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ISOLATION AND STRUCTURE ELUCIDATION OF BIO-ACTIVE COMPOUNDS FROM *BOMBAX CEIBA* LEAF EXTRACT

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Keywords:

Bombax ceiba, β-sitosterol glucoside, Taraxerol, COSY, HMBC

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ABSTRACT: *Bombax ceiba* is a traditional medicinal plant that is beneficial in the treatment of diuretic, hemostatic, antidysenteric, emetic, antipyretic, astringent, and commonly known silk cotton tree, semal, kapok tree. The aim of this study is to isolate and characterize the biologically active compounds from the ethanolic extract of the leaves. The EtOH extract was subjected to silica gel (60-120 mesh) by glass column chromatography, using gradient petroleum-ether, C_6H_6, CH_3OAc, CH_3OH, and purified by preparative TLC (GF_{254}) to yield two pure natural products (taraxerol and β-sitosterol glucoside). The structure was elucidated on the basis of spectroscopic analysis, including ^{1}HNMR, ^{13}CNMR, DEPT-135, COSY, HSQC, and HMBC. The β-sitosterol glucoside shows pharmacological activities such as lowering cholesterol levels, reducing blood pressure, antitumor activity, *etc*. The glucose moiety is highly effective because it is hydrophilic, which inhibits the entry of cholesterol into the esterification of cholesterol. It is the first time isolated from the leaves of this plant.

INTRODUCTION: *Bombax ceiba* belongs to the plant family Malvaceae, which is widely distributed in tropical and subtropical India, Sri Lanka, Pakistan, Australia, Malaysia, Java, Sumatra, *etc*. commonly known cotton tree, red silk cotton and kapok [1]. Various investigations have suggested that extract of *Bombax ceiba* species play a vital role in pharmacological properties including antibacterial, antioxidant [2], antidiabetic [3], hepatoprotective [4], antimicrobial [5], antianxiety [6]. From phytochemical studies, it is confirmed that natural products such as polysaccharides, naphthol, naphtha-quinones, anthocyanins, shamimin, mangiferin, saponins, and steroids are useful in the treatment of various diseases [7, 10].

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Steroids, flavonoids, saponins, polysaccharides have been isolated from this species. Phytosterols are steroidal type saponins which have been isolated from many plants and show interesting pharmacological activities, including reducing the cholesterol level, act as a potent antitumor agent, lowering blood pressure, anti-genotoxicity, apoptosis, and anticancer nutrients [11, 12, 13, 14]. The sterol glucoside has hydrophilic glucose moiety, which plays a vital role in prevention cholesterol entry into esterification of cholesterol [15]. β-sitosterol glucoside **Fig. 1B** is a bioactive compound that decreases nitric oxide production from lip polysaccharides induced RAW 264.7 cells and highly prevents interleukin 6 activities of stimulated macro-phages [16, 17].

β-sitosterol glucoside has been isolated from plant species such as *Pisonia grandis* [18], *Ocimum sanctum*, and *Viola odorata* [19, 20]. Taraxerol **Fig. 1A** is a compound of the saponin group which has been isolated from *Jatropha tanjorensis* [21], *Annona*

reticulata L. [22], *Abroma augusta* [23,] and play a role in prostate cancer [24] and protects the human hepatic LO2 cells [25]. In this study, we report the isolation of a taraxerol (1) and β-sitosterol glucoside (2) from EtOH extract of leaves.

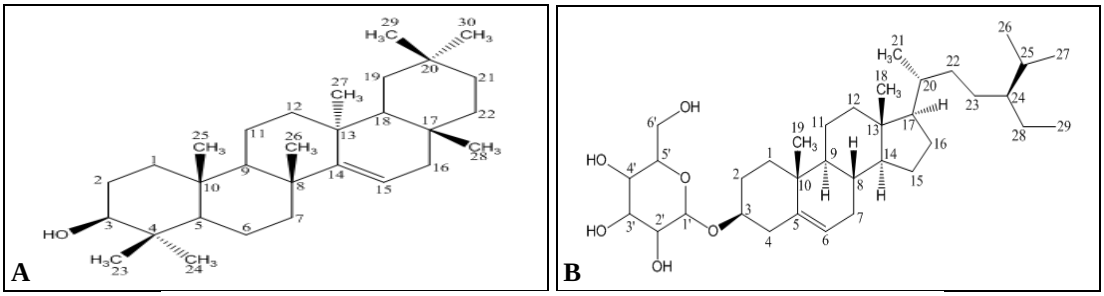

FIG. 1: (A) TARAXEROL AND (B) B-SITOSTEROL GLUCOSIDE

MATERIALS AND METHODS:

Collection and Identification of Plant Materials: The plant leaves of *Bombax ceiba* were collected from the Bisalpur area of district Pilibhit, Uttar Pradesh, India and identified by Professor M. Badruzzaman Siddiqui (Plant Taxonomy and Ethno botany), Department of Botany, Aligarh Muslim University, Aligarh under Acc. No. 31979, AMU, Aligarh.

