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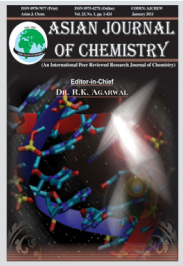

Isolation and Characterization of 22,23-Dihydrostigmasterol (β-Sitosterol) from the Bark of *Polyalthia longifolia* var. *angustifolia*

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The phytosterol namely 17-(5-ethyl-6-methylhaptane-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta(phenanthren-3-ol) has been isolated from methanolic extract of bark of *Polyalthia longifolia* var. *angustifolia*. The structure of the isolated compound was established on the basis of elemental analysis and spectroscopic evidences of IR, UV-vis, ^{1}H NMR, ^{13}CNMR and EIMS.

Key Words: *Polyalthia longifolia* var. *angustifolia*, 22,23-Dihydrostigmasterol, Methanolic extract, Annonaceae.

INTRODUCTION

Polyalthia longifolia var. *angustifolia* Thw. (Annonaceae), is a small tree with linear-lanceolate leaves, 1.0-1.5 cm broad, occurring in Sri Lanka and now grown in tropical parts of India on road side[1]. This plant is the different variety of *Polyalthia longifolia* but very similar to *Polyalthia longifolia* var. *pendula* (PLP) in appearance. The bark of *Polyalthia longifolia* var. *pendula* is used in Indian System of medicine as febrifuge and tonic[2]. The diterpenes[3,4], alkaloids[5,6], steroid[7] and miscellaneous lactones[8] have been isolated from its bark which were reported with various biological activities including antibacterial, cytotoxicity and antifungal activity[9-11]. Another variety *Polyalthia longifolia* var. *angustifolia* (PLA) has not been properly investigated. We have recently reported significant antidiabetic and antioxidant activity of the methanolic extract of *Polyalthia longifolia* var. *angustifolia*[12]. There are no further reports on isolation and characterization of β-sitosterol from the bark of *Polyalthia longifolia* var. *angustifolia*. Hence in the present study, we have made an attempt to isolate, identify and characterize the β-sitosterol from the bark of *Polyalthia longifolia* var. *angustifolia*.

EXPERIMENTAL

The Soxhlet extractor was used for the extraction process for the plant of *Polyalthia longifolia* var. *angustifolia*. The melting point was determined in open capillary tubes (Sisco) and are uncorrected. UV-Vis spectra was recorded on a JASCO, V-630 spectrophotometer. IR spectra was recorded on a Shimadzu-FTIR-8400S spectrophotometer using KBr powder. ^{1}H and ^{13}C NMR spectra of the compound was recorded on a Bruker DRX-500 NMR spectrometer in $CDCl_3$ using TMS as internal standard. The EIMS was recorded on Jeol, GC mate. The TLC was run on silica gel GF_{254} and column chromatography was carried out over silica gel (60-120 mesh, Merck). The elemental analysis of the compound was done with elemental analyzer (Perkin Elmer-2400). Molecular weight of the compound was determined by Rast's method was close to the theoretical value.

The bark of *Polyalthia longifolia* var. *angustifolia* was collected from Bhubaneswar, Orissa in the month of November 2008. The plant was identified by Dr. P.C. Panda, Senior Scientist, Regional Plant Resource Centre, Bhubaneswar, Orissa. A voucher specimen (No. SPS-2) has been preserved in the Pharmacognosy department of our University.

Extraction and isolation of compound: The dried and coarsely ground bark (500 g) was extracted successively with *n*-hexane and methanol in Soxhlet apparatus each for 8 h and the extracts were evaporated in a rotatory evaporator and dried by vacuum pump. The defatted methanolic extract obtained as thick dark brown mass (65 g). The column was eluted with gradient solvent system (*n*-hexane-ethyl acetate). The dried methanolic extract (20 g) was mixed with 60 g silica gel to make the material to get adsorbed in the silica gel. The column was eluted with solvent gradually starting from 100 % *n*-hexane, followed by increasing order of ethyl acetate in *n*-hexane (0, 10, 20, 30, 50, 70 and 95 % ethyl acetate in *n*-hexane) and 25 × 200 mL fractions were collected. After the solvent was

evaporated all the fractions were subjected to TLC analysis. The TLC solvent system for the eluents with100 % hexane, 10 % ethyl acetate:hexane, 20 % ethyl acetate:hexane, 30 % ethyl acetate:hexane, 50 % ethyl acetate:hexane and 60 % ethyl acetate:hexane were found to be 5, 20, 40, 50 and 60 % ethyl acetate:hexane, respectively. On the basis of R_f value, similar fractions were pooled. Among them on the basis of high concentration, 30-40 fractions, eluted with ethyl acetate/hexane (6:4), was taken for purification.

