Functional Groups Determination and Antimicrobial Activity of Isolated Compound from *Alternanthera sessilis*(L.)D.C

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ABSTRACT

In this research, one Myanmar indigenous medicinal plant, namely *Alternanthera sessilis* was chosen for preliminary phytochemical screening and antimicrobial activity tests.The whole plant of *Alternanthera sessilis* gives positive test for alkaloid, flavonoid and phenolic tests. Moreover, the antimicrobial activities of the whole plant of *Alternanthera sessilis* was tested by Agar-well diffusion method on six tested organisms such as *Bacillus subtilis, Staphylococus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Mycobacterium* species respectively. Ethyl acetate extract of the whole plant of *Alternanthera sessilis* responds medium activity on five selected organisms such as *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Mycobacterium* species. Furthermore, the dark green colour amorphous compound (4.4 mg, 0.147%) could be isolated from the whole plant of *Alternanthera sessilis* by using advanced separation methods such as Thin Layer and Column Chromatography. The functional groups present in isolated compound were identified by FT-IR (Fourier Transform Infrared Spectroscopy)spectral data. This pure compound is rechecked for antimicrobial activities using Agar-well diffusion method. Pure compound has low activity on *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* respectively.

Keywords : *Alternanthera sessilis*, Ethyl acetate, Thin Layer Chromatography, Column Chromatography.

1. INTRODUCTION

Myanmar is one of the South-East Asia countries which has a large number of traditional medicinal plants. In Myanmar, the study of traditional plants and their therapeutic used play a very important role in health care system because 70% of its population is in the rural areas and have used traditional medicine for centuries. Most of the people in Myanmar depend on traditional medicinal plants and herbal medicines for the treatment of various diseases.These plants have biologically active principles.[1].Nowadays, resistance to the major drugs used has been a growing problem in the world. Although modern medicine is well developed in the world, large sections of the population in developing countries still rely on the traditional medicinal plants and herbal medicines for their primary health care. During the past decade, traditional systems of medicine have become topic of global interest. Plants used in traditional medicine are more likely to yield pharmacologically active compounds.Today plants are the most exclusive source of drugs for the majority of the world's population.World Health Organization has also urged countries to promote the role of traditional practitioners in the health care systems of developing countries and to allocate financial support for the development of traditional medicinal system.[2]. Moreover, public interest in natural therapies has increased greatly in industrialized countries with expending use of medicinal plants and herbal medicines. Numerous indigenous plants are reputed to be useful for the treatment of various diseases. It is worth while to conduct biological and pharmacological screening for their activities. Many active principles obtained from some of the traditional medicinal plants are currently used world wide.[3]. In this research work, one of indigenous medicinal plants *Alternanthera sessilis*, local name Pa-zun-sa was chemically analyzed. The plant of *Alternanthera sessilis* is widely distributed in Kyaukse Township, Mandalay division, Myanmar.

Botanical Description and Traditional Uses [4]

Botanical Name	– *Alternanthera sessilis* (L.) D.C
Family	– Amaranthaceae
Myanmar Name	– Pa-zun-sa

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Figure 1.The whole plant of *Alternanthera sessilis* (L.) D.C

2. MATERIAL AND METHODS

2.1 Sample Collection

Myanmar indigenous medicinal plant, *Alternanthera sessilis*, was collected from Kyaukse Township, Mandalay Division,Myanmar. The whole plants of *Alternanthera sessilis* were dried in shade and crush into pieces.

2.2 Phytochemical Screening of One Myanmar Indigenous Medicinal Plant [5]

Phytochemical tests were done on the whole plant of *Alternanthera sessilis*, Pa-zun-sa, to determine the presence or absence of organic constituents in it.

2.3 Antimicrobial Activities of *Alternanthera sessilis* [6]

The antimicrobial activities of various extracts such as n-hexane, chloroform ($CHCl_3$), petroleum ether, ethyl acetate (EtOAc), ethanol (EtOH) of selected medicinal plant was tested by Agar-well diffusion method.

