Evaluation of Antimicrobial and Antioxidant Activities of *Avicennia officinalis* L.

Nant Si Si Htay[1], Moe Moe Cho[2]

Abstract

Mangroves have long been a source of astonishment for the laymen and of interest for scientists and are biologically unique, producing a wide array of novel natural products. In the present research, the leaves and roots parts of a mangrove plant, *Avicennia officinalis* L. (Myanmar Name: Thame) belonging to the family Avicenniaceae, collected from Htein Tan Village, Ngaputaw Township, Ayeyarwady Region, Myanmar, were chosen for investigation of some phytoorganic constituents and evaluation of antimicrobial activity, antioxidant activity and estimation of total phenolic content. By column chromatographic separation, three compounds, C-I (an aliphatic ester, 0.02 %), C-II (Lupeol, 0.09 %) and C-III (a triterpene, 0.006 %) were isolated from PE extract of the roots of *A. officinalis*. The crude extracts such as pet-ether, ethyl acetate, ethanol and watery extracts of the leaves and roots of *Avicennia officinalis* (Thame), (TML and TMR) were prepared. The antimicrobial activity of the prepared crude extracts of both TML and TMR was screened on *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* by agar well diffusion method. Among the tested crude extracts, EtOAc extracts of both TML and TMR exhibited the highest antimicrobial activity with inhibition zone diameter range of (ID: 22~33 mm) and (ID: 25~35 mm), respectively against all the tested microorganisms. In the *in vitro*, antioxidant activity screening of 95 % EtOH and watery extract of TML and TMR as well as the isolated compounds by DPPH free radical scavenging assay, both extracts of TMR showed the highest antioxidant activity (IC_{50}: 8.25~8.35 μg/ml). Total phenolic content (TPC) of 95 % EtOH and watery extracts of both TML and TMR was estimated by Folin-Ciocalteau Reagent method. TPC was found to be higher in both extracts of TMR than that in TML. A marked antimicrobial and antioxidant activity of the roots extracts of the plant sample (TMR) was observed which may be attributed to the presence of higher TPC content and other phytochemicals. The plant can be used to control infectious diseases and oxidative stress.

Key words: *Avicennia officinalis* L., Phytoorganic constituents, Antimicrobial activity, Antioxidant activity, Total Phenolic Content

Introduction

Avicennia officinalis L. (Myanmar name: Thame) is commonly available as white mangrove plants in almost all the coastal states of Myanmar. *A. officinalis* is an evergreen tree plant that varies from shrubby stunted individual to tall trees with broad trunk. The literature survey reveals that the seeds are used as maturative poultices, cicatrizant of ulcers and to hasten suppuration of boils and abscesses. The roots are used as aphrodisiac, resin oozing from the bark finds use as contraceptive, bark as diuretic, in the management of skin afflictions especially scabies and also in rheumatism, paralysis, asthma and snake-bites. Its fruits are used as plaster for tumors. Plant decoction with sugar and cumin is used in dealing with dyspepsia (Sharief *et al*., 2014).

In this research work, the Leaves and Roots of *Avicennia officinalis* L. were chosen for isolation of some phytoorganic constituents and investigation of antimicrobial activity, antioxidant activity and total phenolic content.

[1] Dr., Associate Professor, Department of Chemistry, Pathein University
[2] Dr., Associate Professor, Department of Chemistry, Pathein University

Botanical Aspect of *Avicennia officinalis* L.

Family	: Avicenniaceae (Acanthaceae)
Genus	: Avicennia
Botanical name	: *Avicennia officinalis* L.
Myanmar name	: Thame
English name	: Indian Mangrove
Synonyms	: *Avicennia tomentosa*
Parts used	: Leaves and Roots

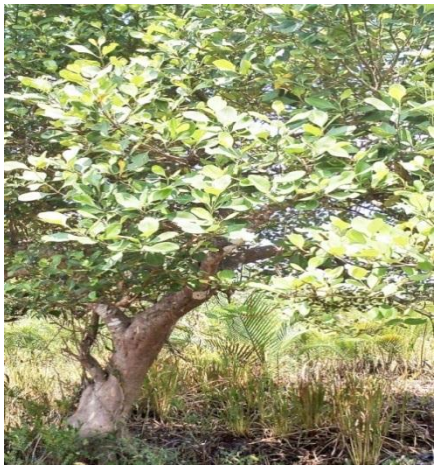


Figure 1 Photograph of the tree of *Avicennia officinalis* L.

