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Isolation of 3β-16α-dihydroxy-5-cholesten-21-al, n-Docosanoic acid and Stigmasterol from petroleum ether extract of stem bark of *Michelia champaca*

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ABSTRACT

This study described phytochemical investigation of the stem bark of Michelia champaca lead to the isolation of 3β-16α-dihydroxy-5-cholesten-21-al, n-docosanoic acid and stigmasterol from petroleum ether extract and were categorized under steroid, long chain fatty acid and triterpenoid constituents. Their structures were established by direct interpretation of their spectral data of IR, [1]HNMR and GC-MS. These phytochemicals have been reported for the first time from the stem bark of Michelia champaca.

Keywords: *Michelia champaca*, magnoliaceae, phytochemicals, isolation.

INTRODUCTION

Plant have been and still are a rich source of many herbal products in major part of India and other countries most of which have been extensively used for traditional human health care systems. The vast majority of people in the world takes care of themselves and uses healing plants that have been used for hundreds of generation. India is a country of vast biodiversity and traditional knowledge for using herbal medicines to cure many ailments [1-3]. *Michelia champaca* Linn (Magnoliaceae) is an evergreen or semi-deciduous, small to medium-sized tree up to 50 m tall. The tree is distributed throughout the Eastern Sub-Himalayan tract zone of West Bengal, Assam, Burma, Western Ghats, South India and widely cultivated in various parts of India and Burma-Yunnan, Indio-China, Siam, Malaya. The stem bark is bole straight, cylindrical, up to 200 cm in diameter, without buttresses; externally bark are smooth, grey to greyish-white and internally yellowish brown, fibrous and crown are conical to cylindrical. The stem bark are used traditionally for the treatment of fever, colic, leprosy, eye disorder, inflammation, antidote

for scorpion and snake venoms, cough, rheumatism, gonorrheoa, cephalagia, gout [4-5]. It has been reported to possess antipyretic, anti-inflammatory [6], antimicrobial [7], leishmanicidal [8] and antidiabetic activities [9]. Earlier workers have reported various constituents from this plant [10-13].

In the present study, we report the isolation and structure elucidation of three compounds from the petroleum ether extract of the stem bark of this plant. Isolated compounds have been characterized as 3β-16α-dihydroxy-5-cholesten-21-al, n-docosanoic acid and stigmasterol, where categorize under steroid, long chain fatty acids and triterpenoids using spectroscopic techniques.

MATERIALS AND METHODS

General Experimental Procedure

IR spectra were recorded on a Perkin-Elmer model 700 IR spectrophotometer using KBr method. ^{1}H NMR (400 MHz) spectra of the compound were recorded at room temperature in $CDCl_3$ using a Bruker AVANCE 400 spectrometer and chemical shift are given in δ (ppm) value relative to TMS as internal standard. Mass spectra were recorded on a MAT 312 spectrophotometer and FAB-MS (positive) data on JEOL SX 102/DA–6000 using Argon/Xenon (6 kv) as a FAB gas. Mass spectra were recorded from 30-450 *m/z*. TLC was carried out using silica gel 60 F254 precoated aluminium sheets (0.2 mm layer thickness, Merck). Column chromatography was performed on silica gel (S.D. fine chemicals Pvt. Ltd., Bombay, 70–300 mesh). The melting points of compounds were determined on a thermoelectrically melting point apparatus. All the chemicals and reagents used were obtained in high purity either from S.D. fine chemicals Pvt. Ltd; Bombay, India and E. Merck Pvt. Ltd., Bombay, India.

Plant material

Fresh stem bark of *Michelia champaca* were collected from local area of Mangalore, Karnataka, in the month of January (2009) and were authenticated by Dr. Noeline J. Pinto, Professor and Head of Botany, Dept St. Agnes College, Mangalore. A voucher specimen of the plant has been deposited at the Department of Botany, St. Agnes College, Mangalore.

