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Phytochemical Studies on *Cissus quadrangularis* Linn.

Achal Thakur[1]*, Vandana Jain[1], L. Hingorani[2] and K.S. Laddha[1]

[1]A-286, Medicinal Natural Products Research Laboratory, Pharmaceutical Division, University Institute of Chemical Technology, Nathalal Parikh Marg, Matunga, Mumbai-400 019. Maharastra, India..
[2]Pharmanza Herbal Pvt. Ltd, Plot No 214, Near Vadadia Patiya, Borsad-Tarapur Road, Kaniya 388435,Dist.Anand, Gujrat,India.

**** For Correspondence :** Achal Thakur E- mail: achalthakur@gmail.com*

ABSTRACT

Cissus quadrangularis Linn. (Family: Vitaceae) is an ancient medicinal plant, named as Hadjod in Hindi. A triterpene δ-amyrin acetate (1), aliphatic acid hexadecanoic acid (**3**) and stilbene glucoside trans-resveratrol-3-O-glucoside (**9**) were isolated for the first time from the stems of *Cissus quadrangularis*, along with previously reported compounds namely, δ- amyrone (**2**) δ-amyrin (**4**), β-sitosterol (**5**), kaempferol (**6**), quercetin (**7**) and resveratrol (**8**). The structure elucidation of the isolated compounds were performed by spectroscopic techniques (IR, UV, ^{1}H-NMR, ^{13}C-NMR and MS) and by direct comparison with literature.

Keywords: *Cissus quadrangularis,* delta-amyrin acetate, hexadecanoic acid, trans-resveratrol-3-O-glucoside

INTRODUCTION

Cissus quadrangularis (Vitaceae), a rambling shrub, characterized by a thick quadrangular fleshy stem, is an edible plant found in hotter parts of India, Sri Lanka, Malaya, Java and West Africa. Commonly known as the "bone setter," the plant is referred to as "Asthisamdhani" in Sanskrit and "Hadjod" in Hindi because of its ability to join bones. The plant has been documented in Ayurveda for its medicinal uses in gout, syphilis, venereal disease, piles, leucorrhoea and as an aphrodisiac. The Siddha system of medicine illustrates its administration for the treatment of piles, diarrhoea and dysentery as well as in kapham (1). Previous studies reported the presence of triterpenoids, steroids, lipids, stilbenes, flavonoids and iridoids (2–6). In this study we report the isolation and characterization of nine compounds in which three compounds namely triterpene δ-amyrin acetate, lipid constituent hexadecanoic acid and stilbene glucoside trans-resveratrol-3-O-glucoside (Piceid), have been reported for the first time from the stems of *Cissus quadrangularis*.

MATERIALS AND METHODS

General procedures

UV spectra was recorded on Jasco V-530 UV/VIS spectrophotometer. IR spectra were measured in KBr using Perkin Elmer FTIR RX-1 infrared spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL Eclipse (300 MHz) spectrometer in CD_3OD. The FAB mass spectra (positive modes) were taken on a micro mass: Q-Tof micro (YA-105). Meting points were recorded on Perkin Elmer DSC (Diffential Scanning Calorimeter) Pyris 6 DSC. TLC and column chromatography were carried out on pre coated silica gel 60 F_{254} plates (0.25mm thick,

Merck, Darmstadt, Germany) and silica gel(60–120 mesh, Merck, Darmstadt, Germany) respectively.

Plant material.

The stems of *Cissus quadrangularis* were collected from the wild location near Mumbai in March 2007. The identities of the collected sample were confirmed by comparison with the authentic sample and by conducting macro and microscopic studies. The voucher specimens (voucher specimen reference number CQ/A) of the plant samples used for this study were deposited in the Department of Medicinal Natural Product Laboratory, University Institute of Chemical Technology, Mumbai, India.

Extraction and isolation.

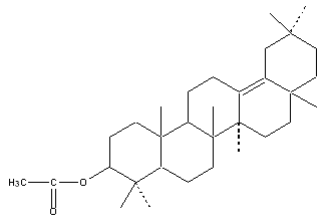
The powdered stem (10 kg) was extracted with methanol yielding a crude extract (605 g) that was partitioned successively with pet ether (60-80 fraction) and ethyl acetate affording pet ether (130 g) and ethyl acetate (58 g) soluble fractions. Pet-ether fraction (130 g) was further fractionated with acetone and acetone soluble fraction was concentrated and mounted on silica bed and eluted with chloroform. The chloroform fractions were combined together and evaporated to dryness under reduced pressure to yield chloroform fraction (26 g).

