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Phytochemical Investigation of *Calophyllum inophyllum* L.

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ABSTRACT: Repeated chromatographic separation and purification of the dichloromethane-methanol (1:1) extract of the stem bark of *Calophyllum inophyllum* led to the isolation of four secondary metabolites which were identified as β-friedelin (**1**), α-friedelan-3-ol (**2**), 4-hydroxyxanthone (**3**) and stigmast-5-en-3-one (**4**) by analysis of their NMR data and comparison with published values.

Key words: *Calophyllum inophyllum*, terpene, steroid, xanthone.

INTRODUCTION

Calophyllum inophyllum L. (Bengali name: Sultan Chapa, English name: Alexandrian laurel balltree, Family: Guttiferae) is a large low-branching, slow-growing evergreen tree native to coastal Asia.[1] Phytochemical studies of various *Calophyllum* species revealed the presence of different triterpenes, steroids and xanthones.[2] The ethnic tribal communities have been using *C. inophyllum* for many generations and information regarding the efficacy remains primarily anecdotal.[1] Therefore, an attempt has been taken to systematically study the chemical constituents of *C. inophyllum* growing in Bangladesh.

MATERIAL AND METHODS

General experimental procedures. The ^{1}H and ^{13}C NMR spectra were recorded using a Bruker AMX-400 instrument and the spectrum was referenced to the residual non-deuterated solvent signal. Vacuum liquid chromatography (VLC) was carried out using Silica gel 60 H (Merck, Germany). Column chromatography (CC) was conducted on silica gel 60 (70-230 mesh, Merck, Germany), whereas PTLC (20 x 20 cm) and TLC (20 x 5 cm) were carried out on silica gel 60 F_{254} on aluminum sheets at a thickness of 0.25 mm (Merck, Germany). Spots on TLC and PTLC plates were visualized under UVGL-58 handheld UV lamp (USA) at 254 and 365 nm and by spraying the developed plates with vanillin-sulfuric acid followed by heating for 5 minutes at 110 ºC.

Plant material. Fresh stem bark of *C. inophyllum* was collected from Barisal. It was identified in Bangladesh National Herbarium, Dhaka, Bangladesh where a voucher specimen (Voucher no. 38712) has been deposited for this collection. The bark was firstly sundried for five consecutive days. Finally, the dried bark was ground into a coarse powder using a grinding machine.

Extraction and isolation. Powdered material (0.8 kg) was soaked in 3.0 L MeOH/DCM (1:1) for a period of 7 days with occasional shaking and stirring. The whole mixture underwent a series of filtrations first with cotton plug followed by Whatman No. 1 filter paper and the filtrate thus obtained was

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concentrated at 40 °C under reduced pressure to obtain crude extract (50.2 gm). A portion of this extract was subjected to VLC for rapid fractionation using petroleum ether, EtOAc and MeOH in order of increasing polarities. VLC fractions 9 and 10 eluted with EtOAc/50-100% MeOH gradient system were mixed and subjected to column chromatography using petroleum ether, CH_2Cl_2 and MeOH in proportion of their increasing polarity. The column fraction eluted with CH_2Cl_2/0-0.2% MeOH gradient system was subjected to PTLC using $CHCl_3$/MeOH (98.5/1.5) which upon two developments gave compound **1** (1.8 mg) and compound **4** (0.9 mg). Solvent treatment of VLC fraction 6 (EtOAc/0-0.5% MeOH) provided compound **2** (5.4 mg). In parallel manner, solvent treatment of the column fraction eluted with petroleum ether/55-67.5% CH_2Cl_2 yielded compound **3** (1.6 mg).