Extraction and Isolation

The fresh stem bark of *M. champaca* was washed with tap water and shade dried at room temperature (28±2°C). The dried stem bark was powdered by electrical blender. Ethanol was used for the extraction of 500 g of plant in the soxhlet apparatus followed by the standard procedure. The plant material was loaded in the body of the soxhlet apparatus and then fitted into a round bottomed flask containing ethanol. The extraction was continued until complete extraction was effected (8 h) and the solvent was removed at the reduced pressure with the help of rotary vacuum evaporator to yield a brownish syrupy consistency. The preliminary phytochemical screenings were performed for testing the different chemical groups present in ethanolic extract using standard procedures [14-15]. The Ethanol extract was then fractioned into petroleum ether soluble fraction (65g), diethyl soluble fraction (40g) and Ethyl acetate soluble fraction (40g). Petroleum ether extract fraction on silica gel column chromatography was eluted with gradient mixture of Petroleum ether: chloroform (95:5, 90:10, 80:20). The elution was monitored by TLC (Silica gel G; visualization: vanillin-sulphuric acid reagent heated at 110°C). Elution carried out with Petroleum ether: chloroform graded mixture yielded Compound **1** (50 mg), Compound **2** (55 mg) and Compound **3** (40 mg) respectively. The compound was then

further purified by preparative TLC on silica gel with the same solvent system to get a pure compound. The IR, ^{1}H NMR and GC-MS analysis of *M. champaca* extract was investigated.

RESULTS

Phytochemical investigations of stem bark of *M. champaca* led to isolation three constituent 3β-16α-dihydroxy-5-cholesten-21-al **1**, n-docosanoic acid **2** from the petroleum ether fraction of ethanolic extract using column chromatography. Its structures have been determined by IR, ^{1}H NMR and MS.

Compound 1

yellow colour, m.p. 139-142°C; IR (KBr) ν_{max} (cm^{-1}); 3431, 2931, 2857, 1712, 594. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 0.09-1.34 (12H, 4×CH_3), 1.63-2.16 (18H, 9×CH_2), 2.05-2.78 (m,8H, 8×CH), 2.16 (t,2H, allylic proton at C-7), 3.87 (s,1H, OH), 3.95 (s, 1H, OH), 5.43 (t, 1H vinylic proton at C-6), 9.85 (d, 1H, CHO). MS (*m/z*); 416 (M^+, $C_{27}H_{44}O_3$), 401, 233, 215, 165, 170, 95, 81, 43 (100%), 41.

Compound 2

white to cream colour crystal, m. p. 74-78, m. p. 74-78°C. IR (KBr) ν_{max} (cm^{-1}); 1711, 1462, 1017. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 0.85-0.89 (m, 3H, terminal methyl), 1.2-1.3 (m, 38H, 19CH_2), 2.1 (t, 1×CH_2, 2H, CH_2 of C-2). MS (*m/z*); 340 (M^+, $C_{22}H_{44}O_2$), 256, 227, 213, 199, 43, 40.

Compound 3

pearl white crystal, m. p. 168°C. IR (KBr) ν_{max} (cm^{-1}); 3433, 2929, 2854, 1634, 1462, 1042, 607. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 0.65-1.1 (m, 18H, 6×CH_3), 1.1-1.26 (m, 18H, 9×CH_2), 1.26-1.86 (8H, 8×CH), 3.59 (1H, CHOH), 5.4 (t, 1H, vinylic protons), 5.2 (2H, allylic protons). MS (*m/z*); 412 (M^+, $C_{29}H_{48}O$), 397, 369, 351, 327, 300, 271.

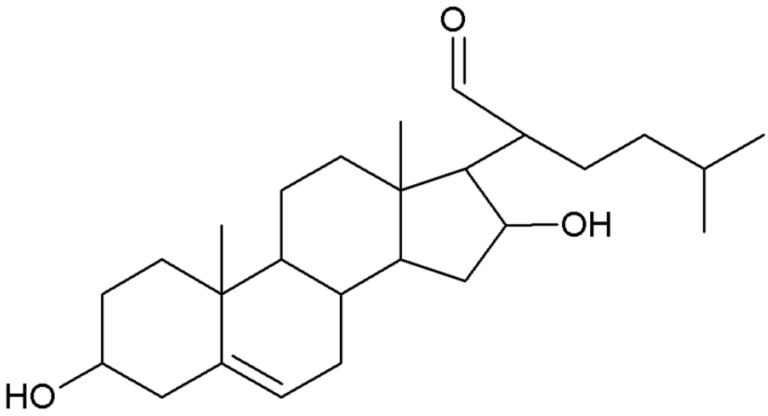
DISCUSSION

Compound **1** designated as 3β-16α-dihydroxy-5-cholesten-21-al, was obtained as yellow crystalline mass from petroleum ether-chloroform (95:5) eluant. It gave a characteristic colour reaction for sterol. Its IR spectrum demonstrated the presence of the hydroxyl group (3431 cm^{-1}), carbonyl group (1712.2 cm^{-1}), C-H stretching in CH_3 (2931 cm^{-1}), C-H stretching in CH_2 (2857 cm^{-1}) and rocking vibration of CH_2 (594.5 cm^{-1}). ^{1}HNMR spectra revealed the presence of four methyl groups between δ 0.09-1.34 and presence of hydroxyl group in 3rd position at δ 3.95. The MS spectra showed the molecular ion peak at *m/z* 416 [M^+] which corresponds to the molecular formula $C_{27}H_{44}O_3$. From the above evidence the compound was determined as 3β-16α-dihydroxy-5-cholesten-21-al.

