medicinal application.

**Keywords :** *Averrhoa bilimbi* L., phytochemical investigation, antimicrobial activity

## Introduction

Medicinal plants have been used as traditional treatments for numerous human diseases in many parts of the world. *Averrhoa bilimbi* L. is long lived perennial evergreen tree widely cultivated in the gardens and fields. Flowering more or less throughout the year (Dassanayake, 1999).

It has been used in the traditional medicine for the treatment of a variety of ailments. Infusions and decoctions of the leaves are used as an antibacterial, antiscorbutic, astringent, postpartum protective medicine, in the treatment of fever, inflammation of the rectum and diabetes.

A leaf infusion is a remedy for coughs and is taken after childbirth as a tonic. The paste of leaves is used in the treatment of itches, boils, skin eruptions, bites of poisonous creatures, rheumatism, cough, cold, mumps and syphilis (Kumari, 2017).

The objectives of this study are to identify and confirm the morphological characters, to examine the solubility test and phytochemical properties, to determine the nutritional value and to evaluate the antimicrobial activity of leaves of *Averrhoa bilimbi* L.

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

## Materials and Methods

### Collection and identification

The specimens of *Averrhoa bilimbi* L. were collected from Hinthada University Campus, especially during the flowering and fruiting period from February to December, 2018. After the collection, the plants were identified with the help of literatures Backer, 1963; Hooker, 1875 and Dassanayake, 1999.

### Preliminary phytochemical investigation on leaves of *Averrhoa bilimbi* L.

The preliminary phytochemical investigation on powdered leaves were carried out to determine the presence or absence of the chemical constituents such as a alkaloid, phenolic compound, flavonoid, steroid, terpenoid, starch, reducing sugar, glycoside, saponin, tannin, α-amino acids, protein and carbohydrate according to the method of British Pharmacopoeia, 1968; Marini Bettolo, 1981; Central Council for Research in Unani Medicine, 1987; Trease and Evans, 2002 and Harborne, 2005.

### Test for Alkaloid

The powdered sample (3 g) was boiled with (50 mL) of methanol and filtered. The filtrate was divided into three portions and tested with 1 % hydrochloric acid and Mayer's reagent, Wagner's reagent and Hager's reagent. The precipitate formed on addition the reagent indicates the presence of alkaloid (Central Council for Research in Unani Medicine, 1987).

### Test for Glycoside

The powdered sample (2 g) was boiled with (25 mL) of methanol for about 20 minutes, allowed to cool and filtered. The filtrate was treated with 1 mL water and sodium hydroxide solution. Yellow green colouration developed within three minutes (Marini Bettolo *et al*., 1981).

### Test for Phenolic Compound

The powdered sample (2 g) was boiled with (25 mL) of methanol and filtered. When the filtrate was treated with 2 mL water and 10 % ferric chloride solution, it gave black colouration, indicating the presence of phenolic compound (Marini Bettolo *et al*., 1981).

### Test for Flavonoid

The powdered sample (2 g) was extracted with (25 mL) of methanol and filtered. When the methanolic extract was treated with a few drops of dilute hydrochloric acid was added followed by a small piece of magnesium. The solution was boiled for a few minutes. The appearance of pink colour indicates the presence of flavonoid (Central Council for Research in Unani Medicine, 1987).

### Test for Steroid and Terpenoid

The powdered sample (2 g) was extracted (25 mL) of with methanol and filtered. When the chloroform and sulphuric acid were added, it furnished a change of colour reddish brown indicating the presence of steroid/ terpenoid (Central Council for Research in Unani Medicine, 1987).

**Test for Tannin**

The powdered sample (2 g) was boiled with (25 mL) of distilled water for about 20 minutes and filtered. The filtrate was treated with a few drops of 5 % ferric chloride and dilute sulphuric acid. Yellow brown precipitate was produced indicating the presence of a tannin (Central Council for Research in Unani Medicine, 1987).

**Test for Saponin**

The powdered sample (2 g) was put into a test tube and (2 mL) of distilled water was added. The mixture was vigorously shaken for a few minutes. Observation was made to see if frothing tool place (Marini Bettolo *et al*., 1981).