Figure 2 Photograph of the roots of *Avicennia officinalis* L.

Description

Avicennia officinalis L. is about 12 m tall tree with smooth lenticles, light coloured but do not have fissured barks. It has 8-20 cm long, pencil like pneumatophores and aerial stilt roots. The Leaves are 8-10 cm long, spoon- shaped, upper side glossy green, underside finely hairy, with salt crystals found on the surface, especially in dry weather. The flower of *A. officinalis* is about 1 cm in diameter, being the largest among all the species of its genus. The flower is orange-yellow, globular in shape with the rancid or fetid smell. The fruits of the plant are 2-3 cm long, oval slightly beaked, smooth velvety and contains a single seed which completely fills the capsule (Thatoi *et al* ., 2016).

Distribution

Avicennia plants have worldwide occurrence. They are densely distributed mangrove species found in both coastal river and sea beds of tropical, temperate regions, subtropical regions of both North and South America including Colombia, Costa Rica, Mexico, Panama, Brazil, Chile; coast of Africa; Middle East; South and South east Asia which includes Myanmar, Coast of India, Bangladesh, Malaysia, Vietnam, Thailand, Indonesia; and coast of Trans-Asia countries Australia and New Zealand (Thatoi *et al*., 2016).

Medicinal Uses of Various Parts of *Avicennia officinalis* L.

The barks, Leaves and fruits of *A. officinalis* have been used as traditional medicine to treat rheumatism, paralysis, asthma, snake-bites, skin disease and ulcer (Job et al., 2015). It has been documented that a resinous substance exuded from the bark act as a contraceptive and apparently can be taken all year long without ill effects (Ganesh et al., 2011).The roots of

A. officinalis have been reported to have aphrodisiac, cicatrizant, diuretic and astringent properties. Unripe seeds are used as poultices onto abscesses, boils, and smallpox sores. The bark is used for skin afflictions, especially scabies. It has been documented that many important phytochemicals are present in *A. officinalis*. Because of the presence of such phytochemicals, *A. officinalis* has been known to possess medicinal properties like analgesic, antioxidant, antimicrobial and anticancer activities (Khushi *et al*., 2016).

Materials and Methods

Sampling of Plant Material and Identification

The leaves and roots of *Avicennia officinalis* L. (Thame) belonging to the family Avicenniaceae were collected from Htein Tan Village, Ngaputaw Township, Ayeyarwady Region, Myanmar, during January and February, 2018. After collection, the scientific name of the plant was identified at the Botany Department, Pathein University. The collected fresh samples of the leaves of *Avicennia officinalis* L. (Thame), (TML), and the root of *Avicennia officinalis* L. (TMR), were washed and air-dried at room temperature for two weeks and then were ground into powder by grinder. The dried powdered samples were separately stored in air-tight containers.

Preparation of Various Crude Extracts

Dried powdered samples of TML and TMR (ca.300 g each) were separately percolated in 95 % EtOH (1 liters) with occasional shaking for one week and filtered. This procedure was repeated three times. The combined filtrate was concentrated under vacuum evaporator to obtain EtOH crude extract. The EtOH crude extract was partitioned between water and pet-ether, PE (60-80°C). After the removal of the aqueous layer, PE soluble extract was obtained. Finally, the aqueous layer was extracted with ethyl acetate, EtOAc. The EtOAc and aqueous layers were separated and concentrated. In this way, non-polar PE extract (PE soluble) and polar extracts (EtOAc, 95 % EtOH and H_2O soluble) from TML and TMR were obtained. These crude extracts were kept for separation of organic constituents and screening of antimicrobial activity, antioxidant activity and estimation of total phenolic content.

