



Phytochemical Screening of *Caralluma lasiantha* Isolation of C₂₁ Pregnane Steroid

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ABSTRACT

Phytochemical screening of *Caralluma lasiantha* was carried out and one C₂₁ pregnane steroid was isolated from chloroform extract. Based on spectroscopic studies (IR, ¹H NMR, ¹³C NMR and ESI-MS) the isolated compound is 3β,14β-dihydroxy-14β-pregn-5-en-20-one which was earlier isolated from other species.

Key words: *Caralluma lasiantha*, Indian traditional medicine, C₂₁ pregnane steroid, Chloroform extract, Phytochemical screening.

INTRODUCTION

In ancient system of medicine like ayurveda, unani and in folkloric medicine, several plants belong to asclepiadaceae are proved to be helpful in healing of disorders. Asclepiadaceae family includes 200 genera and 2500 species. *Caralluma* is one of the genus of asclepiadaceae which grows widely in dry places¹. Certain species *Caralluma* were used as food in emergency needs in Pakistan and India². The word '*Caralluma*' is an Arabian word obtained from 'qarh al-luhum' means wound in the abscess or flesh³. *Caralluma* is also spoken as the synonym of *Boucerosia*, but it varies from *Boucerosia* by its

floral arrangement³. In India species of *Caralluma* are found to be palatable and also considered as a component of traditional medical system. Now a days *Caralluma* is gaining much significance from scientists as it exhibits immunostimulating activities because of presence of flavanoids and saponins⁴. *Caralluma umbellata* Haw also used to treat stomach disorder and pain, which can be evidenced by good antibacterial activity of *Caralluma umbellata* extracts⁵.

Caralluma lasiantha (syn. *Boucerosia lasiantha*) is well known with different local names like Kundeti Kommulu in Telugu and Sirumankeerai

in Tamil⁶. It is a member of Asclepiadaceae family. It is succulent in habit and a familiar indoor ornamental plant⁷. Its growth is found in surrounding places of Anantapur and Chittoor, Andhra Pradesh, India. To reduce body heat, it is used in Indian traditional medicine⁸. Differentiation among various species of *Caralluma* genus is more difficult because of their more intermediary forms in their habitation⁹. Thus, Pavan Kumar *et al*¹⁰ suggested standardized parameters for right differentiation of four *Caralluma* species to determine genuinely. A review article on *C.lasiantha* was published by us¹¹. Anti-proliferative property of *Boucerosia lasiantha* (methanolic extracts) against A431 human skin cancer cells and A375 human malignant melanoma was reported by Madhuri *et al*¹². Madhuri and Siva Rama Krishna¹³ reported the antioxidant activity of methanolic extracts of *Boucerosia lasiantha*. Antihyperglycemic / hypoglycemic activity of methanolic extracts of *Caralluma lasiantha* were investigated by Harsha Kumar¹⁴. In our latest studies, antibacterial activity of extracts of *C. lasiantha* was reported against both Gram (-) bacteria and Gram (+) bacteria¹⁵. Likewise, depending on antifungal activity of extracts of *Caralluma lasiantha* against tested fungi, *C. lasiantha* was recommended as alternate to manage storage fungi by us¹⁶. Ramesh *et al*¹⁷ reported two bisdesmosidic C-21 steroidal glycosides (lasianthoside-A and lasianthoside-B) from *C. lasiantha* (Fig.1) and a known flavone glycoside

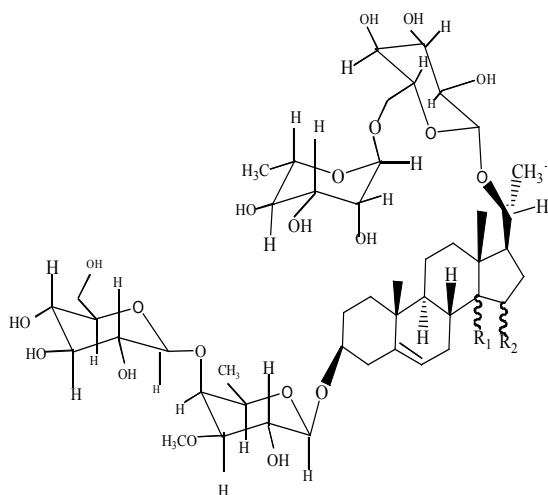


Fig.1: lasianthoside-A and lasianthoside-B
 Lasianthoside-A -R₁, R₂ = Δ^{14-15} ,
 Lasianthoside-B -R₁ = β -OH, R₂ = H

