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Research Article

CHARACTERIZATION OF STEROIDAL NUCLEUS (PHYTOSTEROLS) FROM THE ISOLATED HEXANE EXTRACT OF *BOMBAX CEIBA* L

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ABSTRACT

Purification and characterization of hexane extract isolates of leaves of *Bombax ceiba* resulted in the isolation of steroidal moiety (Phytosterol). The structure of the isolated compound was characterized on the basis of extensive spectral data (^{1}H NMR and GC-MS) and comparison with their literature data.

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INTRODUCTION

Bombax ceiba is commonly known as silk cotton tree and semal which belongs to family Bombacaceae. *Bombax ceiba* is an important medicinal plant of tropical and subtropical India. Its medicinal usage has been reported in the traditional systems of medicine such as Ayurveda, Siddha and Unani. It has wide range of medicinal and pharmacological application. *Bombax ceiba* L. is one of the important plant species is used in various indigenous systems of medicine in India, China and Southeast Asian countries. Almost every part of plant is used as medicine. Some of the ethanomedicinal uses of *B. ceiba* prevalent among different tribes of India have been found possess strong antiinflamatory, antibacterial, antiviral, analgesic, hepatopotective, antioxidant, oxytocic, hypotensive, hypoglycaemic, antiangiogenic, antimutagenic as well as fibrinolysis enhancing activities (Gupta, *et al*. 2004). Mehta & Modi (2010) stated in his research that phytochemical analysis of leaves of *Bombax insigne* Linn. showed the presence of many important classes of phytoconstituents including sterols. Shamimin, a newly discovered flavonol C-glycoside has been isolated as a pale yellow powder from the ethanolic extract of fresh, undried leaves of *B. ceiba*.

This paper describes the detection, isolation and structural elucidation of steroidal compound (phytosterol) in the hexane extract of leaves of *Bombax ceiba* on the basis of extensive spectral properties (NMR) reported from the literature.

MATERIALS AND METHODS

Plant Material

The leaves of plant *Bombax ceiba* were collected, then identified and authenticated from Flora of Maharashtra State - Vol. I and II (Singh & Kartikeyan, 2000; Singh & Kartikeyan, 2001) and Flora of Nagpur District (Ugemuge, 1986).

Detection of Phytosterols (Qualitative Analysis)

Extraction of all samples then done by Soxhlet method with the selected solvent Heaxane. Two standard methods, Salkowski Test (Salkowski, 1872) and Liebermann Burchard's Test (Liebermann, 1803)(Burchard, 1890) were done to determine the presence of sterols for the comparative confirmation with field tests (Krishnaiah, *et al*. 2007). The active extract (Hexane) obtained from leaves of *Bombax ceiba* was subjected to thin layer chromatography (TLC) (Stal, 1965) (Wagner & Bladt, 1995) to find out the number of components present in it. The adsorbent used for preparation of thin layer plate as a stationary phase was Silica Gel G. 15 g powder of Silica Gel G was mixed with 30ml Distilled water. This Silica Gel G suspension was spread with a spreader on thin layer chromatographic glass plates fixed on a stage. The prepared plates were air-dried and activated in an oven at 110°C for 30 min. The activated plates

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then used for the application of samples and standard solution, β-Sitosterol (M P Biomeditech), in Hexane with capillary tubes. The spots of samples and standard solutions were applied on plate, keeping distance of approximately 1cm. The chromatographic glass chamber was saturated with the moistened filter paper by dipping it in selected solvent system: Benzene: Ethyl Acetate (5: 1). The developed plate then derivatize with spraying reagent (20% Antimony Trichloride in Chloroform) for the visualization of phytosterol spots. The Rf values of standard spots and sample spots were calculated.

Rf value $=\frac{\boldsymbol{DistancetravelledbytheCompound}}{\boldsymbol{DistancetravelledbytheSolvent}}$

Isolation

Dried hexane extracted sample was then extracted with Acetone and Acetonitrile. The residue occur kept for boiling at 80°C. for 5-10mins(Kalsait, Khedekar, Saoji, & Bhusari, 2011). The boiled solution then kept in ice bath for 5mins. White floc formed then filtered through filter paper. The dried residue (white powder) then further analysed for the presence of Sterols.

