Chemical and Antimicrobial Investigation from the Bark of *Lagerstroemia speciosa* Linn.

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Abstract

Myanmar is one of the most beautiful countries in the world, blessed with supreme natural environment and resources as well as religious and generous peoples. Peoples in Myanmar have inherited their own traditional medicine and practiced it for over million years of history. Recently medicinal herbalism became more popular than before in the world. Most of the people use the traditional medicinal plants for the treatment of diseases. Therefore, the present work was focused on the bark of the *L. speciosa* Linn. (Pyin-ma) with preliminary phytochemical Investigation, determination of nutrient values and screening of antimicrobial activity. Three isolated compounds (steroid, tannin and terpenoid) were identified by melting point determination, thin layer chromatography and modern spectroscopic method (FT IR). In *vitro* screening of antimicrobial activity was carried out by using agar well diffusion method. It revealed that all the crude extracts (Pet-ether extract; 15 mm~19mm, H_2O extract; 15 mm~20 mm, EtOH extract; 23 mm~26mm and EtOAc extract; 15 mm~20 mm) of plant sample possessed significant antimicrobial activity against six microorganisms such as *Bacillius subtilis, Staphylococcus aureus, Pseudomonas areuginosa, Bacillus pumalis, Candida albican and Escherichia coli*. Only one isolated compound II (tannin); (13mm~15mm) showed the antimicrobial activity against the tested microorganisms.

Keywords: *Lagerstroemia speciosa* L., phytoconstituents, nutrient values, antimicrobial activity

Introduction

The important of medicinal plants and traditional health systems in solving the health care problems of the world is gaining an increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin (Farsworth, 1991). Therefore, in this research work, *Lagerstroemia speciosa* L. (Pyin-ma) was selected for systematic investigation on its antimicrobial activity by using in *vitro* method. The organic constituents present in this selected medicinal plant were isolated and their antimicrobial activities were also investigated.

Materials and Methods

General experimental procedures

The FT IR spectra of all isolated compounds were taken with KBr pellets and recorded on Shimadzu FT IR 8400 Fourier Transform Infrared Spectrometer. The antimicrobial activity of extracts and isolated compoundswere evaluated using agar well diffusion method.

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Plant materials

Lagerstroemia speciosa Linn.was collected from Insein Township, Yangon Division. The sample was authenticated by botanist of Department of Botany, University of Yangon.

Family : Lythraceae
Botanical name : *Lagerstroemia speciosa* Linn.
English name : Queen's flower, Pride of India
Myanmar name : Pyin-ma
Common name : Banaba

Figure 1. Photograph of the *Lagerstroemia speciosa* Linn. (Pyin-ma)

Procedure for Extraction and Isolation of Three Compounds

A glass chromatographic column (60 x 2.5 cm) with a tap attached was clamped so that it was perfectly vertical. The column was packed using solvent system (PE: EtOAc, 40:1 v/v). Ethyl acetate extract 3 g was subjected into slica gel column. Gradient elution was performed successively with (PE: EtOAc, 19:1, 15:1, 9:1, 7:1, 4:1, 2:1 v/v) and successive fractions obtained were monitored by TLC. The fractions which gave the similar appearance on TLC were combined and finally, four main fractions F 1 to F 4 were collected. After removal of the solvents, the fractions F 1, F 3 and F 4 provided solid substances. The solid materials obtained from these fractions were washed and purified with suitable solvents such as pet-ether, ethyl acetate, methanol etc. The three isolated compounds were weighted, their yield percent were calculated and the results were recorded. The colours of the compounds were also noted down. Compound I [colourless crystal, 0.02 % yield, R_f = 0.50; PE : EtOAc (7:1 v/v), m.pt (168.5 °C)], Compound II [white crystal, 0.029 % yield, R_f = 0.48; PE : EtOAc (1:5 v/v), m.pt (253°C)] and Compound III [yellow crystal, 0.008 % yield, R_f = 0.52; PE : EtOAc (1:2 v/v), m.pt (283.5 °C)] were obtained respectively. The detailed procedure was shown in this following flow sheet (figure-2).

