Chemical Constituents and Melanin Promotion activity of *Cissus quadranglaris* Linn

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Abstract

Bio-activity guided isolation of the methanolic extract of the stems of Cissus quadrangularis Linn., resulted three known compounds, lupeol (1), freidalin (2) and β-sitosterol (3). The structures of these compounds were elucidated by physical and spectral data (UV, IR, ^{1}H, ^{13}C NMR and Mass). Different fractions and compounds from the stems were investigated for melanin promotion activity. The compound, lupeol (3) showed five times more potent melanin promotion activity when compared with standard control compound, 3-isobutyl-1-methylxanthine (IBMX).

Key words: *Cissus quadrangularis*, Lupeol, Melanin promotion activity, IBMX.

Introduction

Cissus quadrangularis Linn. (Syn. *Vitis quadranglularis* L.) is belongs to Vitaceae family. It is a rambling shrub usually found in hotter parts of India, Sri Lanka, Malaysia, Java and West Asia. The stem is thick, fleshy, glabrous, and ridged and quadranglular at the nodes. It is referred as "Asthisanhari" in Sanskrit, "hadjod" in Hindi because of its ability to join bones and commonly known as the "bone setter" in English. It is an edible plant; the stems are made into curries, chutneys and eaten in Southern parts of India. The ash of the plant is being used as substitute for baking powder. The stem is useful in piles. The juice of the plant being used to control irregular menstruation, diseases of the ear and in nose-bleeding. A paste of the stem is given in asthma and may be useful for muscular pains, burns, and wounds. A decoction of the shoots with dry ginger and black pepper is given for body pain. The infusion of the plant is anthelmintic[1]. The total plant is considered to be an alternative, aphrodisiacs and anthelmintic, antiasthmatic and is useful in gastrointestinal disorders such as colic and dyspepsia and irregular menstruation[2]. The herb has been used as veterinary medicine. It has been fed to cattle to induce flow of milk[2]. The entire plant is being used in fractures, sprains, rheumatism and irregular growth of teeth, broken horn, anthrax, heamaturia and elephantiasis, dislocation of hip, various wounds and cracked tail[2]. The stem pulp is useful in eye diseases and chronic ulcers. The herb yields an anabolic oxo-steroid which accelerated fracture healing in rats[3]. A few clinical trials have been conducted by using this plant material. It was used as a supplement to treat jawbone fracture and the immobilization period was reduced significantly. An herbal preparation was made in the form of lotion and mouth spray, possessing this herb as a constituent and found to be effective in a variety of inflammatory lesions of the oral cavity including gingivitis and periodontitis[4]. Previous studies reported the presence of several new and known compounds like, lipids[5], stilbenoids[6], triterpenoids[7], steroids[8], iridoids[8] and flavonoids[8,9].

In our continuous interest on the isolation of bioactive secondary metabolites from the plants for personal care applications[10-17], we have undertaken chemical examination of the stems of *Cissus quadranglaris* Linn. We report here the isolation and structure elucidation of three known compounds and melanin promotion activity. The structures of these compounds were characterized by spectral data and co-TLC with an authentic compound. Compound 1 showed potent melanin promotion activity.

Material and Methods

General procedures: Melting points were reported are uncorrected. ^{1}H-NMR, ^{13}C NMR spectra were recorded on Bruker spectrometer in $CDCl_3$, operating at 400MHz for ^{1}H-NMR and 100 MHZ for ^{13}C NMR. EI mass was recorded on Jeol SX 102/DA 600 mass spectrometer. IR spectra were recorded on a Shimadzu IR Prestige 21. UV spectra were recorded on Shimadzu UV spectrophotometer. Column chromatography (CC) was carried on a silica gel column (100-200 mesh). Purity of the samples was checked by TLC on pre-coated aluminum sheets, silica gel 60 F_{254} (20 X 20 cm, 0.2 mm thickness, Merck) and compounds were detected by spraying with 5% sulphuric acid in methanol followed by heating the plates at 110^{o}C for 5 min. The chemical shift values are reported in ppm (δ) units and the coupling constants (*J*) are in Hz. For melanin promotion assay, all chemicals and IBMX were purchased from Sigma (USA).

Plant material: The stems of *Cissus quadrangularis* Linn., (510 g) were collected from Padappai, near Chennai, Tamil Nadu, during January 2009. The plant was authenticated by Dr. P. Santhan, Plant Taxonomist, Durva Herbal Centre, Chennai, India. A voucher specimen of this plant was deposited in Cavinkare Research Centre, Chennai, India.

The powdered stems were exhaustively extracted with methanol (3.0 L) by using soxhlet apparatus for 8 hrs. After evaporation of the solvent under reduced pressure, 32.45 g crude extract was obtained and showed an excellent melanin promotion activity (380% at 50µg/ml). The crude methanolic extract was subjected to silica gel vacuum liquid chromatography (100-200 mesh, 80 g), using solvents such as hexane, hexane : ethyl acetate (19:1, 18:2, 16:4, 1:1), ethyl acetate and methanol to get corresponding fractions 0.4 g (Fr.1), 1.46 g (Fr. 2), 1.50 g (Fr. 3), 1.61 g (Fr. 4) 2.0 g (Fr.5), 5.0g (Fr.6) and 21.0g (Fr.7) respectively. All seven fractions were submitted for biological studies. Fr. 2 showed an excellent melanin promotion activity whereas Fr. 4 showed moderate activity.