General Experimental Procedures: TLC analysis was performed using commercially available glass plates precoated with silica gel (GF_{254}). The melting point was measured, using stuart digital melting point apparatus (SMP10), which are uncorrected. IR spectra were obtained in potassium bromide pellets on a Perkin Elmer spectrometer. ^{1}HNMR and ^{13}CNMR spectra of compound (1) were acquired on a Bruker Avance II spectrometer, operating at 400 MHz for ^{1}HNMR and 100 MHz for ^{13}CNMR in CDCl3 solvent. 1DNMR (^{1}HNMR and ^{13}CNMR), and 2D NMR (HSQC, COSY and HMBC) spectra of compound (2) were recorded in DMSO-d6 solvent on a Bruker Avance Neo 500 MHz NMR spectrometer, operating 500 MHz for ^{1}HNMR and 125 MHz for ^{13}CNMR with TMS as the internal standard.

Extraction and Isolation: The shade air-dried and pulverized foliage (2 Kg) of *Bombax ceiba* plant was extracted with 78% ethanol at room temperature. The ethanolic extract was filtered, and the solvent was totally evaporated under reduced pressure. The dry EtOH extract (85 g) was partitioned between petroleum-ether, benzene, EtOAc and methanol to yield 7 g, 18 g, 28 g and 19 g of extract. The benzene extract (18 g) was chromatographed over a silica gel column using stepwise gradient petroleum-ether/benzene (from 100:0 to 0:100 v/v) to obtain 11 fractions (P1-P11). Fraction P-7 was purified by further, CC, eluting with a mobile phase of petroleum/C_6H_6 (20/80 v/v) to obtain compound 1 (85 mg). Isolated compound with melting point (283 °C) was further, analyzed by spectroscopic methods **Fig. 1A**.

Compound (1): White solid with melting point 283 °C. IR $\bar{\upsilon}_{max}$ (KBr disc) cm^{-1}: 3483 (-OH), 2931 and 2858 (CH), 1636 (C=C), 1469, 1379, 1035. ^{1}HNMR ($CDCl_3$, 400 MHz): δ_H 5.53 (1H, dd, J = 3.3, 7.96, H-15), 3.19 (1H, m, H-3), 0.97 (3H, s, H-23), 0.80 (3H, s, H-24), 0.92 (3H, s, H-25), 1.08 (3H, s, H-26), 0.90 (3H, s, H-27), 0.82 (3H, s, H-28), 0.95 (3H, s, H-29), 0.90 (3H, s, H-30). 13CNMR ($CDCl_3$, 100 MHz): δ_C 158.1 (C-14), 116.9 (C-15), 79.1 (C-3), 55.5 (C-5), 49.2 (C-18), 48.7 (C-9), 41.3 (C-19), 39.0 (C-4), 38.7 (C-8), 38.0 (C-1), 37.7 (C-13), 37.7 (C-17), 37.5 (C-10), 36.6 (C-16), 35.8 (C-12), 35.1 (C-7), 33.7 (C-21), 33.3 (C-29), 33.0 (C-22), 29.9 (C-28), 29.8 (C-26), 28.8 (C-20), 28.0 (C-23), 27.1 (C-2), 25.9 (C-27), 21.3 (C-30), 18.7 (C-6), 17.5 (C-11), 15.4 (C-24), 15.4 (C-25).

The ethyl acetate extract (28 g) was fractionated by glass column chromatography on silica gel, eluting with a gradient of benzene / ethyl acetate (100:0/0:100 v/v) to give fractions B1-B7. Fraction B-3 was separated on CC with the elution of benzene / ethyl acetate (20:60/80:40 v/v) to yield nine subfractions (B-3_1-B-3_9). Subfraction B-3_6 was further, subjected to glass CC on silica gel using gradient benzene / ethyl acetate (40/60 v/v) to obtain compound 2. After isolation, it was purified by preparative TLC to give pure compound 2 (15mg).

The purified amorphous solid was further, characterized by IR, 1DNMR and 2DNMR (summarized in **Table 1** and **Fig. 2**).

