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Assessment of Antitumor Activity of β-sitosterol-β-D-glucoside Isolated and Identified from *Justicia brandegeana*

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Abstract: Several species of Justicia (Acanthaceae) are widely used in folk medicine. Also, the plants are used in treatment of central nervous system disorders and epilepsy. On the other hand, some species of this genus are used popularly in headache and fever treatment, in addition to their role as sedative, analgesic, and anti-tumor effect. This study was carried out for isolation and purification of different metabolites as well as assessment the cytotoxicity effect of the major one; β-sitosterol-β-D-glucoside against different type of cancer cell lines. An aqueous methanol extract of *J. brandegeana* has led to the isolation and identification of six known metabolites: free disaccharides known as 4-O-(α-D-glucopyranosyl)- β-D-glucopyranose (1), methyl-4-hydroxybenzoate (2), p-hydroxybenzoic acid (3), Quercetin 3-O-β-D-4C1-galactopyranoside (4), β-Sitosterol-β-D-glucoside (5) and Lupeol (6) for the first time. Their structure elucidation was based on different spectroscopic analyses. Indeed, Cytotoxic activity of the major constituent namely; β-Sitosterol-β-D-glucoside was evaluated against seven types of cancer cell lines namely; Breast carcinoma (MCF-7), Hepatocellular carcinoma (HepG-2), Colon carcinoma (HCT-116), Lung carcinoma (A-549), Prostate carcinoma (PC-3), Larynx carcinoma HEP-2 and Cervical carcinoma (HELA) cell lines, the results revealed that the cytotoxicity ranged from 0.57-57.25, 3.72-65.08, 5.94-68.51, 8.22-76.15, 0.0-60.13, 0.0-38.62, and 0.0-25.81% in case of breast carcinoma, hepatocellular carcinoma, colon carcinoma, lung carcinoma, prostate carcinoma, larynx carcinoma, and cervical carcinoma cell lines respectively. Thus, *β-sitosterol-β-D-glucoside* could be recommended as anti-cancer (Lung carcinoma) after determining the safe dose for human use.

Keywords: Lupeol, *β*-Sitosterol-β-D-glucoside, *Justicia brandegeana*, Isolation, Bioactive Natural Products Activity

1. Introduction

Family of Acanthaceae family represents a source of some drugs, *Justicia* is the largest one of Acanthaceae comprises several species which are useful in folk medicine for respiratory and gastrointestinal diseases as well as inflammation treatment. On the other hand, the plants are also used in treatments of central nervous system disorder as hallucinogens, sedatives, depressants, as well as treatment of mental disorders and epilepsy. Also, some species of this genus are used popularly in headache and fever treatment, in addition to their role in sedative and analgesic effect, anti-tumor effect, anti-diabetes and HIV protectant [1]. Also, *Phoradendron*

serotinum and *J. spicigera* extracts showed high cytotoxic effects against cell lines of breast cancer (IC50 ≤ 30 microg/mL), and *P. serotinum J. spicigera* showed the highest toxicity effects against human cancer cell lines (MCF-7 and HeLa), respectively. Maria, R. J. *et al*. [2] reported that the *J. spp*. has anticancer effects in HeLa cancer and enhances activity of immune system *in vitro* [3]. However, ethanolic extracts of *J. secunda* has anti-bacterial effect; against *Bacillus cereus* and *Staphylococcus aureus* Gram-+ve bacteria [4, 5]. Moreover, Kumar, S. R. *et al.* [6] recorded that many compounds which were isolated from different plants were biologically active against many diseases. Meanwhile, ethyl acetate extract of *Holoptelea integrifolia* has promising cytotoxic activity against many types of carcinoma [7]. Nowadays, there is a global interest in isolation, purification, and identification of new anticancer materials from plant sources [8]. There are no reports on antitumor effect of methanolic extracts of *J. brandegeana* leaves.