2.4 Extraction and Isolation of Compound from *Alternanthera sessilis*

The whole plant of dried sample (550 g) was percolated with 95% ethanol (1.5 L) for two months. Percolated solution was filtered and evaporated to dryness. The ethanol extract was dissolved in 200 ml of ethyl acetate. This ethyl acetate extract solution was then evaporated. The ethyl acetate crude extract (3.1 g) was chromatographed on silica gel column, eluting with n-hexane and ethyl acetate with various ratios from non-polar to polar produce 195 fractions. Each and every fraction was checked by TLC and 12 combined fractions were obtained. Among them, combined fraction VII was major portion. This combined fraction was checked by TLC for purity. The pure dark green amorphous form (4.4 mg) was obtained. The percent yield is found to be (0.147%) based upon the crude extract. The R_f value of this pure compound is (0.26) by using n-hexane and ethyl acetate (3:2).

2.5 FT-IR Assignments of Pure Bioactive Organic Compound

The FT-IR spectrum of pure compound was measured at the Department of Chemistry, University of Mandalay, Myanmar.

2.6 Antimicrobial Activities of Pure Compound

Antimicrobial activities of pure compound were rechacked by Agar-well diffusion method.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Tests on the whole plant of *Alternanthera sessilis*

Phytochemical Tests on the whole plant of *Alternanthera sessilis* were as shown in table 1.

Table 1. Results of Phytochemical Tests on the whole plant of *Alternanthera sessilis*

No.	Tests	Reagent	Observation	Result
1	Alkaloids	Dragendroff's Reagent	Yellow ppt	+
2	Flavonoids	Conc: HCl, Mg	Reddish brown Solution	+
3	Steroids	Acetic anhydride, Conc: H_2SO_4	–	–
4	Terpene	Acetic anhydride, $CHCl_3$, Conc: H_2SO_4	–	–
5	Phenolic	10% $FeCl_3$ solution	Green blue solution	+
6	Glycoside	10% lead acetate	–	–

(+) = present
(–) = absent

According to this table, the crude extract of *Alternanthera sessilis* shows positive for alkaloids, flavonoids and phenolic tests.

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3.2 Antimicrobial Activities of *Alternanthera sessilis*

The antimicrobial activities of the whole plant of *Alternanthera sessilis* was tested by Agar-well diffusion method on six tested organisms such as *Bacillus subtilis*, *Staphylococus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Mycobacterium* species respectively.

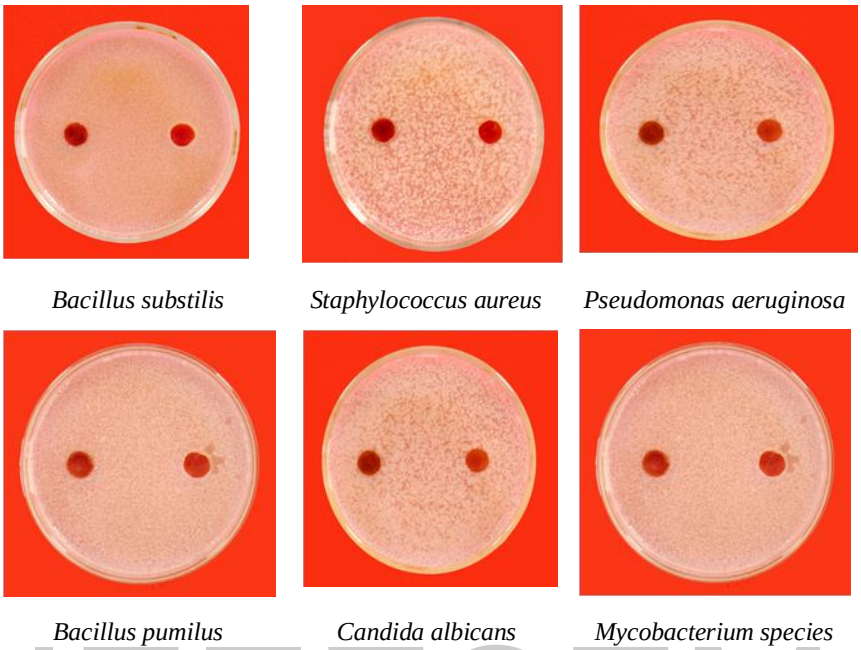

Figure 2. Antimicrobial activities of the whole plant of *Alternanthera sessilis*

