Chemical Examination and Biological Studies on the Bark of *Crataeva nurvala* Buch.-Ham.

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ABSTRACT

In continuation of our investigation on bioactive molecules, the melanin promotion activity-guided fractionation of the methanolic extract of the bark of *Crataeva nurvala*, yielded two known triterpenoid derivatives, lupeol (**1**) and lupenone (**2**). The structure of the compounds were established by physical and spectral data (UV, IR, ^{1}H, ^{13}C NMR and Mass). The compounds were shown potent melanin promotion activity when compared with standard control compound, 3-isobutyl-1-methylxanthine (IBMX).

Key words: *Crataeva nurvala*, triterpenoids, melanin promotion activity.

INTRODUCTION

Crataeva nurvala Buch.-Ham., belongs to the family Capparaceae. It is a medium sized, ornamental, deciduous tree, 6-10 m tall, found either wild or cultivated throughout India. The stem bark is bitter astringent and acrid. In Indian medicine, the stem bark is being widely used as single drug or in compound formulations for the treatment of urinary disorders including urolithiasis, prostatic hypertrophy, blood purifier, skin infections, neurogenic bladder and chronic urinary infections. The stem-bark is also considered anti-inflammatory and also reported to stimulate appetite, bile secretion and bowel movement.[1] It is externally applied to abcess, boils and lymphadenopathy. The leaves are also bitter, acrid, stomachic, depurative, anti-inflmmatory, tonic, antiperiodic and expectorant.[2] The decoction of leaves and stem is used as a remedy for desentery and diarrhoeal diseases by the natives of Taiwan. It is also used as an anticonvulsant in the folklore medicine of the Philippines.[1] Several reports of *C. nurvala* have demonstrated the presence of steroids, terpenoids, fatty alcohols and acids, flavonoids.[3-7] In continuation of our interest on the isolation of bioactive compounds from medicinal plants for cosmetic applications,[8-13] we have undertaken chemical examination of the bark of *C. nurvala*. The present study describes the isolation of two triterpenoids: lupeol (**1**) and lupenone (**2**) from the bark of *C. nurvala*. The crude extract, its fractions and isolated compounds were analysed for melanin promotion activity in B16F10 cells and found that the isolated compounds were shown potent melanin promotion activity.

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MATERIALS AND METHODS

General

Melting points reported are uncorrected. The 400 MHz NMR spectra were recorded on a Bruker AMX 400 in $CDCl_3$ or CD_3OD with TMS an internal standard. The ^{13}C NMR spectra were recorded at 100 MHz in $CDCl_3$ and CD_3OD. IR spectra were recorded on a Shimadzu IR prestige 21; UV spectra were recorded on Shimadzu UV spectrophotometer; GC-MS were on a Jeol SX 102/DA 6000 mass spectrometer. TLC was performed on pre-coated silica gel 60 F_{254} plates (Merck) and the spots were visualized by exposure to iodine vapour or spraying with 5% sulphuric acid in methanol followed by heating the plate at 110ºC for 5 min. The IBMX was purchased from the M/s. Sigma-Aldrich, USA

Plant material

The bark of *C. nurvala* was obtained from bazaar and was authenticated by Dr. P. Santhan, botanist, M/s. Durva Herbal Centre, Chennai. A voucher specimen was deposited in M/s. CavinKare Research Centre, Chennai, India.

Extraction and Isolation

Air-dried bark of *C. nurvala* (1.8 kg) was crushed and coarsely powdered, subjected to an extraction with methanol (3 L)

by using a soxhlet apparatus. After evaporation of the solvent under reduced pressure, 46g of crude methanolic extract was obtained. The crude methanolic extract (45.5g) was suspended in methanol : water (2:8) and partitioned with ethyl acetate and saturated n-butanol to get corresponding fractions 16.1 g and 4.2 g respectively. The EA fraction showed good melanin promotion activity, where as n-butanol fraction showed less activity.

After TLC analysis, the dark brown residue from ethyl acetate fraction (16.0 g) was subjected to silica gel column chromatography, eluted with hexane: ethyl acetate (1:1, 3:1) and ethyl acetate. The homogeneous fractions were combined based on TLC and divided into three major fractions A (5.6 g), B (6.3 g) and C (1.5 g). Fraction A was further purified by silica gel column using a solvent mixture hexane: ethyl acetate (9:1, 7:3) yielded one compound which was re-crystallized with hexane: ethyl acetate to get colorless crystals **2** (230 mg). Fraction B came as brown color solid, which was directly re-crystallized with ethyl acetate and methanol to get colorless amorphous compound **1** (4.65 g).