Properties of isolated compounds. *β*-Friedelin (**1**): White powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.41 (1H, m, H-2a), 2.32 (1H, m, H-2b), 2.26 (1H, m, H-4), 1.77 (1H, dd, *J* = 12.6, 4.2 Hz, H-1), 1.70 (1H, dd, *J* = 12.6, 4.2 Hz, H-1), 1.20 (3H, s, H-28), 1.07 (3H, s, H-27), 1.03 (3H, s, H-26), 1.02 (3H, s, H-30), 0.98 (3H, s, H-29), 0.89 (3H, s, H-25), 0.75 (3H, s, H-24), 0.90 (3H, d, *J* = 6.5 Hz, H-23); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 213.2 (C-3), 59.4 (C-10), 58.2 (C-4), 53.1 (C-8), 42.8 (C-18), 42.1 (C-5), 41.5 (C-2), 41.3 (C-6), 39.7 (C-13), 39.2 (C-22), 38.3 (C-14), 37.4 (C-9), 36.0 (C-16), 35.6 (C-11), 35.3 (C-19), 35.0 (C-30) 32.7 (C-21), 32.5 (C-12), 32.0 (C-28), 31.7 (C-29) 30.5 (C-15), 30.0 (C-17), 28.1 (C-20), 22.3 (C-1), 20.2 (C-27), 18.6 (C-26), 18.2 (C-7), 17.9 (C-25), 14.6 (C-24), 6.8 (C-23).

α-Friedelan-3-ol (**2**): White amorphous powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.49 (1H, m, H-3), 1.03 (3H, s, H-27), 1.03 (3H, s, H-29), 1.01 (3H, s, H-26), 0.98 (3H, s, H-23), 0.94 (3H, s, H-30), 0.86 (3H, s, H-24); ^{13}C NMR (100 MHz, $CDCl_3$): δ 79.0 (C-3), 61.3 (C-10), 53.4 (C-4), 48.7 (C-8), 42.0 (C-18), 41.7 (C-6), 41.7 (C-22), 39.4 (C-5), 39.2 (C-2), 38.7 (C-9), 38.7 (C-13), 38.7 (C-17), 37.1 (C-14), 32.8 (C-11), 32.8 (C-19), 32.2 (C-16), 32.2 (C-21), 32.2 (C-28), 32.2 (C-30), 30.4 (C-12), 30.4 (C-15), 30.4 (C-29), 29.7 (C-20), 19.5 (C-25), 18.3 (C-1), 18.3 (C-26), 18.3 (C-27), 16.8 (C-7), 16.3 (C-24), 10.9 (C-23).

4-Hydroxyxanthone (**3**): Crystals; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (1H, d, *J* = 7.8 Hz, H-8), 7.90 (1H, d, *J* = 7.8 Hz, H-5), 7.79 (1H, t, *J* = 7.8 Hz, H-7), 7.57 (1H, d, *J* = 7.6 Hz, H-1), 7.45 (1H, t, *J* = 7.8 Hz, H-6), 7.37 (1H, d, *J* = 7.6 Hz, H-3), 7.31 (1H, t, *J* = 7.6 Hz, H-2), and 10.90 (1H, s, OH-4).

Stigmast-5-en-3-one (**4**): Colorless crystal; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.37 (1H, m, H-6), 1.20 (3H, s, Me-19), 0.99 (3H, m, *J* = 6.5 Hz, Me-21), 0.93 (3H, t, *J* = 6.5 Hz, Me-29), 0.83 (3H, s, Me-27), 0.82 (3H, s, Me-26), 0.72 (3H, s, Me-18).

RESULTS AND DISCUSSION

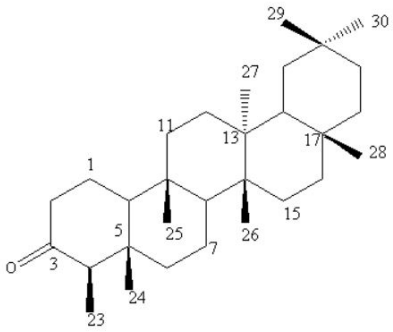
Repeated chromatographic separation and purification of the methanol/CH_2Cl_2 extract of the stem bark of *C. inophyllum* provided four compounds, the structures of which were determined by extensive analysis of NMR spectral data as well as by comparison with published data.

The ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **1** displayed 30 carbon resonances including a carbonyl carbon at δ 213.2. The DEPT experiment indicated that 23 out of the 30 carbon atoms in compound **1** had attached protons. Thus, it exhibited signals for 8 methyls, 11 methylenes, 4 methines and 7 quarternary carbons. These features are suggestive of a triterpenoid skeleton, having a carbonyl group at C-3.