Table 2. Antimicrobial Activities of *Alternanthera sessilis*

Sample	Organisms	Extracts				
		n-hexane	$CHCl_3$	Petroleum ether	EtOAc	EtOH
Alternanthera sessilis	(i) *Bacillus substilis*	–	–	–	–	–
	(ii) *Staphylococcus aureus*	–	(++)	–	(++)	(++)
	(iii) *Pseudomonas aeruginosa*	–	(++)	–	(++)	(++)
	(iv) *Bacillus pumilus*	–	(++)	–	(++)	(++)
	(v) *Candida albicans*	–	–	–	(++)	(++)
	(vi) *Mycobacterium* species	–	(++)	–	(++)	(++)

Agar well – 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

Organisms
(i) *Bacillus substilis*
(ii) *Staphylococcus aureus*
(iii) *Pseudomonas aeruginosa*
(iv) *Bacillus pumilus*
(v) *Candida albicans*
(vi) *Mycobacterium* species

Ethyl acetate and ethanol extracts of *Alternanthera sessilis* responded medium antimicrobial activities on five selected organisms, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albican* and *Mycobacterium* species.

3.3 Extraction and Isolation of Organic Compound from *Alternanthera sessilis*

The whole plant of dried sample (550 g) was percolated with 95% ethanol (1.5 L) for two months. Percolated solution was filtered and evaporated to dryness. The ethanol extract was dissolved in 200 ml of ethyl acetate. This ethyl acetate extract solution was then evaporated. The ethyl acetate crude extract (3.1 g) was chromatographed on silica gel column, eluting with n-hexane and ethyl acetate with various ratios

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from non-polar to polar produce 195 fractions. Each and every fraction were checked by TLC and 12 combined fractions were obtained. Among them, combined fraction VII was major portion. This combined fraction was checked by TLC for purity. The pure dark green amorphous form (4.4 mg) was obtained. The percent yield was found to be (0.147%) based upon the crude extract. The R_f value of this pure compound was (0.26) by using n-hexane and ethyl acetate (3:2).

3.4 FT-IR Assignments of Pure Bioactive Organic Compound

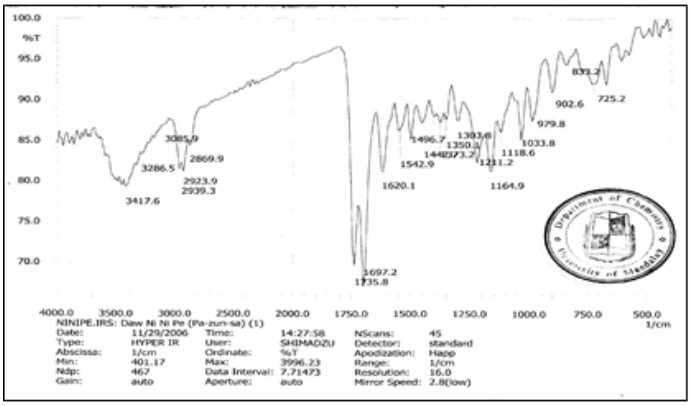

Figure 3. FT-IR spectrum of pure compound

In the FT-IR spectrum, the broad peak which appears at 3417 cm^{-1} shows the (O-H) stretching vibration of alcohol group. The peak at 3286 cm^{-1} indicates (N-H) stretching vibration of amine group. The shoulder at 3085 cm^{-1} gives rise the (C-H) stretching frequency of alkenic group. The bands at 2939 cm^{-1}, 2923 cm^{-1} and 2869 cm^{-1} are due to the unsymmetrical and symmetrical (C-H) stretching vibration of saturated hydrocarbons. The (C=O) stretching vibration bands of carbonyl group could be observed at 1735 cm^{-1} and 1697 cm^{-1}.