The present study was carried out for isolation and purification of different metabolites and assessment cytotoxicity of the major one; β-sitosterol-β-D-glucoside against Breast carcinoma (MCF-7), Colon carcinoma (HCT-116), Hepatocellular carcinoma (HepG-2), Lung carcinoma (A-549), Prostate carcinoma (PC-3), Cervical carcinoma (HELA) and Larynx carcinoma HEP- cell lines.

2. Materials and Methods

2.1. Plant Material

Fresh leaves of *Justicia brandegeana* (Shrimp plant) were collected from Orman Botanic Garden, Giza, during October (2016). Authenticated by Professor Dr. Soad Abdalla Hassan Professor of Taxonomy, Faculty of Science, Ain Shams University. Voucher specimens (Reg. No.: J-1-2016) are kept in herbarium, Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Egypt. The leaves were air-dried and reduced to No. 36 powder and kept in tightly closed container until extraction process.

2.2. General Equipment

^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker NMR spectrometers (400 MHz \MHz for ^{1}H-NMR, 100 MHz \ MHz for ^{13}C-NMR). The chemical shifts δ-values are reported as ppm relative to TMS in DMSO-d_6 and *J*-values are given in Hz. EI-MS were measured on a double focusing sector field MAT 90 mass spectrometer connected to ESI-II ion source (Finnigan, Bremen, Germany). Rotary evaporator (Buchi, G, Switzerland). Glass column for chromatography 100 cm × 3cm, 30 cm × 1.5cm and 50cm × 1cm. Ultraviolet Lamp: UV 254/366 nm, Desaga Heidelberg-Germany, for localization of spots on paper and thin layer chromatogram and follow up the CC fractionation. Mass spectrometer: Varian Mat 711, Finnigan SSQ7000 and OMM7070E. Rectangular glass tanks 50×56×20cm was used for PC 24×24×10 cm for TLC.

2.3. Extraction and Isolation

The air-dried powdered leaves of *J. brandegiana* (500 g) was exhaustively extracted with 70% aqueous methanol (3 x 1L) under reflux at 40-50°C. In each case, the solvent was evaporated to dryness and the yield was 55 g for aqueous methanol extracts. The brownish sticky residue obtained was successively extracted with petroleum ether and methylene chloride for defatting, and then dissolved in the least amount of water and treated with excess of methanol (ten folds for desalting) and filtered, the insoluble fraction proved to contain non- phenolic compounds indicating polysaccharides and inorganic salts kept for further investigation. Dry filtrate (35 g) prepared in water and fractionated on a polyamide 6S (Riedel de Hean AG, Seelze, Hannover, Germany) column (Φ 3 X 100 cm, 200 g) and eluted with water followed by gradient increasing ethanol proportions with decreasing polarity. Elution and fractionation processes (collection of 250 ml portions; each fraction desorbed from the column was then evaporated under reduced pressure) have been followed up and traced by UV-light, CoPC and TLC investigation which showed some spots of phenolic and terpenoids or steroids on using solvent system (Petroleum ether- Benzene- Ethyl acetate 75:25:0.5 v/v); visualized after spraying with EtOH/H_2SO_4 reagent. Ready-made chromatographic plates (20 x 20 cm) coated with silica gel F254 was used for analytical separation and technique was completed ascendingly. Similar fractions were collected and separately reanalyzed and investigated using glass columns of different sizes packed with sephadex LH-20 or silica gel for successive purification.

