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Isolation of Novel Phytoconstituents from the Bark of *Salvadora Oleoides* Decne.

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A phytochemical investigation was conducted on the bark of *Salvadora oleoides* Decne. (Salvadoraceae). Two Sterols compounds, β-sitosterol and stigmasterol, were isolated using chromatographic means. Their structures were established on the basis of extensive spectroscopic (IR, MS, NMR) data analysis and by comparison of the data obtained with those of the literature.

Keyword: *Salvadora oleoides* Decne, β-sitosterol, stigmasterol, NMR.

1. Introduction

Salvadora oleoides Decne. family Salvadoraceae, is evergreen tree or shrubs[1,2]. *Salvadora stocksii* Weght. is commonly used synonyms for *Salvadora oleoides* Decne[3,4,5,6,7]**.** *Salvadora oleoides* Decne is also known as bada pilu or vridhpilu in Hindi[8], It mainly grow in arid and alkaline soil conditions[9], Distributed throughout South Haryana[10] and Rajasthan in India[11]. The leaves of plant is acrid, sweet, sour, appetizer, laxative, carminative[8], stem bark is stimulant[12], alexipharmic; useful in piles, tumors, bronchitis, disease of the spleen, hypoglycaemic, rheumatic pain[13], reported use for its antihyperlipidimic activity[14] and very strong antibacterial use is also reported[15].

Trunk of *Salvadora oleoides* Decne. short, often twisted or bent, up to 2 m in diameter; branches drooping, numerous, stiff, often swollen at forks; Bark grey or whitish-grey. Leaves 3.8- 7.5 cm. by 3-13 mm., glaucous, linear-or ovate-lanceolate, coriaceous and somewhat fleshy, dark greenish-yellow when young, grey when mature petioles 4-6 mm, long. Flowers sessile, greenish-white, minute in paniculate spikes, 2.5-3.8 cm. long, often clustered; calyx cup-shaped, in 4 rounded, obtuse lobes. Fruit a drupe, globose, about 6 cm in diameter, usually yellow when ripe, dark brown or red when dry. Seeds greenish-yellow, about 3 mm in diameter[15].

A variety of Phytoconstituents have been isolated from different parts of *Salvadora oleoides* Decne and their structures elucidated. Like leaves and roots contains alkaloid trimethylamine[16]**,** Seeds contains fatty oil and ethereal oil[16]**,** Phosphatidylethanolamine,

phosphatidylethanolamine, cardiolipin[17].The reported chemical constituents of fruits of *Salvadora oleoides* Decne are dibenzylurea, dibenzylthiourea, stigmasterol[18], oxalic acid and phytic acid[19]. It is also found to be a reach source of calcium[20].

2. Materials and Methods

2.1 Plant Material

The plant *Salvadora oleoides* Decne **[Plate 1]** was collected in the months of April 2008, from Village Pali, District Mahindergarh, Haryana, India and was identified and authenticated by Dr. H. B. Singh Head, Raw Material, herbarium and museum division, NISCAIR, New Delhi Ref.Niscair/Rhmd/Consult/-2008-09/971/02) and a sample has been retained in the department for future reference.

Plate 1: Tree of *Salvadora Oleoides* Decne.

2.1 Extraction and Isolation

The dried bark **[Plate 2]** of *Salvadora oleoides* Decne. (1kg) were ground into a fine powder and soaked in *n*-hexane for six days. The solution was filtered, and the combined filtrates were rotary evaporated under reduced pressure to produce 37g (3.7%) of a brown-gummy extract. The extract was subjected to silica gel column chromatography by eluting *n*-hexane containing increasing percentages of ethyl acetate and the fractions collected were 100 ml each. Fractions 4 and 5 were combined (960 mg) and then subjected to radial chromatography by using increasing polarities of *n*-hexane: ethyl acetate to produce 12.4 mg of β-sitostero. Fractions 6, 7 and 8 were combined (233 mg) and then subjected to silica gel preparative thin layer chromatography using 100: 0 to 10: 90 of *n*-hexane: ethyl acetate to afford 63.5 mg of stigmasterol.

Plate 2: Bark of *Salvadora oleoides* Decne.

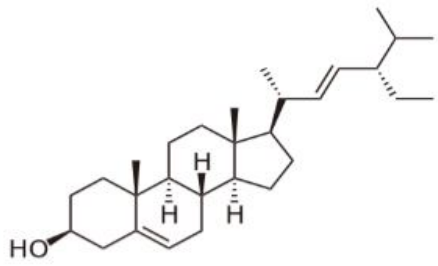
2.2 Spectroscopic Characterization

Different spectroscopic methods were used to elucidate the structure of isolated compound. Among the spectroscopic techniques IR, ^{1}H-NMR, ^{13}C-NMR and GC-MS were carried out. The infra red spectrum was recorded on FTIR Perkin Elmer, ^{1}H-NMR and ^{13}C-NMR spectra were recorded using $CDCl_3$ as solvent on Bruker Advance II 400 NMR and GC-MS spectra were recorded at high resolution on a mass spectrometer (Perkin Elmer Auto system) at spectrometer SAIF Panjab University, Chandigarh, the data are given in m/z values.

