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ISOLATION AND CHARACTERIZATION OF PHYTOSTEROLS FROM *DIEFFENBACHIA AMOENA* LEAF EXTRACT

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Keywords:

Dieffenbachia amoena, β-sitosterol, Stigmasterol, HMBC, COSY

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ABSTRACT: *Dieffenbachia amoena* is a house plant and known dumb cane. The study was performed based on isolation and structure elucidation of phytosterols from the extract of this plant. The CH_3OH crude extract was loaded over the silica gel (60-120 mesh) column, using the stepwise gradient C_6H_6, CH_3OAc, CH_3OH. The fractions were further purified by preparative TLC (GF254) to yield compound (1). Compound (1) was characterized by using various standard spectroscopic techniques such as IR, 1HNMR, 13CNMR, DEPT-135, COSY, HSQC and HMBC. Based on the spectral analysis, it was confirmed that the compound (1) was a mixture of β-sitosterol and stigmasterol. Phytosterols have high medicinal importance and play a vital role in reducing blood cholesterol, a high level of blood cholesterol can cause a risk of cardiovascular disease. Cardiovascular disease is the main problem of the whole world and increasing day by day. We have isolated β-sitosterol and stigmasterol first time from the leaves of the *Dieffenbachia amoena* plant.

INTRODUCTION: *Dieffenbachia amoena*, commonly known as Besar Putih or Dumb Cane, belongs to family Araceae **Fig. 1**. Dieffenbachia is distributed in tropical America and grows in shady, moist, low land of tropical America, Brazil, and north to the islands of the West Indies [1]. It has two types of calcium oxalate crystals (druses and raphides) [2]. Chemicals investigation shows that it has a proteolytic enzyme which possesses poisonous properties [3]. When the leaves extract of the plant comes in contact with the skin, it causes itching, swelling, salivation and Potential of speech loses for near about two days [4].

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Phytosterol is bioactive compounds which are found in cell membranes of all plants [5] and have been isolated from various species of many plants such as Odontonema strictum, *Rubus suavissimus*, *Ageratum conyzoides* and show high medicinal activities and are an essential component of plant cell biofilm [6, 7, 8].

They are mostly similar in structure and biological function to cholesterol [9]. Stigmasterol (stigma) and β-sitosterol (β-sito) are common phytosterols **Fig. 2**, which are primarily used in the human diet and are useful in the treatment of NAFLD (Non-Alcoholic Fatty Liver Disease) [10]. They play a vital role in the regulation of biological processes such as plant growth, modulation of the activity of membrane-bound enzymes, metabolic cycles [11, 12, 13]. Animals, including humans, cannot synthesize phytosterols, therefore, they can be assimilated from food. [14, 15] Both phytosterols play an essential role in lowering blood cholesterol level and

beneficially influence the cardiovascular and immune system in humans and also shows anticancer activity [16, 17]. In the United States, cardiovascular disease is the most common cause of death and over 15 million deaths worldwide by the American Heart Association report in 2017 [18]. Both phytosterol were exhibited various biological activities such as anti-depressant [19], apoptosis [20, 21], uterus [22], anti-cancer [23, 24], anti-Alzheimer's [25], anti-fungal infection [26], immunomodulatory activity [27], inhibitory action on glucoamylase *in-vitro* [28], anti-microbial activity [29], [30], anti-tumour [31], anti-diabetic [32], anti-bacterial 33, anti-allergic [34], and AChE inhibitory activity [35].

In this study, we describe the isolation and characterization of the two significant phytosterols, namely, β-sitosterol and stigmasterol, based on 1H-NMR, 13C-NMR, DEPT-135, COSY, HSQC, and HMBC.