2.4. Fractions

Fraction A (20 g): (100% H_2O); it is a water fraction polyphenol free contains sugars, proteins, and inorganic salts from which compound 1 (45 mg) was obtained by crystallization using methanol. Fraction B&C (30 g): (20% -30% MeOH: H_2O); *n* butanol extract of fraction B was subjected to successive column cellulose (30% MeOH: H_2O), then silica (CH_2Cl_2: MeOH) in ratio (1:8) followed by sephadex LH-20 with S6 solvent system to obtain isolated pure compounds 2 (25 mg) and 3 (32 mg). Fractions D (8 g): (40-50% MeOH) fraction was subjected to column on cellulose (50% MeOH: H_2O), then was fractionated on cellulose with MeOH as an eluent, followed by a Sephadex LH- 20 column using BIW (*n*-BuOH:2-propanol: H_2O, 4:1:5 v/v/v, organic layer) and final sephadex ethanol 100%, to afford pure pure compounds 4 (30 mg) and 5 (200 mg). Fractions E-G (5 g): (60% MeOH: H_2O till 90% MeOH:) fraction was subjected to successive column sephadex LH-20 with S6 solvent system, and final silica gel and using S3; hexane: $CHCl_3$(6:4) yield compound 6 (28 mg).

2.5. Cell Lines and Antitumor Activity Assay

The tested human carcinoma cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Human Breast carcinoma (MCF-7), Hepatocellular carcinoma (Hep G-2), Colon carcinoma (HCT-116), Lung carcinoma (A-549), Prostate carcinoma (PC-3) and Larynx carcinoma (HEP-2) and Cervical carcinoma (HELA). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml Gentamicin. The cells were maintained at 37±1°C in a humidified atmosphere with 5% CO_2 incubator [9] and were Sub-cultured two to three times a week. For antitumor assays, the tumor cell lines were suspended in medium at concentrations 5×10^4 cell/well in corning g 96-well tissue culture plates then incubated for 24 hr. The tested compounds were then added into 96-well plates (six replicates) to achieve eight concentrations for each compound. Six vehicle controls with media or 0.5% DMSO were run for each 96 well plate as a control. After incubating for 24 hr, the Numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plates and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 nmol MTT stock solution (5mg of MTT in 1 ml of PBS) to each well including untreated controls.

The 96 well plates were then incubated at 37±1°C and 5% CO_2 for 4 hours. An aliquot of the media (85µl) was removed from the wells, 50µl of the DEMSO was added to each well and mixed thoroughly using pipit and incubated at 37±1°C for 10 min. Then, the optical density was measured at 590 nm with the micro plate reader (sun rise, TEKAN, inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [1-(ODt/ODc)]×100%. Where, ODt is the mean optical density of wells treated with the tested sample while, ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cells line after treatment with specified compound. The 50% of inhibitory concentration (IC_{50}) [the concentration required to cause toxic effects in 50% of intact cells], was estimated from graphic plots of the dose response curve for each conc. using Graphed Prism software [10, 11].

3. Results

3.1. Chemical Studies

Compound 1 was crystallized from MeOH as buff sweet taste crystals; It gave positive Molisch's reactions indicating its glycosidic nature; soluble in H_2O; R_f value 0.48 in Acetic acid- Water (15:85 v/v). ^{1}H and ^{13}C NMR spectroscopic data (δ in DMSO-d_6 ppm) of compound 1 Table 1.

Compound 2 was crystallized from MeOH as white crystals, single spot R_f =0.92 in *n*-Butanol-Acetic acid- Water (4:1:5 v/v), upper layer (BAW); brown under UV, bluish with $FeCl_3$. ^{1}H-NMR; (300 MHz, CD_3OD): δ 7.83 (2H, d, *J*=8.2 Hz, H-2,6), 6.76 (2 H d, *J*=8.2 Hz, H-3, 5), 3.84 (3H, s, -CH_3). ^{13}C-NMR (75 MHz, CD_3OD): δ 129.41 (C-1), 131.37 (C-2, 6) 116.31(C- 3, 5), 161.02 (C-4), 173.02 (-C=O), 56.03 (-CH_3).

