ISOLATION AND CHARACTERIZATION OF LUPEOL FROM ROOTS OF *LEPTADENIA RETICULATA* AND ITS ANTIMICROBIAL ACTIVITY

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Summary

A pentacyclic triterpenoid lupeol was isolated from the chloroform extract of the root of *Leptadenia reticulata* (Asclepiadaceae). The structure of the compound was determined by spectroscopic analysis (UV, IR, ^{1}H NMR, ^{13}CNMR and FAB-MS). It has showed a significant antibacterial and antifungal activity. It is the first report from the roots of *Leptadenia reticulata*.

Key words: *Leptadenia reticulata,* triterpenoid, lupeol, antimicrobial activity

Introduction

Leptadenia reticulata (Retz.) Wt. & Arn., commonly known as 'Dodi', is an Indian medicinal plant known since 4500 BC. It belongs to the family Asclepiadaceae. The whole plant ameliorates 'tridoshas' (Vatta, Pitta and Kapha), and is of great value in general debility, involuntary seminal discharge, galectogouge and as a stimulant [1], abortifacient, tonic, restorative, bactericidal, antifabrifuge, prostatitis, wound healer and good for mouth ulcer [2]. Roots are used in many Ayurvedic/herbal formulations [3] and diseases of the ear and nose, skin infections and general debility [4]. It is also used for increasing milk-yielding in cattle and to increase the egg laying capacity of hen in poultry industry. Flowers are good for eyesight [5,6].

More than 23 pharmaceutical products are available in the market containing it plant as one of the ingredients. It has great demand in both the local as well as the international market, and sold at Rs 211 per kg of dry powder. Its flowers and tender leaves are used as vegetable [7] and to make bread [8]. Flowers are costly and are sold at Rs 80 per kg. The plant contains leptadenol, hentiacontanol, acetyl alcohol, β-sitosterol, β-amyrin acetate, lupanol3-O-diglucoside, leptidin, quercetin, iso-quercetin, rutin, hyperoside, simiarenol (3-β-hydroxy-E: B-friedehop-5-ene) a rare triterpenoid alcohol, novel pregnane glycosides namely reticulin, calogenin, denticulatin, and 0.5% alkaloids [9-13]. Root is the prime source for Ayurvedic medicine. So, far no detailed phytochemical and biological studies have been carried out on roots of this plant. Since, this plant has good medicinal properties, the present work was undertaken to isolate, purify and identify secondary metabolites. In this paper the isolation and structural elucidation of the lupeol UV, IR, ^{1}H NMR, ^{13}CNMR and FAB-MS and their biological activities being reported.

Material and Methods

Plant material: The *Leptadenia reticulata* (including roots) were collected from Bidar district Karnataka, India, identified with the help of Flora of the Presidency of Madras [14], Flora of Eastern Karnataka [15] and Flora of Gulbarga District [16] and authenticated. A voucher specimen No. HGUG-801 is deposited in the Herbarium of Botany Department, Gulbarga University, Gulbarga.

Extraction and isolation: The roots of *L. reticulata* dried using tray drier under controlled temperature at 40^{0}C and fine powdered (~300 μ) using mechanical pulvarizer, about 500 g powdered raw drugs was successively extracted in a Soxhlet apparatus for 48 h using petroleum ether, chloroform, ethanol (95%) and distilled water. These extracts were concentrated *in vacuo* and only the chloroform extract was subjected to flash column chromatography over silica gel (Merck Kieselgel GF_{254}). The fractions were eluted with solvents of increasing polarity (hexane, chloroform, ethyl acetate and methanol) and a total of 58 fractions of 100 ml were collected The fractions 1 to 5 were obtained from the hexane. Fractions 6 to 14 were collected from the chloroform methanol, (90:10, 50:50, 30:70 and 10:90). Similarly, 15 to 36 fractions were collected from the solvent mixture of hexane ethyl acetate (90:10, 70:30, 50:50, 30:70, 20:80 and

10:90). While, the fractions 37 to 54 were collected from the solvent mixture of chloroform methanol (90:10, 80:20, 60:50, 40:60, 20:80 and 10:90) finally, fractions 55 to 58 were collected from the methanol 100% mobile phase. Then concentrated fractions based on the nature and yield of the compound fractions, 28 to 36 were analyzed by the pTLC using mixture of chloroform and methanol (90:10) solvent system to get lupeol (Rf. value 0.24).