2.3 β-Sitosterol /Stigmasterol

Colourless needles (12.4 mg)/white powder (6.6 mg). IR cm^{-1}: 3393/3434, 2930/2924, 1611/1639, 1457/1457, 1385/1375, 1171/1179. EIMS for β-sitosterol-$C_{29}H_{50}O$

m/z (rel. int.): 414 [M+] (100%), 396 (30.8%), 381 (14.0%), 329 (13.7%), 303 (22.1%), 255 (11.8%), 213 (12.2%), 145 (18.5%), 95 (21.6%), 81 (21.3%), 55 (25.8%), 43 (45.0%). EIMS for stigmasterol-$C_{29}H_{48}O$ m/z (rel. int.): 412 [M+] (39.7%), 351 (13.5%), 314 (7.0%), 300 (25.5%), 271 (38.4 %), 229 (8.6%), 213 (10.6%), 55 (100%). ^{1}H NMR ($CDCl_3$, 400 MHz): 3.53 (1H, m, H-3), 5.36 (1H, d, J = 5.1/7.3 Hz, H-6), 0.69 (3H, s, H-18), 1.02 (3H, s, H-19), 0.93 (3H, d, J = 6.6/6.2 Hz, H-21), 5.16st (1H, dd, J = 15.6, 8.4 Hz, H-22); 5.02st (1H, dd, J = 15.6, 9.2 Hz, H-23), 0.85 (3H, d, J = 7.0/7.3 Hz, H-26), 0.83/0.81 (3H, d, J = 7.0 Hz, H-27), 0.87/0.83 (3H, t, J = 7.7/7.0 Hz, H-29), 1.05-2.32 (others). ^{13}C NMR (CDCl3, 100 MHz): 141.0 (C-5), 121.9 (C-6), 72.0 (C-3), 57.0/57.1 (C-14), 56.3 (C-17), 50.4/50.3 (C-9), 46.1/51.5 (C-24), 42.6/42.3 (C-4, C-13), 40.0 (C-12), 37.5 (C-1), 36.7 (C-10), 36.4/40.7 (C-20), 34.2/138.5 (C-22), 32.2/32.1 (C-8), 32.1si (C-7), 31.9st (C-7, C-25), 31.9/31.7 (C-2), 29.4si (C-25), 28.5/29.3 (C-16), 26.3/129.5 (C-23), 24.5 (C-15), 23.3/25.6 (C-28), 21.3si (C-11), 21.3st (C-11, C-21), 20.1/19.0 (C-27), 19.6 (C-19), 19.3/21.4 (C-26), 19.0si (C-21), 12.2 (C-29), 12.1 (C-18).

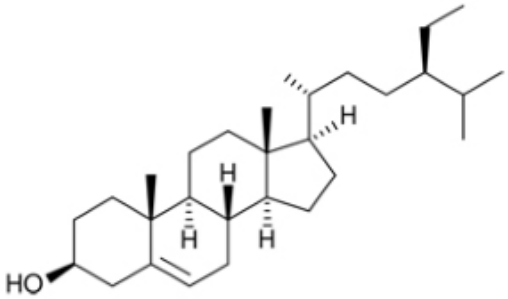


[A] Stigmasterol

3. Results and Discussion

Purification of the *Salvadora oleoides* Decne. bark extracts produced β-sitosterol and stigmasterol. β-Sitosterol **[B]** was isolated as colorless needles. Column chromatography of *n*-hexane extract gave a 139 mg combined fractions, and its preparative thin layer chromatography yielded 6.6 mg of stigmasterol **[A]** as white powder. Spectral data of stigmasterol differed minimally from those of β-sitosterol at and around a *trans*-C_{22}-C_{23} double bond in the former. The EIMS of β-sitosterol/stigmasterol showed the molecular ion m/z 414/412 [M+] that corresponds to the formula $C_{29}H_{50}O/C_{29}H_{48}O$ and in agreement with other previous spectral data. ^{1}H NMR spectrum indicated the presence of six methyl peaks of H-18, H-27, H-29, H-26, H-21 and H-19 that appeared at respective δ 0.69, 0.83/0.81, 0.87/0.83, 0.85, 0.93 and 1.02. The hydroxymethine proton H-3 of β-sitosterol/stigmasterol came out at δ 3.53 as a multiplet. One proton appeared at δ 5.36 as a doublet with coupling constants of 5.1/7.3 Hz represents the endocyclic double bond proton H-6 .Stigmasterol showed two doublet-of-doublets for the other olefinic protons H-23 and H-22 at δ 5.02 (1H, dd, *J* = 9.2, 15.6 Hz) and 5.16 (1H, dd, *J* = 8.4, 15.6 Hz) respectively. The ^{1}H NMR spectral data of stigmasterol are in agreement with those of Hussain et al. **[21]**. The ^{13}C NMR spectrum of β-sitosterol/stigmasterol showed 28/26 signals for the 29 carbons skeleton with the overlapping of 2/6 carbons. The significant carbon signal for the β-sitosterol/stigmasterol would be the C-3 attached to a hydroxyl group and appeared at δ 72.0. The endocyclic carbon-carbon double bond of β-sitosterol/stigmasterol was represented by two signals at δ 121.9 and 141.0 of C-6 and C-5. Other olefinic carbons of stigmasterol appeared at δ 138.5 and 129.5 for C-22 and C-23. The quaternary C-5 signal is shifted to lower field than those of the tertiary C-6, C-22 and C-23. The similarity of the ^{13}C spectral data of β-sitosterol/stigmasterol with those of the published data in[22],[23] confirmed that both two are of the same structure. Stigmasterol

has earlier reported from the fruits of this plant[18] but never have been reported before from the bark of *Salvadora oleoides* Decne. However β-sitosterol was found in many other plants including such as *Zingiber*[24], *Alpinia*[25], *Globba*[26], *Kaempferia*[27], *Costus*[28], *Renealmia*[29], *Aframomum*[30] and *Hedychium*[31], whereas stigmasterol including Zingiberaceae of *Renealmia*[29] and *Alpinia*[32,33].



[B] β-sitosterol

4. Conclusion

The isolation and identification of stigmasterol **[A]** and β-sitosterol **[B]** from the bark of *Salvadora oleoides* was the first ever to be reported from this plant. The work was carried out by utilizing several kinds of chromatographic separation techniques and spectroscopic analyses.

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