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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