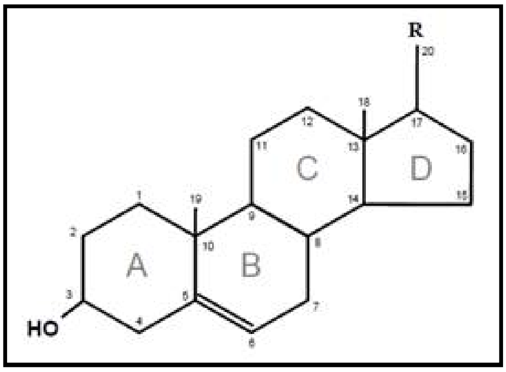

Fig 1 Steroidal Nucleus

Identification of Phytosterol

White powder (7 mg) 1H-NMR δ (ppm) 0.857, 0.880, 0.902 (each 3H, Me-3), 1.999(-OH), 2.045 (1H, H-24), 5.118 (1H, H-22): See Table1, Fig1, Fig 2- (a), (b); GC-MS Retention time in the range 25-38 (m/z) 591.36, 535.31, 316.21, 147.12, 57.06, See Table 2, Fig 3, 4.

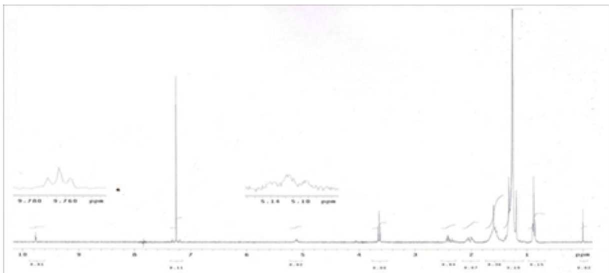

Fig 2 a ^{1}H NMR Peak identification

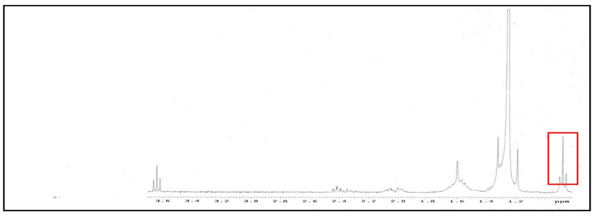

Fig 2 b ^{1}H NMR Peak identification

Table 1 ^{1}H NMR Chemical shift value

```
Sample 4

exp1   s2pul

         SAMPLE                        DEC. & VT
date    Aug 11 2015    dn                      H1
solvent       CDCl3    dof                 -268.4
file /export/home/~    dm                     nnn
vnmr1/2015/Aug/Ext~    dmm                      c
ernal/RTM-Nagpur/S~    dmf                    200
           p14.fid          PROCESSING
    ACQUISITION        lb                    0.10
sfrq        299.950    fn                not used
tn               H1
at            2.000    werr
np            24022    wexp
sw           6006.0    wbs
fb         not used    wnt
bs                2          DISPLAY
pw              3.0    sp                   -49.9
pw              3.0    wp                  3099.8
tpwr             60    vs                   34771
d1                0    sc                       0
tof           900.0    wc                     250
nt             1600    hzmm                 12.40
ct              562    is               171875.64
alock             n    rfl                  651.0
gain              2    rfp                      0
         FLAGS         th                     104
il                n    ins                  1.000
in                n    ai        ph
dp                y
```

```
Sample 4

 Data Collected on: quanta-mercuryh1freq
 Archive directory: /export/home/vnmr1/vnmrsys/data
 Sample directory:
 File: H1

Pulse Sequence: s2pul
```

INDEX	FREQUENCY	PPM	HEIGHT
1	2930.421	9.770	3.6
2	2928.588	9.764	6.8
3	2926.755	9.758	3.2
4	2351.961	7.841	-3.6
5	2351.595	7.840	2.7
6	2198.365	7.329	2.5
7	2177.837	7.261	116.1
8	2158.042	7.195	2.5
9	1535.227	5.118 ←	2.3
10	1098.633	3.663	10.1
11	1092.034	3.641	22.1
12	1085.436	3.619	11.2
13	733.155	2.444	2.7
14	731.322	2.438	2.7
15	725.823	2.420	5.0
16	723.991	2.414	5.0
17	718.492	2.395	3.1
18	716.659	2.389	2.9
19	704.562	2.349	3.2
20	615.850	2.053	3.6
21	613.284	2.045 ←	3.0
22	609.985	2.034	2.1
23	599.721	1.999 ←	3.9
24	479.117	1.597	26.0
25	469.586	1.566	10.7
26	462.987	1.544	8.3
27	416.799	1.390	3.4
28	396.270	1.321	45.6
29	391.138	1.304	23.6
30	376.109	1.254	1325.8
31	356.313	1.188	36.3
32	270.534	0.902 ⇐	13.1
33	263.936	0.880 ⇐	46.0
34	256.971	0.857 ←	16.0
35	-0.000	-0.000	23.2

