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Biological Activities of *Peltophorum pterocarpum* of Myanmar and Isolation of Secondary Metabolites from its Flowers

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Abstract

The isolation of petroleum ether and ethyl acetate crude extracts of *Peltophorum pterocarpum* afforded physcion (**1**), β-sitosterol (**2**), naringenin (**3**), gallic acid (**4**), bergenin (**5**) and 11-O-acetylbergenin (**6**). The isolated compounds **1–6** were identified using UV, FT IR, ^{1}H NMR, ^{13}C NMR, 2D NMR and EI MS as well as comparison with reported data. The antimicrobial activity of pet-ether, ethyl acetate, ethanol and water extracts from flowers, leaves and bark of *P. pterocarpum* was screened against the six microorganisms *Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Escherichia coli* using the agar well diffusion method. All the crude extracts exhibited inhibition zone diameters ranging between 12 mm and 20 mm. The watery and ethanol extracts were free from acute toxicity on albino mice (20–30 g). In the antioxidant DPPH assay, the IC50 values of ethanol and watery extracts of all parts ranged between 0.65 and 2.44 µg/mL, having a pronounced antioxidant property. In the resazurin microplate assay, ethanol extract of flowers, leaves and bark exhibited 15, 62 and 72 % cell death rates on colon cancer stem cells and 8, 27 and 23 % on human lung fibroblast with a 1.5 mg/mL dose.

Keywords:
Peltophorum pterocarpum, acute toxicity, antimicrobial activity, antioxidant DPPH assay, resazurin microplate assay

1. Introduction

The study of traditional medicinal plants and their therapeutics play a very important role in the health care system of Myanmar because most of its population lives in rural areas and has been using traditional medicine for centuries. The plant kingdom constitutes an invaluable source of secondary metabolites which may be important due to their biological properties and their potential use in medicine. In this study, our attention has been focused on *Peltophorum pterocarpum* (DC.) K. Heyne that belongs to the family Caesalpiniaceae and is known as Pan-mèzali (PMZL) in Myanmar. The plant PMZL has been used for the treatment of insom-

nia, skin troubles, ringworm, constipation, stomatitis, dysentery, gargles and tooth powder, eye lotion and embrocation for muscular pains and sores due to its antimicrobial, anti-fungal, anti-inflammatory, antioxidant, anti-diabetic and cardiotonic activities (Oudhia 2003). It is used as an astringent to cure or relieve intestinal disorders after pain at childbirth, sprains, bruises and swelling in traditional medicine (Orwa et al. 2009). The plant is found in tropical Southeast Asia and Northern Asia, Sri Lanka, Thailand, Vietnam, Indonesia, Malaysia, Papua New Guinea, the Philippines and Northern Australia (Sohail 2007). The flower of this plant has been reported to contain phenolic compounds, tannins, anthraquinones, flavonoids and steroids (Jain et al. 2011). Since there is no scientific chemical evaluation of the PMZL flowers of Myanmar, its chemical and biological activities have been investigated in this paper, in which the isolation and identification of six compounds of the flowers of *P. pterocarpum* are reported. In order to compare the potency of different parts of the plant, antimicrobial activity, acute toxicity, antioxidant activity and cytotoxicity of the flowers, leaves and bark of this plant are also investigated.

2. Results and Discussion

2.1 Structure Elucidation of Isolated Compounds

Compound **1** was isolated as an orange crystal and its melting point was found to be 208–209°C. It is soluble in pet-ether, chloroform, ethyl acetate, acetone, methanol and ethanol, but insoluble in water. It is UV active and the R_f value was observed to be 0.5 (PE:EtOAc, 13:1 v/v). Compound **1** was classified as an anthraquinone compound, since the reaction of compound **1** with a 10% ammonia solution produced purple coloration (Akinjogunla 2010). It produced a reddish brown color when treated with a 10% $FeCl_3$ solution, indicating the presence of phenolic OH group. It also provided a yellow color on TLC when treated with 5% H_2SO_4 followed by heating. Its molecular formula, $C_{16}H_{12}O_5$, was determined by EI MS. The UV absorptions maxima at 223, 254, 265 and 286 nm in MeOH indicated the presence of a conjugated double bond due to $\pi \rightarrow \pi^*$ transition. The FT IR spectrum indicated the presence of hydroxyl (3441 cm^{-1}), carbonyl (1743 cm^{-1}) and phenyl (1627, 1566, 1473 cm^{-1}) groups. ^1H NMR spectrum of compound **1** displayed sharp singlets (δ_H 2.41, 3.86) assignable to methyl and methoxy protons. Two doublets (δ_H 7.06, 7.61, each J = 1.6 Hz) were assigned to two meta-coupled aromatic protons. Another two doublets (δ_H 6.67, 7.35) were also assigned as two *meta*-coupled aromatic protons. These couplings were confirmed by ^1H ^1H COSY correlations. Two singlets appearing at δ_H 12.10 and 12.30 were assigned as hydrogen bonded hydroxyl protons, since they appeared at downfield. According to ^1H NMR spectral data, compound **1** contained two meta-coupled aromatic rings connecting with two carbonyl groups, and was thus ascribable as a substituted anthraquinone compound. Furthermore, ^{13}C NMR spectral data of compound **1** showed the presence of 16 carbon signals. The

signals at δ_C 182.0 and 190.8 were ascribed to two carbonyl carbons, and 12 signals appearing between δ_C 106.8 ~ 166.5 were attributed to aromatic carbons. Hence, ^{13}C NMR spectral data assisted compound **1** as an anthraquinone compound. On the basis of HSQC spectral data, the corresponding proton and carbon signals were assigned. The HMBC correlations indicated the attachment of methyl (δ_H 2.41) at C-6 (δ_C 148.4), the methoxy (δ_H 3.86) at C-3 (δ_C 166.5), and two hydroxyl (δ_H 12.30, 12.10) at C-1 and C-8 (δ_C 165.2 ppm, 162.5) respectively. On the basis of 1D and 2D NMR spectral data, the chemical structure of compound **1** (Chart 1) was identified as physcion (Chen 2012).

