

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 -friedelin-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 ¹H and ¹³C 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 F₂₅₄ 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 °C.

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

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filter paper and the filtrate thus obtained was 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, CH₂Cl₂ and MeOH in proportion of their increasing polarity. The column fraction eluted with CH₂Cl₂/0-0.2% MeOH gradient system was subjected to PTLC using CHCl₃/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% CH₂Cl₂ yielded compound **3** (1.6 mg).

Properties of isolated compounds. β -Friedelin (**1**): White powder; ¹H NMR (400 MHz, CDCl₃): 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); ¹³C-NMR (100 MHz, CDCl₃): 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 -Friedelin-3-ol (**2**): White amorphous powder; ¹H NMR (400 MHz, CDCl₃): 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); ¹³C NMR (100 MHz, CDCl₃): 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; ¹H NMR (400 MHz, CDCl₃): 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; ¹H NMR (400 MHz, CDCl₃): 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/CH₂Cl₂ 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 ¹³C NMR spectrum (100 MHz, CDCl₃) 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 quaternary carbons. These features are suggestive of a triterpenoid skeleton, having a carbonyl group at C-3.

The ¹H 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 ¹H NMR and ¹³C 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 ^1H 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

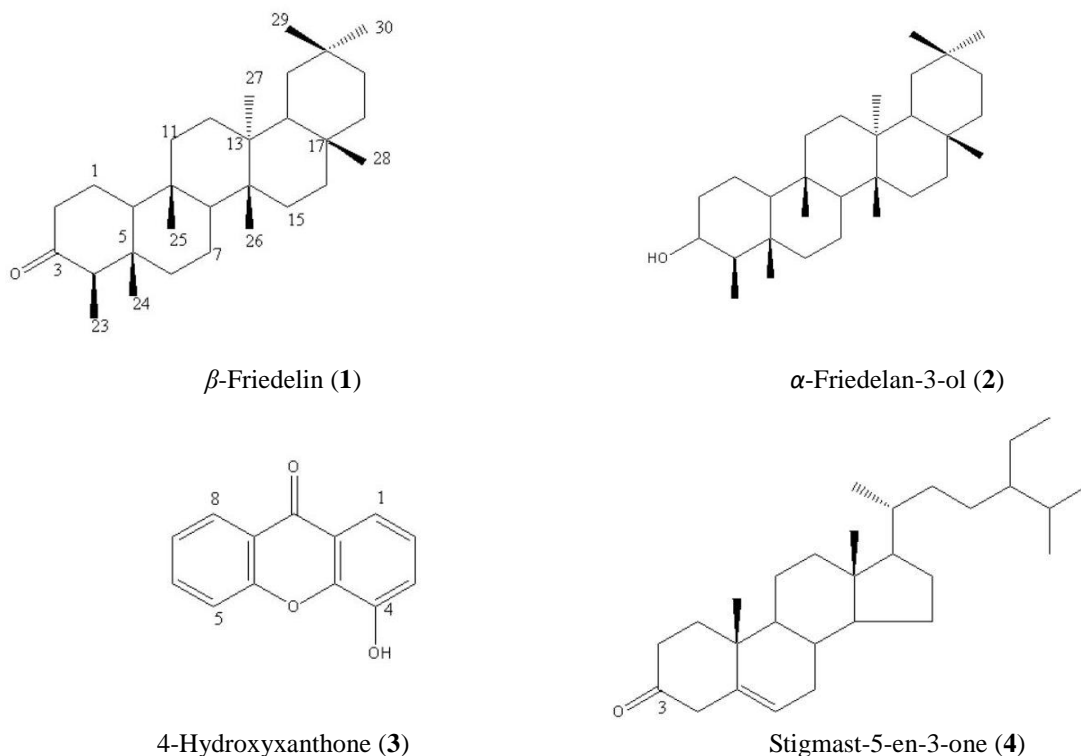


Figure 1. Structures of the compounds obtained from *C. inophyllum*.

