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CHEMICAL INVESTIGATION AND *IN VITRO* CYTOTOXIC ACTIVITY OF *RANDIA DUMETORUM* LAMK. BARK

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ABSTRACT

β-Sitosterol, lupeol and daucosterol have been isolated from ethanolic extract of *Randia dumetorum* bark. β-Sitosterol, Lupeol and Daucosterol have been isolated for the first time from the *Randia dumetorum*. β Sitosterol and Daucosterol were identified by complete interpretation of ^{1}H NMR and ^{13}C NMR spectra using 2D NMR (HSQC, HMBC, DQF-COSY) experiments and Lupeol by direct comparison with authentic sample. The ethanolic extract is subjected to cytotoxic activity against A549 cell (adenocarcinomic human alveolar basal epithelial cells), BE (2) C (Neuroblastonoma Cell Line), Hela, MDA-MB-231 (breast cancer cell line), SK MEL-2 (Human Skin Melanoma Cell) and U87MG cell (Human Neuronale Gliblostoma astrozytom Cell Line) which showed most effective cytotoxic activity at 400 μg/mL concentration on MDA-MB-231 (Breast Cancer Cell Line).

Key words: *Randia dumetorum*, Rubiaceae, Bark, Chemical constituents, Cytotoxic activity.

INTRODUCTION

Randia dumetorum lamk. has been reported as an emetic and antidysentric agent[1]. Different workers have isolated saponins and iridoids from the fruits and bark of this plant[2-10]. However, no sterols have been reported. Herein, we report the isolation of β-sitosterol, lupeol and daucosterol from the bark of *Randia dumetorum* and characterized β-sitosterol and daucosterol by 2D NMR experiments.

EXPERIMENTAL

Dried and powdered stem bark of *Randia dumetorum* was extracted with boiling ethanol and the extract was solublized in methanol and precipitated with acetone. The

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filterate was evaporated to give a residue, which was subjected to column chromatography to give compounds **1, 2** and **3**.

Compound 1 : M.P. 135-137^{o}C, found C,89.11, H, 11.92%, for $C_{29}H_{50}O$ C, 84.05, H, 12.07%; IR: ν^{KBr}_{max} cm^{-1} 3440, 2070, 2959, 2920, 2859, 1640, 1463,1380, 1055. ^{1}H NMR and ^{13}C NMR are given in Table 1.

Compound 2: M.P. 215-216^{o}C. found: C, 84.50; H, 11.73%. Calculated for $C_{30}H_{50}O$ C, 85.51; H, 11.72%. IR ν^{KBr}_{max} cm^{-1} : 3440, 2970, 2959, 2920, 2920, 2589, 1463. NMR (CD_3OD) δ (ppm): 1.68 (3H, s, 20-CH_3), 3.23, 1H, dd, J = 6.2 Hz, 4.57, 4.67 (2H bd, s, 24-Hz), EIMS : m/z : 426 (m^+), 218, 189, 135, 109, 95.

Compound 3: M.P. 289-291^{o}C (decomposed), $[\alpha]_d$-39^{o} C_6H_5N; found C, 72.68; H, 20.22% Calculated for : $C_{35}H_{60}O_6$ C,72.97; H, 10.41%. IR : ν^{KBr}_{max} (cm^{-1}): 3400 (-OH), 1640 (C=C), 878, 880 (β-glycosidic linkage) and 850. ^{1}H NMR and ^{13}C NMR are given in Table 1, NMR spectra were recorded on Bruker DRX-600, operating at 59.2 MHz for ^{1}H NMR and at 150.9 MHz for ^{13}C NMR. C_6H_6-EtOAc (9 : 1), C_6H_6-Me_2CO (9 : 1) and $CHCl_3$-MeOH (95 : 5) were used for TLC and it was visualized by spraying with 5% H_2SO_4.

Cytotoxic activity: Cell viability assay preparation

Different concentration (µg/mL) of ethanolic extracts were prepared in DMSO for determining the cytotoxicity to different cell line. Cells were grown in a 96-well plate in Delbucco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum and antibiotic (Streptomycin, Penicillin-G and Amphotericin). About 1 mL of each cell suspension type (10^5 cells/mL) was seeded in each well and incubated at 37^{o}C for 48 hours in 5% CO_2 for the formation of confluent monolayer. The monolayer of cells in the plate was exposed to various dilutions of extract. The cell viability was measured using MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) with MTT (5 mg/mL) and DMSO. This tetrazolium salt is metabolically reduced by viable cells to yield a blue insoluble formazon product measured at 540 nm spectrophotometrically. Controls were maintained throughout the experiment (untreated wells as control) the assay was performed in triplicate for each of the extracts. The mean of the cell viability values was compared to the control to determine the effect of extract on cells and % cell viability was plotted against concentration of plant crude extract.