Compound **2** (R_f = 0.5, PE:EtOAc, 5:1 v/v, 0.008% yield) was obtained as a colorless needle. It is UV inactive and its melting point was observed to be 138–140 °C. It is soluble in pet-ether, chloroform, ethyl acetate and acetone, but insoluble in ethanol and water. In the Liebermann-Burchard test, it produced a green color. Moreover, the TLC behavior of compound **2** was found to be identical with that of β-sitosterol in any solvent system. Therefore, compound **2** was identified as β-sitosterol and the structure was shown in Chart 1.

Compound **3** (R_f = 0.49, PE:EtOAc, 2:1 v/v, 0.009% yield) was isolated as a pale yellow amorphous and is UV active. The molecular formula $C_{15}H_{12}O_5$ was assigned from the molecular ion peak $[M]^+$ at m/z 272 of the EI MS spectrum. Its melting point was determined to be 250–251 °C. It was soluble in chloroform, ethyl acetate, acetone, methanol and ethanol, but insoluble in pet-ether and water. According to the pink coloration, which was observed after treatment with concentrated HCl and Mg ribbon, and the brown coloration with a 10% $FeCl_3$ solution, compound **3** could be confirmed as flavonoid. The UV spectral data of compound **3** provided the absorption maxima λmax at 290 nm ($\pi \rightarrow \pi^*$) and 328 nm ($n \rightarrow \pi^*$) in MeOH, indicating the presence of a conjugated double bond. The FT IR spectral data of compound **3** indicated the presence of the alcoholic OH and phenolic OH group (3615, 3489, 3309 cm^{-1}), α,β- unsaturated carbonyl group (1689 cm^{-1}), and phenyl ring (1578 cm^{-1}). The ^1H NMR spectral data of compound **3** exhibited one doublet (δ_H 5.84, J = 2.4 Hz) assignable as two meta-coupled phenyl protons on ring A of the flavonoid skeleton. Two doublets (δ_H 7.21, J = 8.4 Hz; 6.79, J = 8.2 Hz) were due to four phenyl protons situated at the 2′, 3′, 5′ and 6′ position of ring B. A doublet of doublet (δ_H 5.23, J = 12.9, 2.8 Hz) was attributed to an oxygenated methine proton, and another two doublets of doublets [δ_H 3.10 (J = 17.1, 12.9 Hz); 2.66 (J = 17.1 and 2.8 Hz)] were due to two methylene protons adjacent to the methine proton of ring C. Absolute configuration at C-2 was confirmed to be *S* based on the coupling constants of the methylene protons $H_{3\alpha,3\beta}$ ($J_{ax\text{-}ax}$ = 12.9 Hz and $J_{ax\text{-}eq}$ = 2.8 Hz). In addition, the ^{13}C NMR spectral data of compound **3** indicated the presence of 13 peaks corresponding to 15 carbons which are attributed to one oxygenated methine carbon (δ_C 78.9), one methylene carbon (δ_C 43.1) adjacent to carbonyl carbon, one carbonyl carbon (δ_C 195.7), three oxygenated aromatic carbons and nine aromatic carbons ranging between

δ_C 95.4 and δ_C 166.4. All of the above-mentioned ^1H and ^{13}C NMR spectral data were consistent with those of reported naringenin (5,7,4′-trihydroxyflavanone) (Jain and Mittal 2012) and the structure was illustrated in Chart 1.

Compound **4** (R_f=0.41, PE:EtOAc, 1:4 v/v, 0.007% yield) was obtained as a pale yellow needle and was UV active. Its melting point was 236–238 °C (PE:EtOAc, 1:5 v/v). It was soluble in chloroform, ethyl acetate, acetone, methanol and ethanol, but insoluble in pet-ether. Compound **4** produced a deep blue coloration with 10% $FeCl_3$, showing the presence of the phenolic OH group. Since it produced a brown coloration with KI and did not produce a pink color with concentrated HCl and Mg ribbon, compound **4** was assumed to be tannin. The UV spectral data of the compound **4** revealed the absorption maxima λmax (223 nm, 272 nm) in MeOH due to π→π* transition. The FT IR spectrum of compound **4** indicated the presence of hydroxyl groups of carboxylic acid and phenol (3550, 3473, 3371, 3273 cm^{-1}), as well as carbonyl (1699 cm^{-1}) groups. The ^1H NMR spectrum of compound **4** showed only one signal excluding solvent peaks in the aromatic region. The singlet peak (δ_H 7.04) was assigned to be two aromatic protons. On the other hand, the ^{13}C NMR spectral data of compound **4** showed 5 peaks corresponding to six aromatic carbons (δ_C 110.3, 121.8, 139.5, 146.2) and one carbonyl carbon (δ_C 170.4). These resonances were also consistent with those of literature values (Gohar et al. 2003). Compound **4** was thus readily identified as gallic acid (Chart 1).

Compound **5** (R_f =0.43, $CHCl_3$:MeOH, 5:1 v/v, 0.018% yield) was isolated as a colorless crystal and was UV active. From the EI MS spectral data, its molecular formula, $C_{14}H_{16}O_9$, was assigned based on the molecular ion peak appearing at m/z 328. Its melting point was found to be 238–239 °C. It was soluble in chloroform, ethyl acetate, acetone, methanol and ethanol, but insoluble in pet-ether and water. Compound **5** also contained the phenolic OH group because it produced a deep blue coloration with 10% $FeCl_3$. The UV spectral data of compound **5** showed the maximum absorption wavelength λ_{max} (231 nm and 271 nm) in MeOH due to π→π* of conjugated double bond. The FT IR spectrum of compound **5** revealed the presence of hydroxyl (3423, 3248 cm^{-1}), carbonyl (1705 cm^{-1}) and aromatic ring (1612, 1529 cm^{-1}). The ^1H NMR spectrum of compound **5** displayed four triplets, two doublets and one multiplet ranging from δ_H 3.21 to 4.96 ppm, which were attributed to one β-glucosyl moiety. The remaining signals at δ_H 3.78 (3H, s) and δ_H 6.99 (1H, s) were assignable to methoxy proton and one aromatic proton. The other five broad signals at δ_H 4.84, 5.38, 5.59, 8.41 and 9.67 ppm were due to hydroxyl protons. The ^1H ^1H COSY correlations [oxygenated methane (δ_H 4.00) to an oxygenated methine proton (δ_H 4.96) and methine proton (δ_H 3.63), methine proton (δ_H 3.59) to an oxygenated methine proton (δ_H 3.83) and methine proton (δ_H 3.21)] inferred the position of a β-glucosyl unit. The ^{13}C NMR resonances of compound **5** were confirmed from APT and HSQC spectral data analysis. The attachment of a glucosyl unit to the isocoumarin core was deduced

