Evaluation of Antimicrobial Activities of Crude Extract and Isolated Organic Compound from the Leaves of *Plantago major* Linn.

Khin Htay Win[1], Aye Mon Thidar Nyo[2], Thidar Khaing[3], Yinn Kay Khaing[4]

Abstract

One of Myanmar indigenous medicinal plants*Plantago major* Linn.belonging to the family plantaginaceae, was collected from Sitarpu, Myintkyina Township, Kachin State. Firstly, phytochemical screening was performed with standard method. The elemental composition of leaf powders wasdetermined by using Energy Dispersive X-ray Fluorescence.Then, the antimicrobial activities of various extracts from the leaves of A-kyaw-paung-tahtaung were tested against on six selected microorganisms by Agar-well diffusion method. In addition, organic compounds were isolated from the leaves of *Plantago major* Linn. by using Thin Layer and Column Chromatographic method. The functional groups of the isolated compound were identified by FT-IR spectroscopy. Finally, the antimicrobial activity of isolated organic compound was determined by Agar well diffusion method. According to the results,theantimicrobial activity of isolated organic compound responds high activity on *Staphylococcus aureus*.

Keywords:*Plantago major* Linn., EDXRF, FT-IR, agar-well

Introduction

In natural product chemistry, a variety of organic compounds isolated from various medicinal plants are very important and useful for medicine. The use of plants based product for disease prevention and treatment has become increasingly popular in ASEAN countries.

Medicinal plants are important for pharmacological research and drug development,not only when plant constituents are used directly as therpeutic agents but also when they are used as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. More herbal extracts have been produced from indigenous plants. Some of these medicines can now be used as substitutes for western drugs.

Myanmar is rich in varities of medicinal plants due to the presence of different climate zones in the country.Myanmar people use herbal medicine tremendously for curing various diseases.The study of traditional medicinal plants and their therapy play a very inportant role in health care system of Myanmar. Plants are not only the major source of energy with foods in most socirties, but also an indispensable source of vitamin and other substance promoting healthy growth (Prajapati, *et al.*, 2003).

Herbal medications were traditionally used for minor problems such as fatigue, aches and pains,menstrul disturbance, disturbance of bowel habit and skin diseases. Nowadays, more and more people are curing their illness with herbal treatment and natural presrenatives that have been effectively used for thousandd of years(Hundley, *et al*., 1986).

[1]Lecturer, Dr, Department of Chemistry, University of Mandalay
[2] Associate Professor, Dr, Department of Chemistry, University of Mandalay
[3]Lecturer, Dr, Department of Chemistry, University of Mandalay
[4]Lecturer, Dr, Department of Chemistry, University of Mandalay

In this research, the leaves of*Plantago major* Linn. was selected to investigate the antimicrobial activity and to isolate the bioactive organic compound.Moreover, the antimicrobial activity of isolated compound was investigated by employing Agar well diffusion method.

Botanical Description

Figure 1.The Plant of *Plantago major* L.

Family name : Plantaginaceae
Botanical name : *Plantago major* L.
Myanmar Name : A-kyaw-paung-tahtaung
English name : Greater Plantain
Parts used : Leaves
Site of Collection : Sitarpu Quarter, Myintkyina Township, Kachin State
Medicinal uses:Inflammatory activity,hyperglycemic activity, dysentery, urinary disease, asthma, analgesic, anti-oxidant

Materials and Methods

Sample Collection

The sample of *Plantago major* L. was collected from Sitarpu Quarter, Myintkyina Township, Kachin State. The leaves of *Plantago major* L. were cut into small pieces and dried in the air, and then it was stored in a well-stoppered bottle and used throughout the experiment.

Phytochemical Investigation of the Leaves of *Plantago major* L.

The phytochemical tests were carried out to detect the presence or absence of organic constituents in the selected plant. (Harbone, 1993)

Determination of Elemental Contents

The mineral contents of the ground and sieved sample of the leaves of *Plantago major* L. was measured by applying Energy Dispersive X-ray Fluorescence (EDXRF) Spectrophotometer at Department of Chemistry, Monywa University.

Determination of Antimicrobial Activities

The antimicrobial activities of the leaves of *Plantago major*L. by using n-hexane, ethyl acetate and ethanol were investigated against*Bacillus subtilis, Staphylococcus aureus, Pseudomonusaeruginosa, Bacillus pumilus, Escherichia.coil* and *Candida albicans species of microorganism* by using Ager-well diffusion method

at Central Research and Development Centre (CRDC), Insein, Yangon.(Lovo V-2010)

Preparation of the Leaves Extract

The dried sample (150 g) was percolated with ethanol 500 mL for 2 months. And then the extracted solution was filtered with filter paper and the residue was washed with small amount of ethanol and filtered again. The filtrate was concentrated at room temperature and leaves extract was obtained. Ethanol extract (3) g was accurately weighed and placed in 250mL beaker and re-extracted with 50 mL of ethyl acetate. The resulting ethyl acetate solution was evaporated at room temperature. Finally, ethyl acetate extract (2) g was obtained.