Isolation of some Organic Constituents

About 3.5 g of PE crude extract of TMR was separated by column chromatography over silica gel adsorbent by eluting with(PE only, PE:EtOAc, 90:1, 80:1, 60:1, 40:1, 20:1, 10:1 5:1,1:1 and finally EtOAc only). Successive fractions obtained were combined on the basic of their behaviour on TLC. Finally fourteen main fractions (F_1 to F_{14}) were collected. Fractions F_1, F_3, F_4, F_5, F_7, F_9, F_{10}, F_{11}, F_{12}, F_{13} and F_{14} were found as mixtures.

Fraction F_2 was evaporated, washed with PE, PE:EtOAc (20:1 v/v) and then crystallized from MeOH, yielded 0.02 % (55.2 mg) of compound-I as colourless crystals. Fraction F_6 was evaporated, washed with PE, PE:EtOAc (10:1 v/v) and then purified by recrystallization from MeOH, to give 0.09 % (218 mg) of compound-II as colourless needle shaped crystals. After removal of the solvents, fraction F_8 provided the solids materials. The solid materials were washed with PE, PE:EtOAc (5:1 v/v) and then purified by recrystallization from MeOH, to give 0.01 % (14.5 mg) of compound III as colourless needle shaped crystals.

Characterization of Isolated Compounds

The isolated compounds were characterized by determination of their physical properties: melting point, R_f values, solubilities in various solvents and some chemical properties: reaction with Liebermann-Burchard reagent, 5 % H_2SO_4, I_2 vapour, 5 % $FeCl_3$, Anisaldehyde sulphuric acid and ethanolic hydroxylamine hydrchloride.

Structural Identification of the Isolated Compounds

The isolated compounds were structurally identified by modern spectroscopic techniques such as UV-visible, FT IR and confirmed by comparison with the reported data and authentic compound.

Investigation of Some Bioactivities

(a) Screening of Antimicrobial Activity of Different Crude Extracts of the Leaves and Roots of Thame

The antimicrobial activity screening of different crude extracts *viz.*, pet-ether, ethyl acetate, ethanol and watery extracts of both TML and TMR was carried out against six species of microorganism *viz.*, *Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* by employing agar well diffusion method at the Pharmaceutical Research Department, Yangon, Myanmar.

Preparation of Samples

Various crude extracts of TML and TMR *viz.*, pet-ether, ethyl acetate, 95 % ethanol and watery extracts (1 g each) were dissolved in 1 mL of their respective solvents such as pet-ether (60-80 ºC), ethyl acetate, 95 % ethanol and distilled water.

Procedure

The antimicrobial activity of the crude extracts was performed by the agar well diffusion assay. The pathogenic test organisms were incubated in trypticase soy broth at an appropriate temperature for 24 h. Nutrient agar medium containing meat extract (0.5 g), peptone (0.5 g), sodium chloride (0.25 g), agar (1.5 g) and 100 mL of distilled water were placed in a breaker and the contents were heated for 30 minutes. The nutrient agar medium was put into sterilized conical flask and plugged with cotton wool and then autoclaved at 121 ºC for 15 minutes. After cooled down to 40ºC, one drop of suspended strain was inoculated to the nutrient agar with the help of a sterilized disposable pipette near the burner. About 20 mL of medium was poured into the sterilized petri dish and allowed to set the medium. Once solidified, the dishes were stored for 2 h in a refrigerator. Four wells of 10 mm diameter each were cut out in the inoculated agar to place extract samples to be tested. The volume of each extracted sample placed in each well was 0.2 mL. The petri dishes were then incubated at 37 ºC for 24 h, and the diameters of clear inhibition zone around the wells were measured. The experiment was done in triplicate and the mean diameter of the inhibition zone was calculated.

(b) Antioxidant Activity

The antioxidant activity of 95 % EtOH and H_2O extracts from both TML and TMR as well as the isolated compounds were determined by DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay.