from the HMBC correlations of H-10b (δ_H 4.96) to C-10 (δ_C 148.1), C-10a (δ_C 116.0), C-6a (δ_C 118.1) and H-4a (δ_H 4.00) to C-6a. The HMBC correlation of aromatic proton (δ_H 6.99) to C-8 (δ_C 150.9), C-9 (δ_H 140.7), C-6 (δ_C 163.4) and C-10a (δ_C 116.0) confirmed the position of this proton to be at C-7 and that of the methoxy group at C-9. Upon careful spectroscopic determination, compound **5** was elucidated to be bergenin (Chart 1). The assigned data were found to be consistent with those of literature values (Dung et al. 2004; Jamal et al. 2009).

Compound **6** (0.005% yield) was obtained as a pale yellow amorphous powder. Its molecular formula was determined to be $C_{16}H_{18}O_{10}$ by EI MS. It was soluble in chloroform, ethyl acetate, acetone, methanol and ethanol, but insoluble in pet-ether and water. Its R_f value was found to be 0.59 with PE:EtOAc (1:5 v/v) and it was a UV active compound. It gave a brown spot on a TLC chromatogram when sprayed with 10% $FeCl_3$, a yellow spot with iodine vapor, bright fluorescence with 10% KOH and a dark-green spot with 5% H_2SO_4 followed by heating. According to chemical tests, the phenolic OH group may be present in compound **6**. The structure of compound **6** was also studied using UV, FT IR, ^{1}H and ^{13}C NMR, as well as 2D NMR spectral data. The UV spectrum of compound **6** showed the maximum absorption wavelength (λ_{max}) at 277 nm and 319 (sh) nm in MeOH, indicating the presence of a conjugated double bond due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions. The FT IR spectrum of compound **6** exhibited the broad absorption band at 3377 cm^{-1} due to O-H stretching vibration of alcoholic and phenolic OH groups. The band at 1712 cm^{-1} indicated the presence of the α,β- unsaturated lactone group. In addition, the appearance of the peak at 1616, 1591 and 1513 cm^{-1} implied the C=C ring skeletal stretching vibration of an aromatic ring. The ^{1}H NMR spectrum of compound **6** showed two triplets, three doublets and one multiplet ranging from δ_H 3.34 to 4.74, which were attributed to one β-glucosyl moiety. The remaining signals at δ_H 2.01 (3H, s), δ_H 3.85 (3H, s) and δ_H 7.01 (1H, s) were assignable to one methyl group, one methoxy group and one aromatic proton. The other three signals at δ_H 3.21, 7.22 and 7.84 were due to hydroxyl protons. ^{13}C NMR spectral data of isolated compound **6** showed sixteen peaks. The ^{13}C NMR resonances of these carbons were confirmed from APT, HSQC and HMBC spectra. The APT spectrum exhibited sixteen signals attributed to two CH_3, one CH_2, six CH, and seven quaternary carbons. The most upfield signals, one methyl and one aromatic methoxy carbons appeared at δ_C 20.4 and 60.3. Most downfield signals at δ_C 171.2 and 164.3 were assigned to the ester CO carbon and carbonyl carbon. Its ^{1}H and ^{13}C NMR spectral data were very similar to those of bergenin (**5**). The significant differences were the disappearance of the hydroxyl proton (δ_H 4.84) and the appearance of additional acetyl protons (δ_H 2.01) and their corresponding carbons [δ_C 20.4 (CH_3), 171.2 (C=O)]. Compound **6** was thus suggested to be an acetate derivative of bergenin (**5**). The attachment of the acetyl group at C-11 was confirmed from HMBC correlations of H-11 (δ_H 4.50, 4.07) to carbonyl carbon of the acetyl group (δ_C 171.2). The assigned data were found to be consistent with

those of literature values (Wang 2008). Therefore, compound **6** (Chart 1) was identified as 11-*O*-acetylbergenin.

2.2 Biological Activities of Crude Extracts and Isolated Compounds of *P. pterocarpum*

In the present study, the antimicrobial activity of pet-ether (PE), ethyl acetate (EtOAc), ethanol (EtOH) and water (H_2O) extracts of PMZL was investigated using the agar well diffusion method against six microorganisms (Fig. 1). Among the tested extracts, ethanol extracts of almost all samples exhibited the most potent activity (20 mm inhibition zone diameter) against most microorganisms. However, the antimicrobial activity of ethyl acetate and water crude extracts was not as potent as that of ethanol extract. The pet-ether crude extracts of flowers and bark could not inhibit the growth of most of the tested microorganisms. Only the petroleum ether extract of leaves was found to be weakly active against five microorganisms excluding *E. coli*. The most resistant microorganism was suggested to be *P. aeruginosa*, since none of the crude extracts of flowers and bark showed an inhibition zone against it. Among the tested microorganisms, *B. pumilus* and *E. coli* were found to be sensitive to ethanol crude extracts, which exhibited several 20 mm inhibition zones against these microorganisms.

The results of an *in vivo* acute toxicity test are shown in Figure 2. The dosages administered were 2–16 g/kg body weight of albino mice. Since no lethality was observed until 2 weeks with the maximum dose, it can be assumed that the water extract and ethanol extract of flowers, leaves and bark of PMZL were free from toxic effects and hence provided safe application of these crude extracts for further *in vivo* biological investigations.