Isolation of Pure Organic Compound

The ethyl acetate extract (2) g was chromatographed on silica gel column as eluting with various rations of n-hexane and ethyl acetate which gave rise to the 180 fractions. Each fraction was checked by TLC and then fractions of the same R_f values were combined, 7 combined fractions were obtained. The combined fractions VI has found to be main portions. Finally, the functional groups in isolated compound were identified by FT- IR spectral data (Silverstein, 2005).

Determination of Antimicrobial Activities of the Isolated Organic Compound

The antimicrobial activities of the isolated organic compound by using n-hexane was investigated against*Bacillus subtilis, Staphylococcus aureus, Pseudomonusaeruginosa, Bacillus pumilus,Escherichia.coil*and *Candida albicans species of microorganism*by using Ager-well diffusion method.(Lovo V-2010)

Results and Discussion

Preliminary Phytochemical Examination of the Leaves of *Plantago major* L.

Phytochemical test were carried out to detect the presence of organic constituents in the leaves of *Plantago major* L. According to the results, the leaves of *Plantago major* L. extract consists of alkaloid, flavonoid, glycoside,saponin, reducing sugar, polyphenol, phenolic, and steroid respectively.

Elemental Contents of the Leaves of *Plantago major* L.

The elemental compositions of the leaves of *Plantago major* L. were determined and the results were shown in table(1). It was found that the amount of potassium, calcium and chlorine are higher than other minerals. Potassium is a healthy mineral as it ensures tomaintain regularheart beats, it also maintains normal body growth and builds protein.Calcium is found naturally in many foods; it is also added to certain products, and supplements are available. Calcium is very essential in muscle contraction, building strong bones and teeth, blood clotting, nerve impulse, transmission, regulating heartbeat. Chlorine is needed for the production of hydrochloric acid in the stomach and in cellular pump functions.

Table 1.Elemental compositions of the Leaves of *Plantago major* L.

Element	Symbol	Result Abundance
Potassium	K	2.003
Calcium	Ca	1.945
Chlorine	Cl	1.535
Silicon	Si	1.407
Aluminum	Al	0.942
Magnesium	Mg	0.635
Sulfur	S	0.296
Iron	Fe	0.240
Phosphourus	P	0.225
Titanium	Ti	0.026
Manganese	Mn	0.009
Strontium	Sr	0.006
Zinc	Zn	0.005
Copper	Cu	0.004
Rubidium	Rb	0.002

Determination of Antimicrobial Activities of the Leaves of *Plantago major* L.

The antimicrobial activities of the leaves of *Plantago major* L. were tested in various solvent systems by using Ager- well diffusion method on six selected organisms.

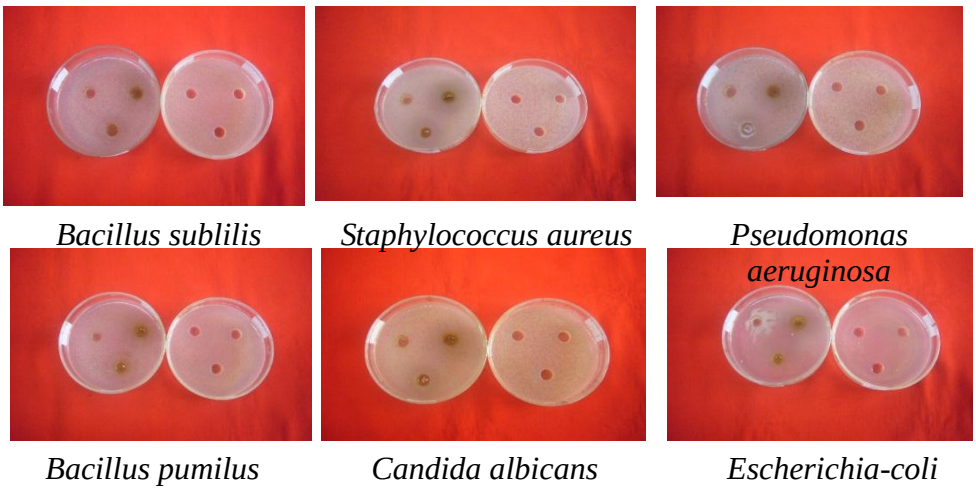

Figure 2.Antimicrobial activities of the leaves of *Plantago major* L.