Compound 3 was obtained from $CHCl_3$-methanol as white needles, single spot R_f = 0.97 in BAW; brown under UV, bluish with $FeCl_3$; m.p. 195°C. IR (ῡ max KBr): cm^{-1} 3400-3000, 1670, 1540, 1510, 1130, 760, 715, etc. ^{1}H-NMR: (300 MHz, Acetone-d_6): δ 7.83 (2H, d, *J* = 8.5 Hz, H-2, 6), 6.85 (2 H d, *J*=8.5 Hz, H-3, 5). UV (λ_{max}, MeOH): nm 230. ^{13}C-NMR (75 MHz, Acetone-d_6): δ 122.39 (C-1), 132.69 (C-2, 6), 116.02 (C-3, 5), 162.76 (C-4), 167.81 (-C=O).

Compound 4: Yellow amorphous powder. R_f-values: 0.48 (BAW), 0.24 (Acetic acid- Water, 15:85 v/v) on PC. Response against spary reagents: dark purple (long/short UV); yellow fluorescence (UV/NH_3); turned to orange/Naturstoff; green color/$FeCl_3$. UV spectral data λ_{max} (nm), MeOH: 259,266 sh, 299sh, 359; (+NaOMe): 272, 327, 410; (+NaOAc): 271, 325, 393; (+NaOAc/ H_3BO_3): 262, 298, 387; (+$AlCl_3$): 275, 303 sh, 433; (+$AlCl_3$ /HCl):271, 300, 364 sh, 402. ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} 12.73 (1H, s, OH-5 hydrogen bonded proton), 7.74 (2H, dd, *J*=2, 8Hz H-2′/6′,), 6.84 (1H, d, *J* = 8Hz, H-5'), 6.40 (1H, brs, H-8′), 6.18 (1H,brs H-6), 5.63 (1H, d, *J* = 7.6 Hz, H-1″), 3.57 (1H, br d, *J* = 11.4 Hz, H-6 ″a), 3.50 -3.20 (4H, m, H-2″, 3″,5″, 6″$_b$, hidden by H_2O-signal), 3.08 (1H, br s, H-4″). ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} 177.84 (C-4), 165.36(C-7), 161.67(C-5), 156.65 (C-2), 156.56 (C-9), 148.58 (C-4′), 145.21 (C-3'), 132.10 (C-3), 121.64 (C-6′), 120.97 (C-1′), 116.31 (C-5′), 115.67 (C-2′), 104.04 (C-10), 99.26 (C-1″), 98.39 (C-6), 94.09 (C-8), 76.16 (C-5″), 74.35 (C-3″), 73.62 (C-2″), 70.01 (C-4″), 60.45(C-6″).

Compound 5: White crystals (200 mg), IR υ KBr max cm^{-1}: 3400, 2920 & 2850, 1705 and 1620, 1445, 1360, 1257, 1160, 1105 & 1020. m.p: 272-274°C, it gave positive Liebermann-Burchard and positive Molisch's reactions indicating its steroidal glycosidic nature, also soluble in a mixture of chloroform and methanol, R_f value 0.48 in Petroleum ether - Ethylacetate (1:9 v/v). IR υ KBr max cm^{-1}: 3400, 2920 & 2850, 1705 and 1620, 1445, 1360, 1257, 1160, 1105 & 1020. ^{1}H & ^{13}C NMR of compound 4 listed in figures 1 & 2 and Table 2.

Compound 6 was colourless crystal m.p. 215 R_f: 0.35,0.93 in *n*-Butanol-Isopropyl alcohol – Water (4:1:5 v/v), upper layer, Chloroform – Methanol- Water (80:20:2), pink-violet colour with Liebermann Burchard test [29]; molecular weight m/z 420 corresponding to molecular formula $C_{30}H_{50}O$. IR *v*, $_{max}$ (CCl_4) cm^{-1}: 3430, 3056, 2929, 2313, 1593, 1435, 1265, 898, 741. ^{1}H NMR ($CDCl_3$, 400MHz): δ 4.70, 4.55(2H, *s*, H-29a, 29b), 3.2(1H, *m*, H-3), 0.77, 0.79, 0.85, 0.94, 0.97, 1.05, 1.65 (each 3H, *s*); ^{13}C NMR ($CDCl_3$, 100 MHz): δ151.0(C-20), 109.33(C-29), 79.0(C-3), 55.30(C-5), 50.44(C-9), 48.71(C18), 48.31(C-19), 48.0(C-17), 42.19(C-14), 40.01(C-8), 39.22 (C-22), 38.76(C-4), 38.71(C-1), 38.05(C-13), 36.71(C10), 35.59(C-16), 34.28(C-7), 29.85(C-21), 27.99(C-23), 27.4(C-2), 27.04(C-15), 25.56(C-12), 21.64(C-11), 20.93(C30), 19.31(C-6), 18.32(C-28), 18.01(C-25), 17.71(C-26), 16.31(C-24), 16.05(C-27).