Melting points were determined on a kolfer hot-stage apparatus and are uncorrected. UV spectrum was taken in MeOH solution using a Perkin-Elmer lambda 9UV/Vis./NIR Spectrometer. IR spectra were recorded on $CHCl_3$ solutions on either a Perkin-Elmer 580 or Philips 9800 FTIR Spectrometer. ^1H NMR and ^{13}C NMR spectra were obtained on Bruker WP 200 SY and AM 200 SY instruments (^1H, 200. 132 MHz; 13C, 50.32 MHz) using TMS as internal standard and $CDCl_3$ as solvent. The Fast Atom Bombardment - mass spectra (FAB-MS) and optical rotations were measured on an optical activity AA-100 Polarimeter in $CHCl_3$ solutions at 20^0C. Petroleum ether specifically refers to the bp $40\text{-}60^0$ fractions were recorded.

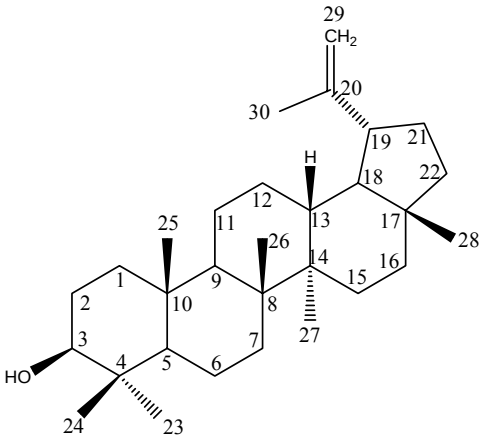
Bioassays: The Soxhlet successive chloroform extract of root and purified fractions of *L. reticulata* were assessed for its antimicrobial activity against a few pathogenic bacteria and fungi using agar well diffusion technique. The pure axenic cultures 10^6 CFU/ml (colony forming units) using 0.8% (w/v) sterile saline by the method of direct microscopic count [17] of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Vibrio cholerae,* and fungi *Aspergillus niger, A. flavus, A. terreus* and *Candida albicans* were procured from the Fungal Biotechnology Laboratory, Department of Botany and Microbiology, Gulbarga University, Gulbarga.

Crude chloroform extract and isolated pure compound (lupeol) was dissolved in distilled dimethyl formamide (DMF) in the concentration of 1, 2, 3 and 4 mg/ml. Streptomycin and Nystatin (1 mg/ml) dissolved in DMF are used as a reference compound (positive control) for bacterial and fungal activity. Further, 100 µl of pure compound were loaded in the peripheral wells of petri plates containing 100, 200, 300 and 400 mg/well concentration. All these plates were incubated after 30 min. in BOD digital incubator at 37^0C for a period of 24 h. the observed marked zone of growth inhibition of bacteria and fungi were measured with the help of a scale and recorded. This experiment was repeated twice with triplicates in order to set reproducibility of data. The data were assayed the mean of triplicates ± standard error. The significant correlation, level at 0.01 and 0.05 level [18].

Results and Discussion

The chloroform extract of the roots of *L. reticulata* afforded one triterpenoid. The isolated compound was identified by spectroscopic analysis as well as by comparison of their spectral data with previously reported values as lupeol [19].