**Test for α-Amino acid**

The powder sample (2 g) was boiled with (25 mL) of distilled water for 20 minutes and filtered. And then, a few drops of each filtrate was spotted on a filter paper using a capillary tube, allowed it to dry and spray with ninhydrin reagent. The filter paper was dried at room temperature and then kept it in oven at 110$^{o}$C for a few minutes, after which the pink colour appears due to the presence of α-amino acid (Marini Bettolo *et al*., 1981).

**Test for Protein**

The powdered sample (2 g) was boiled with (25 mL) of distilled water for about 20 minutes and filtered. To this filtrate a mixture of million's reagent was added. White precipitate deposited and then turned red when heated (Trease and Evans, 2002).

**Test for Reducing Sugar**

The powdered sample (2 g) was boiled with (25 mL) of distilled water for about 20 minutes and filtered. To this filtrate a mixture of Benedict's solution was added and boiled for a few minutes on boiling water bath. Brick red precipitate deposited, when the solution was allowed to cool (Trease and Evans, 2002).

**Test for Starch**

The powdered sample (2 g) was boiled with (25 mL) of distilled water for about 20 minutes and filtered, 2 drops of iodine solution were added to filtrate. Observation was made to see if brown precipitate were formed (Central Council for Research in Unani Medicine, 1987).

**Test for Carbohydrate**

The powdered sample (2 g) was boiled with (25 mL) of distilled water for 20 minutes and filtered. The filtrate was placed into a test tube 5 % α-napthol solution was added and shaken for a few minute. The test tube was kept inclined at an angle of 45$^{o}$ and about 1 mL of concentrated sulphuric acid was slowly introduced along the inner side of the test tube. A red ring was formed between the two layers (Trease and Evans, 2002).

**Solubility test on leaves of *Averrhoa bilimbi* L.**

The solubility characters such as extractive values for the various solvents were determined according to British Pharmacopoeia, 1968.

**Determination of distilled water, acetone, chloroform, ethanol, ethyl acetate, methanol, petroleum ether soluble matter contents**

Distilled water, acetone, chloroform, ethanol, ethyl acetate, methanol, petroleum ether soluble matter contents were determined by the methods given in the British Pharmacopoeia (1968). Five grams of powdered sample was weighed and place in a conical flask, 50 mL of each solvent was added and the flask was stoppered with a cork. The sample was soaked for 3 days. The content was filtered and then the filtrate was taken in a petridish and evaporated to dryness on a water bath at 105$^{o}$C. The experiments was repeated at least three times for each solvents as the above procedure mentioned. The different in weight give the soluble matter contents in each solvent.

**Nutritional property analysis of powdered leaves of *Averrhoa bilimbi* L.**

The nutrient content in powdered leaves of *Averrhoa bilimbi* L. were analyzed by using David Pearson and Kjeldahl method at the Small Scale Industries Department, Ministry of Agriculture, Livestock and Irrigation, North Okkalapa Township, Yangon Division, Republic of the Union of Myanmar.

**Experimental analysis**

The chemical composition of powdered samples was determined: dry matter, by drying at 105$^{o}$C to constant weight; crude fat, by Soxhlet extraction with diethyl ether; crude ash, by incineration n a muffle furnace at 580$^{o}$C for 8 hours; crude protein (N × 6.25) by the Kjeldahl method; carbohydrates were calculated as total carbohydrates (%) = 100 % (moisture + crude protein + crude fat + ash + crude fiber). The fibre components were determined by using the detergent method.

**Antimicrobial activity of various solvent extracts from leaves of *Averrhoa bilimbi* L.**

Various crude extracts of powdered leaves such as acetone, chloroform, ethyl acetate, ethanol, methanol, petroleum ether and distilled· water extracts were used for antimicrobial study. Screening of antimicrobial activity was done by paper disc diffusion assay according to Madigan and Martinko, 2005 at Microbiology Lab, University of Yangon. The six test organisms (four bacterial strains and two fungal strains) were utilized for antimicrobial activity.