The ^1H 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 ^1H 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 ^1H 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 its ^1H 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.

REFERENCES

1. McKee, T.C., Covington, C.D., Fuller, R.W., Bokesch, H.R., Young, S., Cardellina, J.H., Kadushin, M.R., Soejarto, D.D., Stevens, P.F., Cragg, G.M. and Boyd, M.R. 1998. Pyranocoumarins from tropical species of the genus *Calophyllum*: a chemotaxonomic study of extracts in the National Cancer Institute collection. *J. Nat. Prod.* **61**, 1252-1256.
2. Islam, R., Ahmed, I., Sikder, A.A., Haque, M.R., Al-Mansur, A., Ahmed, M., Rasheed, M. and Rashid, M.A. 2014. Chemical investigation of *Mesua nagassarium* (Burm. f.) Kosterm. *J. Basic Applied Sci.* **10**,124-128.
3. Mahato, S.B. and Kundu, A.P. 1994. ¹³C NMR spectra of pentacyclic triterpenoids-a compilation and some salient features. *Phytochemistry* **37**, 1517-1575.
4. Ragasa, C.Y., Ebajo Jr, V., Mariquit, M., Emelina, H., Mandia, R.B. and Urban, S. 2015. Triterpenes from *Calophyllum inophyllum* Linn. *Int. J. Pharmacogn. Phytochem. Res.* **7**, 718-722.
5. Pinto, E., Afonso, C., Duarte, S., Vale-Silva, L., Costa, E., Sousa, E. and Pinto, M. 2011. Antifungal activity of xanthenes: evaluation of their Effect on ergosterol biosynthesis by high-performance liquid chromatography. *Chem. Biol. Drug Des.* **77**, 212-222.
6. Saputra, D.E., Handayani, N. and Wartono, M.W. 2016. Isolation and identification of β -sitosterol and stigmasterol mixture from root bark of slatri (*Calophyllum soulattri* Burm. f). *Alchemy J. Penelitian Kimia* **10**, 87-93.
7. Begum, F., Haque, M., Nahar, K. and Rashid, M. 2015. Secondary metabolites from different extractives of *Stereospermum suaveolens*. *Dhaka Univ. J. Pharm. Sci.* **13**, 31-36.
8. Deddi, P., Noveliandi, N. and Elidahanum, H. 2008. Friedelin, a triterpenoid pentacyclic from the leaves of *Calophyllum soulattri* Burm. f. (Guttiferae). *J. Sains dan Teknologi Farmasi* **13**, 49-52.
10. Isaias, D.E.B., Niero, R., Noldin, V.F., de Campos-Buzzi, F., Yunes, R.A., Delle-Monache, F. and Cechinel-Filho, V. 2004. Pharmacological and phytochemical investigations of different parts of *Calophyllum brasiliense* (Clusiaceae). *Die Pharmazie - Int. J. Pharm. Sci.* **59**, 879-881.
11. Dharmaratne, H.R.W. and Wanigasekera, W.M.A.P. 1996. Xanthenes from root bark of *Calophyllum thwaitesii*. *Phytochemistry* **42**, 249-250.
12. Cao, S.G., Sim, K.Y., Goh, S.H., Xue, F. and Mak, T.C. 1997. Gracilipene: a heterocyclic seco-trisnor-oleanane from *Calophyllum gracilipes* (Guttiferae). *Tetrahedron Lett.* **38**, 4783-4786.
13. Mah, S.H., Cheng Lian Ee, G., Teh, S.S. and Sukari, M.A. 2015. Antiproliferative xanthone derivatives from *Calophyllum inophyllum* and *Calophyllum soulattri*. *Pakistan J. Pharm. Sci.* **28**, 425-429.
14. Cechinel Filho, V., Meyre, Silva, C. and Niero, R. 2009. Chemical and pharmacological aspects of the genus *Calophyllum*. *Chem. Biodiversity* **6**, 313-327.