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Isolation and Characterization of β-sitosterol from *Cissampelos pareira* Linn.

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ABSTRACT

General phytochemical screening of *Cissampelos pareira* (Menispermaceae) revealed the presence of sterols, polyphenolic compounds, saponins, alkaloids etc. The aim of this study is to identify and characterize the bioactive principle from the plant. It has wide folk medicinal uses. The isolation and characterization of Phytoconstituent was done from the chloroform extract of *Cissampelos pareira* Linn. Chloroform extract was subjected to column chromatography and eluted with solvent mixtures of petroleum ether, benzene and chloroform to isolate phytoconstituent. The structure of the isolated compound was established on the basis of elemental analysis and spectroscopic evidences (IR, UV, ^{1}HNMR, ^{13}CNMR, MS). A sterol, stigmast-5-en-3β-ol was isolated from the chloroform extract of the plant. The yield of the compound was 0.0042% w/w, m.p. 136- 138^{0}C, Rf value 0.7 in Toluene: diethylether: Cyclohexane (6:3:1). *Cissampelos pareira* contains β-sitosterol which may be responsible for various pharmacological activities of the plant.

Keywords: *Cissampelos pareira*, β-sitosterol, Menispermaceae, Chloroform extract.

1. INTRODUCTION

Cissampelos pareira of the family Menispermaceae are commonly known as Patha in Ayurveda and have been used for the treatment of fever, urinary problems and skin infections.[1] *Cissampelos pareira* is found very common in semi dry forests of tropics.[2] Various alkaloids and different pharmacological activities of these plants have been reported. Hydrocolloids,[3] Cissampeloflavone,[4] A tropone-isoquinoline alkaloid, pareitropone,[5] Bisbenzylisoquinoline alkaloids, cissamperine with tumor inhibitor activity,[6] trandrine,[7] tropoloisoquinoline alkaloids such as pareirubrine A and B with antileukemic activity[8] have been isolated from *C. pareira Var. hirsuta*. Plant extracts were tested for Antipyretic activity,[9] Chemomodulatory influence of Hirsuta on Gastric cancer and antioxidant system in experimental animal,[10] anti-inflammatory activity,[9] Immunomodulatory activity,[10] antifertility activity, antinociceptive, antiarthritic activity, antibacterial activity,[11] antimalarial activity,[12] diuretic activity,[13] hypoglycemic activity[14] and anticonvulsant activity.[15] A few ethnobotanical reports on treatment of fever, [16] gastrointestinal tract disorders[17] were also investigated. The plant also contains some other phytoconstituents like sterols, saponins, flavonols etc along with alkaloid.[18,19] The present study is an attempt to isolate and characterize β- sitosterol from the aerial parts of *Cissampelos pareira* Linn.

2. MATERIALS AND METHODS

Collection & Authentication of plant Material

The plants of *Cissampelos pareira* Linn were collected from the National botanical research institute, Lucknow, Uttar Pradesh, India and were authenticated by Prof J. P. Shukla, Department of Botany, D.B.S College, Kanpur where a voucher specimen (PH/CP/11) is deposited for further reference.

Extraction and isolation of compound

The shade dried and coarsely powdered leaves were successively extracted with increasing polarity solvents like petroleum ether, chloroform and alcohol using soxhlet apparatus. Chloroform extract was packed in a column containing silica gel 'H' and eluted with solvent mixtures of petroleum ether, benzene and chloroform. All the fractions were monitored on TLC. The fractions collected with petroleum ether: benzene (20:80) was pooled together. The TLC of these fraction was performed by using solvent system Toluene: diethylether: Cyclohexane (6:3:1) which shows a spot at 0.70 Rf value. It was evaporated on a water bath (50- 60^{0}C) to afford a solid residue. The residue was dissolved in a mixture of Chloroform:Ethanol (40:60) with little warming on a water bath. It was left undisturbed in refrigerator when needle shaped crystals was obtained. The characterisation of the isolated compound was done on the basis of phytochemical analysis (Salkowski reaction, Liebermann burchard reaction) and spectroscopic studies. (IR, UV, ^{1}HNMR, 13 C-NMR & FAB-MS).

