Antibacterial Screening on Phytoconstituents of Bark from *Plumeria acutifolia* Poir. (Tayoke Saga)

Thin Thin Sint[1], Aye Aye Tun[2], Maung Maung Htay[3]

Abstract

Systematic investigation on selected traditional medicinal plant, *Plumeria acutifolia* Poir.(bark of Tayoke Saga) was done to screen the antibacterial active constituents. *In vitro* antibacterial activity tests using agar disc diffusion method against *Escherichia coli* ETEC, *Escherichia coli* EPEC, *Klebsiella aeruginosa, Shigella sonnei and Staphylococcus aureus* were chosen for preliminary screening. Ethanol extract showed moderate antibacterial activity and ethylacetate extract showed antibacterial activity against *Staphylococcus aureus* only. Isolation of chemical constituents from active crude extracts was done by solvent partition, successive column chromatographic separation and recrystallization. Two compounds, fulvoplumierin, A, (0.029% yield, mp 150 ºC) and plumericin, B, (0.0003% yield, mp 210 ºC) have been isolated from $CHCl_3$ extract and plumieride, C, (3.5% yield, mp 154 ºC) has been isolated from EtOH extract. Minimum inhibitory concentrations (MIC) of some isolated compounds from that fraction, A, B and C were examined on *S.aureus, E.coli, Proteus morganii, V.cholerae* and *Bacillus substilis.* All compounds showed their potent activity especially on *S.aureus.* B showed the most potent antibacterial activity against *S.aureus* (MIC, 0.016 mg/mL).The antibacterial activity of B is higher than that of A which in turn higher than that of C. Thus, A and B may be main constituents of the plant responsible for antibacterial activity and could be considered as promising antibacterial agent.

Keywords: Antibacterial activity, Minimum inhibitory concentrations (MIC), Escherichia coli, Escherichia coli, Klebsiella aeruginosa, Shigella sonnei

Introduction

There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead modern medicine. The basic molecular structures for synthetic fields are provided from natural sources. This burgeoning worldwide interest in plants reflects recognition of the validity traditional claims regarding the value of in health care. The effects of plant extracts on bacteria, for example, have been studied by a very large number of the different parts of the world (Reedy *et al*., 2001; Erdorul, 2002; Atefl *et al*., 2003).

In Myanmar, most of the people depend on traditional medicinal plants and herbal medicines rather than modern medicines of the treatment of various disorders. Myanmar traditional practitioners use variety of medicine, mostly containing potent medicinal plants available in Myanmar, for the cure of various diseases. These medicines may consist of a single potent plant as well as in combination with other potent plants in different ratios by weight or by volume (San Nyunt Oo, 1993).

Therefore, safe, scientific and systematic development of effective drugs is mandatory to ensure the wellbeing of Myanmar people. The idea of developing drugs from plants as a starting point has always been high in the developing countries since most of them have abundant starting material in their natural flora and scientific expertise is usually available to implement this type of program. To contribute this utility of herbal drug, this work was

[1]Associate Professor, Department of Chemistry, Dagon University
[2] Rector, Bago University
[3] Professor (Retired), Department of Chemistry, University of Yangon

attempted to conduct scientific investigation of constituent of a specific herb and its antibacterial activity.

Plumeria acutifolia Poir. (Tayoke Saga in Myanmar) is a small tree belongs to a family Apocynaceae. Because of their attractive flowers and values of traditional medicine, these plants are very famous in Myanmar (Hundley, 1987). Hot water extract of the bark of *Plumeria acutifolia* was practically found to be a potent remedy in the treatment of malaria which is one of the prevalent diseases in Myanmar (Maung Maung Htay *et al*., 2001). Therefore, some chemical constituents of the bark and latex of this plant was studied and characterized by Maung Maung Htay *et al*. (Khin Myo Sint, 1991; Malar Soe, 1997; Than Than Cho, 1997; Khin Moe Sann, 1997; New Ni Aung, 1997). Especially focus was made on seasonal variation of some chemical constituents present in this plant. However, the bioactivities of this plant and its constituents have not been studied yet. In addition, some spectroscopic data such as mass spectra were lack in their work. In continuation of that work, present study has been investigated the antibacterial activity (MIC), of different crude extracts and some constituents of bark of this plant.

***Plumeria acutifolia* Poir. (Tayoke Saga)**

Family	:	Apocynaceae
Scientific name	:	*Plumeria acutifolia* Poiret
Synonym	:	*Plumeria accuminata* Aiton
	:	*Plumeria rubra* Linn
English name	:	Frangipani Tree or Jasmine Tree or Pagoda Tree
Myanmar name	:	Tayoke Saga

Plumeria, commonly known as frangipani, is a genus of shrubs and trees of the family Apocynaceae, native of tropical America; some as ornamental species are grown in the warmer regions of the world. About eight species are reported from India, but owing to the overlapping of characters in some species, it becomes difficult to fix their identity (The Wealth of India, 1969).

In Myanmar, four species of "Tayoke saga" plant, namely, *Plumeria acutifolia* Poir., *Plumeria rubra* L., *Plumeria alba* L. and *Plumeria obtusa* L. are recorded by Hundley in 1987. Because of their attractive flowers and values of traditional medicine, these plants are very famous in Myanmar (Hundley, 1987).