Table 1. *^{1}H and ^{13}C NMR spectroscopic data (δ in DMSO-d_6 ppm) in comparison between α- and β- maltose.*

Position	α- maltose			β – maltose		
	^{1}H	H(OH)	^{13}C	^{1}H	H(OH)	^{13}C
1	4.88	6.36	92.52	4.2	6.69	97.19
2	3.06	4.72	72.27	2.98	5.06	74.76
3	3.44	5.34	73.29	3.35	5.47	76.80
4	3.09		80.59	3.07		80.06
5	3.21		75.40	3.62		75.45
6	3.61	4.52	60.72	3.47,3.64	4.59	61.00
1`	4.94		101.20	4.9		100.99
2`	3.44	5.43	72.09	3.24	5.45	72.87
3`	3.23	4.68	72.41	3.35	4.64	73.72
4`	3.52	4.78	70.34	3.03	4.89	70.67
5`	3.64		72.94	3.43		73.82
6`	3.26,3.36	4.5	61.25	3.41,3.57	4.60	61.12

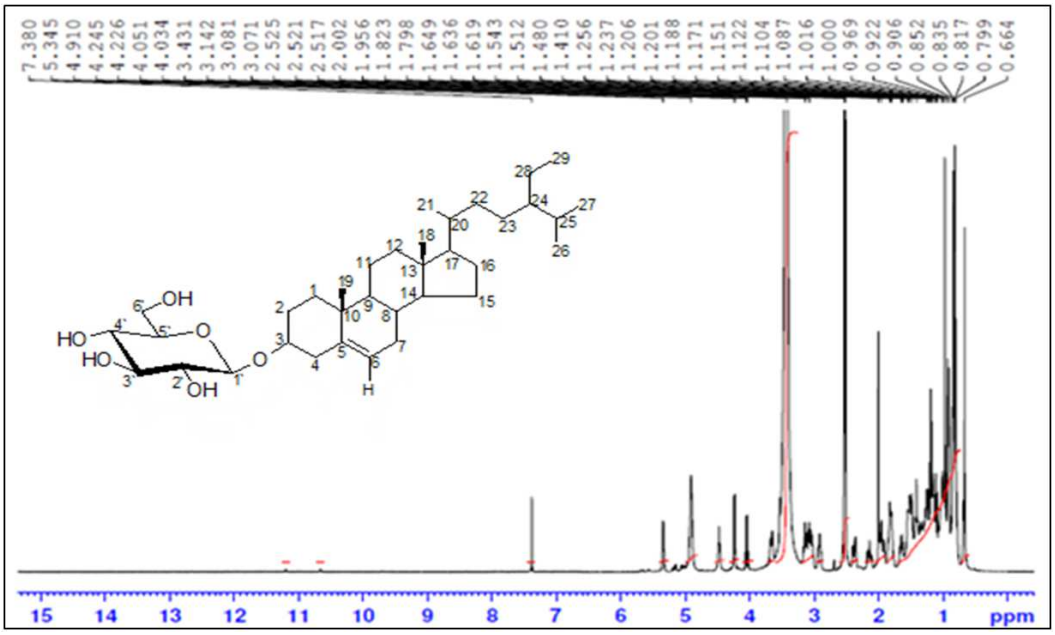


Figure 1. *^{1}H- NMR spectrum of β-sitosterol-β-D-glucoside in DMSO-d_6.*

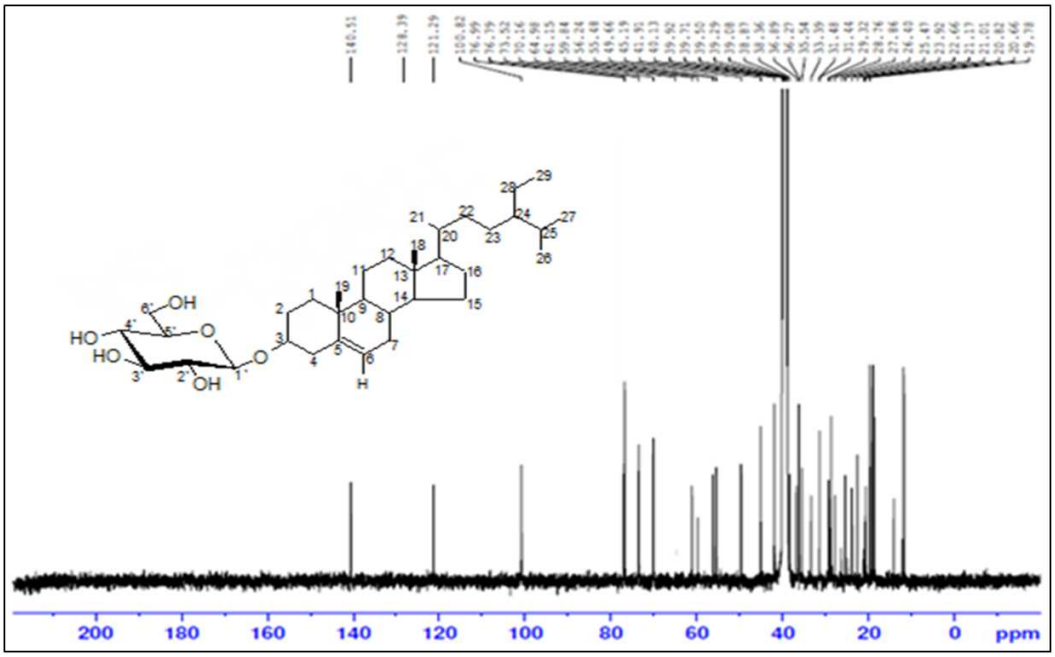


Figure 2. *^{13}C-NMR spectrum of β-sitosterol-β-D-glucoside in DMSO-d_6.*

