Isolation and characterization of stigmast-5-en-3β-ol (β-sitosterol) from the leaves of *Hygrophila spinosa* T. Anders

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Abstract

Purpose: *Hygrophila spinosa* T. Anders (Acanthaceae) commonly known as 'Talmakhana' in Hindi contains a number of phytoconstituents viz. alkaloids, phytosterols, glycosides, amino acids, proteins, phenolic acids, enzymes, vitamins, sugars, minerals, flavonoids, gums & mucilage, terpenoids etc. The objective of the present study was to isolate and characterize phytoconstituents(s) from the chloroform extract of *Hygrophila spinosa* leaves. **Methods:** Chloroform extract was subjected to column chromatography and eluted with solvent mixtures of increasing polarity, composed of petroleum ether, benzene and chloroform to isolate phytoconstituents. The structure of the isolated compound was established on the basis of elemental analysis and spectroscopic evidences (IR, UV, ^{1}HNMR, ^{13}CNMR, MS). **Results:** A sterol, stigmast-5-en-3β-ol was isolated from the chloroform extract of the leaves of the plant. The yield of the compound was 0.0046% w/w, m.p. 136-138^{0}C, λ_{max} in EtOH: 206 nm, R_f value 0.72 in Toluene: Ether: Cyclohexane (5:2:1). **Conclusions:** *Hygrophila spinosa* contains β-sitosterol which may be responsible for various pharmacological activities of the plant.

Keywords: *Hygrophila spinosa*, β-sitosterol, acanthaceae, chloroform extract.

Introduction

Hygrophila spinosa T. Anders (Acanthaceae) has a long history of use in the Ayurvedic and Unani systems of medicine. It contains a number of phytoconstituents viz. alkaloids, phytosterols, glycosides, amino acids, proteins, phenolic acids, enzymes, vitamins, sugars, minerals, flavonoids, gums & mucilage, terpenoids etc [1-6]. The plant is used for the treatment of a number of diseases [1, 7-8]. The present study deals with isolation and characterization of β-sitosterol from the leaves of *Hygrophila spinosa* T. Anders.

Materials and methods

Collection and authentication of the plant material

H. spinosa plants were collected from Berhampur, Orissa, India and botanical identification was done through Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi (Voucher no. BITPcog. 463/07-08). Voucher specimen was preserved in the department for further verification. The leaves were separated from the plant and dried under shade.

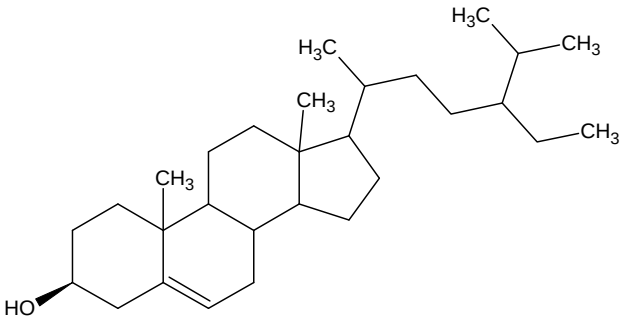
Extraction and isolation of the compound

The shade dried and coarsely powdered leaves were successively extracted with petroleum ether, chloroform and alcohol using soxhlet apparatus. Chloroform extract was chromatographed on a silica gel column and eluted with solvent mixtures of increasing polarity, composed of petroleum ether, benzene and chloroform. All the fractions were monitored on TLC. Fractions collected with petroleum ether: benzene (20:80) were pulled together as these fractions showed a single spot of same R_f value in TLC. It was evaporated in a water bath (50-60^{0}C) to afford a solid residue. The residue was dissolved in a mixture of $CHCl_3$:EtOH (40:60) with little warming on a water bath. It was left undisturbed in refrigerator when needle shaped crystals of stigmast-5-en-3β-ol was obtained. The structure of the isolated compound was established on the basis of elemental analysis and spectroscopic evidences (IR, UV, ^{1}HNMR, ^{13}CNMR, MS). The structure was simulated using ACD/NMR program to obtain the chemical shifts of both proton and carbon.

