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Phytochemical Screening of *Caralluma lasiantha* Isolation of C_{21} Pregnane Steroid

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ABSTRACT

Phytochemical screening of *Caralluma lasiantha* was carried out and one C_{21} pregnane steroid was isolated from chloroform extract. Based on spectroscopic studies (IR, ^{1}H NMR, ^{13}C 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: C*aralluma lasiantha,* Indian traditional medicine, C21 pregnane steroid, Chloroform extract, Phytochemical screening.

INTRODUCTION

In ancient system of medicine like ayurveda, unani and in folkaric 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 places[1]. Certain species *Caralluma* were used as food in emergency needs in Pakistan and India[2]. The word '*Caralluma*' is an Arabian word obtained from 'qarh al-luhum' means wound in the abscess or flesh[3]. *Caralluma* is also spoken as the synonym of *Boucerosia*, but it varies from *Boucerosia* by its floral arrangement[3]. 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 saponins[4]. *Caralluma umbellata Haw* also used to treat stomach disorder and pain, which can be evidenced by good antibacterial activity of *Caralluma umbellata* extracts[5].

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

in Tamil[6]. It is a member of Asclepiadaceae family. It is succulent in habit and a familiar indoor ornamental plant[7]. Its growth is found in surrounding places of Anantapur and Chittoor, Andhra Pradesh, India. To reduce body heat, it is used in Indian traditional medicine[8]. Differentiation among various species of *Caralluma* genus is more difficult because of their more intermediary forms in their habitation[9]. 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 us[11]. 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 Krishna[13] reported the antioxidant activity of methanolic extracts of *Boucerosia lasiantha.* Antihyperglycemic / hypoglycemic activity of methanolic extracts of *Caralluma lasiantha* were investigated by Harsha Kumar[14]. In our latest studies, antibacterial activity of extracts of *C. lasiantha* was reported against both Gram (-) bacteria and Gram (+) bacteria [15]. Likewise, depending on antifungal activity of extracts *of Caralluma lasiantha* against tested fungi, *C. lasiantha* was recommended as alternate to manage storage fungi by us[16]. 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 (Luteoline-4-O-neohesperiodoside) (Fig.2). Steroidal glycosides were extracted from *C.lasiantha* in their studies, using polar solvents like alcohols[18]. 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.

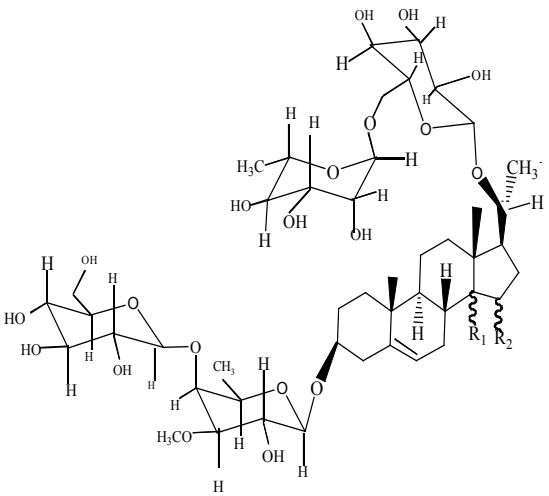

Fig.1: lasianthoside-A and lasianthoside-B
Lasianthoside-A -R_1, R_2 = $\Delta^{14\text{-}15}$,
Lasianthoside-B -R_1 = β-OH, R_2 = H

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 location[20], plant collection[21] and season[22] 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 ^{1}H NMR and ^{13}C 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_{rmax} 3489, 2925, 2854, 1678 and 1458 cm^{-1}
^{1}H NMR ($CDCl_3$): 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.
^{13}C NMR ($CDCl_3$): 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 sterols[25].

RESULTS AND DISCUSSIONS

Nature of extractable phytochemical depends on the polarity of solvents [26]. In the present study, chloroform extracts were taken. A positive test of Libermann-Burchard test exhibits the presence of sterols in the crude mixture[25], 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 ^{0}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.

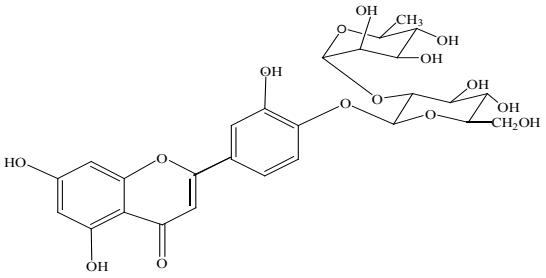

Fig. 2: Luteoline-4-O-neohesperiodoside

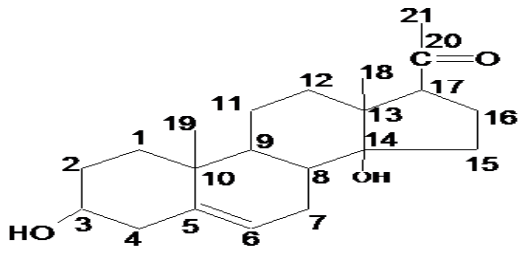

Fig: 3: 3β,14β-dihydroxy-14β-pregn-5-en-20-one

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 ^{1}H-NMR spectrum, one singlet is observed at δ 1.0 due to superimposed signal of methyl protons present on C_{18} and C_{19}. A triplet at δ 5.4 explains the presence of hydrogen on unsaturated carbon (C_6). 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 C_{14} and C_3 respectively. CH proton (i.e., H attached to C_3 which is linked with hydroxyl group) absorbed at δ 2.94. CH_3 and CH protons attached to carbonyl group are shown by peaks with δ 2.26.

Spectral data of ^{13}C-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 C_5 and C_6. 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 C_{14} and C_{17} 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 $C_{21}H_{32}O_3$. The isolated compound was recognized as a typical C_{21} 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 C_{21} steroid was isolated first from *Cynanchum paniculatam* [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 activities [30-35], 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 3β,14β-dihydroxy-14β-pregn-5-en-20-one which was earlier isolated from *Cynanchum paniculatam* and *Caralluma umbellata.*

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