Table 2. *^{1}H &^{13}C NMR of β-sitosterol-β-D-glucoside.*

Position	δ_H	δ_C
1.	1.25(m, 2H)	36.85
2.	1.33(m, 2H)	29.12
3.	3.13(m, 1H)	76.99
4.	2.14(m, 2H)	42.14
5.	-----	140.51
6.	5.34(bs, 1H)	121.29
7.	1.73(m, 2H)	31.41
8.	1.22(m, 1H)	31.46
9.	1.22(m, 1H)	49.83
10.	----	36.27
11.	1.33(m, 2H)	20.21
12.	1.33(m, 2H)	38.20
13.	-----	41.91
14.	1.22(m, 1H)	56.36
15.	1.73(m, 2H)	23.79
16.	1.73(m, 2H)	27.76
17.	1.73(m, 2H)	55.66
18.	0.62(s, 3H)	11.27
19.	0.94(s, 3H)	19.10
20.	1.32(m, 1H)	35.70
21.	0.84(d, 3H, *J*=6.3 Hz)	18.69
22.	1.73(m, 2H)	33.51
23.	1.73(m, 2H)	25.64
24.	1.12(m, 1H)	45.49
25.	2.14(m, 1H)	28.74
26.	0.75(d, 3H/ *J*=7.7 Hz)	18.69
27.	0.73(d, 3H/*J*=1.6 Hz)	18.35
28.	1.33(m, 2H)	22.60
29.	0.77(t, 3H/ *J*=6.9 Hz)	12.29
1′	4.11(d, 1H/ *J*=7.8 Hz)	100.82
2′	3.14(m, 1H)	73.21
3′	3.14(m, 1H)	76.18
4′	3.14(m, 1H)	69.90
5′	3.06(m, 1H)	75.62
6′	2.94(m, 1H)	61.36