In the *in vitro* antioxidant activity study, radical scavenging activity of ethanol and water extracts of flowers, leaves and bark of PMZL and the isolated compounds physcion (**1**), naringenin (**3**), gallic acid (**4**) and bergenin (**5**) were investigated using DPPH assay. The percentage of radical scavenging activity (% RSA) and IC_{50} values of crude extracts, isolated compounds and standard vitamin C are shown in Figure 3 and Figures 4 to 6. It was observed that the higher the concentration of crude extract, the stronger the radical scavenging activity is. A lower IC_{50} value indicates a greater antioxidant activity. Among the crude extracts tested, ethanol extracts of each sample showed the most potent antioxidant activity [IC_{50} µg/mL: 0.68 (Flowers), 1.12 (Leaves), 0.65 (Bark)]. Their potencies were found to be stronger than that of standard vitamin C (IC_{50}: 1.17 µg/mL). Except for water extract of flowers and leaves, the potency of all tested samples was higher than that of standard vitamin C. On the other hand, among the isolated compounds tested, gallic acid (**4**) and bergenin (**5**) were found to possess potent activity (IC_{50} µg/mL: 0.86 and 0.96) in comparison with that of vitamin C. The potency of gallic acid and bergenin was considerable due to the presence of phenolic OH groups.

The cytotoxicity of ethanol extracts of flowers, leaves and bark of PMZL and gallic acid (**4**) were investigated using resazurin microplate assay against colon cancer stem cells (CSC) and human lung fibroblast cells (MRC 5). The cytotoxicity results of treated samples are described in Figures 7 and 8 and Figures 9 to 12. The % inhibition of a 1.5mg/mL dose of ethanol extracts of flowers, leaves, bark and gallic acid (**4**) on the growth of CSC and MRC 5 were found to be 15%, 62%, 72%, 47% and 8%, 27%, 23%, 26% respectively. Among the extracts tested, bark extract showed a greater cytotoxic effect than other crude extracts on colon cancer stem cells. Leaf extract had a greater cytotoxic effect than other crude extracts on human lung fibroblast cells.

3. Materials and Methods

3.1 General Experiment Procedures

The experiment work was conducted at the Department of Chemistry, University of Yangon (UY). The Pan-mèzali flowers were collected from UY campus in April 2010. The plant was identified as *Peltophorum pterocarpum* by an authorized botanist at the Botany Department, UY. The flowers were cleaned by washing them with water and were then air-dried at room temperature. The dried flowers were ground into powder and stored in an air-tight container. Column chromatography was performed using Silica gel 60, 0.063–0.200mm (70–230 mesh, ASTM) (Merck, Germany), and precoated TLC plates (GF_{254} aluminum plates, Merck) were used for thin layer chromatography. All solvents were purified by distillation at their boiling point ranges. The following instruments were used for structure elucidation of isolated compounds: Gallenkamp melting point apparatus, Shimadzu UV-1800 spectrophotometer (Department of Chemistry, UY), FT IR 8400 Fourier Transformed Infrared Spectrophotometer (Department of Medical Research, Lower Myanmar), and INOVA 600 and INOVA 500 (University of Göttingen, Germany).

In the antimicrobial activity test, the selected crude extracts were pet-ether, ethyl acetate, ethanol and water extracts of flowers, leaves and bark of PMZL. The microorganisms chosen were *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli*. For the acute toxicity test, albino mice (20–30g) of both sexes were used as animal models. The 2, 2-diphenyl-1-picry-hydrazyl (DPPH) and the reagents (resazurin, medium, PBS, etc.) necessary for the antioxidant and cytotoxicity assays were obtained from Sigma-Aldrich. The UV-visible spectrophotometer (UV-7504) was applied to measure the absorbance. The cell lines [Colon Cancer Stem Cells (CSC) and Human Lung Fibroblast (MRC 5)] were obtained from the School of Chemical and Life Sciences, Nanyang Polytechnic, Singapore.

3.2 Preparation of Crude Extracts

The dried flower powder of *P. pterocarpum* (ca. 500g) was percolated in 95% ethanol (1 L) for one week and filtered. This procedure was repeated three times. The combined filtrate was concentrated under a vacuum rotatory evaporator to obtain ethanol crude extract. The ethanol crude extract was then successively extracted by partition with pet-ether (60–80°C), ethyl acetate and water. The condensed pet-ether and ethyl acetate extracts were kept for isolation of phytoconstituents.

3.3 Separation and Isolation of Secondary Metabolites

The pet-ether extract (5g) was separated by column chromatography over silica gel with a PE:EtOAc gradient system (49:1 to 5:1, v/v) to produce compound **1** (13mg, 0.003%) and compound **2** (40mg, 0.008%). The EtOAc extract (5g) was also separated by column chromatography, eluting successively with PE:EtOAc (9:1 to 1:9, v/v) to afford seven main fractions (F-I to F-VII). From fractions F-II, F-IV and F-VI, compound **3** (45mg, 0.009%), compound **4** (33mg, 0.007%), compound **5** (88mg, 0.018%) and compound **6** (25mg, 0.005%) were obtained.

3.4 Physicochemical Characterization of Isolated Compounds

The physical properties determined were melting point and solubility in pet-ether, chloroform, ethyl acetate, acetone, methanol, ethanol and water. The chemical tests performed were reactions with 2,4-DNP (Dinitrophenylhydrazine), 10% $KMnO_4$, 10% lead acetate, 10% ammonia solution, 1% $FeCl_3$, Mg ribbon/HCl and Liebermann-Burchard reagent.

3.5 Structural Elucidation of Isolated Compounds

The structures of isolated compounds **1-6** were elucidated and identified using modern spectroscopic techniques such as UV, FT IR, ^1H and ^{13}C NMR, 2D NMR and EI MS spectroscopy.

Physcion (1): orange crystal; mp 208–209°C; UV λ_{max} (MeOH): 223, 254, 265, 286nm; FT IR ν_{max} (KBr): 3441, 2924, 2862, 1743, 1627, 1566, 1473, 1381, 1319, 1280, 1226, 1165, 1103, 1033, 864, 756 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.41 (3H, s, H-16), 3.86 (3H, s, H-15), 6.67 (1H, d, *J*=2.0 Hz, H-2), 7.06 (1H, d, *J*=2.0 Hz, H-7), 7.35 (1H, d, *J*=2.0 Hz, H-4), 7.61 (1H, d, *J*=2.0 Hz, H-5), 12.10 (1H, s, 8-OH), 12.30 (1H, s, 1-OH); ^{13}C NMR ($CDCl_3$): δ 22.1 (C-16), 56.1 (C-15), 106.8 (C-2), 108.2 (C-4),110.3 (C-11), 113.7 (C-14), 121.3 (C-5), 124.5 (C-716), 133.2 (C-13), 135.3 (C-12), 148.4 (C-6), 162.5 (C-8), 165.2 (C-1),166.5 (C-3), 182.0 (C-10), 190.8 (C-9); EI-MS *m/z*: 284 $[M]^+$, Molecular formula $C_{16}H_{12}O_5$.