Compound 2: White amorphous solid was isolated, Melting point 294 °C, IR $\bar{\upsilon}_{max}$ (KBr disc) cm^{-1}: 3402 (-OH), 2934 and 2872 (aliphatic -CH), 1634 (C=C), 1462, 1375, 1072 ^{1}HNMR (DMSO, 500 MHz): δ_H 5.32 (1H, brs, H-6), 4.2 (1H, d, *J* = 7.75, H-1'), 3.6 (2H, dd, *J* = 4.95, 11.3, H-6'), 3.47 (1H, m, H-5'), 3.44 (1H, m, H-3), 3.06 (1H, dd, *J* = 1.9, 5.85, H-3'), 3.02 (1H, dd, *J* = 4, 8.8, H-4'), 2.9 (1H, m, H-2'), 0.66 (3H, s, H-18), 0.96 (3H, s, H-19), 0.83 (3H, d, *J* = 7.25, H-21), 0.89 (3H, d, *J* = 6.35, H-26), 0.82 (3H, d, *J* = 6.7, H-27), 0.80 (3H, d, *J* = 6.4, H-29). ^{13}C NMR (DMSO 125 MHz): δ_C 140.92 (C-5), 121.67 (C-6), 101.23 (C-1'), 77.39 (C-5'), 77.25 (C-3'), 77.2 (C-3), 73.90 (C-2'), 70.59 (C-4'), 61.58 (C-6'), 56.65 (C-14), 55.92 (C-17), 50.08 (C-9), 45.62 (C-24), 42.3 (C-13), 39.70 (C-4), 38.79 (C-1), 37.31 (C-12), 36.69 (C-10), 35.95 (C-20), 33.83 (C-22), 31.90 (C-8), 31.85 (C-2), 29.75 (C-7), 29.19 (C-25), 28.26 (C-16), 25.93 (C-23), 24.35 (C-15), 23.09 (C-28), 21.07 (C-11), 20.19 (C-26), 19.57 (C-21), 19.42 (C-27), 19.09 (C-19), 12.26 (C-29), 12.15 (C-18).

HSQC: C-1 (38.79, 2.37, 2.1; CH_2), C-2 (31.85, 1.94, 1.49; CH_2), C-3 (77.2, 3.44; CH), C-4 (39.70, 1.8, 1.4; CH_2), C-6 (121.67, 5.32; CH), C-7 (29.75, 2.0, 1.48; CH_2), C-8 (31.90, 1.40; CH), C-9 (50.08, 0.9; CH), C-11 (21.07, 1.48, 1.39; CH_2), C-12 (37.31, 1.79, 1.02; CH_2), C-14 (56.65, 0.9; CH), C-15 (24.35, 1.56, 1.03; CH_2), C-16 (28.26, 1.79, 1.23; CH_2), C-17 (55.92, 1.08; CH), C-18 (12.15, 0.66; CH_3), C-19 (19.09, 0.9; CH_3), C-20 (35.95, 1.39; CH), C-21 (19.57, 0.95; CH_3), C-22 (33.83, 1.31, 0.99; CH_2), C-23 (25.93, 1.56, 1.03; CH_2), C-24 (45.62, 0.92; CH), C-25 (29.19, 1.63; CH), C-26 (20.19, 0.89; CH_3), C-27 (19.42, 0.95; CH_3), C-28 (23.09, 1.22, 1.18; CH_2), C-29 (12.26, 0.82; CH_3), C-1' (101.26, 4.2; CH), C-2' (73.90, 2.9; CH), C-3' (77.25, 3.06; CH), C-4' (70.59, 3.02; CH), C-5' (77.39, 3.47; CH), C-6' (61.58, 3.6, 3.4; CH_2).