The chloroform fraction was chromatographed over silica gel (60–120 mesh, 600 g) and eluted with pet ether: ethyl acetate (100:1) yielding compound 1(7 mg), 2 (13 mg), 3 (18 mg), 4 (75 mg) and 5 (155 mg).

The ethyl acetate fraction (55 g) was subjected to repeated chromatography on silica gel (60–120 mesh, 1.5 kg) column. Gradient elution was carried out using ethyl acetate and increasing the polarity with chloroform in 5% stepwise elution to 100% chloroform and then with chloroform and increasing polarity with methanol in 5% stepwise increments to 70% chloroform and 30% methanol to yield two broad fractions EA and EB according to the similarity of the TLC profile. The EA fraction was rechromatographed over silica gel several times, using gradient mobile phase ($CHCl_3$:CH_3OH) yielding compound 6 (15 mg) and 7(152 mg). In a similar way fraction EB when rechromatographed using gradient mobile phase ($CHCl_3$:CH_3OH) resulted in the isolation of compound 8(12 mg) and 9 (6 mg).

RESULTS AND DISCUSSION

Compound 1 was obtained as white crystals and its MS showed a molecular ion peak at m/z 469.6 $(M+H)^+$ consistent with molecular formula $C_{32}H_{52}O_2$ (calculated. 468.75). The IR spectrum showed the presence of ester carbonyl at 1726.1 cm^{-1}, ether O-C=O stretch at 1245.9 cm^{-1}, methylene C- H stretch at 2852.52 cm^{-1}. The ^1H NMR spectrum revealed the presence of eight tertiary methyl groups at δ 0.73, 0.84, 0.87, 0.94, 0.96, 1.01, 1.07 and acetate methyl at 2.04. The multiplet at δ 4.50 is assigned for the proton on the carbon containing acetoxy group.



1: *δ-Amyrin acetate*

The ^{13}C NMR spectrum showed δ 142.67 and 145.21 which confirms the olefinic bond between C 13 and C 18. The signals at δ 171.04 and 21.33 confirmed the presence of acetyl group. On the basis of spectroscopic data and previously reported literature values (7), compound 1 was determined as δ-amyrin acetate.

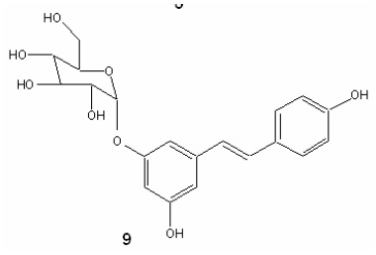
Compound 3 was obtained as white amorphous powder. IR spectrum showed bands for hydroxyl group (3445 cm^{-1}) and carbonyl group (1706 cm^{-1}). The ^1H NMR spectrum displayed a triplet at δ 0.88 for the terminal methyl group. The triplet at δ 1.63 assigned for the methylene proton of C 3 and a triplet at δ 2.34 for methylene proton adjacent to carbonyl group. The ^{13}C NMR spectrum showed the signals at δ 179.7 assigned for the carboxylic carbon and a signal at δ 14.1 for terminal methyl carbon. GC- Mass spectra showed peak at m/z 256 $[M]^+$ corresponding to the molecular formula $C_{16}H_{32}O_2$. Therefore the structure of compound 3 was concluded to be palmitic acid (Hexadecanoic acid).

$$CH_3 (CH_2)_{14} COOH$$

3: *Hexadecanoic acid*

Compound 9 was obtained as off white crystals. Its UV spectrum (216, 305) suggested a stilbene structure. IR spectrum showed bands at 3250 (stretching, O-H), 2822 (stretching, C-H), 1587, 1511 (stretching, C=C) cm^{-1}. The ^1H NMR spectrum revealed the presence of two set of signals. The formal, between δ 3 and 5.3 and is assigned to glucosyl proton. The letter set between δ 6 to 7.5 is constituted by three systems of olefinic and aromatic protons. At δ 7.37 and 6.73, two doublets are assigned to 1-4 disubstituted aromatic ring. Three broad singlets at δ 6.32, 6.55 and 6.88 are assigned to three *meta* related protons of a 1, 3, 5 trisubstituted aromatic ring. A double

doublet at δ 4.63 and δ 5.03 was assigned to protons at 6"a and 6"b respectively. Mass spectrum of trans-piceid showed molecular ion peak at 391 $[M+H]^+$ and intense peak at 229 $[M- 162]^+$. Thus on the basis of spectral evidence and previously reported values (8), structure of compound 9 was elucidated as trans-resveratrol-3-O-glucoside.