β-Sitosterol (2): colorless needle; mp 138–140 °C; FT IR ν_{max} (KBr): 3440, 2939, 2877, 1643, 1458, 1373, 1049, 956 cm^{-1}.

Naringenin (3): pale yellow amorphous; mp 250–251 °C; UV λ_{max} (MeOH): 290, 328 (sh) nm; FT IR ν_{max} (KBr): 3615, 3489, 3309, 3120, 2918, 2835, 1689, 1578, 1512, 1465, 1311, 1249, 1157, 1080, 833, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$/CD_3OD): δ 2.66 (1H, dd, J=17.1, 2.8 Hz, H-3$_\alpha$), 3.10 (1H, dd, J=17.1, 12.9 Hz, H-3$_\beta$), 5.23 (1H, dd, J=12.9, 2.8 Hz, H-2), 5.84 (2H, d, J=2.4 Hz, H-6,8), 6.79 (2H, d, J=8.2 Hz, H-3′,5′), 7.21 (2H, d, J=8.4 Hz, H-2′,6′); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 43.1 (C-3), 78.9 (C-2), 95.4 (C-8), 96.3 (C-6), 102.3 (C-10), 115.4 (C-3′,5′), 127.7 (C-2′,6′), 129.3 (C-1′), 157.1 (C-4′), 163.0 (C-3), 163.6 (C-9), 166.4 (C-7), 195.7 (C-4); EI-MS *m/z*: 272 $[M]^+$, Molecular formula $C_{15}H_{12}O_5$.

Gallic acid (4): pale yellow needle; mp 236–238 °C; UV λ_{max} (MeOH): 223, 272 nm; FT IR ν_{max} (KBr): 3473, 3371, 3273, 3063, 1699, 1620, 1535, 1450, 1334, 1251, 1026, 864, 765 cm^{-1}; ^{1}H NMR (CD_3OD): δ 7.04 (2H, s, H-2,6); ^{13}C NMR (CD_3OD): δ 110.3 (C-2,6), 121.8 (C-1), 139.5 (C-4), 146.2 (C-3,5), 170.4 (C-7).

Bergenin (5): colorless crystal; mp 238–240 °C; UV λ_{max} (MeOH): 231, 271, 317 (sh) nm; FT IR ν_{max} (KBr): 3423, 3248, 2958, 2895, 1705, 1612, 1529, 1467, 1332, 1234,1180, 1091, 1070, 860, 765 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.21 (1H, t, J=9.3 Hz, H-3), 3.48 (1H, m, H-11$_\beta$), 3.59 (1H, t, J=9.3 Hz, H-2), 3.63 (1H, t, J=9.3 Hz, H-4), 3.78 (3H, s, H-12), 3.83 (1H, d, J=10.0 Hz, H-11α), 4.00 (1H, t, J=9.3 Hz, H-4a), 4.84 (1H, brs, 11-OH), 4.96 (1H, d, J=0.5 Hz, H-10b), 5.38 (1H, brs, 3-OH), 5.59 (1H, brs, 4-OH), 6.99 (1H, s, H-7), 8.41 (1H, brs, 10-OH), 9.67 (1H, brs, 8-OH); ^{13}C NMR (DMSO-d_6): δ 59.9 (C-12), 61.2 (C-11), 70.8 (C-3), 72.3 (C-10b), 73.8 (C-4), 79.9 (C-4a), 81.8 (C-2), 109.6 (C-7), 116.0 (C-10a), 118.1 (C-6a), 140.7 (C-9), 148.1 (C-10), 150.9 (C-8), 163.4 (C-6); EI-MS *m/z*: 328 $[M]^+$, Molecular formula $C_{14}H_{16}O_9$.

11-*O*-Acetylbergenin (6): pale yellow amorphous; UV λ_{max} (MeOH): 277, 319 (sh) nm; FT IR ν_{max}: 3377, 2922, 1712, 1616, 1591, 1513, 1464, 1354, 1316, 1233, 1162, 1092, 1046, 867, 762 cm^{-1}; ^{1}H NMR ($CDCl_3$/CD_3OD): δ 2.01 (3H, s, H-14), 3.21 (1H, s, OH), 3.34 (1H, t, J=13.2 Hz, H-3), 3.72 (2H, m, H-2,4), 3.85 (3H, s, H-12), 3.97 (1H, t, J=13.2 Hz, H-4a), 4.07 (1H, dd, J=13.2, 10.8 Hz, H-11β), 4.50 (1H, dd, J=13.2, 1.2 Hz, H-11α), 4.74 (1H, d, J=12.0 Hz, H-10b), 7.01 (1H, s, H-7), 7.22 (1H, s, OH), 7.84 (1H, s, OH); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 20.4 (C-14), 60.3 (C-12), 63.5 (C-11), 70.1 (C-3), 73.1 (C-10b), 73.8 (C-4), 78.8 (C-2), 79.5 (C-4a), 110.3 (C-7), 114.9 (C-10a), 117.5 (C-6a), 140.8 (C-9), 147.5 (C-10), 150.7 (C-8), 164.3 (C-6), 171.2 (C-13); EI-MS *m/z*: 370 $[M]^+$, Molecular formula $C_{16}H_{18}O_{10}$.

3.6 Preparation of Crude Extracts for Biological Activities

The flowers, leaves and bark powders (ca. 10 g) were percolated separately with pet-ether and ethyl acetate at room temperature for 3 days. 200 g of each sample

was percolated with ethanol and boiled with water separately for 3 days. After percolation, the filtrates were filtered through filter papers and concentrated under a vacuum at 45 °C using a rotary evaporator. Finally, the crude extracts were dried on a water bath until attaining a constant weight and were stored in a refrigerator for biological investigations.