FIG. 1: THE SPECIES DIEFFENBACHIA AMOENA

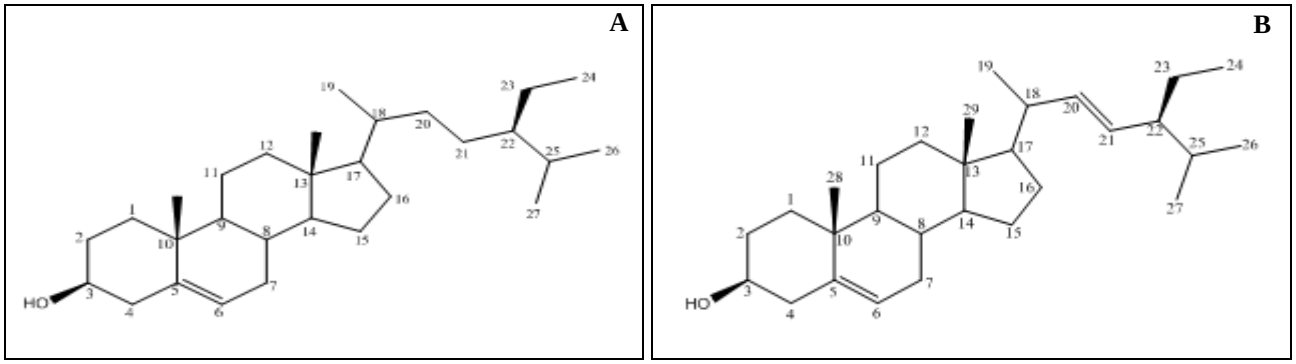


FIG. 2: STRUCTURE OF COMPOUND (1): (A) β -SITOSTEROL (B) STIGMASTEROL

MATERIALS AND METHODS:

Plant Materials: The *Dieffenbachia amoena* leaves were purchased from a nursery, AMU area of district Aligarh, UP, India and authenticated by Professor M. Badruzzaman Siddiqui (Plant Taxonomy and Ethnobotany), Department of Botany, Aligarh Muslim University, Aligarh, India.

General Experimental Procedures: Measurement of the melting point was determined in glass capillaries on Stuart digital melting point apparatus (SMP10), which are uncorrected. Thin-layer chromatography (TLC) was carried out on pre-coated glass plates with silica gel (GF254). Various spectroscopic methods were used in the characterization of the isolated compound (1). The infrared spectrum was recorded in KBr pellets on the Perkin Elmer instrument. 1HNMR, 13CNMR, DEPT, COSY, HSQC, HMBC spectra were determined on a Bruker Avance Neo 500 MHz instrument using $CDCl_3$ solvent, and the chemical shift was reported in ppm with respect to TMS.

Extraction and Isolation: Shade air-dried and pulverized plant leaves (1 Kg) were extracted with 82% methanol for 15 days at room temperature. The CH_3OH extracts were filtered separately and concentrated using rotary evaporators to yield a dark reddish-brown residue (75 g). The CH_3OH extract was fractionated by using benzene and EtOAc solvent to give the benzene extract (18g) and EtOAc extract (22g).

The C_6H_6 extract was subjected by glass column chromatography on silica gel (60-120 mesh) using a petroleum-ether to benzene gradient stepwise (100:0/0:100 v/v), to give eight main fractions (P1-P8). Each fraction collected and monitored by TLC. Fraction P-6 was further chromatographed using a glass column packed with silica gel, eluting with petroleum/C_6H_6 (20/80 v/v) to yield five subfractions (P-6(a)-P-6(e). The subfractions P-6(d) was purified by preparative TLC using gradient petroleum/ethyl acetate (75/25 v/v), to give a compound (1) (55mg). The compound was

visualized single spot when subjected to TLC using various solvent systems such as petroleum/ethyl acetate (90/10 v/v), petroleum/ethyl acetate (75/25 v/v), hexane/ethyl acetate (75/25 v/v), chloroform/ ethyl acetate (80/20 v/v) and it showed to be homogenous compound. The white amorphous solid (60 mg) with melting point 139 ºC was further characterized by IR, 1H-NMR, 13C-NMR, DEPT-135, and 2D-NMR (summarized in **Table 1**).