RESULTS AND DISCUSSION

Column chromatography of ethanolic extract of bark of *Randia dumetorum* gave compounds **1, 2** and **3**.

Compounds **1** and **3** were identified for the first time by interpretation using 2D NMR (HSQC, HMBC and DQF-COSY) experiments and the Compound **2** was identified as lupeol by direct comparasion with authentic sample (Co-TLC and M.M.P).

Compound **1**: It was found to be sterol as it give positive Liebermann-Berchard test[11] and Noller test[12]. It also responded to positive TNM test[13] for unsaturation. The IR spectrum showed characteristic absorption at ν^{KBr}_{max} cm^{-1} 3440 (-OH), 2970, 2959, 2859 (C-H streching), 1440 (C=C streching), 1463, 1380 (gem-dimethyl group) and 1055 (C-O streching). The interpretation of the proton spin-spin coupling pattern was done with the aid of 2D NMR viz. HSQC experiments and is explained in Fig. 1. The ^{1}H NMR and ^{13}C NMR results are tabulated in Table 1. Thus, on the basis of aforementioned spectral data, the structure of compound **1** was assigned as β-sitosterol (Fig. 4), which was further confirmed by direct comparison with an authentic sample.

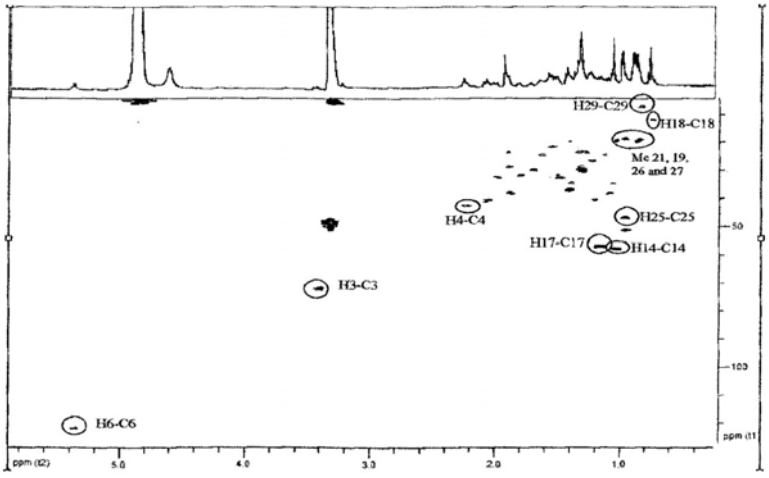

Fig. 1: HSQC spectrum of compound 1

Table 1: ^{13}C and ^{1}H NMR spectroscopic data of samples 1 and 3 in CD_3OD

Position	Compound 1		Compound 3	
	$^{\delta}C$	$^{\delta}H$ (*J* in Hz)[b]	$^{\delta}C$	$^{\delta}H$ (*J* in Hz)[b]
1	38.2	1.09, 1.89, m	38.0	1.12, 1.91, m
2	32.2	1.49, 1.79, m	30.2	1.63, 1.93, m
3	72.4	3.37, m	79.6	3.59, m