Results and discussion

Characterization of the compound

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 tentatively: $C_{29}H_{50}O$ 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. This formula produces 239 hits when searched in http://www.chemspider.com/Search.aspx . Since, the compound gives positive test for steroids so all of the other structures other than steroids were rejected. Based upon the functional group analysis it was found that the nature of oxygen was hydroxyl, also supported by IR spectroscopy (Shimadzu, IRPrestige-21). This implies presence of one double bond in the structure. So, the steroids with other functional groups were rejected. Also on considering the nature of oxygen as hydroxyl and presence of one double bond, the general formula for the compound was C_nH_{2n-6}. Therefore it must be a tetracyclic compound. Based on the melting point and other related data (IR, NMR and Mass) the structure of the isolated compound was proposed as



Stigmast-5-en-3β-ol

Again the compound is a white crystalline compound, m.p. 136-138^{0}C. λ_{max} in EtOH: 206 nm. IR absorptions bands appeared at 3549.99 cm^{-1} (OH), 2935.73 cm^{-1} (CH_2), 2867.38 cm^{-1} (CH), 1637.63 cm^{-1} (C=C), 1063.34cm^{-1} (C-O) (Fig. 1). Mass spectra of this compound suggested that its molecular mass is 414 (M.F. $C_{29}H_{50}O$) having characteristic fragments observed at m/z: 414, 396, 381, 329, 303, 289, 273, 255, 231, 213, 199, 173, 159, 145, 119, 95, 81, 69, 55 (Fig. 2). NMR spectrum of this compound resembled data published in previous studies [9-12]. This compound is having six methyl, eleven methylene and three quaternary carbons with a hydroxyl group. The carbons of alkenes conjugated are at 140.78 ppm (C_5) and 121.72 ppm (C_6) which was confirmed from the ^{13}CNMR. The structure was simulated using ACD/NMR program to obtain the chemical shifts of both proton and carbon. On comparison the experimental data matched with the simulated data which supports the proposed structure of this compound.

