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Lipid Constituents from *Cissus quadrangularis* Leaves

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ABSTRACT

A phytochemical investigation of *Cissus quadrangularis* leaves yielded five additional known compounds including eicosyl eicosanoate, tetratriacontanol, tetratriacontanoic acid, *α*-amyrin and *β*-sitosterol. The characterization of isolated compounds was achieved by chemical and spectral studies (IR, ^{1}H NMR and Mass Spectroscopy).

Keywords: *Cissus quadrangularis,* Vitaceae, Lipids

INTRODUCTION

Cissus quadrangularis Linn. (Family: Vitaceae) is commonly distributed thorough out the hotter parts of India and Sri Lanka and is known as *asthisanhara* in Sanskrit (1, 2). The plant is useful for treatment of bone fracture, diarrhoea, skin disorders and scurvy (3). The plant is reported to contain high amount of dietary antioxidants that includes vitamin C, carotenoids and polyphenols. Ketosteroids, tetracyclic triterpenoid (7-oxo, onocer-8-ene-3*β*, 21*a*-diol), pentacyclic triterpenoids (*δ*-amyrin and *β*-amyrone), stillbene derivatives, *β*-sitosterol and lipids have been reported from aerial parts, specifically from stems (4–7). Literature survey reveals that majority of studies have been carried out on aerial parts or specifically on stems of the plant. Leaves that constitute only 5–8 % of the aerial plant parts (8) have not been explored for its chemical constituents, hence it was thought worthwhile to emphasize on the phytochemical characterization of *C. quadrangularis* leaves. The present investigation deals with the extraction, isolation and characterization of marker constituents from the hexane extract of leaves of *C. quadrangularis.*

MATERIALS AND METHODS

General procedure

Melting point is uncorrected, IR spectra were recorded in KBr and ^{1}H NMR spectra (300 MHz) were measured in $CDCl_3$ with TMS as internal standard. Column chromatography and TLC were carried out using silica gel G (60–120) at room temperature and spots were visualized by exposure to iodine vapors or spraying with Anisaldehyde sulphuric acid reagent.

Plant material

Plant material was collected locally and authenticated by Prof. H. M. Pandit, Botany Dept., Khalasa College, Mumbai, a voucher specimen is deposited in Medicinal Natural Product Research Laboratory, ICT, Mumbai.

Extraction and isolation

Dried and powdered leaves (1.0 kg) were extracted using hexane by Soxhlet extraction. Wax fraction was obtained by adding acetone to the hexane extract. The precipitated wax was further purified by giving washing with cold

acetone. Compounds I-III were obtained by subjecting the wax fraction to column chromatography using varying percentage of chloroform in hexane. Fractions (250 ml) were collected and monitored by TLC. Subjecting the remaining acetone soluble fraction of hexane extract to column chromatography using varying percentage of ethyl acetate in hexane resulted in isolation of compound IV and V.

Eicosyl eicosanoate (I): Fraction 25–30 of the hexane–chloroform (9:1) eluate was purified by preparative TLC (hexane-chloroform 7:3) to give I (15 mg), IR υ_{max} cm^{-1}: 2916, 2850, 1735, 1460, 1180, 730. ^{1}H NMR δ: 0.88 (6H, t), 1.25 [(CH_2)n, br s], 2.3 (2H, t), 4.05 (2H, t). Mass: M^+ 592 ($C_{40}H_{80}O_2$) and m/z 313, 280.

Tetratriacontanol (II): Fraction 58–66 of the hexane-chloroform (7:3) eluate afforded compound II (200 mg). IR υ_{max} cm^{-1}: 3500–3200, 2920, 2850, 1465, 1180, 1050, 725. ^{1}H NMR δ: 0.88 (6H, t), 1.25 [$(CH_2)_n$, br s], 3.64 (2H, t). MS m/z: 476 (M-18).

Acetylation of II: To 20 mg of II, pyridine and acetic anhydride (2 ml each) were added and the mixture was left overnight at room temperature. It was then diluted with water, extracted with diethyl ether, washed successively with dil HCl, H_2O, $NaHCO_3$ solution and water (each 2 × 50 ml) and then dried (Na_2SO_4). Removal of solvent gave a residue, 15 mg, IR υ_{max} cm^{-1}: 2920, 2850, 1720, 1460, 1180, 730. Mass: M^+ 536.

Tetratriacontanoic acid (III): Fraction (75–80), eluted from hexane-chloroform (1:1) yielded compound III, 25 mg. IR υ_{max} cm^{-1}: 3600–3200, 2920, 2850, 1695, 1460, 1180, 730. ^{1}H NMR δ: 0.88 (6H, t), 1.25 [$(CH_2)_n$, br s], 2.35 (2H, t). Mass: M^+ 508 ($C_{34}H_{68}O_2$).