Cont…

Position	Compound 1		Compound 3	
	$^{\delta}$C	$^{\delta}$H (*J* in Hz)[b]	$^{\delta}$C	$^{\delta}$H (*J* in Hz)[b]
4	42.9	2.24, 2.45 m	39.3	2.29, 2.45, m
5	141.2	-	141.7	-
6	122.1	5.34, brd (4.5)	122.1	5.39, brd (4.5)
7	32.6	1.56, 2.00, m	32.4	1.56, 2.00, m
8	32.3	1.49, m	32.7	1.49, m
9	51.3	0.96, m	51.2	0.98, m
10	36.9	-	37.4	-
11	21.9	1.56, m	21.8	1.56, m
12	40.7	1.21, 2.08, m	40.5	1.21, 2.07, m
13	42.8	-	41.1	-
14	57.9	1.05, m	57.7	1.05, m
15	25.0	1.19, 1.63, m	24.6	1.14, 1.63, m
16	29.1	1.33, 1.89, m	28.9	1.88, m
17	57.2	1.17, m	56.8	1.16, m
18	12.0	0.73, s	11.8	0.74, s
19	19.7	1.05, s	19.3	1.07, s
20	37.2	1.40, m	36.8	1.39, m
21	19.1	0.98, d (6.5)	18.9	0.97, d (6.5)
22	34.7	1.07, 1.40, m	34.6	107, 1.39 , m
23	26.9	1.24, m	26.8	1.23, m
24	46.9	0.98, m	46.8	0.98, m
25	30.3	1.70, m	29.9	1.72, m
26	19.1	0.86, d (6.7)	18.9	0.86, d (6.7)
27	20.0	0.88, d (6.7)	19.9	0.89, d (6.7)
28	23.8	1.33, m	23.7	1.32, m
29	11.7	0.88, t (7.4)	11.8	0.88, t (7.4)

Cont…

Position	Compound 1		Compound 3	
	$^{\delta}$C	$^{\delta}$H (*J* in Hz)[b]	$^{\delta}$C	$^{\delta}$H (*J* in Hz)[b]
-OCH$_3$				
Glc-1			102.1	4.39, d (7.6)
Glc-2			74.9	3.17, m
Glc-3			77.7	3.27, m
Glc-4			71.5	3.29, m
Glc-5			77.7	3.37, m
Glc-6			62.1	3.67, dd (11.0, 4.4)
				3.85, dd (11.0, 3.3)

Compound **3**: Compound **3** responded to Liebermann-Burchard test characteristic for sterol and positive TNM test for unsaturation. It gave positive test with Molisch reagent and did not reduce Fehling's solution. It indicated the nature of compound as steroidal glycoside. Its IR spectrum showed characteristic absorption at ν_{max} cm^{-1} 3400 (-OH streching) and 1640 (C=C stretching), 878 and 786 (β-linkage of glucoside)[14]. Hydrolysis of **3** was carried out with 5% sulphuric acid and by extracting the aglycone with $CHCl_3$. It was crystallized from methanol as white needles and identified as daucosterol (Fig. 8) by comparing Co-TLC and MMP with that of an authentic sample. The presence of sugar as glucose was confirmed by co-paper chromatography with an authentic sample. The 2D-NMR (HSQC, HMBC, DQF-COSY) experiments are explained in Fig. 3, 4 and 5. Results of the ^{1}H NMR and ^{13}C NMR are tabulated in Table 1. Thus, on the basis of above aforementioned data, the compound **3** was assigned as daucosterol (Fig. 6).