Sampling of Plant Materials

Pleumeria acutifolia Poir. (Tayoke Saga) used in this study was collected from Zalun Township, Ayeyarwaddy Region, in February, 2004 and identified at the Department of Botany, Dagon University. The stem bark was cut into small parts. After being air dried at room temperature for 2 weeks, these parts were made powder by using grinding machine and stored in air-tight container to prevent moisture changes and contamination.

Phytochemical Investigation of *Plumeria acutifolia* Poir.

Preliminary phytochemical analyses were performed in order to know the different types of chemical constituents present in the bark of *Plumeria acutifolia* Poir. Phytochemical investigation on plant sample was done according to standard procedures.

Investigation of Antibacterial Activity of Crude Extracts from *Plumeria acutifolia* Poir. Bark

Antibacterial activities of crude extracts (PE, $CHCl_3$, EtOAc, EtOH and watery extracts of bark of *Plumeria acutifolia* Poir.) were evaluated by agar disc diffusion method (Finegold *et al.,* 1978; Cruickshank, 1975; Mar Mar Nyein *et al*., 1991). Disc containing plant extracts were dipped in bacteria cultured agar plates and diameter of inhibitory zones were measured after incubation for some time. All antibacterial activity tests were performed at the Department of Medical Research (Lower Myanmar), Yangon.

Preparation of Test Samples

Five extracts were prepared in parallel by extracting plant sample with different solvents, viz, petroleum ether (PE), chloroform ($CHCl_3$), ethyl acetate (EtOAc), ethanol (EtOH) and water.

Dried powdered sample (100 g) was percolated with petroleum ether (60-80 °C) for one week at room temperature. Complete removal of solvent under reduced pressure provided PE extract. The yield percent was calculated on the basis of dried material.

Similarly, different batch of plant samples were extracted using different solvents, viz, $CHCl_3$, EtOAc, EtOH and water as described in the above procedure.

Screening by Agar Disc Diffusion Method

The filter discs (6 mm diameter) were made by punched No.1 Whatman filter paper. The discs were sterilized by autoclaving followed by dry heating at 60 °C for 1 hour. It was then impregnated with concentrated extracts to obtain approximately 20 µg/disc. Prior to adherence on the culture plates, the discs were allowed to dry at 42 °C in incubator.

The bacterial suspension from trypticase soy broth was streak evenly onto the surface of the trypticase soy agar plates with sterile cotton swab. After the inoculums had dried (5 min), the dried discs were placed on the agar with flamed forceps and gently pressed down to ensure proper contact.

The plates were incubated immediately or within 30 min after inoculation. After overnight incubation at 37 °C, the zones of inhibition diameter including 6 mm discs were measured.

Extraction and Isolation of Chemical Constituents from Active Ethanol Extract of Tayoke Saga Bark

Air dried bark powder sample (400 g) was percolated with 95 % EtOH (1200 mL) for one week at room temperature. Ethanol crude extract (32.16 g, 8.02 % yield) was obtained after removal of the solvent. The crude extract was then dissolved in distilled water and partitioned with chloroform (5x100 mL). Removal of chloroform extract (6.0 g, 1.5 % yield). The defatted layer was concentrated under reduced pressure to provide the defatted ethanol extract (25.0 g, 6.25 % yield). These two extracts were used to screen the active chemical constitutions.

Isolation of Chemical Constitutions from $CHCl_3$ Extract of Tayoke Saga Bark

The chloroform crude extract (6.0 g) was dissolved in ethyl acetate and thoroughly adsorbed on silica gel (5 g). the adsorbed material after being dried was transferred to a silica gel column (70 g of silica gel in petroleum ether; column: 2.0 cm in diameter). The column was eluted consecutively with petroleum ether : ethyl acetate (49:1), petroleum ether : ethyl acetate (19:1), petroleum ether : ethyl acetate (9:1), petroleum ether : ethyl acetate (7:1), petroleum ether : ethyl acetate (4:1), petroleum ether : ethyl acetate (3:1) and finally with petroleum ether : ethyl acetate (2:1). A quantity of 10 mL was collected for each fraction and the column chromatography was monitored by TLC using the solvent system of petroleum ether-ethyl acetate mixture in appropriate ratio. The fraction which gave similar TLC pattern were combined together and concentrated. In this way, three major fractions, F_1 to F_3 were obtained.

The fraction F_2 was further purified by crystallization in chloroform-methanol provided compound A as orange needle (11.62 mg, 0.029 % in yield, m.p. 150 °C, R_f = 0.63 in PE : EtOAc, 4:1).

The fraction F_3 was purified by crystallization in methanol provided colourless needles named compound B (1.3 mg, 0.0003 % in yield, m.p. 210 °C, R_f = 0.38 in PE : EtOAc, 4:1).

Isolation of Chemical Constituents from Defatted Ethanol Extract of Tayoke Saga Bark

Defatted ethanol extract (5.0 g) was dissolved in ethanol and thoroughly adsorbed on silica gel (3 g). The adsorbed material after being dried was transferred to a silica gel column (50 g; 2.0 cm in diameter packed in ethyl acetate : ethanol (49:1). The column was eluted consecutively with ethyl acetate : ethanol (19:1), ethyl acetate: ethanol (9:1), ethyl acetate : ethanol (4:1), ethyl acetate : ethanol (3:1), ethyl acetate : ethanol (2:1) and finally with ethyl acetate : ethanol (1:1).