Compound **1:** Colorless amorphous powder, mp: 201-202°C; UV ($CHCl_3$, γ_{max} in nm): 215; IR (KBr, ν_{max} in cm^{-1}): 3340 (hydroxyl), 2850, 1620, 1400, 980, ^1H NMR (CD_3OD): δ 0.75 (3H, s, 24-H), 0.82 (3H, s, 28-H), 0.86 (3H, s, 25-H), 0.94 (3H, s, 27-H), 0.95 (3H, s, 23-H), 1.04 (3H, s, 26-H), 1.69 (3H, s, 30-H), 2.40 (1H, ddd, *J* = 5.6, 110.11.0, 19-H), 3.12 (1H, dd, *J* = 5.1, 11.5 Hz), 4.55 & 4.67 (each 1H, d, *J* = 1.3Hz, 29-H), GC-MS: M/z 426 (36%), 411 (16%), 311 (15%), 257 (11%), 218 (53%), 189 (74%), 135 (79%), 121 (77%), 109 (91%), 95 (100%).

Acetylation of **1**: To a solution of **1** (50 mg) in pyridine (2 ml), Ac_2O (2 ml) was added and the mixture kept for 24 hr at room temperature. After usual work-up yielded mono acetate derivative, **1a** (45 mg).

Compound **1a:** Colorless powder, mp: 164-66°C. ^1H NMR ($CDCl_3$): δ 0.78 (3H, s), 0.83 (3H, s), 0.84 (3H, s), 0.85 (3H, s), 0.94 (3H, s), 1.02 (3H, s,), 1.68 (3H, s), 2.04 (3H, s), 4.46 (1H, dd, *J* = 6.1, 10.1 Hz), 4.57 (1H, s), 4.68 (1H, s). GC- MS: M/z 468 (26%), 453 (12%), 408 (8%), 311 (15%), 218 (42%), 204 (46%), 189 (100%), 121 (80%), 109 (84%), 95 (92%), 81 (71%), 69 (59%).

Compound **2**: Colorless crystals, mp: 164-66°C; UV ($CHCl_3$, γ_{max} in nm): 214; IR (KBr, ν_{max} in cm^{-1}): 2937, 1705 (carbonyl), 1448, 1382, 1016, 869; ^1H NMR ($CDCl_3$): δ 0.80 (3H, s, 28-H), 0.93 (3H, s, 25-H), 0.96 (3H, s, 27-H), 1.03 (3H, s, 23-H), 1.07 (6H, s, 24 & 26-H), 1.68 (3H, s, 30-H), 4.58 (1H, m, 29-H), 4. 70 (1H, d, *J* = 2.2 Hz, 29-H).

Melanin promotion assay

The melanin promotion activity[18] of crude methanolic extract, its fractions, isolated compounds and IBMX (control) were studied in cell lines (B16F10 melanoma cells). The assay method is most precise and reliable. The compound lupeol (**1**) showed potent activity by producing more melanin in the cells than its oxidized compound, lupeonone (**2**). Similarly, another derivative of lupeol, lupeol acetate showed less activity than its parent compound (Table 2)

RESULTS AND DISCUSSION

Repetitive chromatographic steps of the ethyl acetate fraction of the dried bark of *C. nurvala* yielded two known triterpenes [Figure 1].

Compound **1** was obtained as colorless amorphous powder, mp: 201-202°C. It was readily recognized as triterpene derivative from its preliminary NMR spectral data. Its molecular formula was established as $C_{30}H_{50}O$ by GC-MS, M^+ 426. Its IR spectrum showed the presence of hydroxyl group (3340 cm^{-1}) and its UV spectrum showed no characteristic absorption. The ^1H-NMR spectrum clearly showed triterpenoid pattern with six methyl singlets in the up-field at δ 0.75 (Me-24), 0.82 (Me-28), 0.86 (Me-25), 0.94 (Me-27), 0.95 (Me-23), 1.04 (Me-26) and one more methyl appeared as a sharp singlet at δ1.69 (Me-30). The down field shift for Me-30 indicated the presence of double bond between C-20 and C-29. Further, the proton spectrum showed two peaks at δ 4.55 & 4.67 (each 1H, d, *J* = 1.3 Hz, H-29) suggesting the presence of exomethylene protons. Additionally, the spectrum showed signal at 3.12 (1H, dd, *J* = 11.3, 5.1Hz, H-3) which suggested the presence of secondary hydroxyl group.

The ^{13}C NMR spectrum (100 MHz, CD_3OD) [Table1] showed 30 carbon signals. Out of which two double bond carbon signals at δ 150.4, 108.6 and methyl signal at δ19.3 indicates the presence of isopropenyl group in the molecule. The spectrum also clearly showed only one oxygenated carbon at δ 78.1. Further the spectrum showed six methyl, ten methylene, five methine and five quaternary carbon signals. Based on the above spectral data and in comparison with literature data, the structure of the compound **1** has been established as lupeol.[7, 14-16]

Compound **2** was isolated as colorless crystals from hexane: ethyl acetate, mp:168-70°C. It was readily recognized as triterpene derivative from its preliminary NMR spectral data. Its molecular formula was established as $C_{30}H_{48}O$ by GC-MS, M^+ 424. Its IR spectrum showed the presence of carbonyl group (1705cm^{-1}) and its UV spectrum showed