**Table 1 Test organisms utilized to the antimicrobial activities**

| No. | Test Organisms | Source | Diseases |
|---|---|---|---|
| 1. | *Aspergillus flavous* | - | Bronchitis |
| 2. | *Bacillus subtilis* | JAP-0225215 | Pathogenic group, anthrax in animals |
| 3. | *Candida albicans* | IFO-1060 | Skin infection, veginal candidiasis alimentary tract infection |
| 4. | *Escherichia coli* | ATCC-25922 | Cholera, diarrhea and vomiting urinary tract infection |
| 5. | *Pseudomonas fluorescens* | - | Bacteria for leaf blight |
| 6. | *Xanthomonas oryzae* | - | Bacteria for leaf blight |

Madigan and Martinko, 2005

### Preparation of antimicrobial activity test

Paper disc diffusion assay was used according to the method described by Madigan and Martinko, 2005. The assay medium (agar 2.0 g, sucrose 1.0 g, NaCl 0.1 g, yeast extract 0.3 g, distilled water 100 ml, pH 7.0) was utilized for these test organisms. Test organisms (0.3 mL) was added to 100 mL assay medium, then poured into plates. After solidification, about 0.2 mL of crude extracts was impregnated onto the paper disc with the size of 6mm diameter on the test agar plates and these plates were incubated for 24-36 hours at 30$^{o}$C. After 24-36 hours, clear zones (inhibitory zones) surrounding the test discs were measured. These zones indicate the presence of the bioactive compounds which inhibit the growth of test organisms.

## Results

### Morphological characters

Small tree, about 10 meters high. Leaves alternate, unipinnately compound, imparipinnate, terminal leaflet larger than other, 10 to 17 pairs, oblong, 2.0 to 10.0 cm long, and 1.2-1.25 cm wide, the bases subcordate, the margins entire, the tips acuminate, petiolate and exstipulate. Inflorescences are cauliflorous, arising in fascicles from main stem and thicker branches. Flowers are dark red-purple, bracteate, bracteolate, pedicellate, complete, bisexual, regular, actinomorphic, pentamerous, cyclic, hypogynous. Calyx (5), synsepalous, petaloid (reddish green), 5-7 mm long. Petal (5), synpetalous, petaloid (dark red purple), 12-18 mm long and 4 mm wide. Stamens 5+5, the filament free and unequal, the anther two whorl, 10-12 mm long, the shorter 4-5 mm long, dithecous, extrorse, dorsifixed, longitudinal dehiscence. Ovary (5), syncarpous, pentalocular, axile placentation, one ovule in each locule, the stigma 5 fid. Fruit berry fleshy with 5 blunt longitudinal ridges, greenish yellow when ripe. Seeds, flattened disc like, smooth and white.