The resulting dark green residue of Fr. 2 showed solid in nature and its TLC showed mixture of two compounds. The fraction was purified by small silica gel column eluted with hexane: chloroform (1:1) and obtained two compounds, 1 (60 mg) and 2 (65 mg). The moderately active fraction, Fr.4 was found to be solid in nature, crystallized with methanol and obtained compound 3 (305 mg). All three compounds were submitted for biological activity and compound 1 showed melanin promotion activity.

Results and Discussion

Compound 1 (Lupeol): Crystallized from hexane: ethyl acetate, mp: 194-99^{o}C. UV ($CHCl_3$, γ_{max} in nm): 215nm; IR (KBr, ν_{max} in cm^{-1}): 2850, 1620, 1400, 980; ^{1}H NMR ($CDCl_3$): δ 0.77 (3H, s, H-24), 0.82 (3H, s, H-28), 0.86 (3H, s, H-25), 0.94 (3H, s, H-27), 0.95 (3H, s, H-23), 1.04 (3H, s, H-26), 1.69 (3H, s, H-30), 2.40 (1H, ddd, *J*=5.6, 11.0, 11.0 Hz, H-19), 3.20 (1H, dd, *J* =5.1, 11.5 Hz, H-3), 4.57 (1H, d, *J* =1.3 Hz, H-29), 4.69 (1H, d, *J*= 1.3 Hz, H-29); ^{13}C NMR ($CDCl_3$): δ 38.6 (C-1), 27.3 (C-2), 78.9 (C-3), 38.9 (C-4), 55.2 (C-5), 18.2 (C-6), 34.2 (C-7), 40.7 (C-8), 50.3 (C-9), 37.1 (C-10), 20.8 (C-11), 25.0 (C-12), 38.7 (C-13), 42.7 (C-14), 27.9 (C-15), 35.4 (C-16), 42.9 (C-17), 48.2 (C-18), 47.9 (C-19), 150.9 (C-20), 29.7 (C-21), 39.9 (C-22), 27.9 (C-23), 15.3 (C-24), 16.0 (C-25), 15.9 (C-26), 14.5 (C-27), 17.9 (C-28), 109.3 (C-29), 19.3 (C-30). EI-MS (m/z): 426 [M]$^{+}$, 411, 408, 393, 218, 207, 189.

Compound 2 (Friedalin): Crystallized from hexane, mp: 258-60^{o}C. UV ($CHCl_3$, γ_{max} in nm): 215 nm, IR (KBr, ν_{max} in cm^{-1}): 2855, 1710 (C=O), 1461 and 1380, 980; ^{1}H NMR ($CDCl_3$): δ 0.73 (3H, s), 0.88 (3H, d, *J*= 6.7 Hz), 0.87 (3H, s), 0.96 (3H, s), 1.00 (3H, s), 1.01 (3H, s), 1.05 (3H, s), 1.18 (3H, s), 2.39 (1H, m); ^{13}C NMR ($CDCl_3$): δ 22.30 (C-1), 41.51 (C-2), 213.28 (C-3), 58.19 (C-4), 42.13 (C-5), 41.51 (C-6), 18.24 (C-7), 53.07 (C-8), 37.41 (C-9), 59.42 (C-10), 35.72 (C-11), 30.54 (C-12), 38.26 (C-13), 39.67 (C-14), 32.44 (C-15), 36.09 (C-16), 29.97 (C-17), 42.74 (C-18), 35.43 (C-19), 28.16 (C-20), 32.73 (C-21), 39.25 (C-22), 6.82 (C-23), 14.64 (C-24), 17.93 (C-25), 18.65 (C-26), 20.24 (C-27), 32.07 (C-28), 35.06 (C-29),

31.68 (C-30). EI-MS (m/z): 426 $[M]^+$, 411, 398, 302, 273, 218, 205, 163, 123, 95, 69, 44.

Compound 3 (β-sitosterol): Crystallized from methanol, mp: 137-40°C. UV ($CHCl_3$, γ_{max} in nm): 214 nm; IR (KBr, ν_{max} in cm^{-1}): 3500-3300 (OH), 2940, 2860, 1640, 1380, 1060, 1020, 970, 960, 800; ^1H NMR ($CDCl_3$): δ 0.68 (3H, s), 0.82 (3H, d, *J* =7.5 Hz), 0.84 (6H, d, *J* =7.8 Hz), 0.92 (3H, d, *J*= 5.08 Hz), 1.01 (3H, s), 3.51 (1H, br m), 5.36 (1H, br s), ^{13}C NMR ($CDCl_3$): δ 37.24 (C-1), 31.56 (C-2), 71.79 (C-3), 42.29 (C-4), 140.75 (C-5) 121.7 (C-6), 31.85 (C-7), 31.85 (C-8), 50.12 (C-9), 36.49 (C-10), 21.07 (C-11), 39.76 (C-12), 42.30 (C-13), 56.75 (C-14), 24.28 (C-15), 28.23 (C-16), 56.04 (C-17), 11.87 (C-18), 19.38 (C-19), 36.13 (C-20), 19.02 (C-21), 33.92 (C-22), 29.11 (C-23), 45.82 (C-24), 26.05 (C-25), 18.76 (C-26), 19.81 (C-27), 23.05 (C-28), 11.97 (C–29). EI-MS(m/z): 414 $[M^+]$, 396, 381, 273, 255, 231, 213.