TABLE 1: NMR SPECTROSCOPIC DATA (1H 500 MHZ AND 13C 125 MHZ) OF THE ISOLATED COMPOUND (1) RECORDED IN $CDCl_3$.a

Atom	Type	δ_C	δ_H	^1H-^1H COSY	HMBC
1	CH_2	37.24	1.83 (Ha1), 1.06 (Hb1)	------	------
2	CH_2	31.64	1.95 (Ha2), 1.83 (Hb2)	H-3	------
3	CH	71.83, 76.6	3.53 (m)	Ha2, Ha4	------
4	CH_2	42.31	2.28 (Ha4), 2.23 (Hb4)	H-3	------
5	C	140.7	-----	-----	3, 6, 7, 10
6	CH	121.7	5.35 (bd-s)	Ha7, Hb7	-----
7	CH_2	31.89	1.99 (Ha7), 1.45 (Hb7)	H-6	-----
8	CH	31.9, 32.8	1.83 (m)	-----	-----
9	CH	50.1, 51.1	0.90 (m), 1.52 (m)	-----	-----
10	C	36.5	-----	-----	-----
11	CH_2	21.08	1.50 (m)	Ha12	-----
12	CH_2	39.8, 39.7	1.85 (Ha12), 1.13 (Hb12)	H-11	-----
13	C	42.27	-----	-----	-----
14	CH	56.8, 56.9	0.9 (m)	-----	-----
15	CH_2	26.0, 24.4	1.15 (m)	-----	-----
16	CH_2	28.3, 28.8	1.83 (Ha16), 1.27 (Hb16)	-----	-----
17	CH	56.04, 56.1	1.02 (m)	-----	13, 18
18	CH	36.2, 40.5	1.20 (m), 2.0 (m)	H-20	-----
19	CH_3	19.0, 18.3	0.92 (d) [J = 6.1]	-----	17, 18, 20
20	CH_2	33.9, 138.3	1.28 (Ha20), 1.02 (Hb20), 5.14 (1H, m)	Hb21, H-18	-----
21	CH_2	24.4, 129.3	1.57 (Ha21), 1.06 (Hb21) 5.0 (1H, m)	Ha20	-----
22	CH	45.82	0.9 (m)	-----	-----
23	CH_2	23.1, 25.4	1.20 (Ha23), 1.0 (Hb23)	-----	-----
24	CH_3	12.0, 12.2	0.83 (t) [J = 7.6]	-----	22
25	CH	29.14	1.72 (m)	H-26	-----
26	CH_3	19.8, 20.2	0.81 (d), [J = 6.9]	H-25	-----
27	CH_3	19.4, 19.0	0.77 (d) [J = 6.1]	-----	-----
28	CH_3	18.8, 15.4	0.80 (s)	-----	-----
29	CH_3	11.9, 12.0	0.68 (s)	-----	12, 13, 14