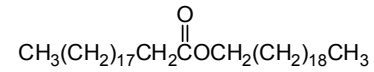
a-Amyrin (IV): Fraction (70–85) eluted from hexane-ethyl acetate (9:1) resulted in compound IV (100 mg), further purified by recrystallization with acetone. IR υ_{max} cm^{-1}: 3450-3200, 2916, 2846, 1461, 1360, 1035, 725. ^{1}H NMR δ: 0.770 (3H, d), 0.793 (3H, s) 0.830 (3 H, s), 0.872 (3H, br s), 0.940 (3H, s), 0.969 (3H, s), 1.000 (3H, s), 1.072 (3H, s), 1.90 (2H, m), 3.218 (1H, q) 5.128 (1 H, t). Mass: M^+ 426 ($C_{30}H_{50}O$).

β-Sitosterol (V): Eluted in hexane-ethyl acetate (8:2) fractions 100–110 afforded compound V, 120 mg, identified by comparison with an authentic sample (melting point, co-TLC).

RESULTS AND DISCUSSION

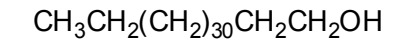
Compounds I-V were isolated by silica gel chromatography followed by preparative TLC of wax fraction and acetone soluble fraction obtained from hexane extract of *C. quadrangularis* leaves.

Compound I, melting point 67–69 °C, had IR absorption bands at 2916 and 2850 (long chain aliphatic group), 1735 (ester carbonyl group) and 730 cm^{-1} (long chain). ^{1}H NMR spectrum of the compound showed a triplet at δ 0.88 for terminal methyl group, a broad singlet at δ 1.25 for − (CH_2)n, a triplet at δ 2.3 for –CH_2-O-C(=O)- and a triplet at δ 4.05 for –CH_2-C(=O)-O- proton, suggesting a long chain aliphatic ester. A $[M]^+$ at m/z 592 suggested the molecular formula as $C_{40}H_{80}O_2$. Further fragment ions at m/z 313 and 280 suggest the acid and alcohol moiety of C_{20} chain length. Thus on the basis of above data and comparing the literature values (7), compound I is characterized as eicosyl eicosanoate [I].



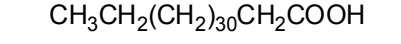
I: Eicosyl eicosanoate

Compound II, melting point 90-92 °C, possessed IR bands at 3500-3200, 2920, 2850, 1050, 730 cm^{-1} for a long chain aliphatic alcohol. ^{1}H NMR displayed a triplet δ 0.88 for terminal methyl group, a broad singlet at δ 1.25 for -(CH_2)n and methoxyl protons adjacent to hydroxyl group resonated at δ 3.64 as a triplet. A $[M]^+$ was absent in its mass spectrum instead it has showed [M-H_2O] at m/z 476 (characteristic of long chain alcohols which gives M-18 peak), suggesting the molecular formula as $C_{34}H_{70}O$. On acetylation it afforded a mono acetate, $(M)^+$ at m/z 536($C_{36}H_{72}O_2$). The compound II is characterized as tetratriacontanol.



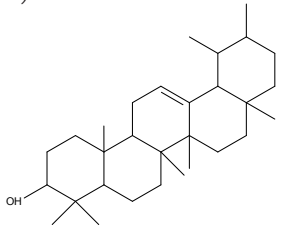
II: Tetratriacontanol

Compound III, melting point 96–98 °C possessed IR bands at 3620–3200, 1695, 730 cm^{-1} for long chain aliphatic carboxylic acid. The ^{1}H NMR displayed a triplet at δ 0.88 for terminal methyl group and the methoxyl protons adjacent to carbonyl group resonated at δ 2.35 as a triplet. Presence of $[M]^+$ at m/z 508 ($C_{34}H_{68}O_2$), indicates the acid to be tetratriacontanoic acid.



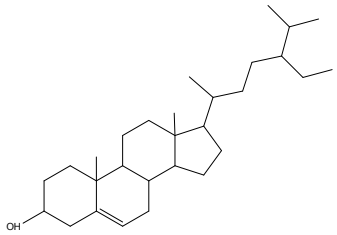
III: Tetratriacontanoic acid

Compound IV, melting point 178-182 °C, has been isolated from acetone soluble fraction. The compound was confirmed as *a*-amyrin by comparing the spectral data (^{1}H NMR and MS) with the literature values (9, 10).



IV: *a*-Amyrin

Compound V (*β*-sitosterol) melting point 130–135 °C, has been isolated from acetone soluble fraction and characterized by comparison with an authentic sample.



V: *β*-sitosterol

CONCLUSION

In conclusion, from the leaves of C. *quadrangularis* five additional marker constituents namely eicosyl eicosanoate, tetratriacontanol, tetratriacontanoic acid, *a*-amyrin and *β*-sitosterol have been isolated and characterized by spectral analysis and by comparison with the authentic samples. The isolated compounds can serve as important marker constituents for standardization of *C.quadrangularis* extract and its formulations.

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