A quantity of 10 cm^3 was collected for each fraction and the column chromatography was monitored by TLC using the solvent system of ethyl acetate and ethanol mixture. The fraction which gave similar TLC pattern were combined together and concentrated. In this way, three major fractions, F_4-F_6, were obtained.

The fraction F_5 after crystallization in ethyl acetate – methanol provided compound C as colourless needles (2.80 g, 3.5 % in yield, m.p. 154 °C, R_f = 0.25, EtOAc : EtOH, 9:1).

Determination of Minimum Inhibitory Concentration (MIC) of Isolated Compounds

Microorganisms are tested for their ability to produce visible growth in a series of microplate wells of broth (broth microdilution) containing serial two fold dilutions of the antimicrobial agent (Cruickshank *et. al*., 1975; Finegold and Martin, 1982). The lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism is known as the MIC.

Bacteria samples: Eight strains were employed in this study. They included three types of *Staphylococcus aureus*, two types of *Escherichia coli*, *Proteus morganii*, *Vibrio cholera* and *bacillus subtilis*. Strains were obtained from the Department of Medicinal Research (Lower Myanmar), Yangon. The minimum inhibitory concentration (MIC) values against bacterial strains were investigated using a broth micro-dilution method.

Broth Microdilution Assay

To determine the MIC of pure compounds (A and C), 5 mg of each sample was dissolved in minimum amount (ca 0.25 mL) of EtOH and diluted with trypticase soy broth

(ca. 7.75 mL) to obtain a working solution containing 0.625 mg/mL of pure compound. Using aseptic technique, trypticase soy broth (100 µL) was introduced into 96 wells of microdilution trays {12 (No. 1-12) x 8 (A-H)}. Then, the sample solution (100 µL) was introduced to all of the first wells (A-1 through G-1) so as to obtain 0.313 mg/mL of pure compound. By using multi-channel pipette (8-channels) and Titertek micro-titration equipment, 100 µL of the mixture was used for downstream serial dilution up to 10 consecutive wells. The last 100 µL of individual 10^{th} wells were discarded. By this two-fold serial dilution method, ten concentrations of pure compound (0.313, 0.156, 0.078, 0.039, 0.0195, 0.0098, 0.0049, 0.0025, 0.0013, 0.0006 mg/mL) were prepared individually in 1^{st} well through 10^{th} well. While transferring the content of each well, the mixture was thoroughly mixed with multi-channel pipette. The last two wells (11^{th} and 12^{th}) serve as positive growth control (broth plus inoculum) and a negative control (broth alone). Then, inoculum (20 µL) was introduced to its respective wells and the microplates were incubated at 37 °C for 18 hours. Prior to the spectrophotometric recordings, the mixtures were allowed to mix thoroughly by gently rocking the plates mechanically on a shaking machine. Growth of microorganisms was determined by an automated microplate reader (Bio Rad) at a wave length of 450 nm. From each and every well, 0.02 µL of broth suspension was inoculated onto nutrient agar, incubated at 37 °C for 18 hours and the growth of the respective organisms was recorded. The concentration of the compound in the last well with no growth of bacteria on nutrient agar was the minimum inhibitory concentration of the tested sample.

Compound B (1.3 mg) was dissolved in minimum amount (ca 0.25 mL) of EtOH and diluted with trypticase soy broth (ca 4.75 mL) to obtain a working solution contain 0.26 mg/mL of compound B. By two-fold serial dilution method, ten concentrations of compound (0.13, 0.065, 0.0325, 0.0163, 0.008, 0.004, 0.002, 0.001, 0.0005, 0.00025 mg/mL) were prepared individually in 1^{st} well through 10^{th} well.

The photographs of microdilution tray used in the measurement of MIC and agar plates used to predict the minimum bactericidal concentration (MBC) of individual samples are shown in Figure 17. The values of MIC of isolated compounds are reported in Table 3.

Results and discussions

Phytochemical Investigation of *Plumeria acutifolia* Poir.

Preliminary phytochemical tests were carried out on stem bark of plant samples. It was found that alkaloids, carbohydrates, glycoside, saponins, flavonoids, α-amino acid, phenolic compounds, steroids, terpenoids and tannins were found to be present in the stem bark of *P. acutifolia* Poir.

Investigation of Antibacterial Activity of Various Crude Extracts

Antibacterial activities of crude extracts were investigated by agar disc diffusion method. Five extracts were used to test antibacterial activity against six micro-organisms, namely, 2 species of *Escherichia coli, Kiebsiella aeruginosa, Shigella sonnei* and 2 species of *Staphylococcus aureus*. Ethanol extract showed moderate antibacterial activity on tested strains.