The data were analyzed by DEPT-135, COSY, HSQC, and HMBC

Compound (1): white solid isolated from petroleum/benzene(20/80) fraction, Melting point 139 ºC, IR $\bar{\upsilon}_{max}$ (KBr) cm^{-1}: 3434 (OH), 2934 and 2867 (CH), 1640 (C=C), 1463 (CH2), 1378 (OH def), 1058. 1HNMR (CDCl3, 500 MHz): δH 5.35 (1H, bd-s, H-6), 5.14(1H, m, H-21), 5.0 (1H, m, H-20), 3.53 (1H, m, H-3), 0.92 (3H, d, J= 6.1, H-19), 0.83 (3H, t, J= 7.6, H-24), 0.81 (3H, d, J= 6.9, H-26), 0.77 (3H, d, J= 6.1, H-27), 0.80 (3H, s, H-28), 0.68 (3H, s, H-29). 13C NMR ($CDCl_3$ 125 MHz): δC 140.72 (C-5), 121.73 (C-6), 71.83, 76.6 (C-3), 56.76, 56.9 (C-14), 56.1, 56.04 (C-17), 50.12, 51.1 (C-9), 45.82 (C-22), 42.31 (C-4), 42.27 (C-13), 39.8, 39.7 (C-12), 37.24 (C-1), 36.5 (C-10), 36.18, 40.5 (C-18), 138.3, 33.94 (C-20), 31.9, 32.8 (C-8), 31.89 (C-7), 31.64 (C-2), 29.14 (C-25), 28.8, 28.3 (C-16), 26.05, 24.4 (C15), 129.3, 24.36 (C-21), 25.4, 23.06 (C-23), 21.08 (C-11), 20.2, 19.8 (C-26), 19.4, 19.0 (C-27), 19.0, 18.3 (C-19), 18.78, 15.4 (C-28), 12.2, 12.0 (C-24), 12.0, 11.9 (C-29). HSQC: C-1 (37.24, 1.83, 1.06; CH_2), C-2 (31.64, 1.95, 1.83; CH_2), C-3 (76.6, 71.83, 3.53; CH), C-4 (42.31, 2.28, 2.23; CH_2), C-6 (121.73, 5.35; CH), C-7 (31.89, 1.99, 1.45; CH2), C-8 (32.8, 31.91, 1.83; CH), C-9 (51.1, 50.12, 0.90; CH), C-11 (21.08, 1.50; CH_2), C-12 (39.77, 1.85, 1.13; CH_2), C-14 (56.8, 56.76, 0.9; CH), C-15 (26.05, 24.4, 1.15; CH_2), C-16 (28.8, 28.25, 1.83, 1.27; CH_2), C-17 (56.04, 1.02; CH), C-18 (40.5, 36.18, 1.20; CH), C-19 (19.03, 81.3, 0.92; CH_3), C-20 (138.3, 33.94, 5.0, 1.28, 1.02; CH_2), C-21 (129.3, 24.36, 5.14, 1.57, 1.06; CH_2), C-22 (45.82, 0.9; CH), C-23 (25.4, 23.06, 1.20, 1.0; CH_2), C-24 (12.2, 12.0, 0.83; CH_3), C-25 (29.14, 1.72; CH), C-26 (20.2, 19.82, 0.81; CH_3), C-27 (19.4, 2.77; CH_3), C-28 (18.78,15.4, 0.80; CH_3), C-29 (12.0, 11.86, 0.68; CH_3).

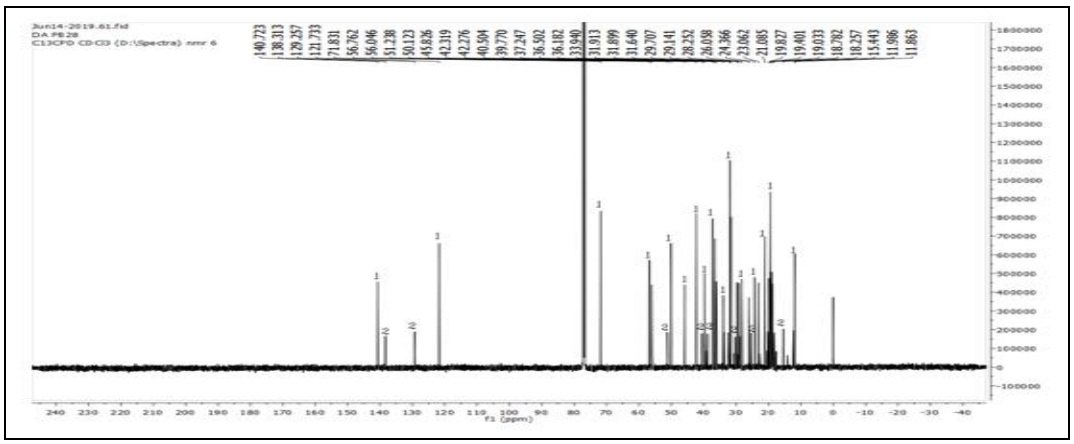
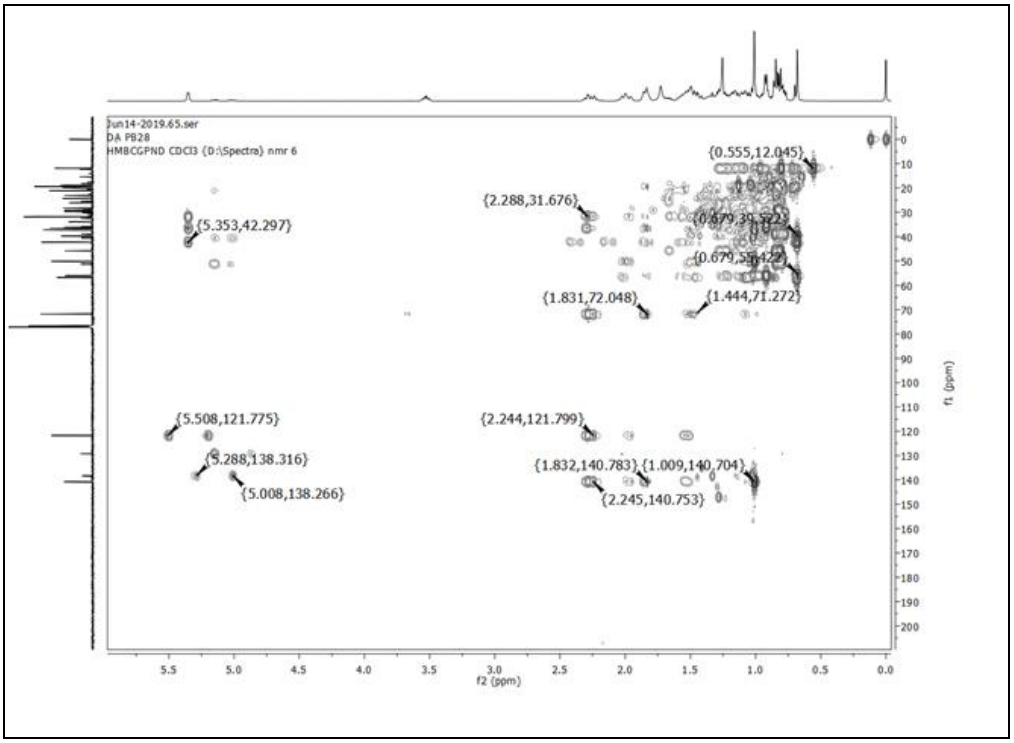