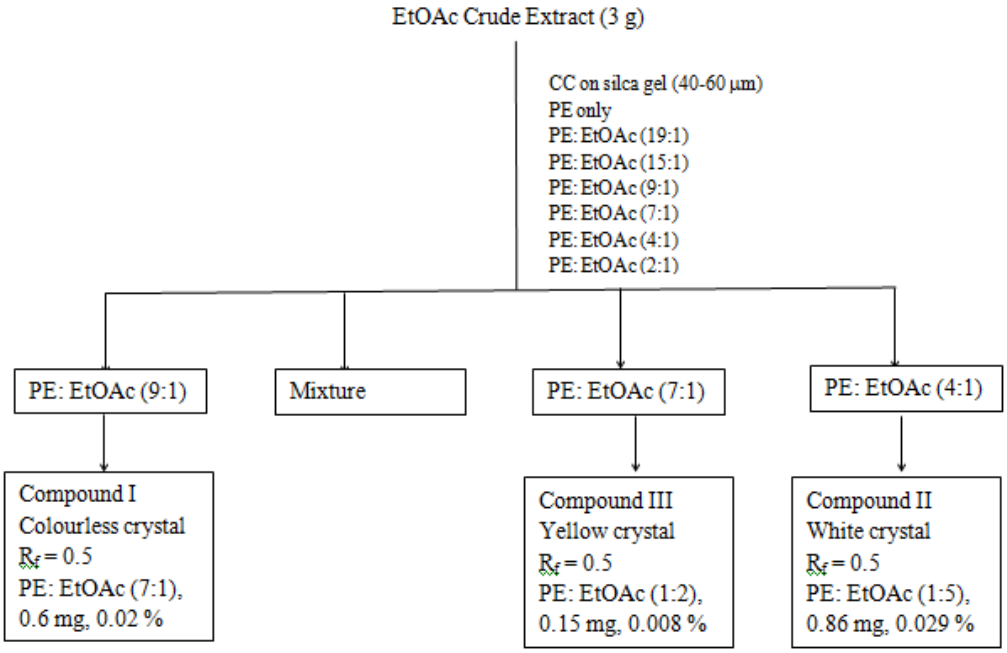

Figure 2. Procedure for the isolation of phytochemical constituents from EtOAc crude extract of Pyin-ma bark

Results and Discussion

Identification of Isolated Compounds I, II and III

Isolated compound I was identified by the modern spectroscopic method, FT IR spectroscopy. In the FT IR spectrum, the bands at 3633-3288 cm^{-1} appeared due to the presence of O-H stretching of alcohols and phenols. The band at 3047 cm^{-1} appeared due to the presence of C-H stretching of aromatic compounds. The absorption band at 2947 cm^{-1} was attributed to C-H stretching of CH_2 and CH_3. The bands at 1553, 1457 cm^{-1} represent the C=C stretching of phenyl group. The present of C-O stretching of cyclic alcohol and C=C stretching of 1, 2, 4- trisubstitued benzene ring was indicated by the bands at 1217, 1056, 429, 877 cm^{-1} respectively. Therefore, Compound I may be steroid compound.

The FT IR spectrum of compound II, the band appeared at 3517 cm^{-1}, 2559 cm^{-1} showed the presence of carboxylic acid group (-COOH). The peaks at 3356 and 3241 cm^{-1}were attributed to OH stretching vibration of phenolic –OH group. The absorption bands at 1722 and 1595 cm^{-1} represent the C=O stretching of carbonyl group and C=C stretching of olefinic groups respectively. The presence of in plane bending vibration and C-O stretching of cyclic alcohols was indicated by the bands at 1371 and 1077 cm^{-1} respectively. The band at 962 cm^{-1} showed the C-H bending vibration. So, Compound II may be tannin compound.