(Luteoline-4-O-neohesperidoside) (Fig.2). Steroidal glycosides were extracted from *C.lasiantha* in their studies, using polar solvents like alcohols¹⁸. We have reported for first time the isolation of stigmasterol from *C.lasiantha*¹⁹. Thorough literature shows that no extractions from *C.lasiantha* were carried out using solvents having intermediate polarity. Hence, phytochemical screening of chloroform extracts of *C.lasiantha* is carried out in the present study.

MATERIALS AND METHODS

All chemicals used in the present studies are Analytical Reagent grade, Merck India Co. Ltd. If necessary, chemicals are purified by using standard procedures. Geographical location²⁰, plant collection²¹ and season²² affect the active constituents of the plants which play an important role in exhibiting the biological activities by extracts of plants. Fresh plants of *Caralluma lasiantha* (Asclepiadaceae) are collected from Gooty, Anantapur District, Andhra Pradesh, India in February 2012. A voucher specimen of it was deposited in Herbarium, Department of Botany, Sri Krishna Devaraya University, Anantapur.

Isolation and identification

Stems and roots were dried under shade, powdered and sieved (sieve No.14). Then the powder was stored in air tight containers. The weighed quantity of powder was extracted with successive solvent extraction in soxhlet extractor by using solvents of varying polarity (Hexane, Chloroform, and Methanol). Last traces of solvent were removed by applying vacuum to concentrate all the extracts^{23, 24}. Later, the crude extracts are purified by recrystallization. Melting point was recorded on a Fisher–John apparatus. The IR spectra were recorded on an IFS-120H spectrometer. The ¹H NMR and ¹³C NMR spectra were obtained on Bruker 300MHz, 75MHz spectrometer, using TMS as an internal standard. ESI-MS was recorded on a ZAB-HS mass spectrometer and HREIMS was recorded on the Agilent Technologies 6510 Q-TOF LC/MS.

Spectral data

I.R.: ν_{max} 3489, 2925, 2854, 1678 and 1458 cm^{-1}
¹H NMR (CDCl₃): δ 5.42 (1H, t), 4.46 (1H, s), 3.53 (1H, s), 2.94 (1H, t), 2.259 (8H, S), 1.84 (3H, t), complex pattern peaks at δ 1.50, 1.27 and 1.0.
¹³C NMR (CDCl₃): δ 206.4 (C-20), 138.9 (C-5),

121.3 (C-6), 84.3 (C-14), 71.4 (C-3), 62.8 (C-17), 48.6 (C-13), 45.5 (C-4), 40.7 (C-10), 38.5 (C-8), 37.0 (C-9), 36.4 (C-2), 35.9 (C-1), 33.9 (C-15), 32.7 (C-12), 31.2 (C-7), 26.8 (C-21), 24.0 (C-11), 20.4 (C-19), 19.6 (C-18), 15.0 (C-16).

Mass (ESI-MS): (m/z) 355 (M+ Na)⁺, 315.2, 301, 297.2, 279, 199, 139, 101.1

Libermann-Burchard test

The extract was boiled after treating with few drops of acetic anhydride. On the addition of concentrated sulphuric acid to the above cooled solution, a brown ring was formed at the junction of two layers as well as the upper layer forms green colour to confirm the presence of sterols²⁵.