Tests for alcohol

4g of cerric ammonium nitrate was dissolved in 10ml of 2N HNO_3 on mild heating. A few crystals of isolated compound were dissolved in 0.5ml of dioxane. The solution was added to 0.5ml of cerric ammonium nitrate reagent and diluted to 1ml with dioxane and shaken well. The developed yellow to red color indicates the presence of an alcoholic hydroxyl group.[20]

Tests for steroid

Salkowski reaction

A few crystals were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to the solution. A reddish color was seen in the upper chloroform layer. [20]

Liebermann burchard reaction

A few crystals were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to it followed by addition of 2-3 drops of acetic anhydride. Solution turned violet blue and finally green. [20]

3. RESULTS AND DISCUSSION

Phytochemical analysis (Salkowski's test and Lieberman-Burchard test) of the compound confirms its steroidal nature. The elemental analysis (Elementar, Vario EL III) revealed that the compound contains 83.86% of C, 12.25% of H and 3.89% of O. The N % was found to be nil. Now based on the number of O (1 or 2) in the proposed compound the molecular weight could be: 411.30 or 822.62 respectively. So, Based upon the number of O the formula could be $C_{29}H_{50}O$ or $C_{58}H_{100}O_2$. From ^{13}CNMR and ^{1}HNMR the number of C and H was found to be near to the first formula i.e. $C_{29}H_{50}O$. The exact molecular mass for the formula was found to be 414.7.

The IR absorption spectrum showed absorption peaks at 3373.6cm^{-1} (O-H stretching.); 2940.7 cm^{-1} and 2867.9 cm^{-1} (aliphatic C-H stretching); 1641.6 cm^{-1} (C=C absorption peak); other absorption peaks includes 1457.3 cm^{-1} (CH_2); 1381.6 cm^{-1} (OH def), 1038.7 cm^{-1} (cycloalkane) and 881.6 cm^{-1}.

^{1}HNMR ($CDCl_3$, 400MHz): ^{1}HNMR has given signals at δ 3.2(1H, m, H-3), 5.26 (1H, m, H-6), 5.19(1H, m, H-23), 4.68(1H, m, H- 22), 3.638(1H, m, H-3), 2.38(1H, m, H-20), 1.8-2.0 (5H, m) ppm. Other peaks are observed at δ 0.76-0.89 (m, 9H), 0.91-1.05 (m, 5H), 1.35-1.42 (m, 4H), 0.69-0.73 (m, 3H), 1.8-2.00 (m, 5H), 1.07-1.13 (m, 3H), 1.35-1.6 (m, 9H) ppm.

^{13}CNMR ($CDCl_3$, 100MHz): ^{13}CNMR has given signal at 150.98, 145.2 (C-5), 139.8 (C-22), 121.7, 118.89(C-6), 79.03 (C-3), 55.3(C-14), 55.18(C-17), 50.45 (C-9), 48.3 (C-9), 40.8 (C-20), 40.1(C-12), 39.2 (C-13), 38.9 (C-4), 38.6 (C-12), 37.18 (C-1), 37.12 (C-10), 36.3 (C-8), 35.59(C-20), 34.29 (C-22), 34.24 (C□7), 32.66 (C-8), 29.86 (C-25), 29.71 (C-16), 28.41 (C-2), 28.1 (C-15), 27.4 (C-28), 26.1 (C-11, 26), 21.6 (C-27), 19.32 (C-19), 17.71 (C-21), 15.6 (C-18, 29).

FAB-MS spectroscopy showed the molecular ion peaks at 414 that correspond to molecular formula, $C_{29}H_{50}O$. Ion peaks were also observed at m/z 367, 271, 255, 229,189, 175, 161, 133, 121, 105, 107, 95, 81, 69, 55, 41.

Based on the melting point and other related data (IR, NMR and Mass) the structure of the isolated compound was proposed as

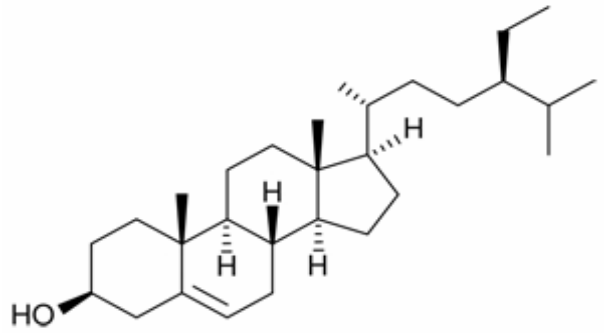

Figure 1: β-sitosterol ($C_{29}H_{50}O$; Mol.Wt: 414.71)

4. CONCLUSION

From the above study, β-Sitosterol was isolated and characterized from chloroform extract of *Cissampelos pareira* Linn. and this is a phytosterol. β-Sitosterol reduce carcinogen induced cancer of the colon. It shows antiinflammatory, anti-pyretic, antiarthritic, anti-ulcer, insulin releasing and oestrogenic effects and inhibition of spermatogenesis. Beta-sitosterol is mainly known and used for its cholesterol lowering property. But studies have shown that the phytochemical may have other health benefits: easing symptoms of benign prostatic enlargement, reducing risk of cancer and prevention of oxidative damage through its antioxidant activity.

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