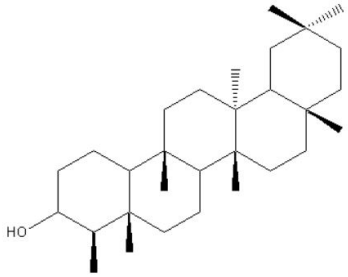
The ^{1}H NMR spectrum of compound **1** revealed the presence of seven methyl singlets at δ 1.20, 1.07, 1.03, 1.02, 0.98, 0.89, 0.75 and a methyl doublet at δ 0.90 (*J = 6.5)* which were assignable to Me-28, Me-27, Me-26, Me-30, Me-29, Me-25, Me-24 & Me-23 respectively. The methylene protons at C-1, C-2 and methine proton at C-4 appeared at δ 1.70 & 1.77, δ 2.32 & 2.41 and 2.26, respectively.

The ^{1}H NMR and ^{13}C NMR data were found to be identical to those reported for *β*- friedelin. Thus, compound **1** was identified as *β*- friedelin (**1**).[2, 3]

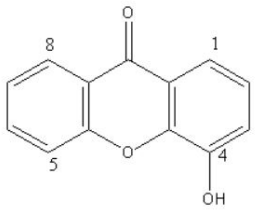
The ^{13}C NMR spectrum of compound **2** was similar to that of compound **1.** However, the carbonyl group signal observed in the ^{13}C NMR spectrum of compound **1** was absent in the spectrum of compound **2** and in fact it was replaced by an oxymethine carbon resonating δ 79.0. This was further supported by the appearance of ^{1}NMR signal at δ 3.49 (1H, m, H-3), which could be assigned to the oxymethine proton at C-3. On this basis, compound **2** was characterized as α-friedelan-3-ol (**2**), which was confirmed by comparison with previously reported values.[4]



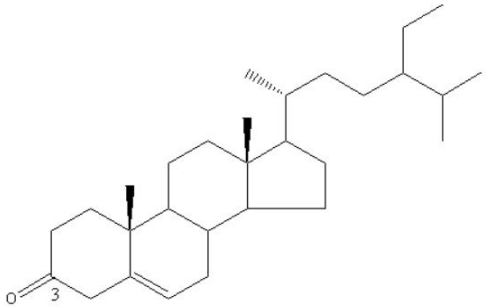
β-Friedelin (**1**)



α-Friedelan-3-ol (**2**)



4-Hydroxyxanthone (**3**)



Stigmast-5-en-3-one (**4**)

Figure 1. Structures of the compounds obtained from *C. inophyllum.*

The ^{1}H NMR spectrum of compound **3** displayed signals for seven aromatic protons at δ 8.40 (1H, d, J = 7.8 Hz, H-8), 7.90 (1H, d, J = 7.8 Hz, H-5), 7.79 (1H, t, J = 7.8 Hz, H-7), 7.57 (1H, d, J = 7.6 Hz, H-1), 7.45 (1H, t, J = 7.8 Hz, H-6), 7.37 (1H, d, J = 7.6 Hz, H-3), 7.31 (1H, t, J = 7.6 Hz, H-2), characteristic for the xanthone derivatives. The ^{1}H NMR spectrum also showed a broad singlet at δ 10.90 ppm which was attributed to the hydroxyl group proton at C-4. Hence, compound **3** was identified as 4-hydroxyxanthone (**3**), which was further confirmed by comparison with previously published values. [5]

The ^{1}H NMR spectrum of compound **4** allowed solving the structure typical for a steroidal molecule. The identity of this compound was established by direct comparison of it ^{1}H NMR spectrum with that of an authentic sample as well as by co-TLC. Thus, it was confirmed as stigmast-5-en-3-one (**4**).[6, 7]

Although, α-friedelan-3-ol (**2**), 4-hydroxyxanthone (**3**), and stigmast-5-en-3-one (**4**) have been reported from *C. inophyllum*; this is the first report of occurrence of β- friedelin (**1**) from this plant. However, β- friedelin (**1**) was previously found in *C. soulattri*, *C. brasiliense*, *C. thwaitesii*, *C. moonii*, *C. cordato-oblongum*, and *C. mucigerum* [8-12], whereas, 4-hydroxyxanthone (**3**) has been reported from *C. soulattri* and *C. brasiliense*.[13,14] Hence, this supports close relationship between these *Calophyllum* species.

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