3.7 Evaluation of Antimicrobial Activity using the Agar Well Diffusion Method

The antimicrobial activity was screened using the agar well diffusion method at the Central Research and Development Center (CRDC), Yangon. The microorganisms selected were *B. subtilis, S. aureus, P. aeruginosa, B. pumilus, C. albicans* and *E. coli*. Nutrient agar was prepared according to the method described by Cruickshank (1975). Briefly, nutrient agar was boiled and 20–25 mL of the medium was poured into a test tube, plugged with cotton wool and autoclaved at 121 °C for 15 min. Then the tubes were cooled down to 60 °C and poured into a sterile petri dish, and 0.1 mL of spore suspension was also added to the dishes. The agar was allowed to set for 30 min, after which a 10 mm plate agar well was made with the help of a sterilized cork borer. After that, about 0.1 mL of sample was introduced into the agar well and incubated at 37 °C for 24 hours. The inhibition zone (clear zone) appeared around the agar well, indicating the presence of antimicrobial activity. The extent of antimicrobial activity was measured from the zone of inhibition diameter. The results are described in Figure 1.

3.8 Evaluation of Acute Toxicity using the Litchfield and Wilcoxon Method

The acute toxicity effect of crude extracts was studied using the Litchfield and Wilcoxon method (1949) at the Pharmacology Research Division, Department of Medical Research (DMR), Lower Myanmar, Yangon. The total number of 310 adult mice, weighing (20–30 g), were fasted for 18 hours and divided into 31 groups, each group containing 10 animals. The mice of groups 1 to 15 were orally administrated with different doses of water extracts, and those of groups 16 to 30 were treated with different doses of ethanol extracts. The dosages administered were 2, 4, 8, 12 and 16 g/kg body weight respectively. The mice of group 31 were treated with normal food and water and considered as the control group.

After administration of extracts, the animals were separately housed in the standard aluminum cages and allowed access to food and water. The animals were observed for the first six hours continuously for mortality behavioral changes if any, and observation was extended to every 24 hours for 14 days. The mortality during this period was noted as a nil or percentage of deaths. Each study was carried out separately. The results obtained from the acute toxicity study are described in Figure 2.

Microorganisms	Types of Micro-organisms	Sample	Inhibition Zone Diameters (mm)			
			PE	EtOAc	EtOH	H_2O
Bacillus subtilis	Gram (+)ve	Flowers	—	15	20	15
		Leaves	16	15	12	—
		Bark	—	18	15	15
Staphylococcus aureus	Gram (+)ve	Flowers	—	15	20	18
		Leaves	15	16	14	13
		Bark	—	18	15	15
Pseudomones aeruginosa	Gram (—)ve	Flowers	—	—	—	—
		Leaves	13	17	14	17
		Bark	—	—	—	—
Bacillus pumilus	Gram (+)ve	Flowers	—	15	20	18
		Leaves	13	20	20	16
		Bark	—	20	20	18
Candida albicans	Gram (+)ve	Flowers	—	16	13	13
		Leaves	13	15	20	16
		Bark	—	18	18	—
Escherichia coli	Gram (—)ve	Flowers	—	15	20	18
		Leaves	—	14	20	18
		Bark	—	20	20	18
Agar well - 10 mm				(—) = not detected		

Fig. 1: Results from antimicrobial activity

No. of mice per group	Dosage g/kg (b.wt)	Flowers		Leaves		Bark	
		No. of Death per tested mice	% of death	No. of Death per tested mice	% of death	No. of Death per tested mice	% of death
10	16 g/kg (AE)	0/10	0	0/10	0	0/10	0
10	12 g/kg (AE)	0/10	0	0/10	0	0/10	0
10	8 g/kg (AE)	0/10	0	0/10	0	0/10	0
10	4 g/kg (AE)	0/10	0	0/10	0	0/10	0
10	2 g/kg (AE)	0/10	0	0/10	0	0/10	0
10	16 g/kg (EE)	0/10	0	0/10	0	0/10	0
10	12 g/kg (EE)	0/10	0	0/10	0	0/10	0
10	8 g/kg (EE)	0/10	0	0/10	0	0/10	0
10	4 g/kg (EE)	0/10	0	0/10	0	0/10	0
10	2 g/kg (EE)	0/10	0	0/10	0	0/10	0

Note: AE-Aqueous extract, EE-95% Ethanol extract

Fig. 2: Results from the evaluation of acute toxicity

3.9 Evaluation of Antioxidant Activity using DPPH Free Radical Scavenging Assay

The antioxidant activity was investigated using the DPPH method at the Department of Chemistry, University of Yangon. The effect of tested samples on DPPH radical was determined using the method by Marinova and Batchvarov (2011). Briefly, 1.5 mL each of 60 µM DPPH (2, 2-diphenyl-1-picrylhydrazyl) and a test sample solution (Five different doses: 10, 5, 2.5, 1.25 and 0.625 µg/mL) were mixed. The reaction mixtures were shaken for 30 min and the absorbance was measured at 517 nm using a UV spectrophotometer. For the control solution, 1.5 mL of methanol was used instead of the sample solution. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\%\ Radical\ Scavenging\ Activity\ (\%RSA) = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

where $A_{Control}$ is absorbance of control solution and A_{Sample} is absorbance of tested sample solution. IC_{50} was calculated using the linear regressive excel program. The results are described in Figure 3 and Figures 4–6.