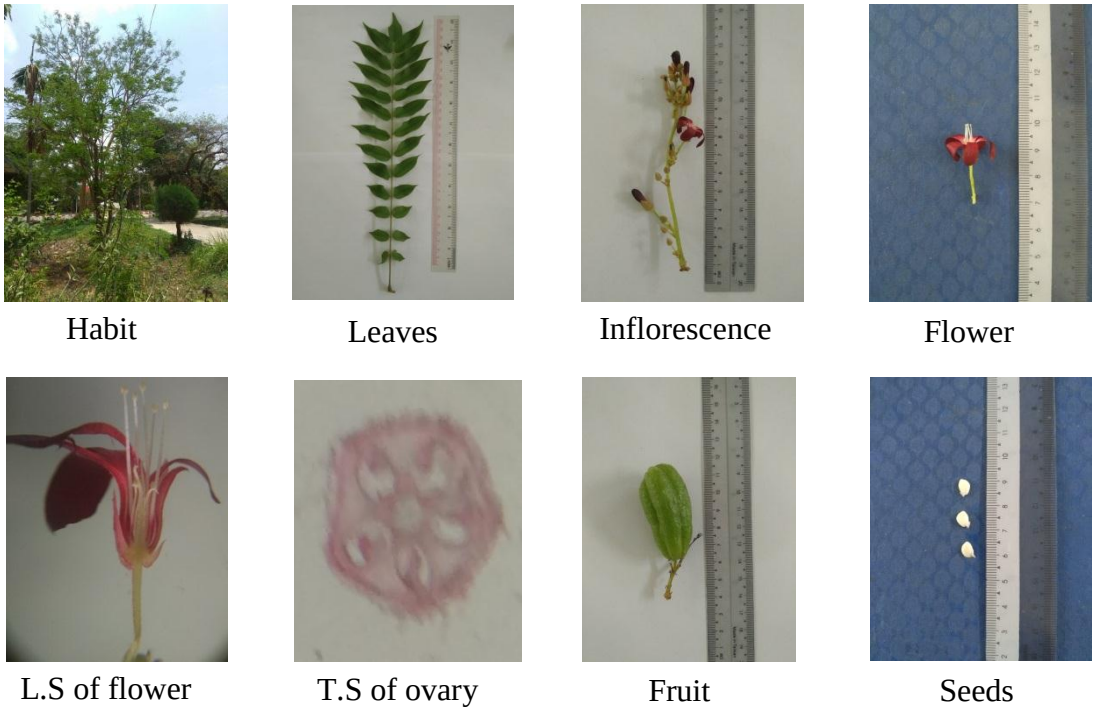



**Figure 1** Morphological characters of *Averrhoa bilimbi* L.

**Preliminary phytochemical investigation on leaves of *Averrhoa bilimbi* L.**

The preliminary phytochemical test of powdered leaves of *Averrhoa bilimbi* L. indicated that the presence of alkaloid, glycoside, phenolic compound, flavonoid, steroid, terpenoid, tannin, saponin, α-amino acid, protein, reducing sugar, starch and carbohydrate. The results were shown in Table (2) and Figure (2).

**Table 2 Preliminary phytochemical investigation on leaves of *Averrhoa bilimbi* L.**

| No. | Tests | Extracts | Test reagents | Observation | Results |
|---|---|---|---|---|---|
| 1. | Alkaloid | Methanol | 1 % HCl + Mayer's reagent | White ppt. | + |
| | | Methanol | 1 % HCl + Wagner's reagent | Brown ppt. | + |
| | | Methanol | 1 % HCl + Hager's reagent | Yellow ppt. | + |
| 2. | Glycoside | Methanol | 1 mL $H_2O$ + NaOH | Yellow green color | + |
| 3. | Phenolic compound | Methanol | 2 mL $H_2O$ + 10 % $FeCl_3$ | Black color | + |
| 4. | Flavonoid | Methanol | Mg coil + HCl (dil) | Pink color | + |
| 5. | Steroid/ Terpenoid | Methanol | $CHCl_3$ + $H_2SO_4$ (conc.) | Reddish brown color | + |
| 6. | Tannin | Water | 5 % $FeCl_3$ + $H_2SO_4$ (dil) | Yellow brown ppt. | + |
| 7. | Saponin | Water | Shaken with 2 mL $H_2O$ | Frothing | + |
| 8. | α-amino acid | Water | Ninhydrin reagent | Pint spot. | + |
| 9. | Protein | Water | Millon's reagent (heated) | White ppt. turned red when heated | + |
| 10. | Reducing sugar | Water | Benedict's solution | Brick red ppt. | + |
| 11. | Starch | Water | Iodine | Brown ppt. | + |
| 12. | Carbohydrate | Water | 5 % α napthol sol: + $H_2SO_4$ (conc.) | Purple ring | + |

(+) = Present

**Figure 2** Preliminary phytochemical investigation on leaves of *Averrhoa bilimbi* L.

**Solubility test on leaves of *Averrhoa bilimbi* L.**

The solubility of powdered leaves were investigated to determine amount of total solids soluble in various solvents. The solubility of powdered leaves were found to be mostly soluble in methanol and least soluble in chloroform. The results were shown in Table (3) and Figure (3 and 4).

**Table 3 Solubility test on leaves of *Averrhoa bilimbi* L.**

| No. | Solubility properties | Content in % |
|---|---|---|
| 1. | Distilled water | 5.07 |
| 2. | Ethanol | 5.20 |
| 3. | Methanol | 6.80 |
| 4. | Ethyl acetate | 2.73 |
| 5. | Acetone | 1.73 |
| 6. | Petroleum ether | 2.00 |
| 7. | Chloroform | 1.26 |