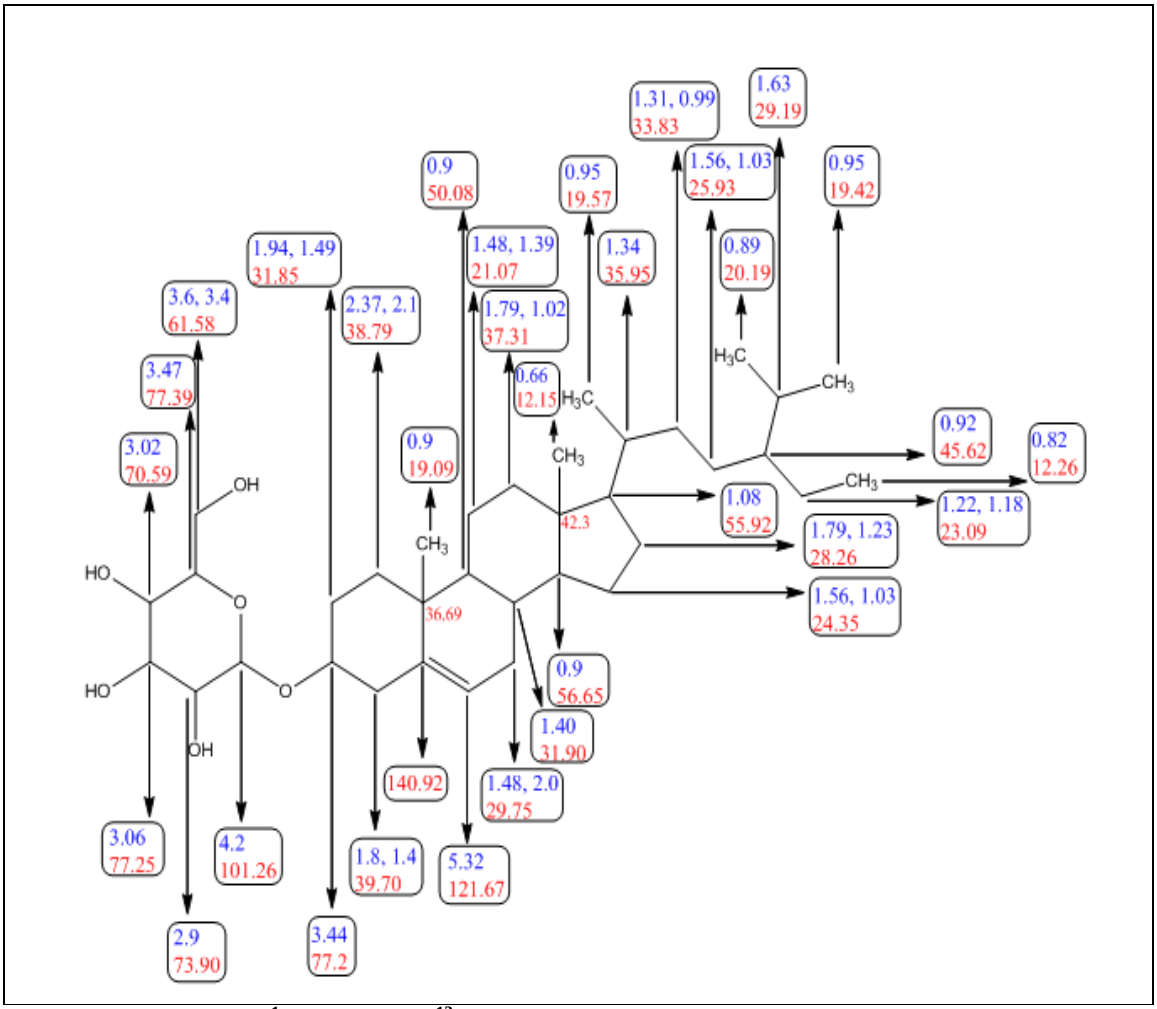

FIG. 2: CHARACTERISTIC ^{1}HNMR AND ^{13}C-NMR PEAK ASSIGNMENT OF β-SITOSTEROL-GLUCOSIDE

TABLE 1: ^{1}HNMR, ^{13}CNMR AND ^{2}D NMR DATA FOR COMPOUND (2)[a]