FIG. 3: 13C-NMR SPECTRA FOR COMPOUND (1)



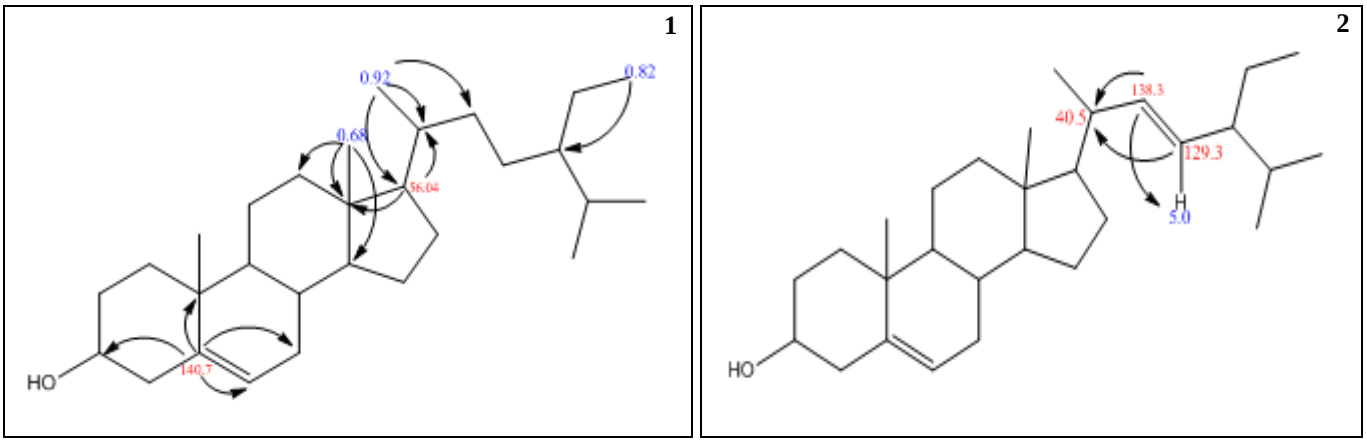


FIG. 4: HMBC CORRELATIONS FOR COMPOUND (1). β-SITOSTEROL (1) AND STIGMASTEROL (2)