3.2. β-sitosterol-β-D-glucoside Activity

Because there are no reports on anticancer of *β-sitosterol-β-D-glucoside* extracted from of *J. brandegeana* leaves. That's why the present study assessed the cytotoxicity of *β-sitosterol-β-D-glucoside* from *J. brandegeana* leaves against breast carcinoma, hepatocellular carcinoma, colon carcinoma, lung carcinoma, prostate carcinoma, larynx carcinoma and cervical carcinoma cell lines.

When comparing the cytotoxicity percentages in different types of carcinoma cell lines exposed to *β-sitosterol-β-D-glucoside* from *J. brandegeana* leaves. The results indicated that the cytotoxicity ranged from 0.57-57.25, 3.72-65.08, 5.94-68.51, 8.22-76.15, 0.0-60.13, 0.0-38.62 and 0.0-25.81% in case of breast carcinoma, hepatocellular carcinoma, colon carcinoma, lung carcinoma, prostate carcinoma, larynx carcinoma and cervical carcinoma cell lines respectively, according to the concentration which were used (Table 3 & Figure 3).

On the other hand when comparing the viability of the different carcinoma cell lines exposed to the serial concentration of *β-sitosterol-β-D-glucoside*, it was ranged between 42.75-99.43% in case of breast carcinoma, 34.08-96.28% in case of Hepatocellular carcinoma, 31.49-94.06% in case of colon carcinoma, in case of lung carcinoma 23.85-91.78%, 39.87-100% in case of prostate carcinoma, 61.38-100% in case of larynx carcinoma and 74.19-100% in case of cervical carcinoma (Table 4), cell lines according to concentration which were used compared with negative control.

Table 3. *Cytotoxicity percentages in different types of carcinoma cell lines exposed to β-sitosterol-β-D-glucoside from J. brandegeana leaves methanolic extract.*

Type of Carcinoma	Sample conc. (µg)					
	100	50	25	12.5	6.25	3.12
	Cytotoxicity% (Growth inhibition%)					
Breast carcinoma	57.25	35.79	20.66	10.14	4.88	0.57
Hepatocellular carcinoma	65.08	41.57	27.31	15.86	9.35	3.72
Colon carcinoma	68.51	54.78	46.14	31.21	17.57	5.94
Lung carcinoma	76.15	62.86	51.27	35.44	19.06	8.22
Prostate carcinoma	60.13	29,08	14.81	5.79	1.27	0.0
Larynx carcinoma	38.62	17.36	8.11	2.68	0.0	0.0
Cervical carcinoma	25.81	14.75	5.88	1.27	0.0	0.0
Untreated control	0.0	0.0	0.0	0.0	0.0	0.0

Table 4. *Viability percentages in different types of carcinoma cell lines exposed to of β-sitosterol-β-D-glucoside from J. brandegeana leaves methanolic extract.*

Sample conc. (µg)	Breast Carcinoma	Hepatocellular carcinoma	Colon carcinoma	Lung Carcinoma	prostate carcinoma	Larynx carcinoma	cervical carcinoma
	Viability%						
100	42.75	34.08	31.49	23.85	39.87	61.38	74.19
50	64.21	58.43	45.22	37.14	70.92	82.64	85.25
25	79.34	72.69	53.86	48.73	85.19	91.89	94.12
12.5	89.86	84.14	68.79	64.56	94.21	97.32	98.73
6.25	95.12	90.65	82.43	80.94	98.73	100	100
3.125	99.43	96.28	94.06	91.78	100	100	100
Untreated Control	100.00	100.00	100.00	100.00	100	100	100.00

