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Phytochemical Investigation of *Schleichera oleosa* (Lour.) Oken Leaf

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Abstract

Schleichera oleosa (Lour.) Oken. is a medicinal plant of Bangladesh with enormous traditional applications in folk medicine. The current study was designed to isolate the secondary metabolites by successive chromatographic separation of *n*-hexane and dichloromethane soluble fractions of a methanol extract of leaves of *S. oleosa*. A total of four compounds were separated and identified as 5,7-dihydroxy-4'-methoxyflavone, stigmasterol, lupeol and betulinic acid. The structures of the isolated compounds were elucidated by analysis of their ^{1}H NMR data and comparison with published values.

Key words: *Schleichera oleosa*, phytochemical, chromatography, NMR.

Introduction

Bangladesh is an excellent reservoir of medicinal plants which is the vital source of medicine for mankind (Rashid *et al*., 2015). More than 500 different species of medicinal plants grow in Bangladesh. A major part of these species is used in folk medicines for the cure of various ailments (Uddin *et al*., 2015). Medicinal values of these plants are attributed to the bioactive molecules which can exert substantial biological effects on the human body. These natural compounds formed the basis for the development of modern pharmaceutical drugs. Moreover, plant-derived natural products have attracted the attention of scientists due to their minimal side effects. Therefore, the research interest in the phytochemical studies of medicinal plants is rising in order to discover the bioactive leads (Cragg and Newman, 2013; Veeresham, 2012 and McChesney *et al*., 2007).

Schleichera oleosa (Lour.) Oken (Family: Sapindaceae) is a deciduous, evergreen, medium sized tree which is widely distributed in the Indian subcontinent and Southeast Asia. The plant is locally familiar as Kushum (Thind *et al*., 2011). Traditionally it is used as folk medicine for several ailments such as pain, infection and dysentery (Pokhrel *et al*., 2015). Kusum oil, a seed oil of *S. oleosa*, is used for the treatment of rheumatism, skin diseases and also for promoting hair growth (Palanuvej and Vipunngeun *et al*., 2008). Bark extract was reported to cure gynecological disorder, inflamed skin and ulcers (Mohapatra and Sahoo, 2008). Previous phytochemical study led to isolation of phenolic compounds, fatty acids, tannins, hydroxyl sterols and triterpenoids from this plant (Goswami and Singh, 2017). Bioactive triterpenoids isolated from *S. oleosa* bark exhibited antimicrobial potentials against bacterial and fungal pathogens (Ghosh *et al.*, 2011). Hydroxylated sterols isolated from the bark of *S. oleosa* showed potent anticancer properties (Pettit *et al*., 2000). As part of our continuous study on medicinal plants of Bangladesh (Fahad *et al*., 2020;

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Islam *et al*., 2019; Moniruzzaman *et al*., 2019) we studied the chemical constituents of *S. oleosa* and we, herein, report the results of our phytochemical investigation of this plant species.

Materials and Methods

Collection and preparation of plant material: The leaves of *S. oleosa* were collected from Botanical Garden, Mirpur, Dhaka-1216, Bangladesh and was authenticated (Accession no. DACB 63763) in Bangladesh National Herbarium, Mirpur, Dhaka-1216, Bangladesh. After collection, the plant samples were washed and dried at room temperature. The dried leaves were grinded to a coarse powder. The powdered sample (500 g) of *S. oleosa* was soaked in methanol for few days. The filtrate was concentrated to dryness with a rotary evaporator. About 5g of the dried methanolic extract of *S. oleosa* was subjected for Kupchan partitioning (Van-Wagenen *et al*., 1993) to yield *n*-hexane, dichloromethane and ethyl acetate soluble fractions.

Isolation of chemical compounds: An aliquot of the dichloromethane soluble materials (300 mg) was subjected to gel permeation chromatography over Sephadex (LH-20) from which fifty nine sub-fractions was collected, each 20.0 ml. Preparative TLC screening of sub-fractions 49-53 over silica gel using toluene: ethyl acetate (80:20) provided compound **1**. On the other hand, the dichloromethane soluble fraction (500 mg) was fractionated by column chromatography (CC) over silica gel (Kieselgel 60, mesh 70-230) using a mixture of *n*-hexane and ethyl acetate in order of increasing polarities. Preparative TLC of column fractions 56-59 and 45-51 over silica gel using toluene in ethyl acetate (98:2) as the developing solvent afforded compound **2** and compound **3**, respectively.

Properties of isolated compounds

5,7-dihydroxy-4′-methoxyflavone (**1**)*:* (0.5 mg, 0.01% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (2H, d, *J* = 8.0 Hz, H-2′/H-6′), 7.03 (1H, d, *J* = 8.0 Hz, H-3′/H-5′), 6.60 (1H, s, H-3), 6.46 (1H, d, *J* = 2.0 Hz, H-6/H-8), 6.30 (1H, d, *J* = 2.0 Hz, H-6/H-8), 3.90 (3H, s, -OCH_3, C-4′).

Stigmasterol (**2**)*:* (2.9 mg, 0.058% yield); ^{1}H NMR (400 MHz, $CDCl_3$) spectral data was identical to published values (Pierre and Moses, 2015).

Lupeol (**3a**)*:* ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.71 (1H, br. s, H_a-29), 4.59 (1H, br. s, H_b-29), 3.20 (1H, m, H-3), 1.69 (3H, s, H_3-30), 1.02 (3H, s, H_3-25), 0.96 (3H, s, H_3-23), 0.93 (3H, s, H_3-26), 0.82 (3H, s, H_3-28), 0.78 (3H, s, H_3-24).