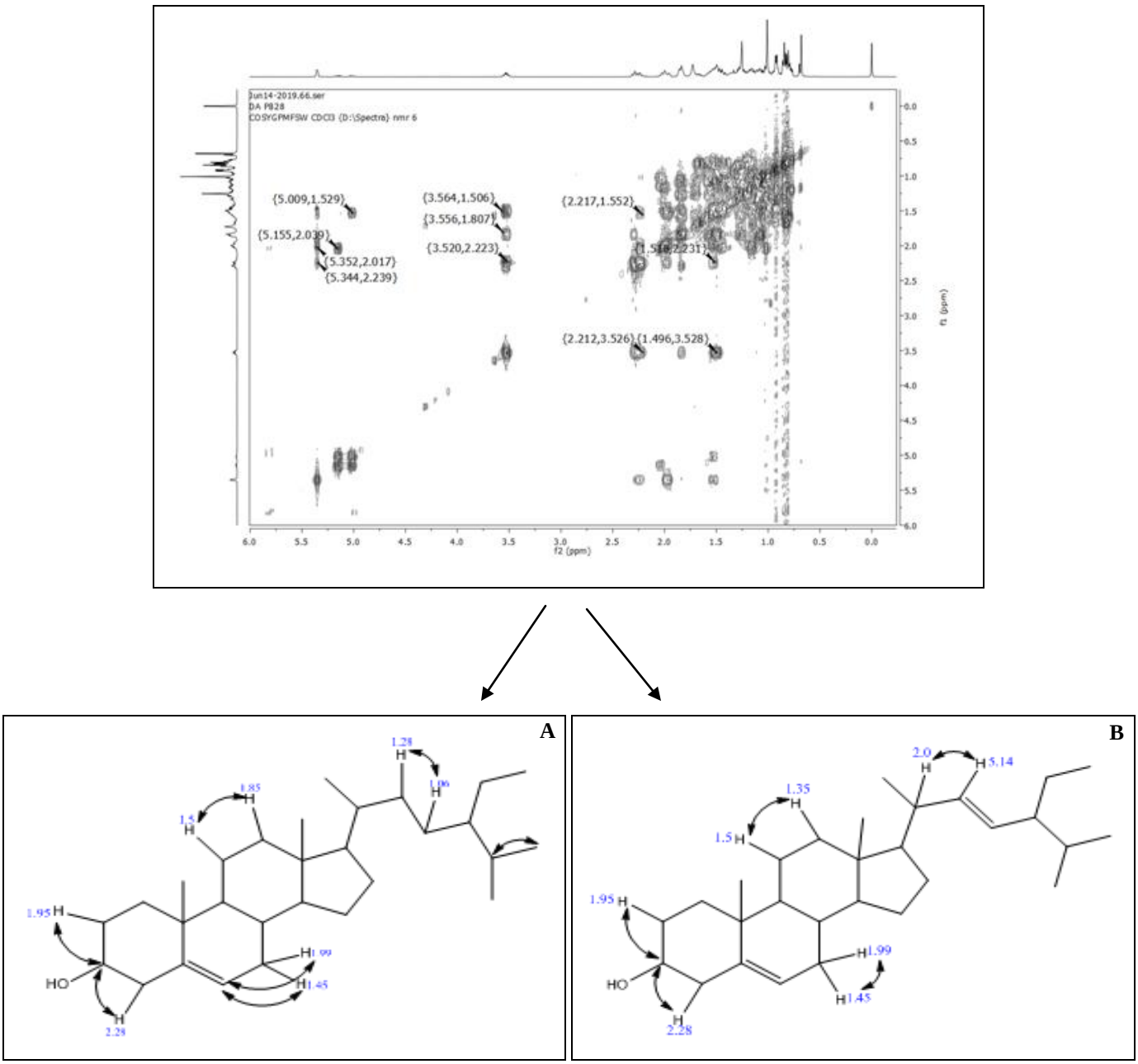


FIG. 5: COSY CORRELATIONS FOR COMPOUND (1). β-SITOSTEROL (A) AND STIGMASTEROL (B)