9: *Trans-resveratrol-3-O-glucoside*

The structures of other known compounds isolated from *Cissus quadrangularis* were identified by comparison of their spectroscopic data with reported values from literature as δ-amyrone (2), δ-amyrin (4), β-sitosterol (5), kaempferol (6), quercetin (7) and resveratrol (8).

δ-Amyrin acetate:

$C_{32}H_{52}O_2$, white crystal, IR ν_{max} (KBr) cm^{-1}: 1726.1 (stretching, ester, C=O), 1245.9 (stretching, ester, O-C=O), 2852.52 (stretching C-H). ^{1}H NMR: H-23- 0.94 (3H, s), H-24- 0.73 (3H, s), H-25-0.84 (3H, s), H-26-0.1.01 (3H, s), H-27-1.07 (3H, s), H-28-0.96 (3H, s), H-29-0.87 (6H, s), H-30- 0.87 (6H, s), 2.04 (3H,s, OAc),H-3- 4.50 (1H, m). ^{13} C NMR: 39.78 (C-1), 34.81 (C-2), 80.94 (C-3), 37.13 (C-4), 55.56 (C-5), 18.14 (C-6), 26.67 (C-7), 41.69 (C-8), 51.12 (C-9), 37.35 (C-10), 23.52 (C-11), 26.90 (C-12), 142.67 (C-13), 46.76 (C-14), 25.95 (C-15), 36.82 (C-16), 34.71 (C-17), 145.21(C-18), 40.75 (C-19), 33.33 (C-20),37.70 (C-21), 38.60 (C-22), 21.11 (C-23), 27.65(C-24), 16.77 (C-25), 17.80 (C-26), 23.56 (C-27), 25.25 (C-28), 23.69 (C-29), 34.51 (C-30), 171.04 (C=O), 21.33 (O-C-CH3). FAB-MS (*m/z*): 469.6 $[M+1]^+$.

Hexadecanoic acid:

$C_{16}H_{32}O_2$, white amorphous, mp. 69 ^{0}C, IR ν_{max} (KBr) cm^{-1}: 3445.8 (stretching, O-H), 2971.3, 2848.0 (stretching, C-H), 1706.2 (stretching, C=O).^{1} H NMR: H- 16, 0.88 (3H, t), H-3/15, 1.26 (br.s), H-3, 1.63 (2H, t), H-2, 2.34 (2H, t).^{13} C NMR: 179.72 (C-1), 33.99 (C-2), 24.68 (C-3), 29.38 (C-4), 29.71 (C-5-C11), 29.44 (C-12), 29.25 (C-13), 31.93 (C-14), 22.7 (C-15), 14.13(C-16). GC-Mass (*m/z*): 256 $[M]^+$.

Trans-resveratrol-3-O-glucoside (Piceid):

$C_{20}H_{22}O_{8,}$ Off white crystals, UV (MeOH) λ_{max}: 216, 305; IR (KBr) ν_{max} (cm^{-1}) 3250, 2822, 1587, 1511. ^{1}H NMR (CD_3OD, 300MHz): 7.37 (2H, d, H-2', H-6'), 7.05 (lH, s, Hb), 6.99 (lH, d, Ha), 6.88 (1H, *s*,H-2), 6.73 (2H, d, H-3', H-5'), 6.32 (1H, *s*, H-6),6.55 (1H, *s*, H-4), 5.29 (1H, d, Glc H-1"), 5.03 (IH, *dd*, Glc H-6a"), 4.63 (1H, *dd*, Glc H-6b"), 3.18-3.70 (4H, m, Glc H-2", H-3", H-4". H-5"); ^{13}C-NMR (300 MHz, CD3OD): 60.77(C-6"), 69.81(C-4"), 73.34(C-2"), 76.74(C-3"), 77.18(C-5"), 100.69(C-1"), 102.79(C-4), 104.79(C-2), 107.22(C-6), 115.59(C-3',5'), 125.28(C-7), 128.02 (C-2',6'), 128.62(C-8), 139.43 (C-1'), 146.65(C-1), 157.36(C-4'), 158.41(C-5), 158.93(C-3). FAB-MASS spectrum. FAB-MS (m/z) : $[M+H]^+$ 391, 229 $[M-162]^+$.

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