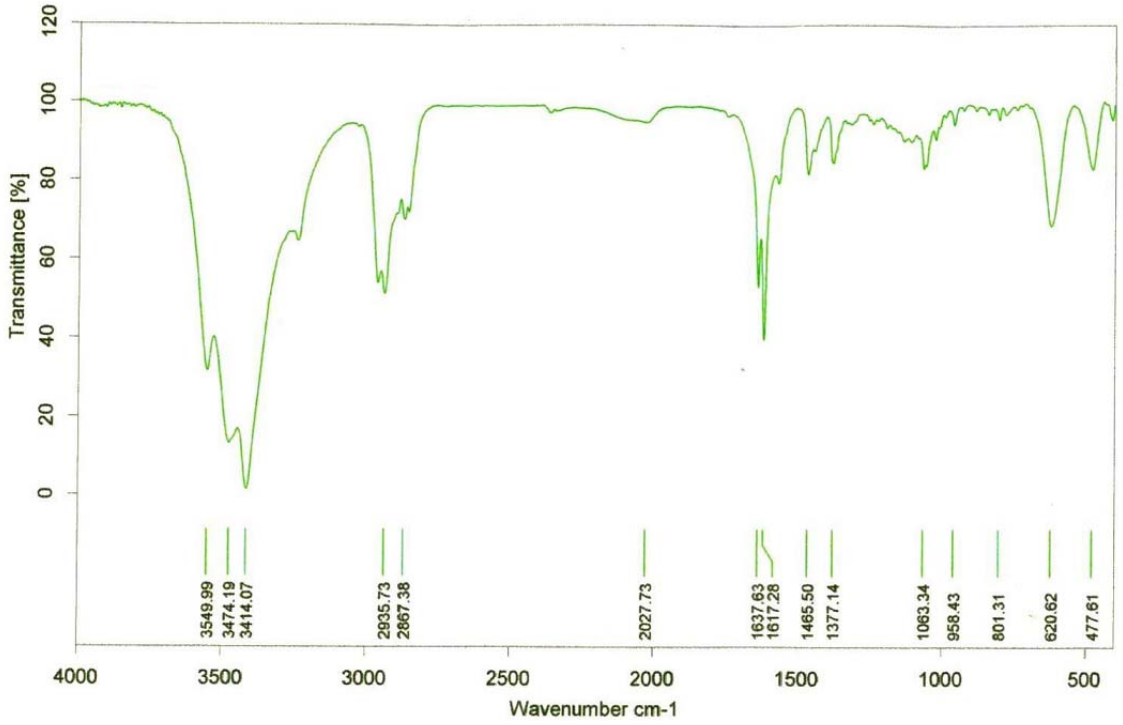


Fig. 1: IR spectrum of the compound

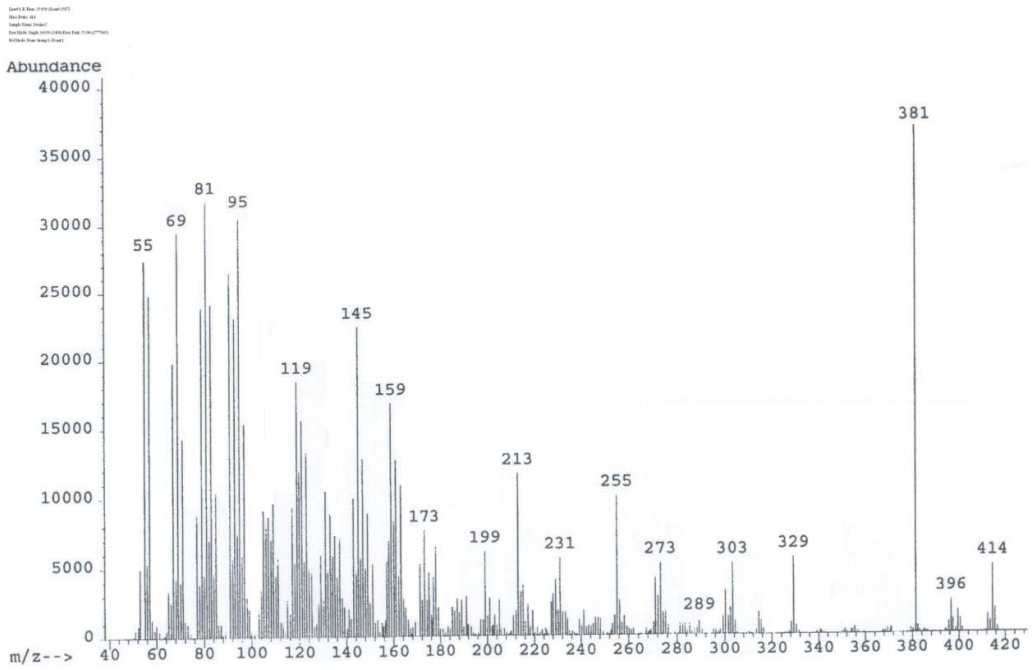


Fig. 2: Mass spectrum of the compound

Table 1: Comparative chemical shifts of proton NMR of the compound

Position	Group	δ_H (ppm)	
		Obtained	ACD/ChemSketch (Product Version: 10.00)
1	CH_2	1.47	1.38;1.13
2	CH_2	1.56	1.57;1.32
3	CH	3.52	3.25
4	CH_2	2.28	2.23;1.98
5	C	-	-
6	CH	5.36	5.37
7	CH_2	2.03	2.04;1.79
8	CH	1.67	1.45
9	CH	1.48	1.44
10	C	-	-
11	CH_2	1.52	1.52;1.27
12	CH_2	1.49	1.49;1.24
13	C	-	-
14	CH	1.50	1.4
15	CH_2	1.60	1.60;1.35
16	CH_2	1.84	1.60;1.35
17	CH	1.49	1.47
18	CH_3	0.68	1.26
19	CH_3	1.02	1.16
20	CH	1.64	1.64
21	CH_3	0.94	1.06
22	CH_2	0.88	1.25
23	CH_3	1.04	1.25
24	CH	1.50	1.46
25	CH	1.65	1.82
26	CH_3	0.83	1.01
27	CH_3	0.85	1.01
28	CH_2	1.04	1.29
29	CH_3	0.88	0.96
30	OH(at C_3 position)	2.00	2.00

Table 2: Comparative chemical shifts of ^{13}CNMR of the compound

Position	Group	δ_C (ppm)	
		Obtained	ACD/ChemSketch (Product version: 10.00)
1	CH_2	37.28	30.1
2	CH_2	31.69	31.8
3	CH	71.82	71.7
4	CH_2	42.33	41.5
5	C	140.70	140.9
6	CH	121.72	121.9
7	CH_2	31.69	30.0
8	CH	31.93	31.9
9	CH	50.17	50.8
10	C	36.52	37.8
11	CH_2	21.10	22.7
12	CH_2	39.80	37.2
13	C	42.33	44.0
14	CH	56.79	56.5
15	CH_2	24.37	27.7
16	CH_2	28.25	27.3
17	CH	56.09	58.3
18	CH_3	11.86	20.7
19	CH_3	19.40	23.0
20	CH	36.52	36.1
21	CH_3	18.79	19.4
22	CH_2	33.98	33.9
23	CH_3	26.14	29.9
24	CH	45.88	46.1
25	CH	28.91	31.7
26	CH_3	19.80	21.0
27	CH_3	18.79	21.0
28	CH_2	23.10	25.8
29	CH_3	11.99	12.2

Conclusion

Stigmast-5-en-3β-ol (β-Sitosterol) was isolated and characterized from chloroform extract of *H. spinosa* leaves and this is a phytosterol. β-Sitosterol reduce carcinogen-induced cancer of the colon. It shows anti-inflammatory, 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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