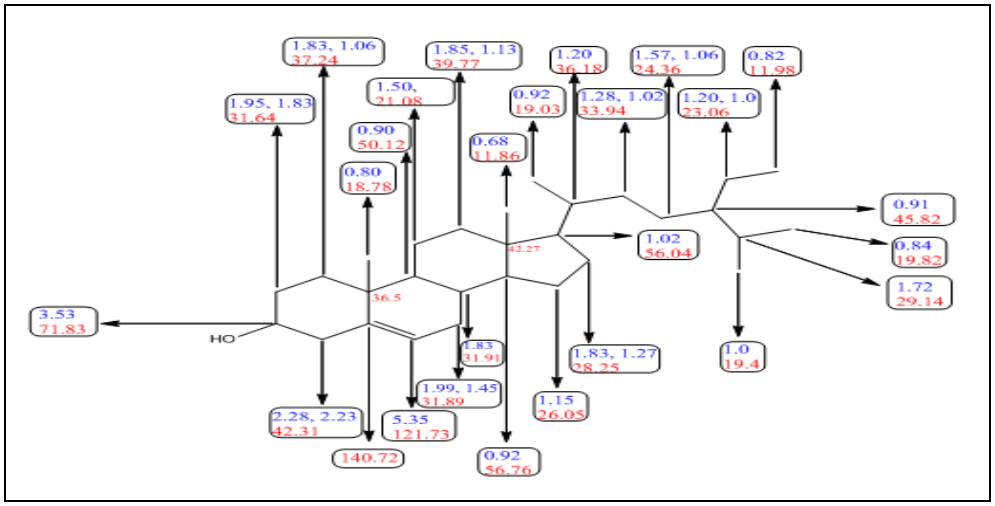


FIG. 6: CHARACTERISTIC 1H-NMR AND 13C-NMR PEAK ASSIGNMENT OF β-SITOSTEROL

RESULTS AND DISCUSSION: Compound (1) was isolated as a white amorphous solid with melting point 139 ºC. The ^{1}H-NMR and 13C-NMR spectra of compound 1 shows a broad signal at δ_H 5.35 (1H) and 13C-NMR at δ_C 121.7, indicating the presence of a double bond. The other signals were observed at δH 3.53 (1H) and δC 71.83 corresponding hydroxyl groups. A 13C-NMR spectrum of compound 1 is shown in **Fig. 3**.

In HMBC, the 2J and 3J correlations between δH 0.68 and δC 39.8 (C-12), 42.27 (C-13) and 56.8 (C-14) suggested the presence of a methyl group in position (C-29) and 3J correlations between δH 0.82 and δC 45.8 supporting its placement at C-24 **Fig. 4**. The correlations observed between δH 1.28 and 1.06 in the COSY spectrum suggested the presence of methylene group at C-20, C-21, and connectivity of methine proton δH 2.0 (1H) to alkene proton δH 5.14 (1H) indicating the location of alkene at C-20 **Fig. 5**. Thus, compound (1) is a mixture of β-sitosterol and stigmasterol. Spectra have shown that β-sitosterol has a maximum portion.

Isolation of β-sitosterol is very difficult because β-sitosterol and stigmasterol have same R_f value. The difference between two compounds is only at position C-20, and C-21, where β-sitosterol has a single bond and stigmasterol, has a double bond at this position. Furthermore, the literature reveals that it is very difficult to obtain β-sitosterol in pure form [36, 37]. Characteristic NMR peak assignment of β-sitosterol summarized in **Fig. 6**.

CONCLUSION: These compounds were the first time reported from the leaves of Dieffenbachia amoena. Both phytosterols were reduced risk of heart diseases. The various spectroscopic techniques (IR, 1HNMR, 13CNMR, DEPT-135, COSY, HSQC, HMBC) were confirmed that the isolated compound was mixtures of β-sitosterol and stigmasterol.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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