In the FI IR spectrum, the strong and broad bands appeared at 3448 and 3317 cm^{-1} showed the presence of OH stretching vibration of carboxylic acid group. The peak at 2916 cm^{-1} was attributed to C-H stretching vibration of $–CH_3$ and $–CH_2$ groups. The absorption band at 1728 cm^{-1} confirmed that the compound III contains –COOH group which was appeared due to C=O stretching of carboxylic acid group. The band at 1628 cm^{-1} appears due to C=C stretching vibration of olefinic group. The absorption peaks at 1458 and 1381 cm^{-1} are probably due to asymmetric C-H bending, The C-O stretching vibration of cyclic alcohol was observed by the appearance of absorption at 1180 cm^{-1} and 1057 cm^{-1}. The compound II may be terpenoid.

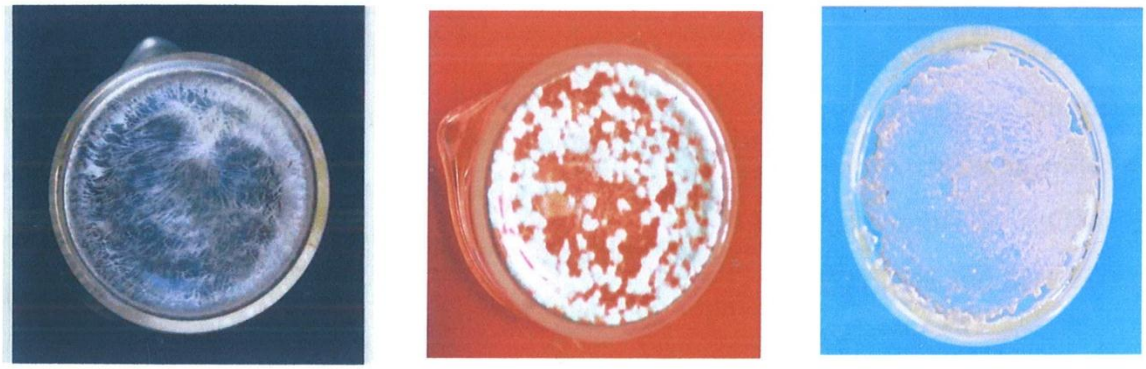

Figure 3 Photographs of crystalline forms of isolated compound I, II and III

Table 1. Classification of Isolated Compounds

No.	Compound	Solvent	Reagent	Observation	Remark	R_f
1	Compound I	PE	I_2 vapour	Brown		
			Libermann	Blue	Steroid	0.5
			Buchard, Δ, 5 % H_2SO_4	Purple		
2	Compound II	EtOAc	1 % FeCl3	Deep Blue	Phenolic OH	
			Mg/HCl	No Pink colour	Flavonoid absent	0.48
			KI	Pink	Tannin	
			5 % H_2SO_4	Yellow	Tannin	
3	Compound III	PE	I_2 vapour			
			5 % H_2SO_4	Yellow		
			Libermann	Purple	Terpenoid	0.52
			Buchard, Δ	Pink		

Determination of Nutrient values

Some chemical analysis such as moisture, ash, fibre, protein, fat and carbohydrates were quantitatively determined and the results obtained are summaried in Table 3.

Moisture Content

The moisture content was determined by electric oven method (Raghuramulu, 1983). The moisture content was found to be (7.87 %) which is less content, therefore the sample powder can stored for a long period without any growth of mould.

Ash Content

The ash content was determined by the method given in "The Chemical Analysis of Foods". The ash content of sample was found to be (4.59 %).

Crude Fibre Content

Crude fibre was determined by crude fiber estimation method (Raghuramulu, 1983) and (31.12 %) of crude fibre content was found in this sample.

Protein Content

The nitrogen content was determined by Macro Kjedahl method and then this nitrogen content was multiplied by 6.25 to give protein content (0.95 %).

Fat Content

Fat content in the dried powdered bark was determined by the Soxhlet extraction method using petroleum ether. The percent content of fat was resulted to be (0.14 %).