1. Distilled water
2. Ethanol
3. Methanol
4. Ethyl acetate
5. Acetone
6. Petroleum ether
7. Chloroform

**Figure 3** The solubility test of various solvents of *Averrhoa bilimbi* L.

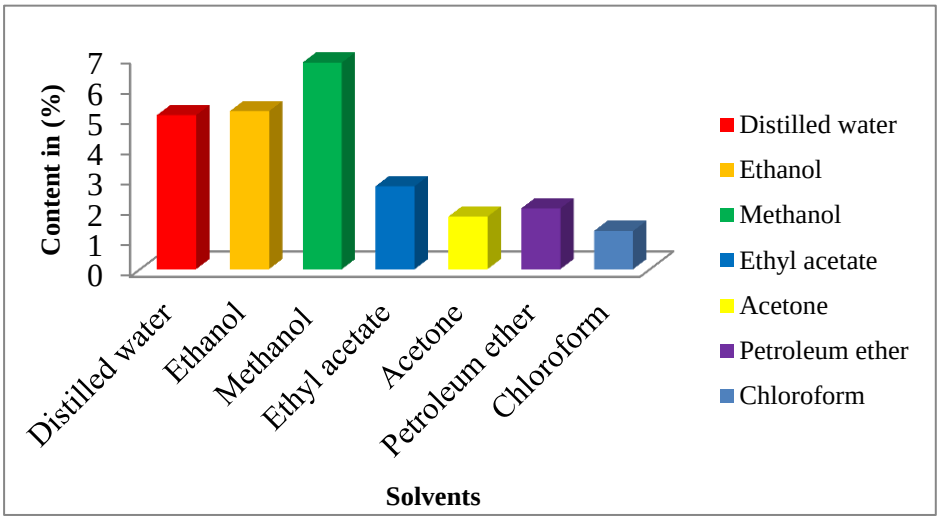



**Figure 4** The solubility test of various solvents of *Averrhoa bilimbi* L.

**Nutrient analysis of powdered leaves of *Averrhoa bilimbi* L.**

The nutrient content in powdered leaves were determined by using David Pearson and Kjeldahl method. In this analysis yielded protein, moisture, ash, fat, fiber and carbohydrate. The results were shown in Table (4) and Figure (5) and (6).

**Table 4 Nutrient content of powdered leaves of *Averrhoa bilimbi* L.**

| No. | Components | Values (%) |
|---|---|---|
| 1. | Protein | 12.28 |
| 2. | Moisture | 9.53 |
| 3. | Ash | 5.93 |
| 4. | Fat | 3.34 |
| 5. | Fiber | 21.95 |
| 6. | Carbohydrate | 46.97 |

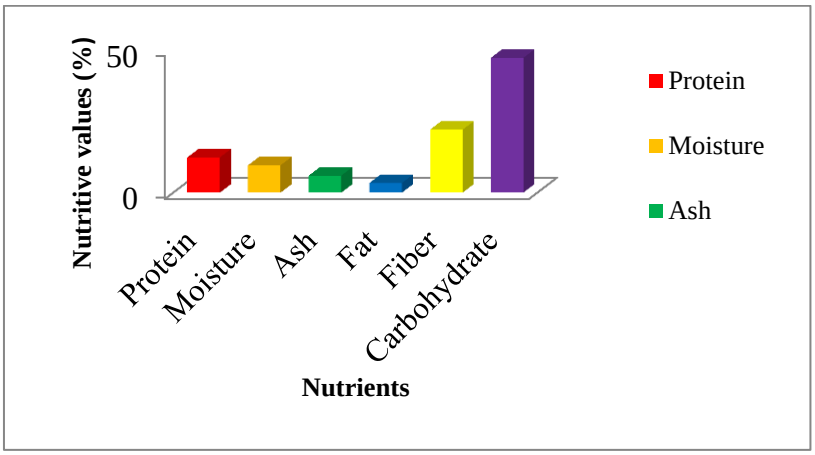
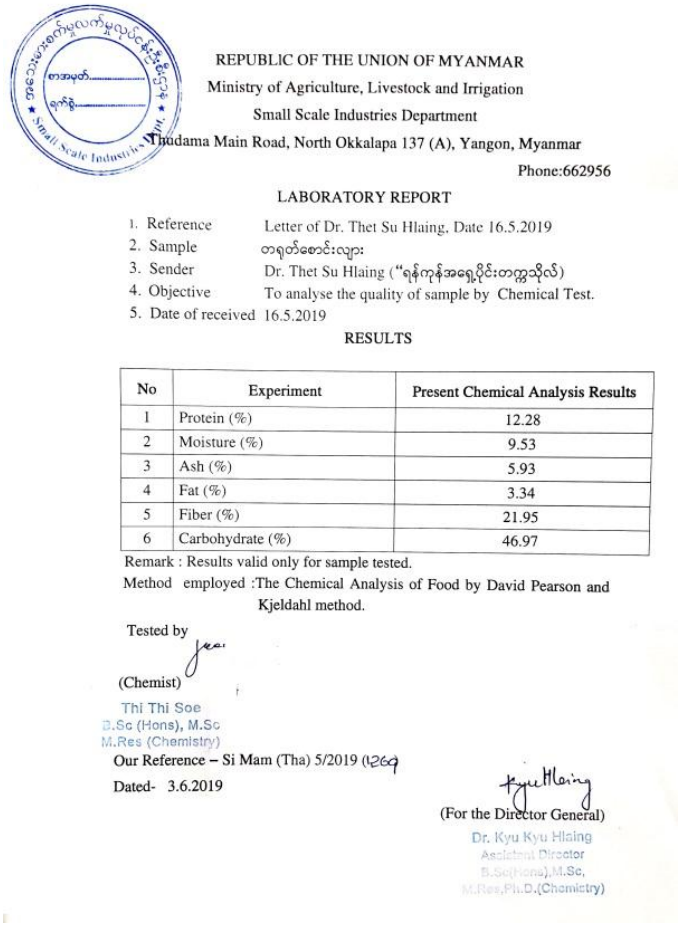