Agar disc diffusion method is based on the diameter of the inhibitory zone that is proportional to the logarithm of the concentration of the antibacterial agent under constant experimental conditions (culture medium composition, thickness of agar, inoculums size,

Table (1). Antibacterial Activity of Crude Extracts on Different Bacterial Strains

No.	Type of Bacteria	Diameter of Inhibition Zone(mm)				
		PE	$CHCl_3$	EtOAc	EtOH	H_2O
1	*Escherichia coli* ETEC	-	-	-	**14**	-
2	*Escherichia coli* EPEC	-	-	-	**14**	-
3	*Klebsiella aeruginosa*	-	-	-	**14**	-
4	*Shigella sonnei*	-	-	-	**12**	-
5	*Staphylococcus aureus* DMR -ML 96	-	-	14	**15**	12
6	*Staphylococcus aureus DMR - M 20*	-	-	-	**15**	-

disc diameter = 6 mm no zone of inhibitor = (-)

incubation time and temperature, etc). When comparing different antibacterial agents to known concentration, the inhibitory zone diameter is taken as a measure of the antibacterial activity. The larger the diameter, the higher the antibacterial activity of test agents.

Studies on the Physicochemical Properties of Isolated Compounds

Three purified compounds (A, B and C) were isolated from the ethanol extract of dried bark powder of *P. acutifolia*. The yield percent and melting points of these isolated compounds are reported in Table 2.

Melting Point of isolated compounds were measured not only to investigate the purity but also to make comparison with reported data in literature. Table 3.6 present the observed melting points of all isolated compounds alongside with those reported data in literature.

Table (2).Yield and Melting Points of Isolated Compounds from *P. acutifolia* Poir. Bark

Compounds	Yield (%)	Melting point (°C)		Remark
		Observed	*Literature	
A	0.029	150	151-152	Fulvoplumierin
B	0.0003	210	211-212	Plumericin
C	3.5	154	156-158	Plumieride

*Marck Index, 1967

Studies on Spectroscopic Data and Identification of Isolated Compounds

Compound A

Compound A provided colour reaction to anisaldehyde and Libermann-Buchard reagents. Therefore, compound A may be a terpenoid compound.

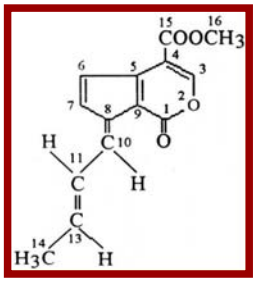
The UV spectrum of isolated compound A in methanol is presented in Figure 1. The wavelength of maximum absorption λ_{max} was observed at 226.72, 262.36 and 364.14 nm indicating the presence of long conjugated system. The FT-IR spectrum of compound A is presented in Figure 2. = C-H Stretching vibration of olefinic system was found at 3.37 cm^{-1}. Absorption bands at 2962 and 2869 cm^{-1} represent -C-H stretching vibration of aliphatic hydrocarbon. >C=O stretching vibration of lactone system was observed at 1747 cm^{-1} as a strong band. The absorption band at 1717 cm^{-1} was attributed to C=O stretching vibration of ester. The absorption band at 1653 and 1593 cm^{-1} represent C=C stretching vibration. -C-H Bending vibration of aliphatic hydrocarbon was found at 1433 and 1385 cm^{-1}. The strong band at 1294 cm^{-1} was due to -C(O)-O-C stretching vibration of ester. In addition, -C-O-C- stretching vibration of cyclic ester group was observed at 1094 cm^{-1}. The absorption bands at 978 and 960 cm^{-1} indicated =C-H out of plane bending of alkenic group.

^{1}H NMR reveals the number of protons and their environment present in a chemical compound. ^{1}H NMR spectrum of compound A (Figure 3) was taken in $CDCl_3$ and recorded on 400 MHz. A total of 12 protons could be observed from it. A doublet (3H) appeared at 2.02 ppm was attributed to methyl proton on C-14 which coupled to C-13 proton with coupling constant 6.8 Hz. A singlet (3H) at δ 3.9 ppm was due to the $–OCH_3$ protons attached to C-15. Multiplet (1H) centered at 6.55 ppm were attributed to =CH proton of C-11 which coupled to each proton of C-10 and C-13 with ^{3}J coupling constant (13.6 Hz) and C-14 methyl protons with allylic coupling constant (^{4}J). The olefinic proton of C-6 and C-7 appeared as two doublets (each 1H), respectively, at δ 7.22 and 7.33 ppm which coupled each other with coupling constant 5.36 Hz. The olefinic proton of C-10 appeared as a doublet (J=11.72 Hz, 1H) at δ 7.95 ppm. In addition, a singlet (1H) at δ 8.28 ppm was due to the =C-H proton attached to C-3.

The molecular structure of compound A was further confirmed by its ^{13}C NMR spectrum (Figure 4). A total of 14 carbons were observed. Two peaks appeared at δ 19.5 (C-14) and 52 (C-16) ppm were due to the one methyl and one methoxy carbon atoms. Six peaks appeared at δ 156 (C-3), 145.5 (C-13), 143 (C-10), 130 (C-6), 127 (C-11) and 127 (C-7) ppm were due to the six methane carbon atoms. Four signals at δ 150 (C-5), 136 (C-8), 113 (C-4) and 109 (C-9) could be assigned as quaternary carbon of olefinic moiety. Remaining two carbons of carbonyl group appeared at 165 (C-1) and 156.5 (C-15) ppm. Table 3.9 represents ^{1}H and ^{13}C NMR peaks assignment of isolated compound A. Confirmation for the presence of one methyl, one methoxy and six methane carbon in this compound was further made form DEPT spectrum (Figure 5).