The peak at 1620 cm^{-1} shows the (C=C) stretching vibration of alkenic group. The (N = N) stretching vibration band could be determined at 1542 cm^{-1}. The band of 1496 cm^{-1} shows the (C-N) stretching vibration band. (C-H) in plane bending vibration of allylic hydrocarbons is observed at 1442 cm^{-1}. The peaks at 1303 cm^{-1} and 1211 cm^{-1} show (C-C-O) stretching vibration of ester groups.The peaks at 979 cm^{-1}, 902 cm^{-1} and 833 cm^{-1} imply the (C–H) out of plane bending vibration of trans or E and cis or Z alkenic groups.

3.5 Antimicrobial Activities of Pure Organic Compound

Antimicrobial activities of pure organic compound were tested by Agar-well diffusion method.

Staphylococcus aureus | *Pseudomonas aeruginosa* | *Candida albicans*

Figure 4. Antimicrobial activities of the isolated pure compound

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Table 3. Antimicrobial Activities of Pure compound

Sample	Organism		
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Candida albicans*
Pure Compound	(+)	(+)	(+)

Agar well – 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

According to this results, the pure compound has low activity on *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*.

4. CONCLUSION

In this research, *Alternanthera sessilis* was collected from Kyaukse Township, Mandalay Region, Myanmar. The phytochemical investigation of the whole plant of *Alternanthera sessilis* was done. The whole plant of *Alternanthera sessilis* gives positive for alkaloid, flavonoid and phenolic tests. Furthermore, the antimicrobial activity of whole plant of *Alternanthera sessilis* were tested by Agar well diffusion method. Ethyl acetate and ethanol extracts of *Alternanthera sessilis* responded medium antimicrobial activities on five selected organisms, such as *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albican* and *Mycobacterium* species. Hence, the ethyl acetate extract of *Alternanthera sessilis* was chosen for detailed chemical analysis. In the experimental work, the ethyl acetate extract of *Alternanthera sessilis* was separated by modern separation methods, such as Thin Layer and Column Chromatography. The pure dark green amorphous compound (4.4 mg) was obtained. The yield percent is found to be (0.147%) based upon the ethyl acetate crude extract. In the FT-IR spectrum, the broad peak which appears at 3417 cm^{-1} shows the (O-H) stretching vibration of alcohol group. The peak at 3286 cm^{-1} indicates (N-H) stretching vibration of amine group. The shoulder at 3085 cm^{-1} gives rise the (C-H) stretching frequency of alkenic group. The bands at 2939 cm^{-1}, 2923 cm^{-1} and 2869 cm^{-1} are due to the unsymmetrical and symmetrical (C-H) stretching vibration of saturated hydrocarbons. The (C=O) stretching vibration bands of carbonyl group could be observed at 1735 cm^{-1} and 1697 cm^{-1}. The peak at 1620 cm^{-1} shows the (C=C) stretching vibration of alkenic group. The (N = N) stretching vibration band could be determined at 1542 cm^{-1}. The band of 1496 cm^{-1} shows the (C-N) stretching vibration band. (C-H) in plane bending vibration of allylic hydrocarbons is observed at 1442 cm^{-1}. The peaks at 1303 cm^{-1} and 1211 cm^{-1} show (C-C-O) stretching vibration of ester groups.The peaks at 979 cm^{-1}, 902 cm^{-1} and 833 cm^{-1} imply the (C–H) out of plane bending vibration of trans or E and cis or Z alkenic groups. Furthermore, this pure compound was rechecked by antimicrobial activity using Agar-well diffusion method. Pure compound has low activity *on Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* respectively.

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