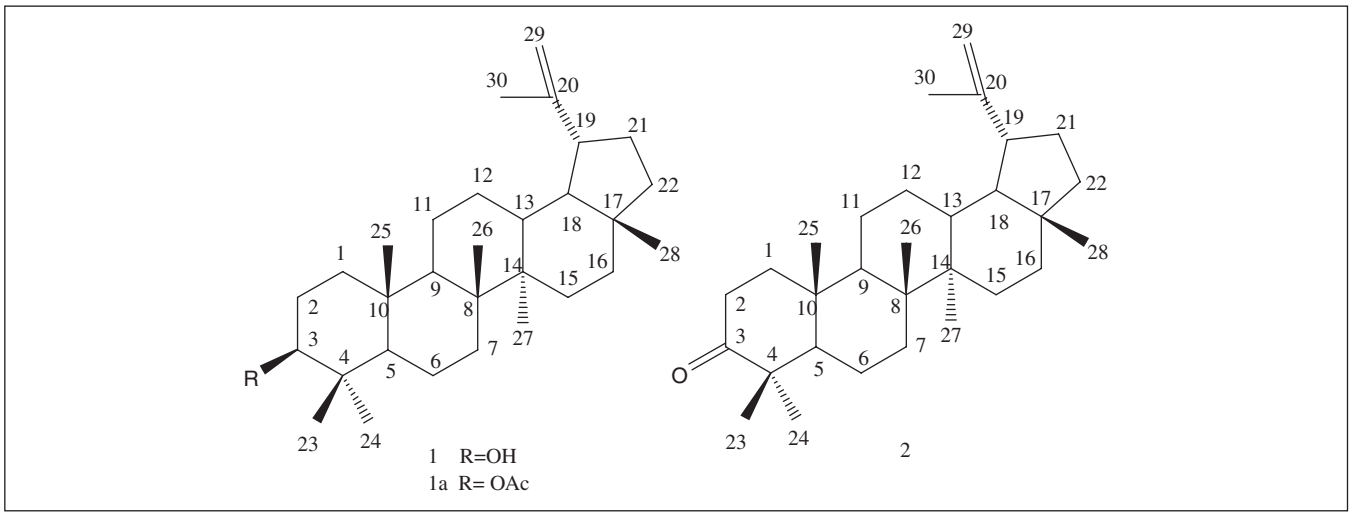

Figure 1: Compounds from *C. nurvala*

Table 1: ^{13}C NMR Spectroscopic data for compound 1 in CD_3OD and compound 2 in $CDCl_3$ (100 MHz)

Position	1	2
C-1	38.4	34.1
C-2	27.1	39.9
C-3	78.1	218.1
C-4	38.6	47.2
C-5	55.3	54.8
C-6	17.9	19.6
C-7	34.0	33.5
C-8	40.5	40.7
C-9	50.4	49.9
C-10	36.8	36.8
C-11	20.5	21.4
C-12	25.0	25.1
C-13	38.0	38.1
C-14	42.4	42.9
C-15	27.1	27.3
C-16	35.2	35.4
C-17	42.5	42.8
C-18	48.3	48.1
C-19	47.9	47.2
C-20	150.4	150.8
C-21	29.4	29.7
C-22	39.5	39.6
C-23	27.9	26.6
C-24	15.1	21.2
C-25	16.0	15.9
C-26	15.9	15.8
C-27	14.5	14.4
C-28	17.9	18.0
C-29	108.6	109.3
C-30	19.3	19.2

Table 2: *In-vitro* Melanin promotion activty

Compound/Fraction	Concentration (μg/ml)	%Melanin promotion
Methanolic extract	75	97.5
Ethyl acetate fraction	25	108
n-Butanol fraction	35	20
IBMX (Control)	15	70
Lupeol (**1**)	15	401
Lupenone (**2**)	15	150
Lupeol acetate (**1a**)	15	245

no characteristic absorption. The ^1H-NMR spectrum clearly showed triterpenoid pattern with six methyl singlets at δ 0.80 (Me-28), 0.93 (Me-25), 0.96 (Me-27), 1.03 (Me-23), 1.07 (Me-24), 1.07 (Me-26), a vinyl methyl signal at δ 1.68 (Me-30) and an exomethylene group signals at δ 4.58 (1H, m, H-29), 4. 70 (1H, d, *J* =2.2 Hz, H-29).

The ^{13}C NMR spectrum (100 MHz, $CDCl_3$) [Table1] showed clearly 30 carbon signals. Out of which one carbonyl signal at δ 218.7 and two double bond signals at δ 150.8 and 109.3. No oxygenated carbon signal was observed in the spectrum. By comparing the compound **1** and **2** spectral data, the former contains hydroxyl group and later contains keto group in the molecule, remaining all other signals are more or less same. The compound **2** might be an oxidized version of compound **1**. The structure of compound **2** was deduced from the comparison of its spectral with those of literature and identified as lupenone.[17]

CONCLUSION

The present investigation has helped to identify the major source for lupeol and also potent melanin promoter. Lupeol fatty acid esters have been reported for melanogenesis

promotion activity by Japanese Scientists but not lupeol and lupenone. The study of melanin promotion activity is first time for this plant.

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