The molecular mass and molecular formula of compound A was determined from its EI-MS spectrum (Figure 6). Parent ion peak at m/z 224 was also the base peak. Therefore, the molecular mass of compound A was deduced as 244 which was consistent with the molecular formula $C_{14}H_{12}O_4$.

From the above observations, the isolated compound A was identified as fulvolmierin, $C_{14}H_{12}O_4$.



Fulvolmierin (A), $C_{14}H_{12}O_4$

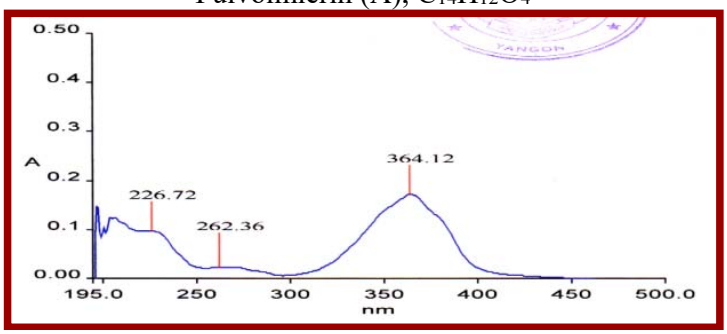


Figure (1). UV spectrum of isolated compound A

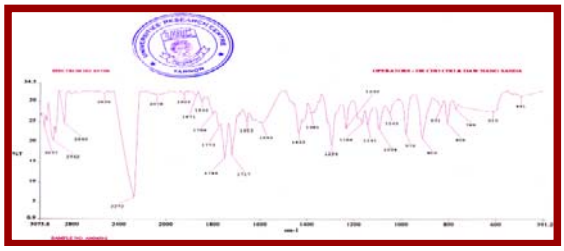


Figure (2). FT - IR spectrum of isolated compound A

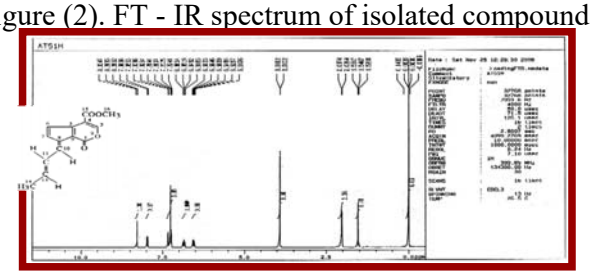


Figure (3). **^{1}H NMR spectrum of isolated compound A**

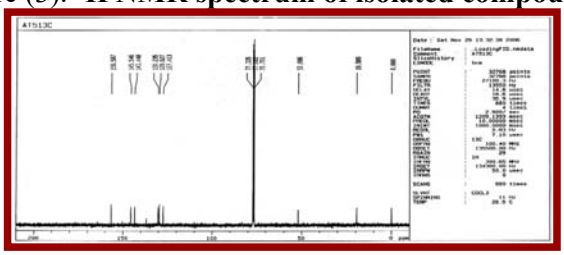


Figure (4). ^{13}CNMR spectrum of isolated compound A

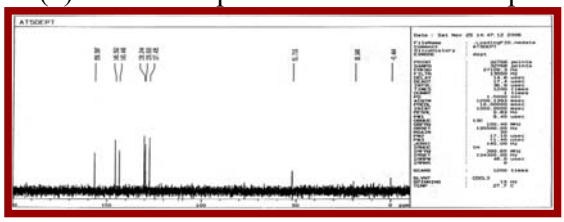


Figure (5). DEPT spectrum of isolated compound A

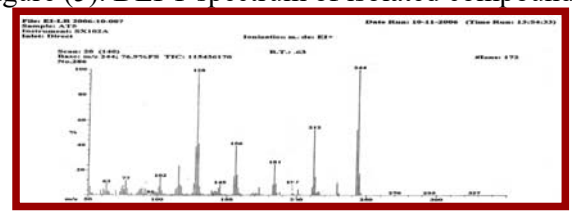


Figure (6). Mass spectrum of isolated compound A

Compound B

Compound B provided colour reaction to anisaldehyde and Libermann-Büchard reagents. Therefore, compound B may be characterized as a terpenoid.

Compound B in methanol has λ_{max} 214.93 nm indicating the presence of enone moiety. Figure 7 represents the ultraviolet spectrum of compound B. The FT-IR spectrum of compound B is also presented in Figure 8. A band at 3035 cm^{-1} was due to = CH stretching vibration of olefinic system. Antisymmetric and symmetric stretching vinration of aliphatic C-H was found at 2958 and 2868 cm^{-1}. >C = O stretching of lactone group was observed at 1758 cm^{-1} and that of ester was observed at 1716 cm^{-1}. Absorption band at 1591 cm^{-1} was attributed to C = C stretching vibration in olefin. Deformation vibration of aliphatic C-H of –CH_3 group was observed at 1402 cm^{-1}.C-O-C stretching vibration of ester and cyclic ester

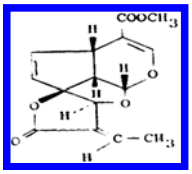
were observed at 1267 and 1085 cm^{-1}. = C-H out of plane bending of alkenic group was observed at 978 and 760 cm^{-1}.