The sample solutions were prepared by dissolving 2 mg of each sample in 10 mL of 95 % EtOH under vigorous shaking. The mixture solution was filtered and the stock solution was obtained. Desired concentrations (40.0, 20.0, 10.0, 5.0, 2.5, 1.25, 0.625 μg/mL) of sample solutions were prepared from this stock solution by serial dilution with appropriate amount of 95 % EtOH. Ascorbic acid was used as standard in 0.625-40 μg/ml solutions. The control solution (1.5 mL of 60 μM DPPH in 1.5 mL of EtOH solvent), blank solution (1.5 mL of sample solution in 1.5 mL of EtOH) and test sample solution (1.5 mL of sample solution in 1.5 mL of 60 μM DPPH) were prepared and allowed to stand at room temperature for 30 min. After 30 min, the absorbances of these solutions were measured at 517 nm by using UV-1800 spectrophotometer. Absorbance measurements were done in triplicate for each

solution and then mean values so obtained were used to calculate percent inhibition of oxidation by the following equation.

$$\% \text{ Oxidative Inhibition} = \frac{A_{DPPH} - (A_{Test\ sample} - A_{Blank})}{A_{DPPH}} \times 100$$

A_{DPPH} = absorbance of DPPH in 95 % EtOH solution
$A_{Test\ sample}$ = absorbance of (sample + DPPH) solution
A_{Blank} = absorbance of (sample+ 95 % EtOH) solution

(c) Determination of total phenol content of the Leaves and Roots of Thame samples by Folin-Ciocalteu method

Total phenolic content (TPC) in 95 % EtOH and H_2O extracts were determined by Folin-Ciocalteu method (Rekha *et al*., 2012). The test sample solution (1 mg/mL) was prepared by dissolving 1 mg of respective crude extract in 0.5 mL each of distilled water and methanol. Each crude extract samples (0.5 mL) was added into 5 mL of 10 % FC reagent and incubated for 5 min at room temperature. To each tube, 4 mL of 1 M Na_2CO_3 was added and the tubes were kept at room temperature for 30 min and the absorbance of reaction mixture was read at 765 nm. Similarly, standard Gallic acid solutions with the concentration of (1000, 500, 250, 125, 62.5 and 31.25 μg/mL) were prepared and the absorbance of each reaction mixture was read at 765 nm. A standard curve was plotting by the absorbance against concentration of Gallic acid. The blank solution was prepared as above procedure by using distilled water instead of sample solution. Total phenolic content was estimated as μg Gallic acid equivalents per milligram (μg GAE/mg) of crude extract.

Results and Discussion

Identification of Isolated compounds

Compound-I (An aliphatic ester)

An aliphatic ester, colourless crystals, 0.02 % yield, melting point=125-126 °C. The R_f = 0.61 in PE:EtOAc (40:1 v/v) solvent system, brick red spot on TLC by spraying with 5 % H_2SO_4 followed by heating, a yellow spot with iodine vapour, violet spot with anisaldehyde-sulphuric acid followed by heating, yellow colouration with Liebermann Burchard reagent, and gave deep red colouration on testing with ethanolic hydroxylamine hydrochloride reagent which is the test forester and so it could be classified as an ester. It is UV inactive.

FT IR (KBr, ν cm^{-1}): 2917 cm^{-1} and 2846 cm^{-1}(asym & sym. stretching vibration of $–CH_2$ and $–CH_3$), 1735 cm^{-1}(C=O stretching vibration of R-C(O)-O-R), 1475 cm^{-1} and 1461 cm^{-1}(C-H bending vibration of alkyl groups), 1169 cm^{-1}(C (O)-O-C stretching vibration of aliphatic ester), 727 cm^{-1} and 717 cm^{-1}($–(CH_2)_n$ bending vibration of alkyl groups)
All the results such as melting point, R_f value and chemical behavior on TLC, compound-I was classified as an aliphatic ester.

Compound-II (Lupeol)

Lupeol (A terpenoid), colourless needle shape crystals, 0.09% in yield, melting point= 213-214 ºC, R_f = 0.53 in PE:EtOAc (10:1 v/v) solvent system, purple colour spot on TLC chromatogram while spraying with 5 % H_2SO_4 followed by heating, a yellow spot with iodine vapour, violet spot with anisaldehyde-sulphuric acid followed by heating and violet colouration with Liebermann Burchard reagent and so it could be classified as a terpenoid. No absorption was observed in the readily accessible UV region. The melting point of compound-II (213-214 ^{0} C) was similar to that of lupeol (215-216 ^{0} C) (Saratha *et al.*, 2011). The R_f value of compound-II was found at 0.53 in the solvent system of PE:EtOAc

(10:1 v/v) and identical with that of authentic lupeol in any solvent system and they also give the same behavior on TLC.