3.10 Evaluation of Cytotoxicity of Crude Extracts and Gallic Acid using Resazurin Microplate Assay

The cytotoxicity of ethanol extracts of flowers, leaves and bark of PMZL against colon cancer stem cells and human lung fibroblast was studied using resazurin microplate assay at the School of Chemical and Life Sciences, Nanyang

Tested samples	% RSA (mean±SD) in different concentration (µg/mL)					IC_{50} (µg/mL)
	0,625	1,25	2,5	5	10	
Flowers (water)	12.72±2.49	40.81±6.51	63.84±1.25	71.74±3.81	91.27±1.14	1,75
Flowers (EtOH)	48.46±5.19	65.92±3.81	75.06±2.49	89.69±0.38	93.85±1.26	0,68
Leaves (water)	16.04±3.81	35.99±5.19	50.71±1.23	73.40±3.81	89.86±0.52	2,44
Leaves (EtOH)	31.01±2.88	55.69±2.69	81.55±1.32	88.94±0.38	91.02±0.25	1,12
Bark (water)	35.99±1.44	52.62±2.49	80.88±3.81	89.44±0.38	91.69±0.80	1,15
Bark (EtOH)	49.29±6.28	72.57±2.49	87.45±2.50	91.02±0.66	92.27±0.25	0,65
Physcion	35.41±0.35	39.78±0.88	43.89±0.53	48.79±1.06	55.65±0.18	5,88
Naringenin	23.94±3.30	33.50±5.19	48.46±3.81	57.61±2.49	65.92±1.44	2,92
Gallic acid	44.72±1.05	58.55±0.49	66.06±1.63	76.41±1.33	86.52±1.98	0,86
Bergenin	44.31±6.28	55.11±2.49	59.02±3.10	65.92±3.81	72.57±2.49	0,96
Standard Vitamin C	25.20±1.40	53.58±0.88	65.53±1.13	74.82±0.59	83.32±0.78	1,17

*Fig. 3: % RSA (Radiacal Scavenging Activity) and IC_{50} Values of water and ethanol extracts of flowers, leaves, bark, and isolated compounds (**1**, **3**, **4**, **5**) and Standard Vitamin C*

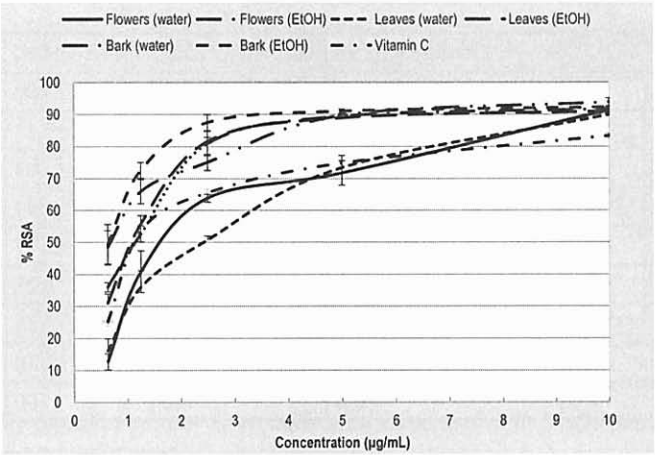

Fig. 4: Radical Scavenging Activity of different concentrations of water and ethanol extracts from flowers leaves and bark of Pan-mèzali

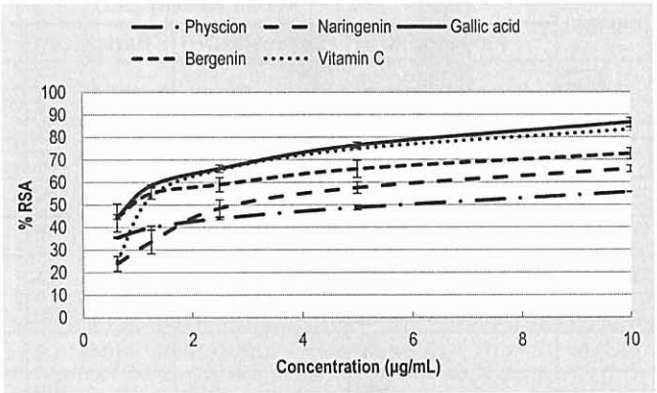

Fig. 5: Radical Scavenging Activity of different doses of compounds isolated from flowers of Pan-mèzali and Standard Vitamin C

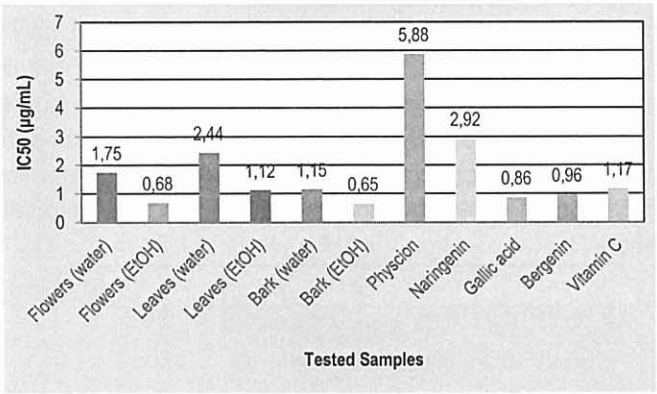

Fig. 6: A bar paragraph of IC_{50} of extracts and four isolated compunds of Pan-mèzali and Standard Vitamin C

Concentration (mg/mL)	% Cell viability ±SD			
	Flowers (EtOH)	Leaves (EtOH)	Bark (EtOH)	Gallic acid
1,50	85± 1.24	38±5.78	28±6.84	53±10.67
0,75	96± 1.07	90±4.54	92±0.47	83±10.83
0,38	100± 0.83	99±0.48	97±1.54	90±4.49
0,19	101± 0.83	102±1.00	101±2.51	97±1.06
0,09	101 ± 2.19	104±0.73	99±1.77	99±1.24
0,05	104± 3.45	104±1.66	101±2.25	102±1.15
0,02	105± 0.70	106±1.81	102±1.35	102±0.41
0,01	107± 0.78	109±0.90	106±1.76	104±0.51
0	100	100	100	100

Fig. 7: Cytotoxic effect of crude extracts of Pan-mèzali and gallic acid on colon cancer stem cells

Concentration (mg/mL)	% Cell viability±SD			
	Flowers(EtOH)	Leaves(EtOH)	Bark(EtOH)	Gallic acid
1,50	92±2.28	73±1.77	77±4.75	74±4.79
0,75	93±0.47	92±3.53	94±0.48	89±2.91
0,38	95±1.32	98±0.43	94±0.32	94±2.04
0,19	96±2.79	99±2.49	96±0.14	98±1.06
0,09	97±1.69	100±0.8	100±0.79	100±1.19
0,05	100±1.91	103±1.62	101±2.16	101±0.46
0,02	100±2.21	104±1.68	101±1.09	101±0.68
0,01	105±1.69	105±0.30	104±1.00	104±2.00
0	100	100	100	100