**Figure 5** Nutrient composition of powdered leaves of *Averrhoa bilimbi* L.



REPUBLIC OF THE UNION OF MYANMAR
Ministry of Agriculture, Livestock and Irrigation
Small Scale Industries Department
Thudama Main Road, North Okkalapa 137 (A), Yangon, Myanmar
Phone:662956

LABORATORY REPORT

1. Reference — Letter of Dr. Thet Su Hlaing, Date 16.5.2019
2. Sample — တရုတ်စောင်းလှာ
3. Sender — Dr. Thet Su Hlaing ("ရန်ကုန်အရှေ့ပိုင်းတက္ကသိုလ်)
4. Objective — To analyse the quality of sample by Chemical Test.
5. Date of received — 16.5.2019

RESULTS

| No | Experiment | Present Chemical Analysis Results |
|---|---|---|
| 1 | Protein (%) | 12.28 |
| 2 | Moisture (%) | 9.53 |
| 3 | Ash (%) | 5.93 |
| 4 | Fat (%) | 3.34 |
| 5 | Fiber (%) | 21.95 |
| 6 | Carbohydrate (%) | 46.97 |

Remark : Results valid only for sample tested.
Method employed :The Chemical Analysis of Food by David Pearson and Kjeldahl method.

Tested by
(Chemist)
Thi Thi Soe
B.Sc (Hons), M.Sc
M.Res (Chemistry)

Our Reference – Si Mam (Tha) 5/2019 (1264)
Dated- 3.6.2019

(For the Director General)
Dr. Kyu Kyu Hlaing
Assistant Director
B.Sc(Hons),M.Sc,
M.Res,Ph.D.(Chemistry)

**Figure 6** Nutrient composition of powdered leaves of *Averrhoa bilimbi* L.

## Antimicrobial activity of leaves of *Averrhoa bilimbi* L.

In this investigation, chloroform, ethyl acetate, ethanol and methanol extracts observed against on *Aspergillus flavous*, *Bacillus subtilis*, *Escherichia coli* and *Xanthomonas oryzae*, Acetone, distilled water and petroleum ether extracts did not show antimicrobial activity on all tested organisms. The results were shown in Table (5) and Figure (7).

**Table 5** Antimicrobial activity of various solvent extracts from leaves of *Averrhoa bilimbi* L.

| Solvents / Test organisms | Acetone | $CHCl_3$ | EtOAc | EtOH | MeOH | PE | DW |
|---|---|---|---|---|---|---|---|
| *Aspergillus flavous* | - | - | 24 mm | - | - | - | - |
| *Bacillus subtilis* | - | 36 mm | 26 mm | - | - | - | - |
| *Candida albicans* | - | - | - | - | - | - | - |
| *Escherichia coli* | - | 24 mm | 16 mm | 14 mm | 16 mm | - | - |
| *Pseudomonas fluorescences* | - | - | - | - | - | - | - |
| *Xanthomonas oryzae* | - | 30 mm | 30 mm | 16 mm | 22 mm | - | - |