FT IR (KBr, ν cm^{-1}): 3308 cm^{-1} (stretching vibration of O-H group of alcohol), 2943 cm^{-1} and 2873 cm^{-1}(asy. and sym. C-H stretching vibration of $–CH_2$- and $–CH_3$ groups), 1636 cm^{-1} (C=C stretching vibration). 1453 cm^{-1} and 1379 cm^{-1}(bending vibration of CH_2 and CH_3), 1042 cm^{-1} (C-O stretching vibration of cyclic alcohol)

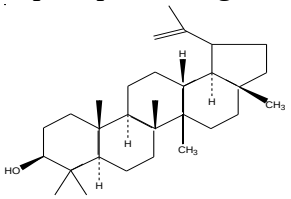
The observed FT IR spectral data of compound-II were also studied by comparing with those of reported lupeol (Jain and Bari et al., 2012) and are tabulated in Table 1.

Table 1. FT IR Spectral Data of Isolated Compound-II and Lupeol*

Wave number (cm^{-1})		Band assignment
Compound-II	**Lupeol***	
3308	3311	ν_{O-H} of alcoholic O-H
2943,2873	2946,2870	ν_{C-H} (asym & sym) of $–CH_2$ and $–CH_3$ group
1638	1638	$\nu_{C=C}$ olefinic group
1453, 1379	1464	δ_{C-H} of (asym & sym) of $–CH_2$, $-CH_3$ group
1042	1035	ν_{C-O} of CH-OH, cylic alcohol

***Jain and Bari *et al.*, 2010

From the physicochemical properties, melting point, R_f value and FT IR spectral data, isolated compound-II was identified as lupeol possessing the following molecular structure.



Lupeol($C_{30}H_{50}O$)

Compound –III (A triterpene)

A triterpene, colourless needle shape crystals, 0.006 % in yield, melting point= 135-136 °C, R_f value= 0.57 in PE:EtOAc (5:1 v/v) solvent system, brick red spot on TLC chromatogram while spraying with 5 % H_2SO_4 followed by heating, a yellow spot with iodine vapour, violet spot with anisaldehyde-sulphuric acid followed by heating and pink colouration with Liebermann Burchard reagent and so it could be classified as a triterpene. It is UV inactive.

FT IR (KBr, ν cm^{-1}): 3352 cm^{-1} (stretching vibration of O-H group of alcohol), 2921 cm^{-1} and 2853 cm^{-1}(asy. and sym. C-H stretching vibration of $–CH_2$- and $–CH_3$ groups), 1717 cm^{-1} (C=O stretching vibration of cyclic six membered ketone), 1636 cm^{-1} (C=C stretching vibration). 1450 cm^{-1} and 1375 cm^{-1}(bending vibration of $-CH_2$ and $-CH_3$), 1041 cm^{-1} (C-O stretching vibration of cyclic alcohol), 877 cm^{-1} (bending vibration of =CH) All of above results indicated that compound III may be classified as a triterpene compound.

***In vitro* Antimicrobial activity of some crude extracts from the leaves and roots of Thame by agar well diffusion method**

Among the tested crude extracts of TML, EtOAc extract, EtOH extract and PE extract exhibited antimicrobial activity (inhibition zone diameter range, ID: 11~ 33 mm) against all the tested microorganisms but watery extract showed the least activity against all the tested microorganisms except *Escherichia coli* (ID: 11 mm~13 mm). It was found that EtOAc extract of TML exhibited the most pronounced antimicrobial activity (ID: 22 mm ~ 33 mm). In the case of TMR, EtOAc extract showed the most potent antimicrobial activity (ID: 25~35 mm) against all the tested microorganisms followed by EtOH extract (ID: 13~27 mm), PE extract (ID: 11~13 mm) against all the tested microorganisms except

Escherichia coli and *Pseudomonas aeruginosa* but watery extract showed the least activity (ID: 11~12 mm) against *Bacillus pumilus, Candida albicans,* and *Staphylococcus aureus*. Among the tested crude extracts of TML and TMR, EtOAc extract of TMR showed the most potent activity (ID: 25 mm ~ 35 mm) against all the tested microorganisms. The observed microbial inhibition zone diameters are summarized in Table 2.