Purification of the compound: Mixed fraction 30-40, after concentration was taken for thin layer chromatographic study using various solvent systems. Among them the ethyl acetate:hexane (9.5:0.5) gave good resolution in the TLC studies and this solvent system was selected for preparative TLC. The concentrated pool was taken in methanol and the solution was spotted in the preparative TLC plates. The spotted plates were kept in completely saturated chamber of selected mobile phase. After development of chromatogram, the plates were exposed to iodine vapour and the band (R_f = 0.104) was identified and scrapped out from the plates. The collected powder was suspended in *n*-hexane and filtered through Whatman filter paper. The filtrate was concentrated and the isolated product [mixed fraction (30-40) was obtained as white crystalline solid (65 mg)]. The compound was highly soluble in *n*-hexane, chloroform, ethyl acetate and methanol. The process of isolation was repeated to get substantial amount for analytical purpose. The isolated compound was then subjected for characterization through UV-vis, FTIR, NMR, mass spectroscopic techniques.

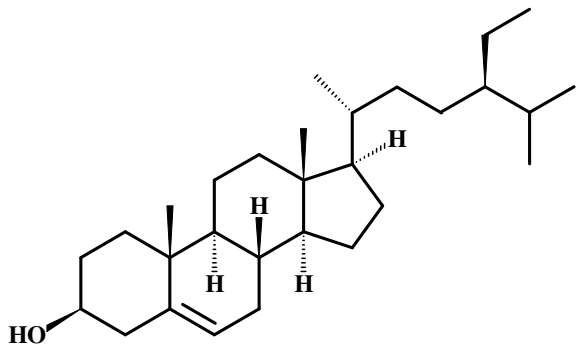
RESULTS AND DISCUSSION

The compound is a white crystalline compound with m.p. 136-138 ºC and λ_{max} at 210 nm (MeOH). 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. The N % was found to be nil. Mass spectra of this compound suggested that its molecular mass is 414 and was supported by the molecular weight calculated by Rast's procedure. The IR spectrum exhibited broad peak centered at 3409 cm^{-1} suggesting the presence of hydroxyl group which was substantiated by chemical identification tests[13]. The ^{13}C NMR spectrum suggested the presence of approximately 27-29 number of carbon atoms (Table-1). Based upon the above the molecular formula is proposed to be $C_{29}H_{50}O$. The mass spectrum exhibited fragmentation at m/z 273, characteristic of Δ^5 sterol. It also contained fragments at m/z: 414, 396, 381, 329, 303, 289, 255, 231, 213, 199, 173, 159, 145, 119, 95, 81, 69 and 55. IR absorptions bands appeared at 3409.57 cm^{-1} (OH), 2925.45 cm^{-1} (CH-unsaturated), 2853.23 cm^{-1} (CH-aliphatic), 1515.82 cm^{-1} (C=C), 1047.52 cm^{-1} (C-O). The ^1H NMR spectrum corresponds to the data of stigmasterol (Table-1). Presence of tertiary (δ ppm: 0.80 and 0.65), secondary (δ ppm: 0.090, 0.83 and 0.81) and primary (δ ppm: 0.84) methyl groups were indicated from the ^1H NMR spectrum. The multiplets were observed at 4.84 (1H) and 5.36 ppm (2H) for olefinic protons. A singlet was observed at 3.2 ppm which could be assigned to the alcoholic proton. The ^{13}C NMR showed peaks at 140.754 and 121.704 ppm assignable to carbons of conjugated alkene. The secondary alcoholic carbon was indicated by the peak at 71.7 ppm. The other observed data were very close to the data previously reported for characterization of stigmast-5-en-3β-ol[14-17].

TABLE-1
^1H AND ^{13}C NMR DATA OF THE COMPOUND

Positions	Groups	^{13}C NMR (δ ppm)	^1H NMR (δ ppm)
1	CH_2	37.24	–
2	CH_2	31.63	–
3	CH	71.78	3.44 d (11, 10, 5, 4)
4	CH_2	42.27	2.19 m
5	C	140.75	–
6	CH	121.70	5.38 br. d
7	CH_2	31.89	–
8	CH	31.89	–
9	CH	50.12	–
10	C	36.49	–
11	CH_2	39.76	–
12	CH_2	42.27	2.19 m
13	C	28.24	–
14	CH	56.75	–
15	CH_2	24.29	–
16	CH_2	28.24	–
17	CH	56.04	–
18	CH_3	11.85	0.61 s
19	CH_3	19.39	0.91 s
20	CH	36.12	–
21	CH_3	18.77	0.85 d (6.4)
22	CH_2	33.93	2.01-1.74 m
23	CH_3	23.05	1.30-0.69 m
24	CH	45.82	–
25	CH	29.14	–
26	CH_3	19.81	0.78 d (6.4)
27	CH_3	19.02	0.76 d (6.4)
28	CH_2	23.05	–
29	CH_3	11.85	0.75 t (6.4,6.4)

In conclusion, β-sitosterol was isolated and characterized from the bark of *Polyalthia longifolia* var. *angustifolia*. Further studies are desirable to evaluate its pharmacological activity.



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