RESULTS AND DISCUSSIONS

Nature of extractable phytochemical depends on the polarity of solvents²⁶. In the present study, chloroform extracts were taken. A positive test of Libermann-Burchard test exhibits the presence of sterols in the crude mixture²⁵. By repetitive column chromatography, the crude extract of *C. lasiantha* (using petroleum ether as a solvent) was purified using silica gel (230-400 mesh) and a mixture of petroleum ether and acetone as an eluent. An amorphous white solid compound (M.Pt: 190-200

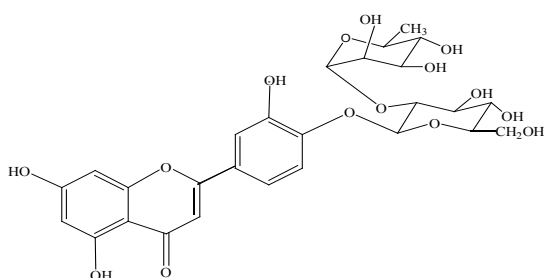


Fig. 2: Luteoline-4-O-neohesperidoside

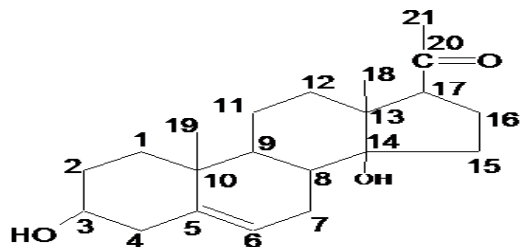


Fig. 3: 3β,14β-dihydroxy-14β-pregn-5-en-20-one

°C) was obtained as one of the product from the mixture. The isolated phytochemical was confirmed as steroid from a positive TLC test. The spot on TLC plate was eluted with a solvent mixture of hexane; ethyl acetate (7:35) and sprayed with 10% sulphuric acid to give a dark pink spot. R_f value was found to be 0.42.

I.R. spectrum shows the presence of carbonyl group (C=O str: 1678 cm⁻¹), skeletal unsaturation (C=C str: 1458 cm⁻¹) and a hydroxyl group (O-H str: 3489 cm⁻¹). In the ¹H-NMR spectrum, one singlet is observed at δ 1.0 due to superimposed signal of methyl protons present on C₁₈ and C₁₉. A triplet at δ 5.4 explains the presence of hydrogen on unsaturated carbon (C₆). Two singlets at higher δ values (4.46 and δ 3.53) show the attachment of an electronegative atom like oxygen to proton indicating the presence of a hydroxyl group attached to C₁₄ and C₃ respectively. CH proton (i.e., H attached to C₃ which is linked with hydroxyl group) absorbed at δ 2.94. CH₃ and CH protons attached to carbonyl group are shown by peaks with δ 2.26.

Spectral data of ¹³C-NMR spectrum supported the proposed structure (Fig.3). A peak above δ 200 (δ 206.4) shows the presence of a carbonyl group. Peaks in between δ 120 to 140 (138.9 and 121.3) exhibit the presence of unsaturation between C₅ and C₆. Presence of peaks in the higher side of alkanes absorption range (δ 0 to 80) at 84.3 and 71.4 shows the attachment of hydroxyl group to C₁₄ and C₁₇ respectively, from elemental analysis (C-75%; H-9.6%; O-14.4%) and mass analysis (m/z: M+Na⁺ = 355), molecular formula was found to be C₂₁H₃₂O₃. The isolated compound was recognized as a typical C₂₁ steroidal skeleton with a carbonyl group. On ESI-MS fragmentation, a series of product ions were generated at m/z 315.2, 301, 297.2, 279, 199, 139, 101.1 which explain the fragmentation pattern including loss of hydroxyl group in the form of water molecules.

The structure of isolated compound is proposed as 3β,14β-dihydroxy-14β-pregn-5-en-20-one (Fig.3) by comparing its spectral data with those available in literature^{27, 28, 29}. The present C₂₁ steroid was isolated first from *Cynanchum paniculatum*²⁷. Based on its presence in non-polar extracts of *Caralluma umbellata*, Ramesh *et al*²⁹ suggested that

it is the precursor for glycosides like carumbelloside I & II. It was also concluded that the present C21 steroid can be helpful as a marker for *Caralluma* genus. As different moieties and functional groups present on synthetic / natural molecules direct their pharmacological activities³⁰⁻³⁵, structure activity relationship between such activities and this isolated molecule can be further studied.

CONCLUSION

C21 steroid was isolated from chloroform extract of *Caralluma lasiantha*. The isolated compound is 3 β ,14 β -dihydroxy-14 β -pregn-5-en-20-one which was earlier isolated from *Cynanchum paniculatum* and *Caralluma umbellata*.

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