Paper disc size = 6 mm

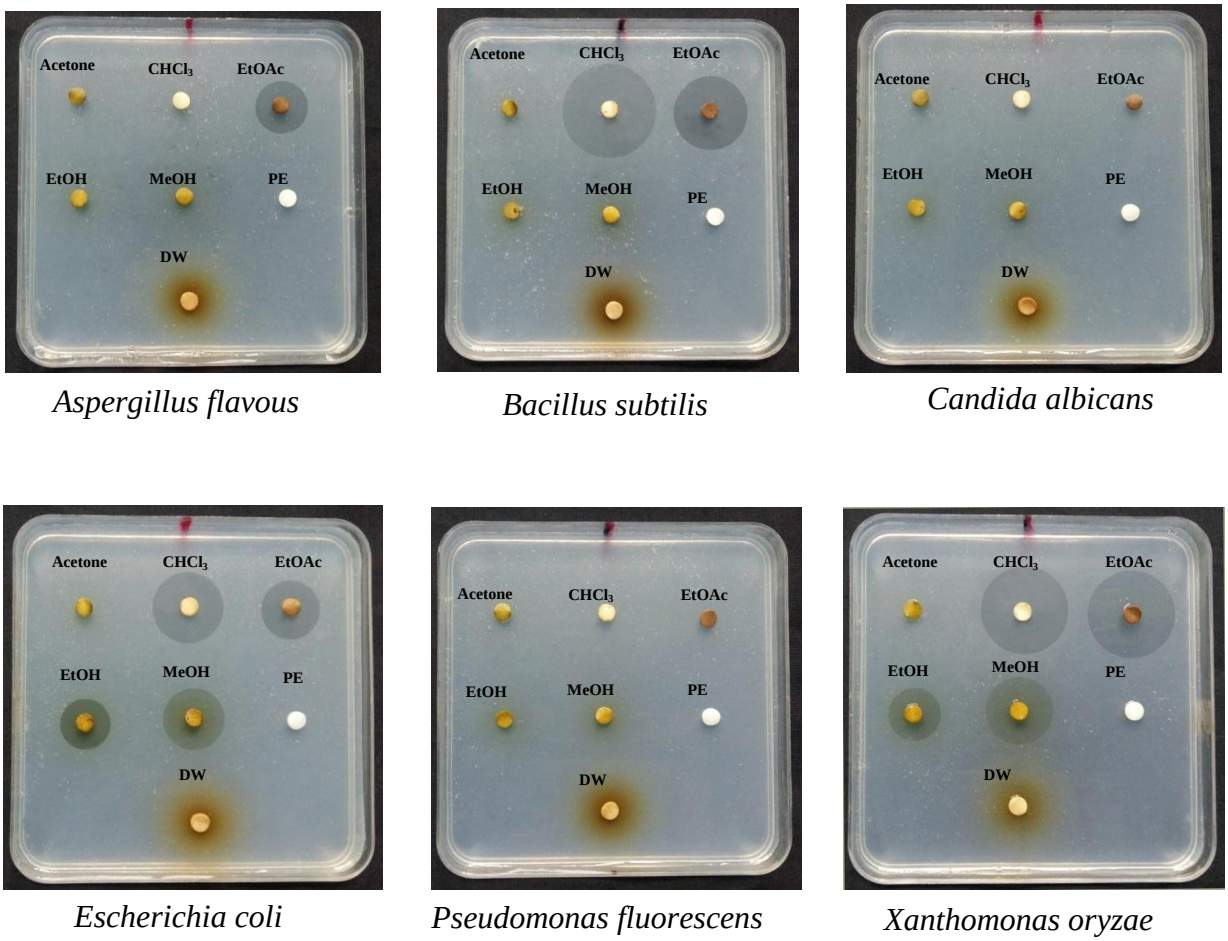



**Figure 7** Antimicrobial activity of various solvent extracts from leaves of *Averrhoa bilimbi* L.

## Discussion and Conclusion

In this research, morphological characters, preliminary phytochemical tests, physicochemical properties, nutritional values and antimicrobial activities on the powdered leaves had been undertaken.