Table 2. Inhibiton Zone Diameters of Various Crude Extracts from the Leaves and Roots of Thame Against Six Species of Microorganisms by Agar Well Diffusion Method

Microorganisms	Types of Microorganisms	Sample	Inhibition Zone Diameters (mm)			
			Watery	PE	EtOAc	95 % EtOH
Bacillus pumilus	Gram (+) ve	TML	13	11	26	23
		TMR	12	12	30	14
Bacillus subtilis	Gram (+) ve	TML	12	14	33	16
		TMR	-	12	30	17
Candida albicans	Fungus	TML	11	13	29	17
		TMR	12	13	30	27
Escherichia coli	Gram (-) ve	TML	-	11	22	18
		TMR	12	-	26	17
Pseudomonas aeruginosa	Gram (-) ve	TML	12	11	27	18
		TMR	-	-	25	13
Staphylococcus aureus	Gram (+) ve	TML	13	11	25	16
		TMR	11	11	35	14

Agar well diameter = 10mm, 10mm~ 14mm (+)
15mm~19mm (++), 20mm above (+++)
(-) = no zone of diameter

TML = Thame leaves
TMR = Thame roots

Antioxidant Activity

The antioxidant activities of 95 % EtOH and H_2O extracts of TML and TMR as well as the isolated compounds were studied by DPPH (1,1-diphenyl-2-pricryl hydrazyl) free radical scavenging UV spectrophotometric assay method. The antioxidant activity was expressed as 50 % oxidative inhibitory concentration (IC_{50}). From the results, it was found that the antioxidant activity of both 95 % EtOH extract of TMR (IC_{50}: 8.25 µg/mL) and water extract of TMR (IC_{50}: 8.35 µg/mL) were higher than that of 95 % EtOH and watery extracts of TML (IC_{50}: 16.10 and 19.95 µg/mL). The lower IC_{50} values show the higher radical scavenging activity and so it was found that antioxidant activity of isolated compounds was in the order of compound-II, lupeol (IC_{50} = 15.51 µg/mL) > compound-III, a triterpene (IC_{50} = 17.05 µg/mL) > compound-I, an aliphatic ester (IC_{50} = 35.33 µg/mL). The % oxidative inhibition and IC_{50} values of watery and 95 % EtOH extracts of TML and TMR as well as the three isolated compounds are summarized in Tables 3 and 4. From the observation, it can be inferred that although all the tested crude extracts and the isolated compounds showed antioxidant activity, which was lower than standard ascorbic acid (IC_{50} = 1.17 µg/mL).

Table 3. Percent Oxidative Inhibition and IC_{50} Values of Crude Extracts from the Leaves and Roots of Thame and Standard Ascorbic Acid

Extracts	Oxidative Inhibition %(mean ± SD) in different conc: (µg/mL)							IC_{50} (µg/mL)
	0.625	1.25	2.5	5	10	20	40	
TML(watery)	29.13±4.87	34.69±0.49	35.19±1.26	38.40±3.07	39.45±1.88	50.18±0.045	55.78±0.002	19.95
TML(EtOH)	32.10±1.77	33.52±0.70	34.70±1.40	36.86±0.60	40.51±1.68	55.67±0.008	61.45±0.001	16.10
TMR(watery)	32.59±0.95	33.15±2.15	37.79±0.47	42.18±0.54	53.68.±2.17	62.12±0.027	67.35±0.006	8.35
TMR(EtOH)	35.56±1.99	33.09±6.10	38.40±1.30	43.23±0.85	50.83.±0.67	66.76±0.004	69.45±0.004	8.25
Ascorbic acid	14.04±2.09	54.83±2.48	72.44±3.83	77.13±1.47	87.4±2.3	91.12±2.24	95.32±2.19	1.17