Table 2. Results of Nutrient Values for *Lagerstoremia speciosa* L. (Pyin-ma)

No.	Principal Contents	Nutrient Value Results (%)
1	Moisture Content	7.87
2	Ash Content	4.59
3	Fibre Content	31.12
4	Protein Content	0.95
5	Fat Content	0.14
6	Carbohydrate Content	55.33

Screening of Antimicrobial Activity

Antimicrobial activities of the plant crude extracts (PE, 70 % EtOH, EtOAc and H_2O) and two isolated compounds; Compound I (steroid) and Compound II (tannin) were investigated against six species of microorganisms by employing agar well diffusion method (Mounyr, 2016). The samples were tested on six species of bacteria including *Bacillius subtilis*, *Staphylococcus aureus*, *Pseudomonas areugionosa*, *Bacillius pulmalis*, *Candida albican* and *Escherichia coli*. The results were shown in figure 4, 5 and table 2. In *vitro* screening of antimicrobial was carried out by using the agar well diffusion method. It revealed that all the crude extracts (PE extract; 15 mm~16 mm, EtOH extract; 23 mm~26mm, EtOAc extract; 15 mm~20 mm, H_2O extract; 15 mm~20mm) of plant sample possessed significant activityagainst microorganisms such as *Bacillius subtilis*, *Staphylococcus aureus*, *Pseudomonas areuginosa*, *Bacillus pumalis*, *Candida albican* and *Escherichia coli*. Only one isolated compound II, tannin (13mm~15mm) shows the antimicrobial activity against the tested microorganisms.