Atom	Type	δ_C	δ_H	^{1}H-^{1}H COSY	HMBC
1	CH_2	38.79	2.37 (Ha_1), 2.1 (Hb_1)	Hb_2	----------
2	CH_2	31.85	1.94 (Ha_2), 1.49 (Hb_2)	H-3	----------
3	CH	77.2	3.44 (m)	Ha_2, Hb_2, Ha_4, Hb_4	----------
4	CH_2	39.70	1.8 (Ha_4), 1.4 (Hb_4)	H-3	----------
5	C	140.92	----------	--------	----------
6	CH	121.67	5.32 (brs)	Ha_7, Hb_7	----------
7	CH_2	29.75	2.0 (Ha_7), 1.48 (Hb_7)	H-6	----------
8	CH	31.90	1.40 (m)	---------	----------
9	CH	50.08	0.9 (m)	Hb_{11}	10, 11
10	C	36.69	----------	----------	----------
11	CH_2	21.07	1.48 (Ha_{11}), 1.39 (Hb_{11})	H-9, Hb_{12}	----------
12	CH_2	37.31	1.79 (Ha_{12}), 1.02 (Hb_{12})	Ha_{11}	----------
13	C	42.3	----------	----------	----------
14	CH	56.65	0.9 (m)	Ha_{15}	----------
15	CH_2	24.35	1.56 (Ha_{15}), 1.03 (Hb_{15})	H-14, Ha_{16}	----------
16	CH_2	28.26	1.79 (Ha_{16}), 1.23 (Hb_{16})	Ha_{15}	----------
17	CH	55.92	1.08 (m)	----------	20
18	CH_3	12.15	0.66 (s)	----------	11, 17
19	CH_3	19.09	0.96 (s)	----------	----------
20	CH	35.95	1.39 (d) [J = 4.25]	Hb_{22}	22
21	CH_3	19.57	0.83 (d) [J = 7.25]	----------	17, 20
22	CH_2	33.83	1.31 (Ha_{22}), 0.99 (Hb_{22})	H-20	----------
23	CH_2	25.93	1.56 (Ha_{23}), 1.03 (Hb_{23})	----------	----------
24	CH	45.62	0.92 (m)	H-25	----------
25	CH	29.19	1.63 (m)	H-24	24
26	CH_3	20.19	0.89 (d) [J = 6.35]	----------	25, 27
27	CH_3	19.42	0.82 (d) [J = 6.7]	----------	25
28	CH_2	23.09	1.22 (Ha_{28}), 1.18 (Hb_{28})	----------	----------
29	CH_3	12.26	0.80 (d) [J = 6.4]	----------	28
1’	CH	101.26	4.2 (d) [J = 7.75]	H-2’	----------
2’	CH	73.90	2.9 (m)	H-1’, H-3’	----------
3’	CH	77.25	3.06 (dd) [J = 1.9, 5.85]	H-2’	----------
4’	CH	70.59	3.02 (dd) [J = 4, 8.8]	----------	----------
5’	CH	77.39	3.47 (m)	H-6’	----------
6’	CH_2	61.58	3.6 (dd) [J = 4.95, 11.3]	H-5’	----------

[a]Spectra run at 500 MHz (^{1}HNMR) AND 125 MHz (^{13}CNMR) IN DMSO

RESULTS AND DISCUSSION: Compound 1 was isolated as a white solid with a melting point of 283 °C. IR spectra show a broad absorption band at 3483 cm^{-1}, which authenticates the presence of hydroxyl groups (-OH) and absorption observed at 1636 cm^{-1}, indicating C=C stretching vibrations. The ^{1}HNMR spectra showed a signal at δ_H 5.53 (1H, dd, J = 3.3, 7.9) reveal proton that is attached to a double bond carbon. The eight singlets (δ_H 0.80, 0.82, 0.90, 0.90, 0.92, 0.95, 0.97, 1.08; 3H × 8, s) were displayed in ^{1}HNMR spectra, confirming eight methyl groups (-CH_3). The ^{13}CNMR and DEPT spectrum indicate eight methyls, ten methylene, four methine, and six quaternary carbons. Thus, the structure of compounds (1) was defined as taraxerol. Compound 2 was isolated as a white amorphous solid with a melting point of 294 °C. IR spectra were showing a broad absorption peak at 3402 cm^{-1}, which confirming of -OH group and absorption band at 2850 and 2934 cm^{-1} indicates CH stretching of methyl and methylene groups. ^{1}HNMR and ^{13}CNMR in **Table 1** and **Fig. 5** and **Fig. 6** spectra revealed six methyl signals at δ_H 0.66 (s), 0.96 (s), 0.80 [d, J = 6.4], 0.82 [d, J = 6.7], 0.83 [d, J = 7.25], 0.89 [d, J = 6.35] and δ_C 12.15, 19.09, 19.57, 20.19, 19.42, 12.26, indicating six methyl groups (-CH_3). ^{13}CNMR, DEPT have resolved six methyls (-CH_3), 11 methylene (CH_2), nine methines (CH), three quaternary (C) and six glucose moiety carbon [5 methine carbon (CH) and one methylene carbon (CH_2)] which were also confirmed by HSQC spectra. Cross-peaks in the COSY spectrum shown in **Fig. 3** were observed between H-1’ (δ 4.2) and H-2’ (δ 2.9) and between H-5’ (δ 3.4) and H-6’ (δ 3.6) and between H-2’ (δ 2.9) and H-3’ (δ 3.06) in glucose moiety.