Table 4. Percent Oxidative Inhibition and IC_{50} Values of Isolated Compounds and Standard Ascorbic Acid

Compounds	Oxidative Inhibition %(mean ± SD) in different conc: (µg/mL)							IC_{50} (µg/mL)
	0.625	1.25	2.5	5	10	20	40	
Compound-I	11.43±1.35	15.56±2.15	20.33±0.33	25.54±0.57	34.32±2.65	41.89±2.21	50.31±0.08	35.33
Compound-II	25.43±1.67	32.56±0.65	35.16±1.78	38.18±0.43	43.33±0.97	55.23±1.22	59.45±1.98	15.51
Compound-III	21.22±0.43	30.12±1.34	33.56±1.88	41.22±1.45	45.12±2.87	51.22±0.87	55.34±2.73	17.05
Ascorbic acid	14.04±2.09	54.83±2.48	72.44±3.83	77.13±1.47	87.4±2.3	91.12±2.24	95.32±2.19	1.17

Total Phenolic Content of Various Crude Extract from the Leaves and Roots of Thame by Folin-Ciocalteu (FC) Method

The total phenolic content in EtOH and watery extracts of both TML and TMR samples were estimated by Folin-Ciocalteu method. Gallic acid was used to construct a standard calibration curve for total phenol. Total phenolic content (TPC) was expressed as microgram of Gallic acid equivalent per milligram of crude extract (µg GAE/mg). Standard calibration curve of Gallic acid was prepared by measuring the absorbance of various amount of Gallic acid in the range of 1000 to 31.25 µg/ml. As shown in Table 5 and Figure 3, the prepared Gallic acid standard curve gave a straight line, which obeyed Beer's Lambert law, with the linear regressive equation of $y = 0.0038x + 0.2232$, $R^2 = 0.9995$. From the standard curve it was found that with the increase in concentration of Gallic acid, the complexation would also increase and as a result increase in the absorbance is observed.

The results of total phenolic content of various crude extracts from TML and TMR were presented in Table 6. From the result, TPC in both EtOH extract (350.95 µg GAE/mg) and watery extracts (277.26 µg GAE/mg) of TMR was found to be much higher than those in both extract of TML (166.74 µg GAE/mg and 103.58 µg GAE/mg).

Table 5. The Absorbance of Gallic Acid Standard Solution at λ_{max}765 nm

No	Concentration (µg/mL)	Absorbance at λ_{max} 765 nm
1		0.35
2	31.25	0.44
3	62.5	0.72
4	125	1.19
5	250	2.05
6	500	4.00
	1000	

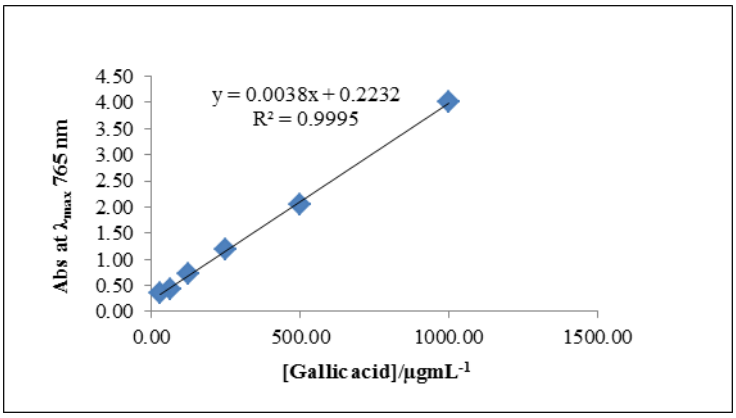


Figure 3. A plot of Gallic acid standard curve

Table 6. Total Phenolic Content (TPC) of 95 % EtOH and watery extracts from TML and TMR

No	Samples	TPC(µgGAE/mg±SD)
1	EtOH (TML)	166.74±0.033
2	Watery(TML)	103.58±0.011
3	EtOH (TMR)	350.95±0.030
4	Watery(TMR)	277.26±0.002

Conclusion

The present study on *Avicennia officinalis* L. (Thame) provides the following information.