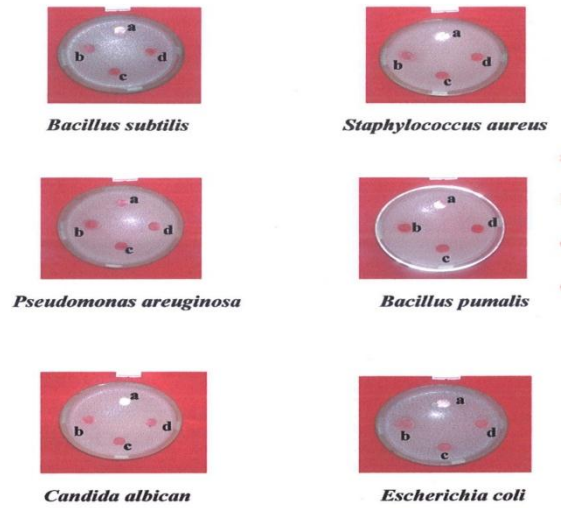

Figure 4. Effect of Isolated compounds on six microorganisms

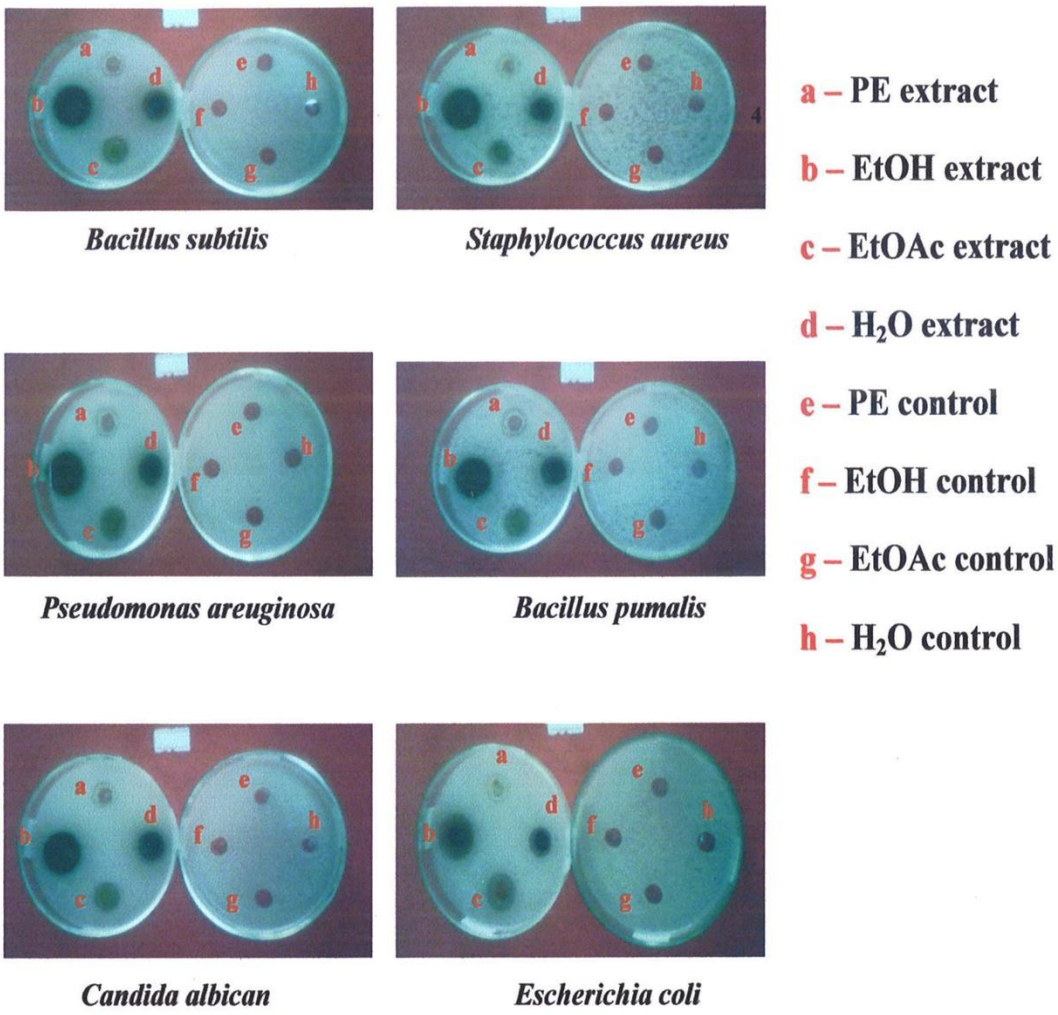

Figure 5. Effect of Pyin-ma bark extracts on six microorganisms

Table 3. Results for Antimicrobial Activity of Crude Extracts and Isolated Compounds from *Lagerstroemia speciosa* L. (Pyin-ma) Bark

Samples	Solvents	Organisms					
		1	**2**	**3**	**4**	**5**	**6**
Pyin-ma bark	PE	15 mm (++)	15 mm (++)	16 mm (++)	16 mm (++)	16 mm (++)	15 mm (++)
	EtOH	24 mm (+++)	26 mm (+++)	24 mm (+++)	24 mm (+++)	24 mm (+++)	23mm (+++)
		17 mm (++)	15 mm (++)	16 mm (++)	16 mm (++)	15 mm (++)	20 mm (+++)
	H_2O	20 mm (+++)	15 mm (++)	18 mm (++)	16 mm (++)	16 mm (++)	15 mm (++)
Compound I	PE	-	-	-	-	-	-
Compound II	MeOH	13 mm (+)	14 mm (+)	14 mm (+)	14 mm (+)	13 mm (+)	15 mm (++)

1. *Bacillus subtilis*
2. *Staphylococcus aureus*
3. *Pseudomonas areuginosa*
4. *Bacillus pumilis*
5. *Candida albican*
6. *Escherichia coli*

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

Conclusion

From the present study of physicochemical and antimicrobial activity investigations on the bark of the *Lagerstroemia speciosa* L. (Pyin-ma),it is potentially useful for the medicinal formulation to treat burn infection, fever, food poinsoning otamycosis, agonal infection, diarrheoa and skin diseases since. It has bacterial action against *Staphylococcus aureus* responsible for fever, food poisoning, skin disease, *Pseudomonas aerugionosa* for burn infection, *Bacillus substilis* for agonal infection and *Escherichia coli* responsible for diarrhea.

References

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