Betulinic acid (**3b**)*:* ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.71 (1H, br. s, H_b-29), 4.59 (1H, br. s, H_a-29), 3.25 (1H, m, H-3), 3.02 (1H, m, H-19), 1.70 (3H, s, H-30), 0.99, 0.92, 0.89, 0.78, 0.76 (5s, each 3H, all tertiary -CH_3).

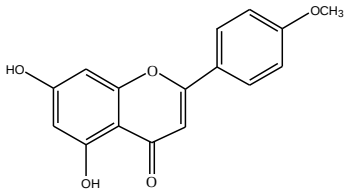
Results and Discussion

Successive chromatographic separation and purification of *n*-hexane and dichloromethane soluble materials of *S. oleosa* leaf extract yielded a total of four compounds (Figure 1). The structures of these compounds were established as 5,7-dihydroxy-4'-methoxyflavone (**1**), stigmasterol (**2**), lupeol (**3a**) and betulinic acid (**3b**) by analyses of their ^{1}H NMR spectroscopic data as well as by comparison with published reports.

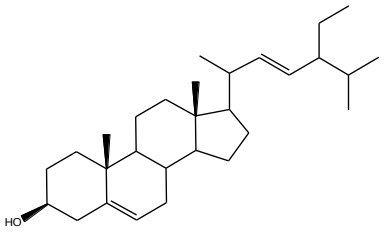
The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **1** displayed a pair of ortho-coupled (*J* = 8.0 Hz) doublets at δ 7.03 and 7.85 which indicated that the "B" is substituted at C-4′ and thus these were assigned to H-3′ & H-5′ and H-2′ & H-6′, respectively. The singlet at δ 3.90 which obviously indicated that, a methoxy group was present in the structure at C-4′ position. The spectrum also showed two meta-coupled doublets (*J* = 2.0 Hz) at δ 6.30 and 6.46 which were assigned to H-6 and H-8 or *vice versa*. The lone singlet of one proton intensity at 6.60 could be ascribed to H-3. All these ^{1}H NMR data of compound **1** are in close agreement with the published value of 5,7-dihydroxy-4′-methoxyflavone or acacetin (Gomes *et al*., 2011). Therefore, compound **1** was characterized as 5,7-dihydroxy-4′-methoxyflavone (Figure 1).

The ^{1}H NMR spectral features of compound **2** were in close agreement with the ^{1}H NMR spectrum recorded for stigmasterol previously isolated in our laboratory (Sufian *et al*., 2015; Chowdhury *et al*., 2013). Therefore, compound **2** was identified as stigmasterol (Figure 1).

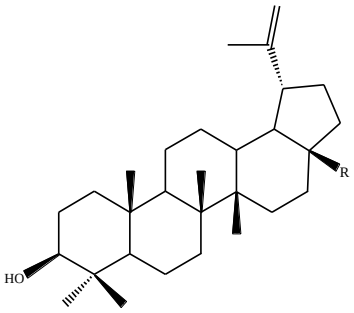
The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **3** displayed signals assignable to a mixture of two closely related triterpenoids *i.e*- lupeol (**3a**) and betulinic acid (**3b**). The ^{1}H NMR spectral signal assignable to compound **3a** revealed a multiplet at δ 3.20, suggesting the presence of an oxymethine proton, H-3 in the triterpene nucleus. The ^{1}H NMR spectrum displayed five three proton singlets at δ 0.78, 0.82, 0.93, 0.96 and 1.02 which were assigned to the methyl groups at C-24, C-28, C-26, C-23 and C-25, respectively. The spectrum also revealed two broad singlets at δ 4.71 (1H, br. s, H_a-29) and 4.59 (1H, br. s, H_b-29), together with an allylic methyl proton signal at δ 1.69 (3H, s, H_3-30) confirmed an isopropenyl functionality. Therefore, compound **3a** was characterized as lupeol (Figure 1). This was further confirmed by comparing its ^{1}H NMR data with the published values (Fahad *et al*., 2020; Sufian *et al.,* 2015).



5,7-dihydroxy-4′-methoxyflavone (**1**)



Stigmasterol **(2)**



R= -CH_3, Lupeol (**3a**)
R= -COOH, Betulinic acid (**3b**)

Figure 1. Compounds isolated from *Schleichera oleosa.*

The ^{1}H NMR spectral data assignable to compound **3b** showed five methyl singlets at δ 0.76, 0.78, 0.89, 0.92, 0.99, a vinylic methyl group at δ 1.70 (3H, s) and a pair of exomethylene protons at δ 4.59 (1H, br. s) and δ 4.71 (1H, br. s). The spectrum also displayed a multiplet at δ 3.25 which could be ascribed to the oxygenated proton of C-3 (H-3). On the basis of the spectral data, compound **3b** was characterized as betulinic acid (Figure 1) which was further confirmed by comparing its ^{1}H NMR data with the published report (Haque *et al*., 2013).

Conclusion

Phytochemical investigation of the *n*-hexane and dichloromethane soluble fractions of *S. oleosa* leaf extract yielded a total of four compounds which were characterized as 5,7-dihydroxy-4′-methoxyflavone (**1**), stigmasterol (**2**), lupeol (**3a**) and betulinic acid (**3b**) on the basis of their spectral data. Further studies are necessary to explore the biological activities of the isolated metabolites and to relate these with the traditional uses of this plant.

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