^{1}H NMR spectrum of compound B is shown in Figure 9. A singlet at δ 7.4 ppm was interpreted as a proton of C-13. A triplet at δ 6.0 ppm was identified as a proton on C-6 which coupled to each proton of C-5 and C-7 with same coupling constant (*J* = 4 Hz). Two doublets each representing one proton at δ 5.65 and 5.56 ppm were attributed to C-7 and C-1. A singlet (2H) at 3.7 ppm was due to the $–OCH_3$ proton attached to C-15. A doublet (3H) appeared at 2.09 ppm is attributed to methyl proton on C-14 which coupled to C-13 proton with coupling constant 7.32 Hz. Moreover, remaining methane protons of C-5, C-9 and C-10 appeared as a *dd* (1H) at 4.01; a triplet (1H) at 3.42; and a singlet (1H) at 5.1 ppm; respectively.

The number of carbons present in compound B were investigated from its ^{13}C NMR spectrum which is shown in Figure 10. A total of 15 carbons were observed.

Molecular weight of compound b was confirmed from its EIMS spectrum which is shown in Figure 11. From these data, it could be deduced that the molecular weight of compound B was 290 consistent with molecular formula of $C_{15}H_{14}O_6$.

On the basis of observed melting point, spectroscopic data and by comparing with literature data, the isolated compound B was assigned as plumericin.



Plumericin (B), $C_{15}H_{14}O_6$

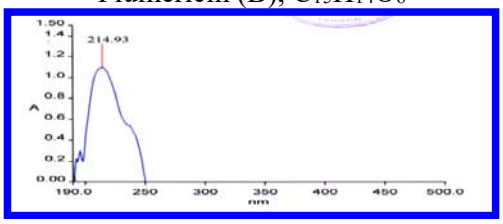


Figure (7). **UV spectrum of isolated compound B**

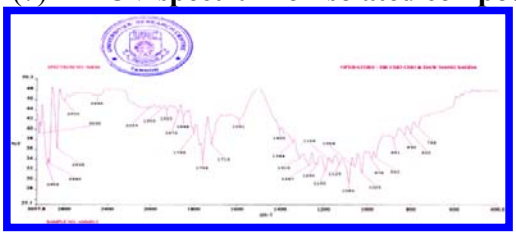


Figure (8). FT - IR spectrum of isolated compound B

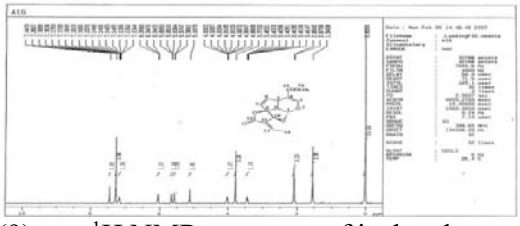


Figure (9). ^{1}H NMR spectrum of isolated compound B

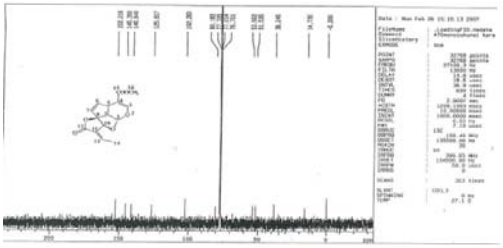


Figure (10). ^{13}CNMR spectrum of isolated compound B

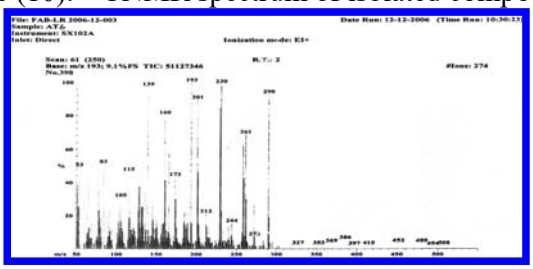


Figure (11). Mass spectrum of isolated compound B

Compound C

Ultraviolet spectrum of compound C was taken using EtOH and described in Figure 12. Wavelength of maximum absorption at 225 nm was consistent with reported value of plumieride (217-238 nm) (Marck Index, 1967).

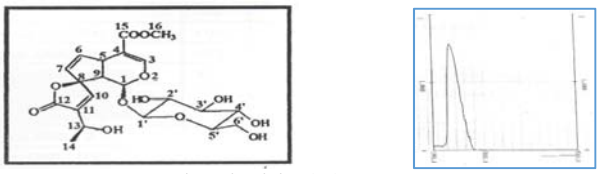
FT-IR spectrum of compound C is described in Figure 13. A broad band centered at 3379 cm^{-1} was due to O-H stretching vibration of alcohol. Antisymmetric and symmetric stretching vinration of aliphatic C-H was found at 2909 and 2851 cm^{-1}. >C = O stretching of lactone group was observed at 1755 cm^{-1} and that of ester was observed at 1697 cm^{-1}. Absorption band at 1633 cm^{-1} was attributed to C = C stretching vibration in olefin. Bending vibration of aliphatic C-H of $–CH_3$ group was observed at 1436 cm^{-1}. C-O-C stretching vibration bands of ester were observed at 1288 and 1266 cm^{-1}. C-O stretching of sugar group was observed at 1099, 1076 and 1039 cm^{-1}. = C-H out of plane bending vibration of alkenic group was observed at 865 and 786 cm^{-1}.