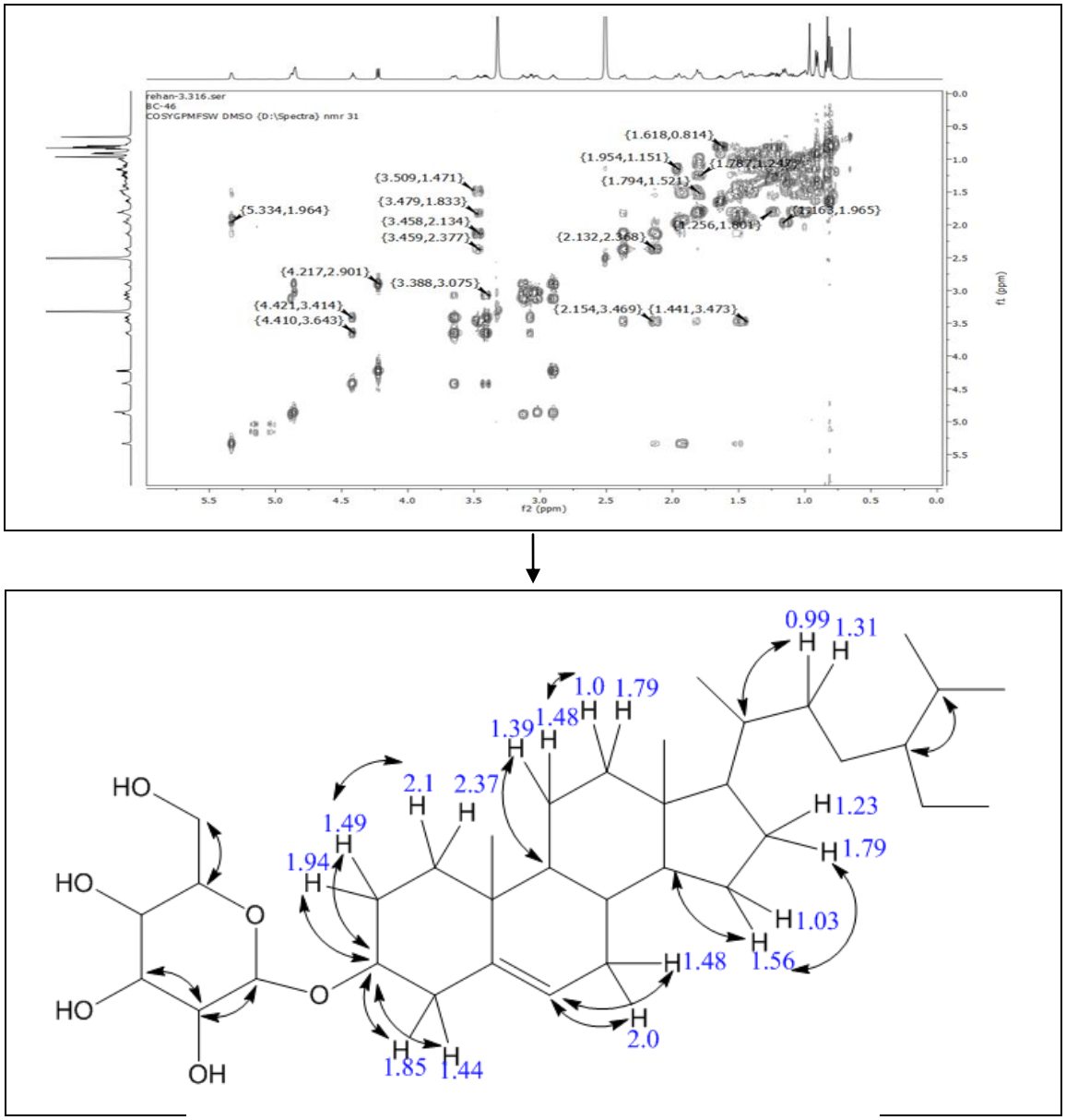

FIG. 3: COSY CORRELATION SPECTRA OF COMPOUND (2)