Melanin promotion activity: The melanin promotion activity[18] of different fractions of methanolic extract along with its crude extract and IBMX (control) were studied in cell lines (B16F10 melanoma). The assay method is most precise and reliable. The crude methanolic extract, fractions 2 and 4 and lupeol (1) were showed significant activity by producing the more melanin in the cells (table 1).

Conclusion

Friedalin and β–sitosterol[19] have already been reported from this plant while lupeol[20] is being reported for the first time from this plant. The study of the melanin promotion activity is also first time for this plant.

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Table-1: Intro Melanin Promotion Activity

Compound / fraction	Concentration (μg/ml)	% Melanin promotion
Methanolic extract	50	380
Fr.2	25	150
Fr.4	25	53
IBMX (Control)	15	70
Lupeol (**1**)	15	401
Friedalin (**2**)	50	Not Active
β-Sitosterol (**3**)	50	Not Active

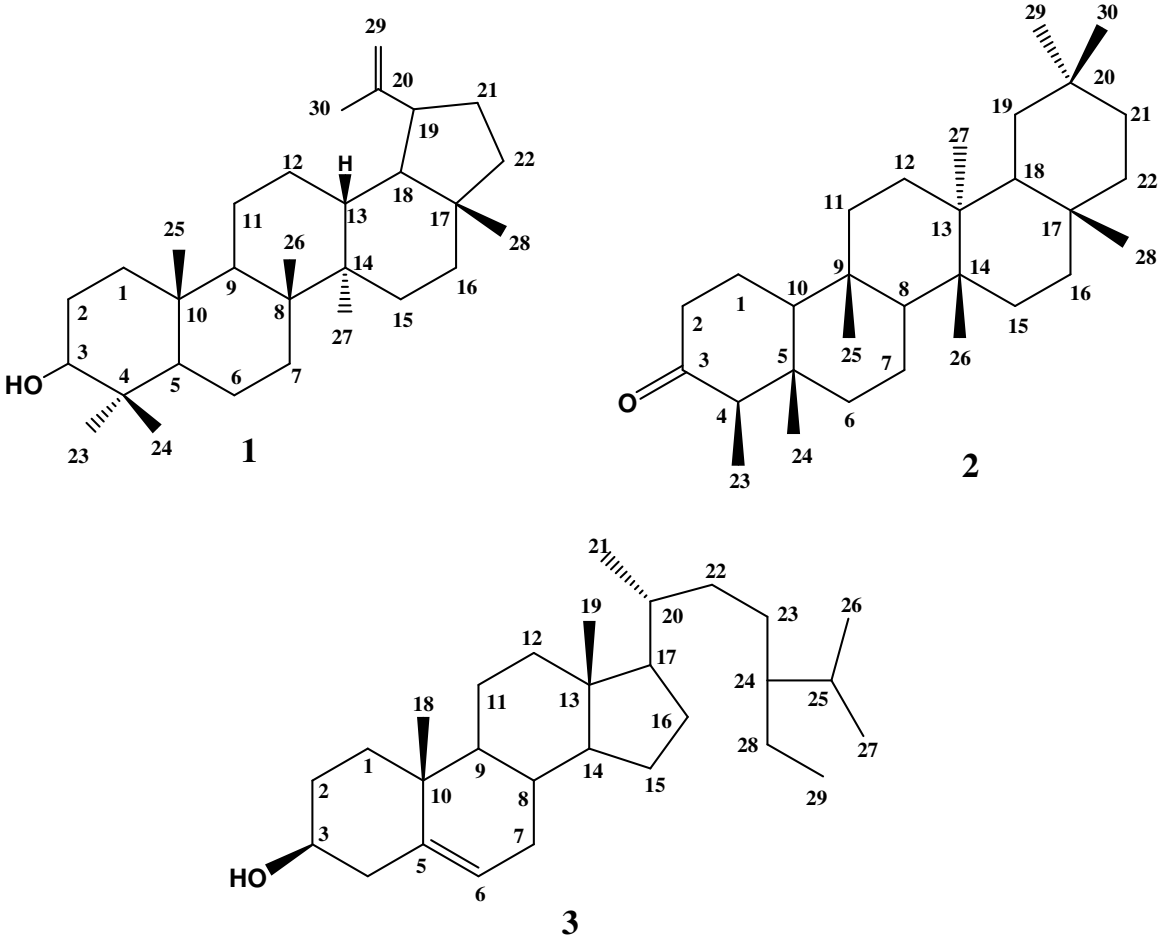

Fig 1: Structures of three compounds isolated from *Cissus quadrangularis*