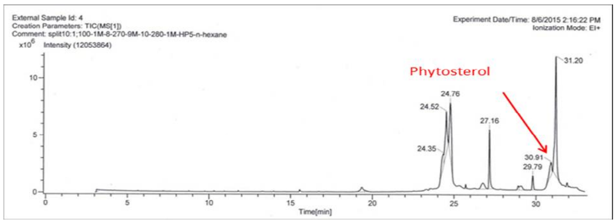

Fig 3 GC of unknown isolate

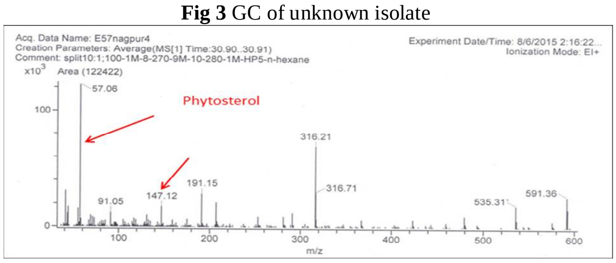

Fig 4 MS of unknown isolate

Table 2 GC-MS RT (min) of unknown isolate

Peak Number	Time [min]	Type	Peak Width(FWH [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	3.12	BB	0.1953	1348731.55	231837.23		3.10	3824	3.31	192893
2	19.13	BV	0.0740	265176.23	47680.91		18.93	84480	19.13	85922
3	19.33	VV	0.1778	4857445.35	394487.27		19.13	85922	19.52	88619
4	19.52	VB	0.1464	1086715.52	116226.26		19.52	88619	19.84	90851
5	24.35	BV	0.1078	12385487.57	1040198.81		24.09	664530	24.36	2539431
6	24.52	VB	0.0983	26836719.18	3579249.84		24.36	2539431	24.64	4414045
7	24.76	BB	0.1438	39665587.59	4724771.09		24.66	4427065	24.93	1071518
8	25.71	BB	0.0487	1331108.51	368192.72		25.62	419839	25.92	373681
9	26.77	BB	0.2408	7698867.20	516614.74		26.48	374314	27.05	389397
10	27.16	BB	0.0614	23216743.71	5201843.30		27.06	386684	27.45	406700
11	28.93	BV	0.0878	1923103.17	368850.35		28.77	303136	28.97	305625
12	29.11	VB	0.2207	4450736.43	350227.50		28.97	305625	29.37	310605
13	29.79	BB	0.0783	6456820.15	1258858.96		29.69	311238	29.96	342268
14	30.91	BB	0.1424	12361739.07	1155369.37		30.60	568638	31.00	1941133
15	31.20	BB	0.0896	71713551.07	10481361.87		31.02	2040228	31.45	954544
16	31.90	BV	0.0721	1728310.38	356025.61		31.82	620991	32.00	583103
17	32.03	VB	0.0745	300754.77	62228.74		32.00	583103	32.14	553634

RESULTS AND DISCUSSION

Compound was isolated as a white powder. The GC-MS (Mass spectral) data of Compound (Ret. Time: 30.90 to 30.91, m/z: 147.12, 57.06) gave a molecular formula $C_{24}H_{38}O_4$ suggesting Steroidal nucleus, which was supported by the ^1H NMR spectral data. ^1H NMR spectral data of compound exhibited 3 methyl singlets were appeared as 3 methyl triplet at δ 0.875 ppm, δ 880 ppm, δ 0.902 ppm. Other protons appeared at δ 1.999 ppm, δ 2.045 ppm (H-24), δ 5.118 ppm (H-22). The proton corresponding to the H-3 was steroidal moiety (Slomp & Mackellar, 1962; Sadikun, *et al*., 1996; Habib, *et al*., 2007; Azizudin & Choudhary, 2008). Liebermann-Burchard reaction indicated isolated compound having a steroidal skeleton. The physical and spectral data are consistent to the reported literature values (Sureshkumar, *et al*., 2012).

CONCLUSION

Steroidal moiety was isolated from hexane extract isolates obtained from the leaves of *Bombax ceiba.* The structures of the isolated compound was identified and characterized as Phytosterol compound by comparing with the spectral data reported in the literature.

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