In morphological study, *Averrhoa bilimbi* L. is small tree. The leaves are unipinnately compound, alternate, imparipinnate, petiolate and exstipulate. The inflorescences are cauliflorous, arising in fascicles from main stem and thicker branches. The flowers are dark red-purple, pentamerous and hypogynous. Stamens 5+5, free and unequal. Ovary (5), axile placentation and stigma 5 fid. The fruits are berry fleshy with 5 blunt longitudinal ridges. The seeds are flattened disc like, smooth and white. These characters are in agreement with those mentioned by Backer, 1963; Hooker, 1875 and Dassanayake, 1999.

Alkaloid, glycoside, phenolic compound, flavonoid, steroid, terpenoid, tannin, saponin, α-amino acids, protein, reducing sugar, starch and carbohydrate were found in preliminary phytochemical tests.

In solubility properties, the powdered leaves were most soluble in methanol and least soluble in chloroform. This result showed that methanol should be the solvent of choice.

In the nutritional property analysis, the protein content (12.28 %), moisture (9.53 %), ash (5.93 %), fat (3.34 %), fiber (21.95 %) and carbohydrate (46.97 %) were obtained from the

powdered leaves. This data indicated that the leaves are rich source of carbohydrate, fiber, protein and are low in fat. According to the literatures, carbohydrate may serve as supplements for energy, as they have potentials to improve the health status of its users. Its fiber content can help to enhance gastrointestinal function, prevents constipation and may reduce cholesterol content. Therefore *Averrhoa bilimbi* L. has valuable nutritional components and could be a complementary nutrient.

The antimicrobial activities of various crude extracts such as acetone, chloroform, ethyl acetate, ethanol, methanol, petroleum ether and distilled water were determined by using paper disc diffusion method with six test microorganisms. These test organisms were *Aspergillus flavous*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. In this result, chloroform, ethyl acetate, ethanol and methanol extracts showed antimicrobial activity against on *A. flavous*, *B. subtilis*, *E.coli* and *X. oryzae*. Chloroform extract exhibited a maximum inhibition zone of (36 mm) and (30 mm) against *E. coli* and *X. oryzae*. Ethyl acetate extract showed a maximum zone of inhibition against *X. oryzae* (30 mm) followed by *B. subtilis* (26 mm). Ethanol extract against *E. coli* (14 mm) and *X. oryzae* (16 mm). Methanol extract showed antimicrobial activity against on *X. oryzae* (22 mm) and *E. coli* (16 mm).

The results found in this study concerning the activity of *Averrhoa bilimbi* L. leaves extracts are in agreement with other previous works which found significant antimicrobial activity leaves methanol and ethanol extracts against *Escherichia coli* and *Xanthomonas oryzae*. The antimicrobial activities may be due to strong occurrence of active compounds i.e. saponins, tannins, alkaloids, steroids, phenols and flavonoids. Results of this finding confirmed the use of *Averrhoa bilimbi* L. as traditional medicine. This indicated that the plant have potentially antimicrobial properties and could be used in the development of novel antimicrobial agents.

## 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. Thida Htoo, Professor, Department of Botany, East Yangon University, for her beneficial advices and constant encouragement.

## References

Backer, C. A., (1963). **Flora of Java** (Vol.I), Noordhoof. Groningen Company, Netherlands.

British Pharmacopoeia, (1968). **Physicochemical standards of Unani formulations**. The pharmaceutical press, London.

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

Dassanayake, M. D., (1999). **A Revised Handbook to the Flora of Ceylon**. (Vol, XIII), Brookfield.

David-Pearson, (1976). **The chemical analysis of foods.** Churchill Livingstone, New York.

Harborne, J. B., (2005). **Phytochemical Methods, A guide to Modern Technique of Plant Analysis**. Champman and Hall, London.

Hooker, J. D., (1875). **The Flora of British India**. (Vol.I) L. Reeve and Co. Ltd. London.

Kirtikar, K. R. and B. D. Basu, (1935). **Indian medicinal plants**, (Vol. IV), The Parabasi Press.

Kjeldahl, J., (1883). **New Method for the Determination of Nitrogen.** Chem-News.

Kumari, S.C., (2017). **International Journal of All Research Education and Scientific Methods**. Vol. 5, Telangana, India.

Madigan, M. and J. Martinko, (2005). **Brock Biology of Microorganisms**, 11th ed, Prentice Hall.

Marini-Bettolo, G. B., (1981). **Plant Screening by Chemical and Chromatographic Procedure Under Field Condition,** Journal of Chromatography, (Vol.I).

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