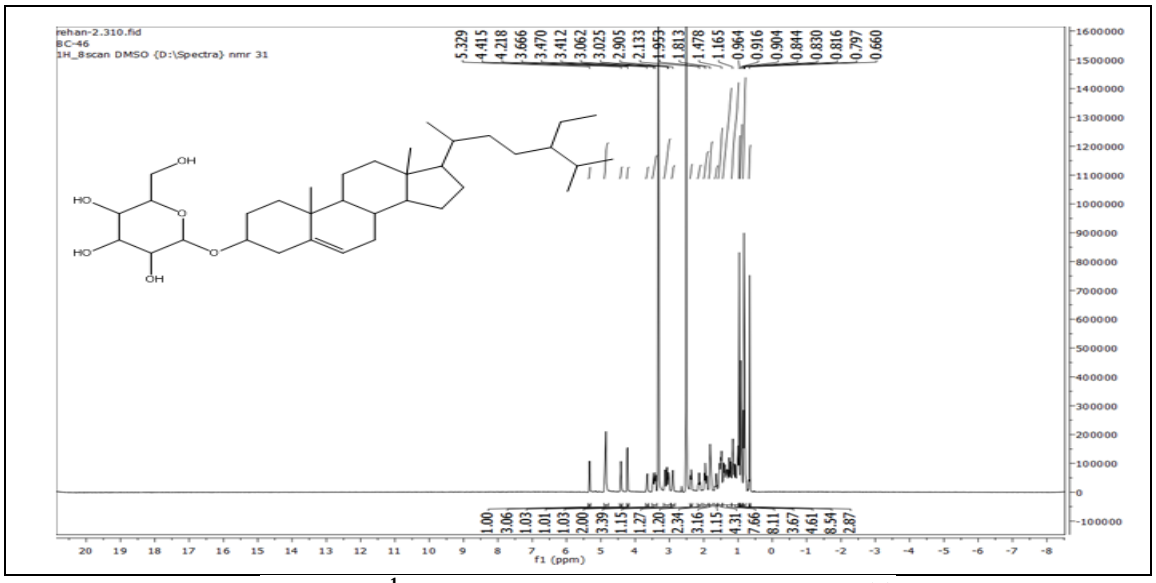

FIG. 4: ^{1}H-NMR SPECTRUM OF COMPOUND (2)