By using silica gel column chromatographic separation, three compounds, compound-I (an aliphatic ester, colourless crystals, 0.02 %, mpt = 125-126 °C), compound-II (lupeol, colourless needle shape crystals, 0.09 %, mpt = 213-214 °C) and compound-III (triterpene, colourless needle shape crystals, 0.006 %, mpt = 135-136 °C) were isolated from PE extract of TMR. The antimicrobial activity of PE, 95 % EtOH, EtOAc and watery extracts from both samples were screened on *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* by agar well diffusion method. Among the tested crude extracts of both TML and TMR, EtOAc extract exhibited the highest antimicrobial activity followed by EtOH extract and PE extract against all the tested

microorganisms, but watery extract showed the least activity. In the *in vitro* antioxidant activity screening of 95 % EtOH and watery extract of TML and TMR as well as the isolated compounds by DPPH free radical scavenging assay, both extracts of TMR showed higher antioxidant activity (IC_{50}: 8.25~8.35 μg/ml) than that of TML (IC_{50}: 16.10 ~ 19.95 μg/ml). Although all isolated compounds possess antioxidant potency, which was lower than standard ascorbic acid. The TPC contents in both watery and EtOH extracts of TMR were found to be much higher than that in TML.

The results suggest that phenolic compounds and other phytoconstituents present in the plant contribute significantly to the antioxidant activity and so application of the plants as a remedy may exert several beneficial effects by virtue of its antioxidant activity. The results also confirm the validity of the use of the leaves and root of *Avicennia officinalis* as traditional medicines and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens.

Acknowledgements

I would like to acknowledge the Department of Higher Education (lower Myanmar), Ministry of Education, Myanmar, for the provision of opportunity to do this research. My deepest gratitude is extended to Dr Win Naing, Rector, Dagon University, for allowing me to participate in the 2nd Myanmar-Korea Conference on Useful Plants. I am very grateful to Dr Myat Myat Moe, Professor and Head, Department of Botany, Dagon University and the board of Editors, Dagon University Research Journal, Dagon University, for giving me the opportunity to submit the research article and invaluable suggestions.

References

Ganesh, S. and J.J. Vennila. (2011). " Phytochemical Analysis of *Acanthus ilicifoliu*s and *Avicennia officinalis* by GC-MS", *Research Journal of Phytochemistry*, **5**(1): 60-65

Jain, P.S. and S.B. Bari. (2010). "Isolation of lupeol, stigmasterol and campesterol from petroleum ether of woody stem of *Wrightia Tinctoria*". A*sian Journal of Plant Sciences*, **9** (3), 163-167

Job, N., S. Manomi and P. Rosamma.(2015). "Isolation and characterization of endophytic fungi from *Avicennia officinalis*". *International Journal of Research in Biomedicine and Biotechnology*, **5** (1), 4-8

Khushi, S., M. Hasan, MD. and A. S. M. Monjur-Al-Hossain. (2016). "Medicinal activity of *Avicennia officinalis*: Evaluation of phytochemical and pharmacological properties*". Saudi Journal of Medicical and Pharmaceutical Sciences*, **2** (9), 250-255

Rekha, C., and M. Poornima. (2012). "Ascorbic acid, total phenolic content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits". *International Journal of Pharmaceutical Research*, **1** (2), 303-310

Saratha, V., S. I. Pillai and S. Subramanian. (2011). "Isolation and characterization of lupeol, a Triterpenoid from *calotropis gigantea* Latex". *Int. J. Pharm. Sci. Rev, Res.*, **10**, 54-57

Sharief, MD. N., A.S.P.S. Veni and V. U. M. Rao. (2014). "Quantification of Phytochemicals and Antibacterial Activity of Fruit Extract of *Avicennia officinalis*". *Asian Journal of Pharmaceutical and Clinical Research*, **7**(2), 127-130

Thatoi, H., D. Samantaray, and S. K. Das. (2016). "The genus *Avicennia*, a pioneer group of dominant mangrove plant species with potential medicinal values: a review". *Frontiers in Life Science*, **9** (4), 267-291