Fig. 8: Cytotoxic effect of crude extracts of Pan-mèzali and gallic acid on human ung fibroblast

Polytechnic, Singapore. Approximately 2 ×104 cells/well were seeded in a 96-well plate, and dilution of extracts (100 µL/well) were added and incubated at 37°C with 5% CO_2 for 24 hours. After incubation, 100 µL of resazurin solution was added to each well and the plate was incubated for 2–4 hours. The fluorescence signal was measured using the Spectra Max M5 multi-detection microplate reader (Molecular Devices, USA) at the excitation and emission wavelength of 535 nm and 590 nm. The% inhibition of cell growth was calculated using the following equation:

$$\%\ Inhibition = \left[1 - \frac{FU_T}{FU_C}\right] \times 100$$

where FU_T and FU_C are the mean of the fluorescent unit from the treated and untreated (control) groups.

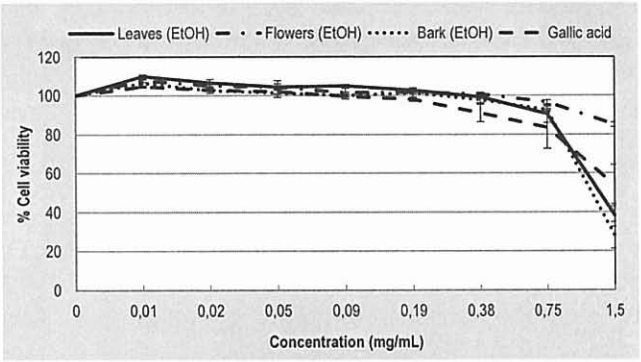

Fig. 9: Viability of colon cancer stem cells after exposure with crude extracts of Panmèzali and gallic acid

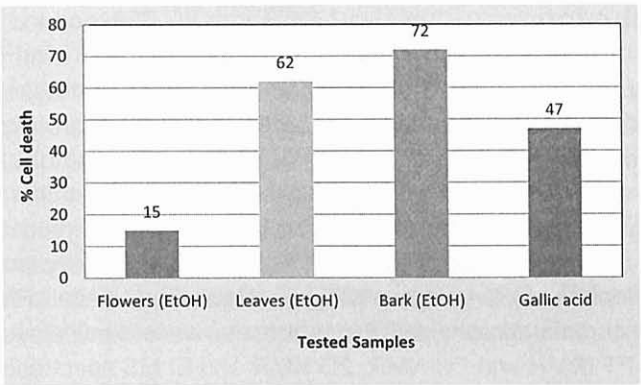

Fig. 10: % Cell death of colon cancer stem cells after exposure with 1.5 mg/mL of tested samples

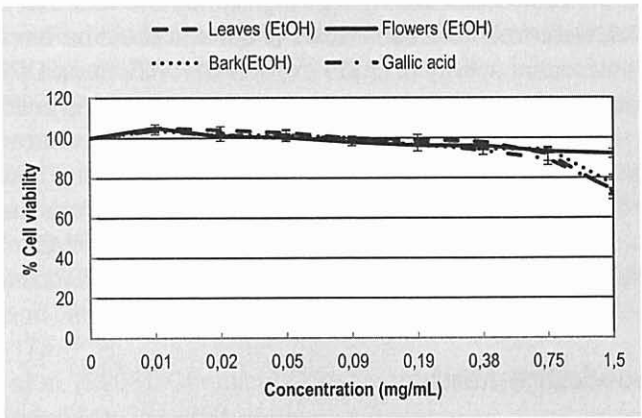

Fig. 11: Viability of human lung fibroblast cells after exposure wih crudeextracts of Panmèzali and gallic acid

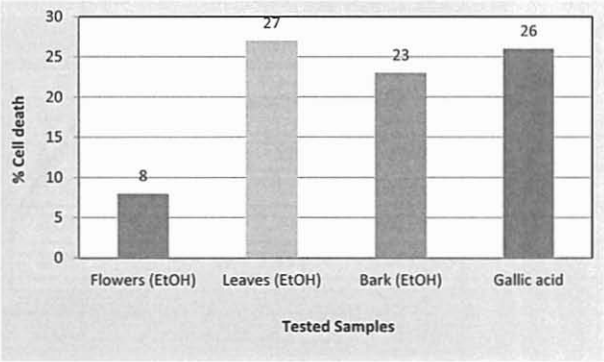

Fig. 12: % Cell death of human lung fibroblast cells after exposure with 1.5mg/mL of testes samples

4. Conclusion

The following inferences could be deduced from the overall assessment of the chemical and biological investigations of *Peltophorum pterocarpum* (DC.) K. Heyne (Pan-mèzali).

Six secondary metabolites, physcion (**1**), β-sitosterol (**2**), naringenin (**3**), gallic acid (**4**), bergenin (**5**) and 11-*O*-acetylbergenin (**6**) were successively isolated from the flowers of *Peltophorum pterocarpum* (DC.) K. Heyne (Pan-mèzali, PMZL) using silica gel column chromatography, and their structures were identified using the combination of UV, FT IR, ^{1}H and ^{13}C NMR, 2D NMR and EI MS spectroscopic methods, and also through comparison with the reported data. The pet-ether, ethyl acetate, ethanol and water extracts of PMZL were found to possess pronounced antimicrobial activity against both Gram positive and Gram negative bacteria, and also against fungus. They could be applicable as broad-spectrum antimicrobial agents. The results obtained from an acute toxicity test highlighted the fact that PMZL flowers, leaves and bark were free from acute toxic effect and could be employable safely. Screening of antioxidant activity of crude extracts of PMZL using DPPH assay showed that the various parts of PMZL also possessed pronounced antioxidant activity. In the *in vitro* resazurin cytotoxicity assay, the ethanol crude extracts of PMZL also exhibited a mild cytotoxic effect on colon cancer stem cells and human lung fibroblast with a 1.5mg/mL dose. The present biological investigations have thus contributed to understanding the medicinal importance of the plant PMZL. It can be practically applicable for diseases related to microbial infection, free radicals and cancer.

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