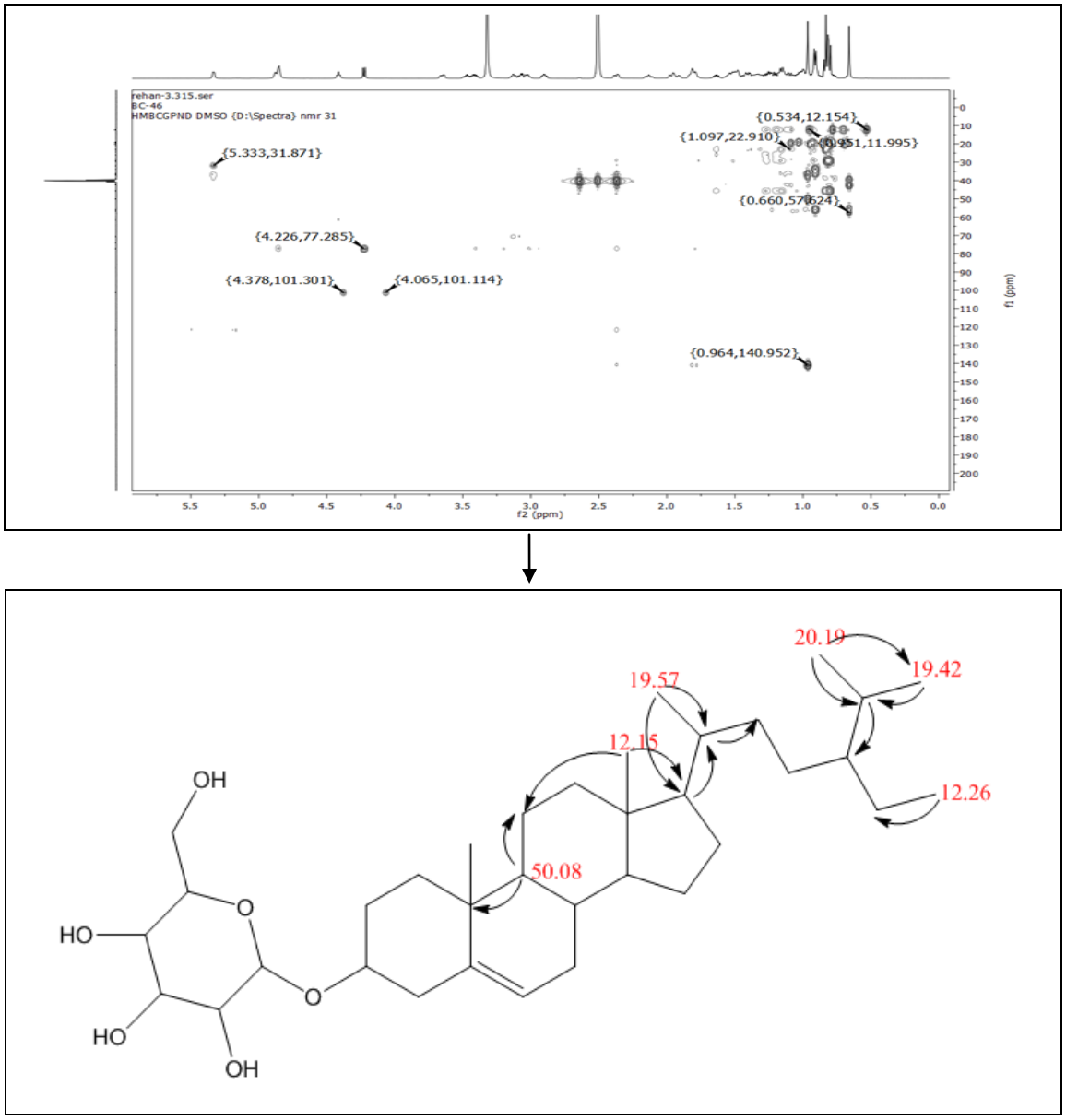

FIG. 5: HMBC CORRELATION SPECTRA OF COMPOUND (2)

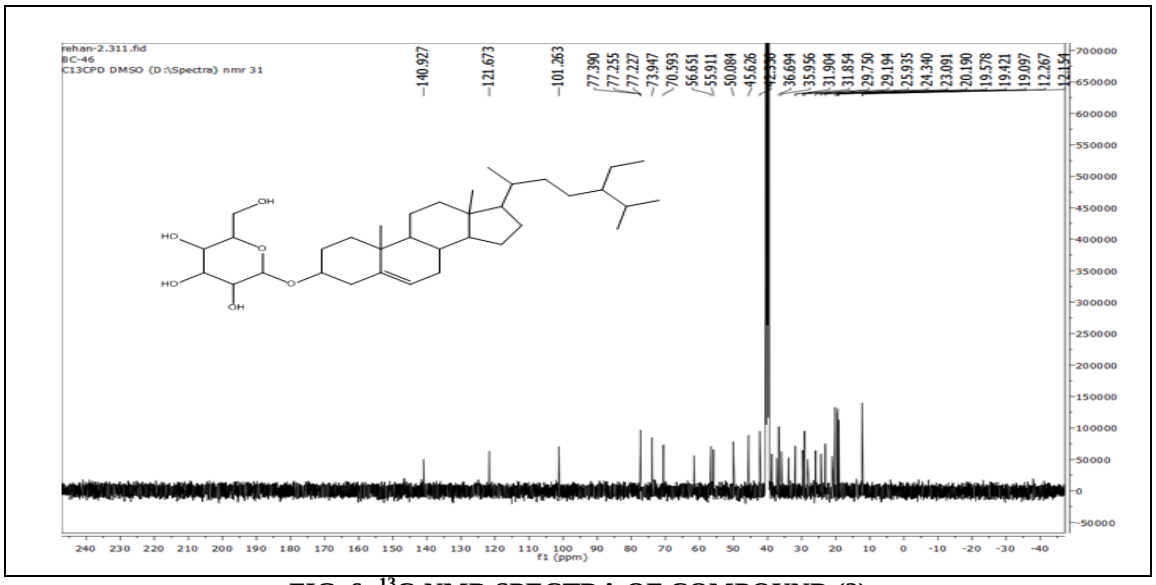

FIG. 6: ^{13}C-NMR SPECTRA OF COMPOUND (2)

^{1}H-^{1}H COSY was also shown correlations between H-3 (δ 3.4) and H-2 (δ 1.49), H-2 (δ 1.94), H-4 (δ 1.44), H-4 (δ 1.85) and between H-6 (δ 5.3) and H-7 (δ 1.48), H-7 (δ 2.0).

The HMBC correlations in **Fig. 4** were performed between C-21 (δ 19.5) and C-17 (δ 55.9), C-20 (δ 35.9), indicating the location of methyl groups (-CH_3) at C-21 and the correlation between C-18 (δ 12.1) and C-17 (δ 1.08), C-11 (δ 21.07), confirming the position of a methyl group at C-18. Thus, the structure of compound 2 was identified as a β sitosterol-3-O-glucoside and first time isolated from the leaves of this plant**.**

CONCLUSION: ^{1}HNMR, ^{13}CNMR, DEPT-135, and 2D NMR methods were revealed that isolated compounds were taraxerol and β-sitosterol glucoside. Both show high medicinal activities. Important role β-sitosterol-glucoside is reducing cholesterol levels because glucose moiety is hydrophilic β-sitosterol glucoside is a steroidal saponin which first time reported from the leaves of *Bombax ceiba*.

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CONFLICTS OF INTEREST: Nil

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