Table 2.Antimicrobial Activities of the Leaves of *Plantago major* L.

Samples	**Extracted Solvents**	**Inhibition zone diameter (mm)**					
		I	**II**	**III**	**IV**	**V**	**VI**
A-kyaw-paung-htataung	n-hexane	-	-	-	-	-	-
	EtOAc	15 (++)	15 (++)	15 (++)	17 (++)	14 (+)	13 (+)
	EtOH	13 (+)	13 (+)	13 (+)	12 (+)	-	12 (+)

Agar-well -10mm

10mm-14mm (+)

15mm-19mm (++)

20mm- above (+++)

According to this table (2), the ethyl acetate extracts of the leaves of A-kyaw-paung-tahtaung responds medium activities on *Bacillus subtilis, Staphylococcus aureus, Pseudomonusaeruginosa, Bacillus pumilus*and low activity on *Escherichiacoil*and *Candida albicans*organisms. Ethanol extract gave low activities on all tested organisms, except *Candida albicans*.

Organisms
I = *Bacillus subtilis*
II = *Staphylococcus aureus*
III = *Pseudomonas aeruginosa*
IV = *Bacillus pumilus*
V = *Candida albicans*
VI = *Escherichia coli*

Assignment of Functional Groups of Isolated Organic Compound

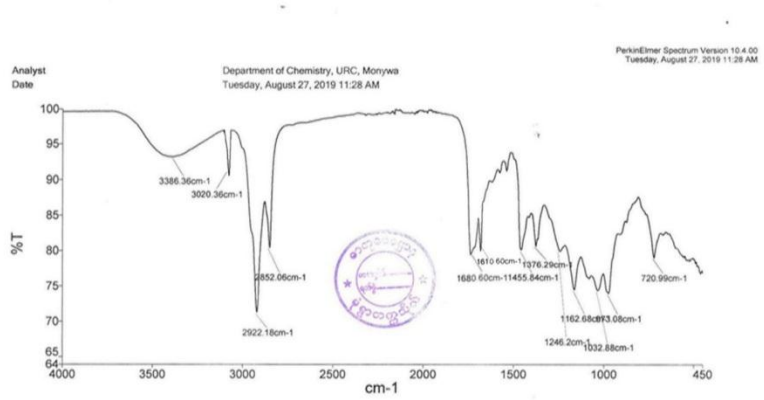

Figure 3. Fourier Transform Infrared Spectrum of Isolated Organic Compound

The FT-IR spectrum of an isolated organic compound was measured at Department ofChemistry, Monywa University as shown in (Figure. 3).

In this spectrum, the band which appears at 3386.36 cm^{-1} indicates O-H stretching vibration of hydroxy groups. The peak at 3020.46 cm^{-1} imply C-H stretching vibration of sp^2 hydrocarbon. The peak at 2922.18, 2852.06 cm^{-1}signifies C-H stretching vibration of sp^3 hydrocarbon. The peak at 1680 cm^{-1} shows C=O stretching vibration of carbonyl group. The peak at 1610 cm^{-1} indicates C=C stretching vibration of aromatic benzene ring. The peak at 1376.29 cm^{-1} give C-H bending vibration of methyl group. The peak at 1246.2 cm^{-1} C-O stretching vibration of carbonyl group. The peak at 1032.88 cm^{-1} shows C-O-C stretching vibration of ether group. The peak at 720.99 cm^{-1} give C-H bending vibration of cis or zalkenic group.

According to FT-IR assignments, the isolated organic compound consists of hydroxyl groups,sp^2 hydrocarbon, sp^3 hydrocarbon, carbonyl group, aromatic benzene ring, methyl group, ether group, and C-H bending vibration respectively.

Determination of Antimicrobial Activities of the Isolated Organic Compound

The antimicrobial activities of ethyl acetate extract of the isolated organic compound wasdetermined by using Ager-well diffusion method on six selected organisms. The results are tabulated in table 3.