^{1}H NMR spectrum of compound C is shown in Figure 14. Two singlets at δ 7.49 and 7.2 ppm were attributed to two = CH protons of C-3 and C-10. Two doublets at 5.5 (J = 5.6 Hz) and 5.1 ppm (J = 4.5 Hz) represent = C-H proton of C-7 and a proton of C-1. A *dd* at δ 6.3 ppm indicated C-6 proton. In addition, multiples centered at δ 4.29 ppm represent a proton of C-13 bearing –OH group. Two triplets appeared at 2.9 (J = 8 Hz) and 2.5 ppm (J = 1.65 Hz) represent two C-H protons of C-5 and C-9. For 3 protons of C-16 as a singlet was observed at δ 3.69 ppm. A doublet peak at δ 4.5 ppm was attributed to the proton of C-1. Three *dd* at δ 2.8, 3.4 and 3.8 ppm indicated C-3, C-4 and C-2 protons. Two multiplets centered at δ 3.02 and 4.35 ppm represent protons of C-6′ and C-5.

^{13}C NMR spectrum of compound C is described in Figure 15. The presence of 21 carbons including two carbonyl carbons, six double bonded carbons and a glucose unit were investigated in this compound.

The molecular mass of compound C was also determined from its EI-MS spectrum described in Figure 16. From this spectrum, the molecular mass (m/z) was 470 which consisted with molecular formula $C_{21}H_{26}O_{12}$.

On the basis of all spectroscopic data, compound C was assigned as plumieride with the following molecular structure.



Plumieride (C), $C_{21}H_{26}O_{12}$

Figure (12). UV spectrum of isolated compound C

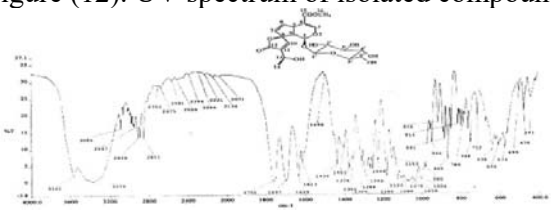


Figure (13). FT - IR spectrum of isolated compound C

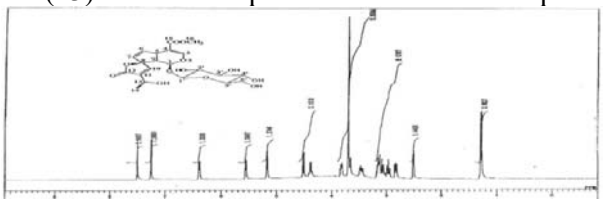


Figure (14). ^{1}H NMR spectrum of isolated compound C

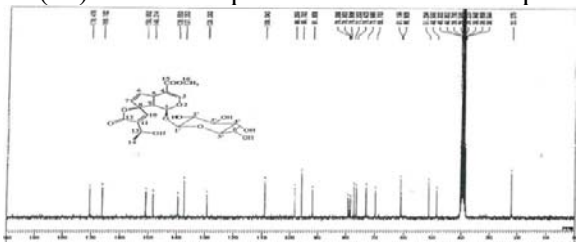


Figure (15). ^{13}C NMR spectrum of isolated compound C

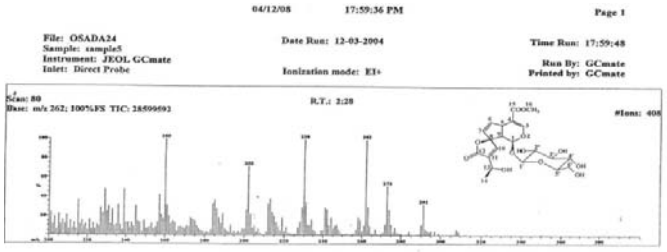


Figure (16). Mass spectrum of isolated compound C

Determination of Minimum Inhibitory Concentration (MIC) of Isolated Compounds

The antibacterial potencies of three compounds isolated from the bark of *P. acultifolia* Poir. Were investigated on *Proteus morganii*, *Escherichia coli, Staphylococcus aureus, Vibrio cholera and Bacillus subtilis.* Results are shown in Table 3.

It was found that MIC values of compound B were lower than that of compound A and C for all tested microorganisms revealing the superiority in antibacterial activity of compound B over A and C. in addition, compound A (fulvoplumierin) was found to possess more potent antibacterial activity (lower MIC) than C (plumieride).

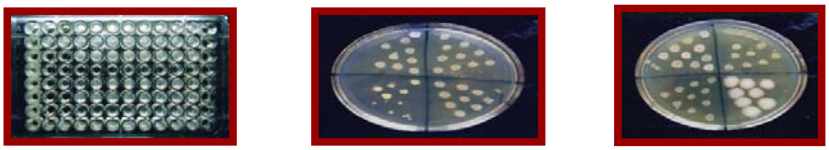


Figure (17). Microbroth dilution tray for determination of MIC of isolated compound B

Table (3). MIC values of Isolated Compounds from Tayoke Saga Bark.