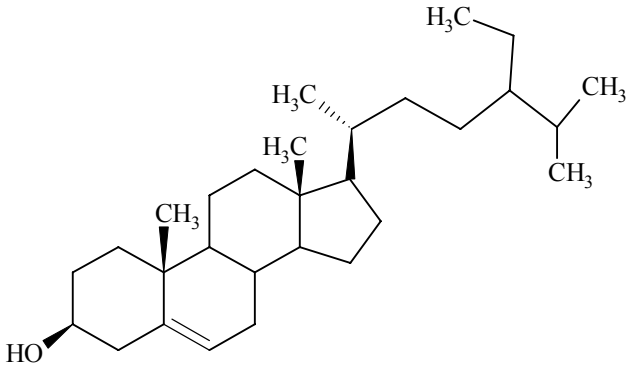

Fig. 2: Structure of compound 1

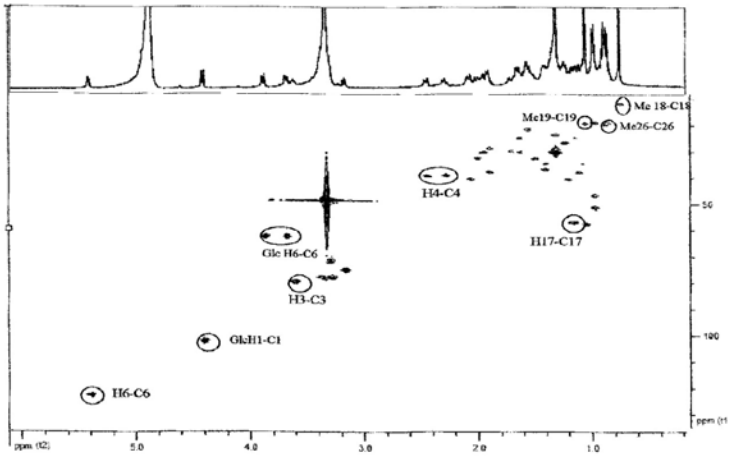

Fig. 3: HSQC spectrum of compound 3

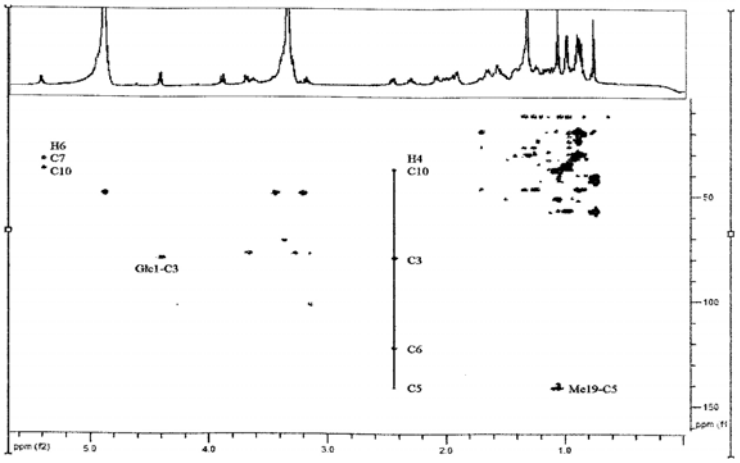

Fig. 4: HMBC spectrum of compound 3

Cytotoxic activity of crude extract

Cell viability assay on normal A549 cell (Adenocarcinomic Human Alveolar Basal Epithelial cells) showed that % age of cell viability was found to be 97.35% at a concentration of 100 µg/mL with respect to control, which decreased to 23.87% with increase in concentration to 800 µg/mL.

Cytotoxic activity on BE(2)C (Neuroblastoma cell line derived from human bone marrow) with the extract showed a potent cytotoxic activity against BE-(2)C cell line extract showed % cell viability of 83.41 % at concentration 100 µg/mL, which decreased to 20.14% with increasing concentration to 500 µg/mL.

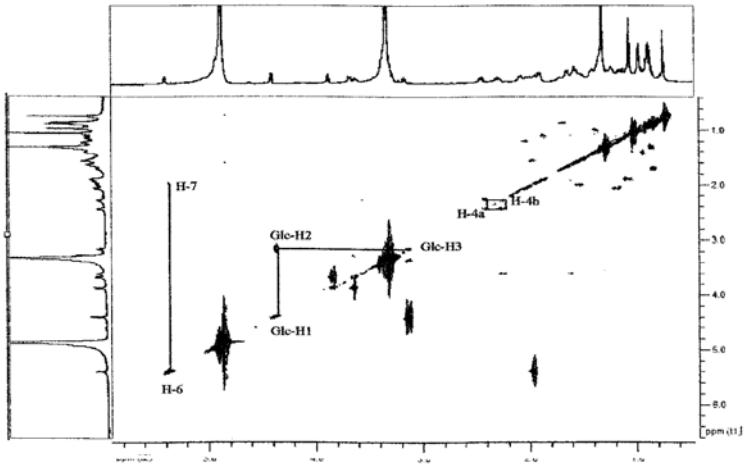

Fig. 5: DQF-COSY spectrum of compound 3

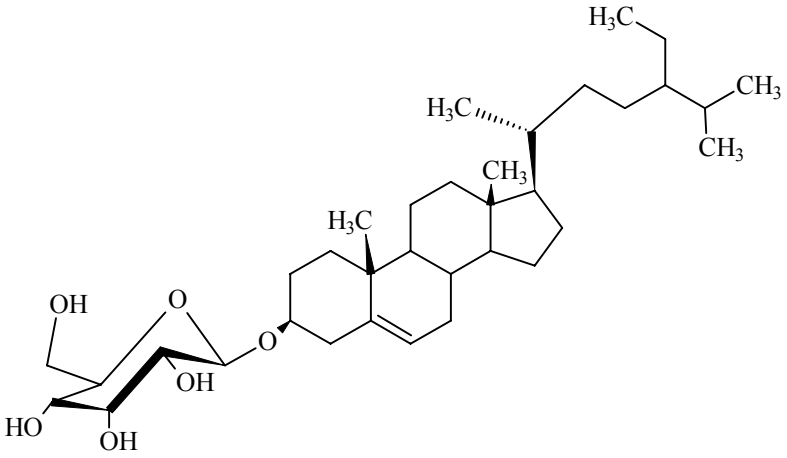

Fig. 6: Structure of compound 3

Inoculation of Helacell line, which are immortalized cell line, result with extract showed % cell viability of 92.06% with extract concentration 50 µg/mL, which decreased to 43.3% with increasing concentration to 800 µg/mL.