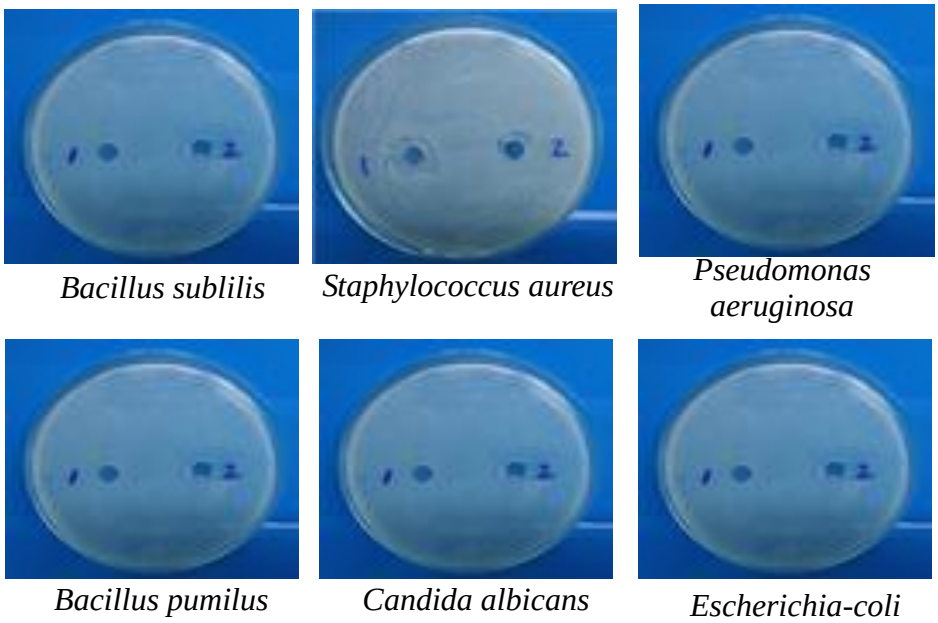

Figure 4. Antimicrobial activities of the Isolated Organic Compound

Table 3.Antimicrobial Activities of the Isolated Organic Compound

Samples	Extracted Solvents	Inhibition zone diameter (mm)					
		I	II	III	IV	V	VI
Isolated Organic Compound	EtOAc	–	30 (+++)	–	–	–	–

Agar-well -10mm

10mm-14mm (+)

15mm-19mm (++)

Organisms

I = *Bacillus subtilis*
II = *Staphylococcus aureus*
III = *Pseudomonas aeruginosa*
IV = *Bacillus pumilus*
V = *Candida albicans*
VI = *Escherichia coli*

20mm- above (+++)

In accordance with these results,the ethyl acetate extracts of the isolated organic compound gavehigh activities on *Staphylococcus aureus*and do not respondon remaining five organisms.

Conclusion

In this research,the leaves of *Plantago major* L., one of the most abundant and widely distributed medicinal crops in the world was investigated. *Plantago major* L responded a variety of constituents such as alkaloids, steroid, reducing sugar, saponin, phenolic compound, glycoside, and polyphenol in phytochemical tests.The presence of these phytochemicals confirms this sample had medicinal value.

From the EDXRF data, the highest amount of potassium and calcium were observed and the lowest amountsidentified were copper and rubidium. Andthen, organic compounds were isolated from the leaves of *Plantago major* L. by using Column and Thin Layer Chromatographic separation methods.According to FT-IR spectrum of isolated organic compound, it consists of hydroxy group, sp^2 hydrocarbon, sp^3 hydrocarbon, carbonyl group, aromatic benzene ring, methyl group, carbonyl group, ether group and Cis or Zalkenic group.

Moreover, antimicrobial activities of crude extract and isolated organic compound were tested by Agar-well diffusion method on six selected organisms. In which , ethyl acetate extracts of the crude extract responds medium activities on *Bacillus subtilis, Staphylococcus, Pseudomonas aeruginos and Bacillus pumilus*but ethyl acetate extract of isolated organic compound responds high activities on *Staphylococcus aureus and do not responds on other microorganisms.*

Plantago major L plays an important role in the management of certain ailments and diseases such as ulcers, bacterial and viral infections, diarrhoea, pain and inflammation. The biological activities and medicinal properties of *Plantago major* L mainly depends on the activities of the responsible active chemical constituents.

References

W.H.O (1999) "W.H.O Monographs on selected Medicinal Plants"Geneva,1.

Harbone.J.B., 1973, "Phytochemical methods; Aguide to modern technique of plant analysis". Chapman and Hall New York.279.

Hundley, HG and Chit KoKo, 1986. "List of trees.Shrubs.Herbs and Principal climbers etc.," 3rd Edition, Union of Burma, Government of printing and stationary, Yangon.

Lovo,V, Patial A, Phatak and Chandra N,(2010) "Free Radical, Antioxidants and Functional Foods Inpact on Health, Pharmacogn Rev.

PacakovaRNDr.Vera, CsC, 2008/2009, "Chromatography in biochemistry". Faculty of Natural Sciences, Charles, University in Prague.

Prajapati N.D, S.S.Purobit, AK. Sharma and J.Kuma, 2003."A Hand Book of Medicinal Plant" Agrobios, India.

Silverstein R.M,F.XWebster,D.J.Kielme(2005). "Spectrometric Identification of Organic Compounds,7thedotion ,John Wiley and Sons,Inc.,USA