No.	Organism	MIC value (mg/mL)		
		A	B	C
A	*Proteus morganii*, DMR 1D1	> 0.313	0.065	0.313
B	*Escherichia coli*, DMR 1D5	> 0.313	> 0.13	> 0.313
C	*Staphylococcus aureus*, DMR 234	0.313	0.13	> 0.313
D	*Staphylococcus aureus* DMR, 1D 15	0.313	0.0163	> 0.313
E	*Staphylococcus aureus*, DMR ML 86	0.156	0.13	> 0.313
F	*Escherichia coli*, DMR 1 D 16	> 0.313	0.065	> 0.313
G	*Vibrio cholerae* DMR 1 D 93	> 0.313	0.065	> 0.313
H	*Bacillus subtilis* DMR B-5	> 0.313	> 0.13	> 0.313

All these test samples especially showed their potent activity against *Staphylococcus aureus*. Indeed, compound B showed the most potent antibacterial activity (lowest MIC, 0.0163 mg/mL) on *Staphylococcus aureus* DMR ID 15 which is notoriously resistant to penicillin and many other antibiotics. Control of penicillin resistant *Staphylococcus aureus* in hospital is now particularly important in wound infections. Moreover, the spread and carriage of Staphylococcus aureus is far more important in the population. Therefore, it is expected that isolated compounds (A and B) were hoped to be used in prevention of the *Staphylococcus aureus* infections.

It was also suggested that isolated compounds (A or/and B) could be used in the formulation of traditional medicine used in the treatment of boils and pimples, wound infection, pneumonia, septicemia, food intoxication and toxic shock syndrome which are caused by *Staphylococcus aureus*.

Conclusion

In vitro antibacterial activity of crude extracts revealed that ethanol and ethyl acetate extracts of Tayoke Saga bark showed moderate amtibacterial activity on tested strains.

Three compounds fulvoplumierin (A) (0.029 % yield, mp. 150 ºC), plumericin (B) (0.0003 % yield, mp. 210 ºC) and plumieride (C) (3.5 % yield, mp. 154 ºC) were isolated from ethanolic extract of *P. acultifolia* Poir. And their structures were identified by UV, FT-IR, ^{1}H and ^{13}C NMR and MS spectroscopy.

All isolated compounds showed mild to moderate antibacterial activity against tested organisms, however, A and B showed potent activity on *Staphylococcus aureus*. Minimum

inhibitory concentration (MIC) values of compound A and B, respectively, on V are 0.156 and 0.0163 mg/mL. Compound B was found to be more potent than the compound A.

Therefore, A and B may be main constituents of the plant responsible for antibacterial activity and could be considered as promising antibacterial agent.

Acknowledgements

The authors wish to thank Rector Dr U Win Naing, Pro-Rector Dr Daw Nu Nu Yee and Dr Daw Nay Thwe Kyi, Dagon University and Professor Dr Daw Cho Cho Win, Head of Department of Chemistry and Professor Dr Daw Hnin Hnin Than, Department of Chemistry, Dagon University for their kind provision of the opportunity to submit this research paper.

References

Atefl, D.A. and Endorul O.T., (2003). "Antimicrobial Activities of various medicinal and commercial plant extracts", *Turk. J. Biol*., **27**, 157-162

Cruick S.R., Duguid, J.P., Marmior, B.P., and Swain, R.H.A., (1975). Medical Microbiology, Curchill Livingstone Ltd., London

Endorul, O.T., (2002). "Antibacterial activities of some plant extracts used in folk medicine," *Pharmaceutical Biol*., **40**, 269-273

Finegold, S.M., Martin, W.J. and Scott, E.G., (1978). Diagnostic Microbiology, The C. V. Mosby Co. London

Hundey, H.G.,(1987). List of Trees, Shrubs, Herbs and principal Climbers, etc. 4th Revised Edition, Forest Department, Yangon

Mar Mar Nyein, Chit Maung, Mya Bwin and Tha,S.J. (1991). "In vitro Testing of Various Indigenous Plant Extracts on Human Pathogenic Bacteria," *Myanmar Hlth. Sci. Res.J.,* **3**, 89-99

Maung Maung Htay, Tin Tin Aye, Khin Myo Sint and David Tin Win, (2001). "Identification of Fulvoplumierin Isolated from the Bark of *Plumeria acutifolia* Poir. An Antimalarial Drug of Traditional Medicine in Myanmar." *AU.J.Tech*., **5**(1), 5-8

New Ni Aung, (1997). The study of the chemical components of the bark of *Plumeria acutifolia* Poir., MSc (Thesis), Department of Chemistry, University of Mawlamyine "

Reddy, P.S., Jamil, K. and Madhusudhan, P., (2001). Antibacterial activity of isolate from Piper longum and Toxus baccata," *Pharm. Biol*., **39**, 236-238

San Nyunt Oo, (1993), Traditional Medicine Formulary 1000, (Myanmar Version), Ah Thine Ah Wine Press, Yangon

Wealth of India, (1969), A Dictionary of Indian Raw Materials and Industrial Products, Raw Materials, vol VIII, CSIR, New Delhi, 164-165