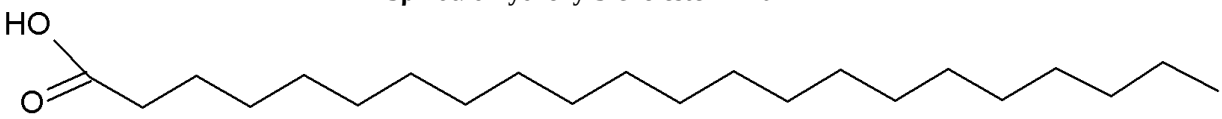
Compound **2,** a long chain fatty acid named n-Docosanoic acid was obtained as white to cream colour crystal from petroleum ether-chloroform (90:10) eluant. The IR spectrum showed characteristic band for carbonyl group (1711 cm^{-1}) and presence of C-H deformation in CH_3 and CH_2 at 1462.2 cm^{-1} and 1017.8 cm^{-1}, respectively. The ^{1}HNMR signal of compound 2 displayed terminal methyl protons at δ 0.85, CH_2 protons at δ 1.2-1.3, and presence of CH_2 protons adjacent

to carboxylic group at δ 2.17. The MS spectra showed the molecular ion peak at *m/z* 340 [M^+] corresponding to the molecular formula $C_{22}H_{44}O_2$. Comparison of spectra data with the known fatty acid supported it's characterization as n-Docosanoic acid.

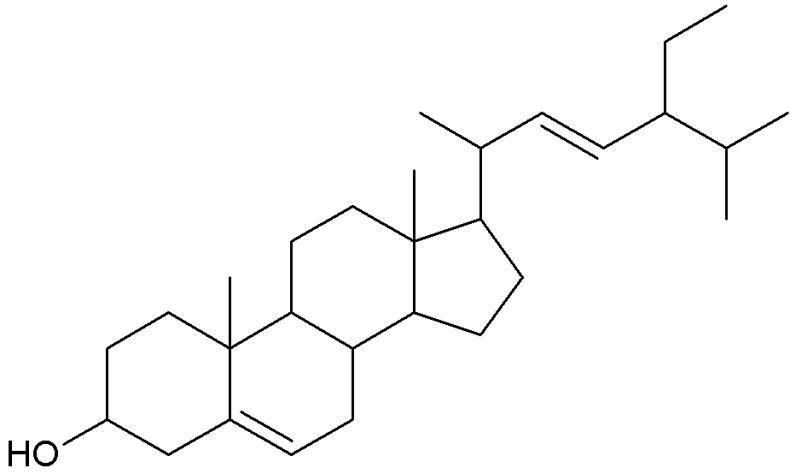
Compound **3**, named stigmasterol showed positive response to Libermann-Burchard test and Salkowski test. The IR spectrum displayed strong absorption at 3433 cm^{-1} and presence of C-H stretching in CH_3 (2929.0 cm^{-1}) and CH_2 (2854.6 cm^{-1}), C=C stretching (1634.5 cm^{-1}), C-H deformation in gem dimethyl (1462.6 cm^{-1}) and C-O stretching of secondary alcohol (1042.4 cm^{-1}). The ^1H NMR spectra of this compound exhibited the presence of six methyl group at position 18, 19, 21, 26, 27 and 29 in between δ 0.65 to 1.1. The ^1H NMR spectra also showed vinylic proton at δ 5.4 and allylic proton at δ 5.2 The MS spectra showed the molecule ion peak at *m/z* 412 [M^+] corresponding to the molecular formula $C_{29}H_{48}O$. Its identity was also confirmed by co-chromatography with an authentic sample (Sigma Chemicals, USA). The structures of phytochemicals isolated from peteroleum ether extract of *Michelia champaca* stem bark are given in Figure 1.



3β-16α-dihydroxy-5-cholesten-21-al



n-docosanoic acid



Stigmasterol

Figure 1. Phytochemicals isolated from stem bark of *Michelia champaca*

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