Lupeol (1) is white coloured amorphous powder with mp 214-216^{o} $[\alpha]_D$ + 30.4^{o} (C, 0.58 in $CHCl_3$). IR spectrum exhibited hydroxyl [υ_{max}: 3610, 1020 cm^{-1}] and exomethylene [υ_{max}: 3070, 1640, 887 cm^{-1}] absorption. The mass spectrum displayed a molecular ion [M^+] peak at *m/z* 426 corresponding to $C_{30}H_{50}O$ together with fragments at *m/z* 411 [M^+-15] and 408 [M^+-18] which were due to the loss of methyl group and a molecule of water from the molecular ion peak. The mass spectrum also showed a base peak at *m/z* 41 [$C_3H_5^+$] arising from the loss of the side chain of lupeol. The ^{1}H NMR spectrum exhibited six tertiary methyl singlets at [δ_H: 0.75, 0.77, 0.80, 0.92, 0.94 and 1.02], a methane group at [δ_H: 1.66 (br d, *J*=0.5 (Hz)], a secondary carbinol group at [δ_H: d*H*: 3.20 (dd, *J*=9.6 and 6.2 Hz)] and an exomethylene group at [δ_H: 4.58 (^{1}H, d, *J*=0.4 Hz) and [δ_H: 4.65 (^{1}H, dq, *J*=0.4 and 0.5 Hz)] typical of pentacyclic triterpenoid [20, 21] of the lupeol (1).



Lupeol (Lup-20 (29)-en-3β-ol)

The structural assignment of lupeol (1) was further substantiated by its ^{13}C NMR spectrum which showed seven methyl groups at [δ_C:28.0 (C-23), 19.3 (C-30), 18.0 (C-28), 16.1 (C-25), 15.9 (C-26), 15.4 (C-24), 14.5 (C-27)], an exomethylene group at [δ_C: 150.8 (C-20), 109.3 (C-29)] and a secondary hydroxyl bearing carbon at [δ_C:78.9 (C-3)], in addition to ten methylene, five methine and five quaternary carbons. The shielding of C-23 methyl of lupeol could be due to the influence of the adjacent C-3 hydroxyl group. These data were in close agreement with those reported for lupeol [22, 23] and further confirmed the identity of the compound.

Antimicrobial activity: Antibacterial activity of crude chloroform extract has displayed significant to moderate and dose dependent antibacterial activity. The extract of root is active against *E. coli, S. aureus, P. aeruginosa* and *V. cholerae.* The significant zone of inhibition at 300 µg/well recorded against *V. cholerae*. The isolated pure compound (lupeol) had shown significant antibacterial activity especially against *P. aeruginosa, S. aureus* and *V. cholerae* at the concentration of 300 µg/well. While, at 200 µg/well, *E. coli* showed good activity.

The successive crude chloroform extract of *L. reticulata* root have showed significant antifungal activity against the *Aspergillus niger, A. flavus, A. terreus* and *Candida albicans.* The maximum zone inhibition was observed in *A. niger* at 300 µg/well. The isolated pure fraction (lupeol) exhibited significant antifungal activity against the *Aspergillus niger, A. flavus, A. terreus* and *Candida albicans*. The maximum zone inhibition was observed in *A. flavus A. niger* and *A. terreus* at higher concentration of extracts (200 µg/well). While, more significant activity *C. albicans* at lower concentration at 100 µg/well.

Earlier reported antibacterial and antifungal activities using whole plant [24] alcohol and aqueous extract of *L. reticulata* was carried out. The alcoholic (90%) and aqueous (50%) extracts of leaves and roots are active against *Trichophyton rubrum* [25]. Further activity on antibacterial of phenolic and non phenolic fractions of the plant against 18 organisms. Herbinol, an herbal antiseptic cream which includes *L. reticulata* as an ingredient showed significant antimicrobial activity against some common microbes causing septicemia [26]. Thus the results obtained in our investigation clearly indicated that, significant zone of inhibition of antimicrobial activity when compared to crude chloroform extract pure compound (lupeol) exhibited marked zone of inhibition.

This is the first report of the isolation of the pentacyclic triterpenoid lupeol from the roots of *Leptadenia reticulata.* Its analysis may result in the isolation of a few more biologically active compounds. The crude chloroform extract and isolated pure compound (lupeol) exhibited antimicrobial activity against both bacteria and fungi. Lupeol has given significant zone of inhibition against both bacteria and fungi. Denoting that lupeol might be responsible for the antibacterial activity of the roots as reported in earlier literature.

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