Cytotoxic activity of extract on MDA-MB-231 (breast cancer cell line) showed that % age viability was 85.75% on treating cell line at 50 µg/mL, which decreased to only 29.99% with further increasing concentration to 400 µg/mL and again increased to 35.24% with the concentration of 800 µg/mL showing that extract is most effective at concentration 400 µg/mL.

Cell viability assay on SK-MEL-2 (Human Skin Melonoma Cell Lines) showed that extract provided viability of 96.24% at concentration of 100 µg/mL, which decreased to 53.57 at 500 µg/mL concentration of crude extract. Inoculation of U87 MG- Human Neuronale Gliblostoma (Astrozytom) cell line showed % cell viability of 96.97% at concentration of 100 µg/mL, which decreased to 40.44% at 500 µg/mL concentration.

These results show that ethanolic extract was most effective against MDA-MB-231 (breast cancer cell line) at concentration of 400 µg/mL. Comparative cytotoxic effect of ethanolic extract on above mentioned cell lines are shown in Fig. 7 and Fig. 8.

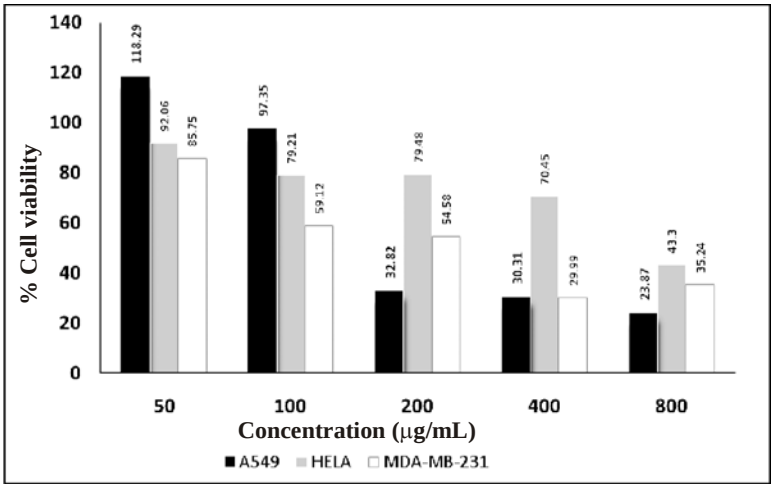

Fig. 7: Comparative cytotoxic effect of ethanolic extract on different cell lines

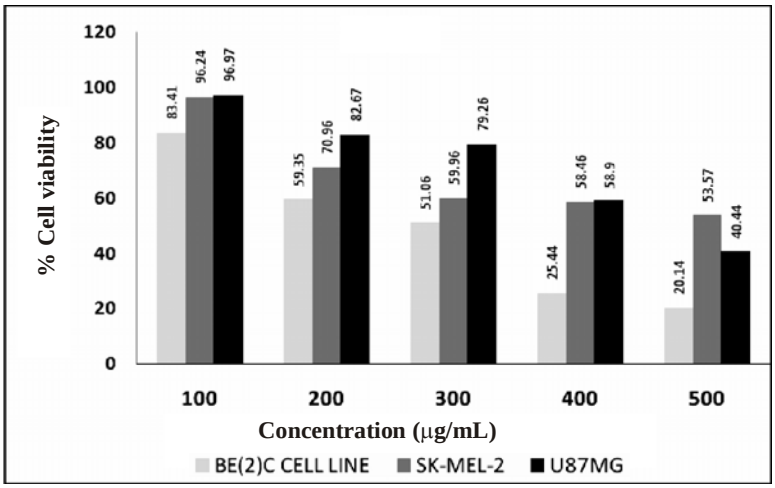

Fig. 8: Comparative cytotoxic effect of ethanolic extract on different cell lines

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