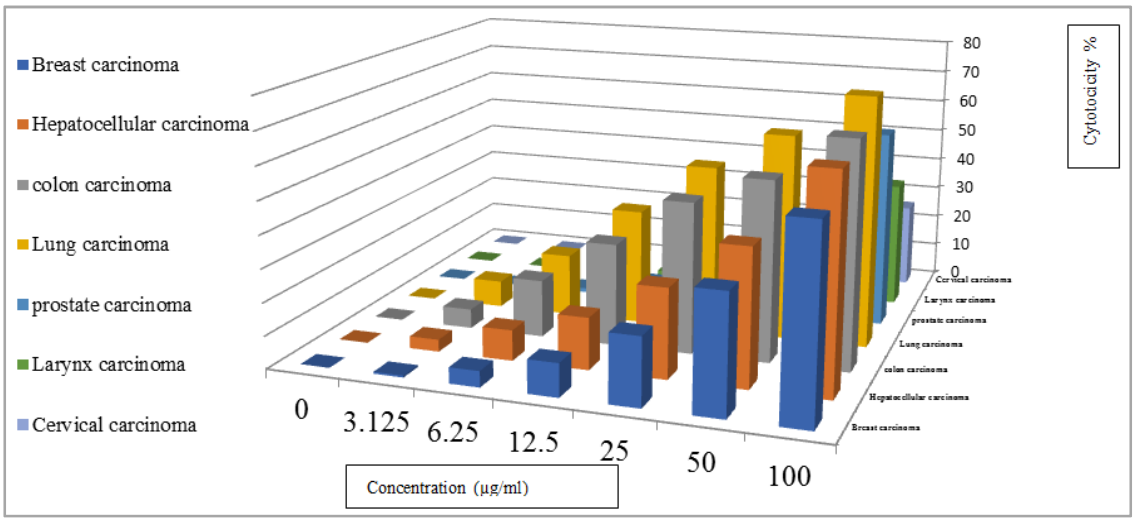


Figure 3. *Cytotoxicity percentage effect in different types of carcinoma cell lines exposed to of β-sitosterol-β-D-glucoside from J. brandegeana leaves methanolic extract.*

When compared the inhibitory index of methanolic extracts of *β-sitosterol-β-D-glucoside* from *J. brandegeana* leaves methanolic extract against breast carcinoma, hepatocellular carcinoma, colon carcinoma, lung carcinoma, prostate carcinoma, larynx carcinoma and cervical carcinoma cell lines, the Inhibitory fifty concentration (IC_{50}) was 83.1, 67.3, 36.2, 24.0, 83.7, 100 and 100 µg/ml respectively (Table 3 & Figure 4). On the other hand, when comparing among the different carcinoma types according to inhibitory index, it was found that the lung carcinoma cell lines is the most susceptible cell line, thus it was taken as standard susceptible cell line and given the arbitrary index value of 100 units. Therefore, the inhibitory index values of another cell lines; breast carcinoma, hepatocellular carcinoma, colon carcinoma, prostate carcinoma, larynx carcinoma and cervical carcinoma were 28.88, 35.66, 66.29, 28.67, 24.0 and 24.0% respectively, as cytotoxic as the cytotoxicity of the *β-sitosterol-β-D-glucoside* against lung carcinoma cell line (Table 4 & Figure 5).

Relative potency level can also be used as a conventional method in comparing the degree of cytotoxicity effect of *β-sitosterol-β-D-glucoside* of *J. brandegeana* to different types of cell lines. The relative potency levels of tested materials were expressed as the number of folds, compared with the least effective compound or least affected carcinoma cell line included in the experiment. Hence, the numbers of folds representing the potency levels can be obtained by dividing the IC_{50} of *β-sitosterol-β-D-glucoside* in case of larynx carcinoma and/or cervical carcinoma, which considered as the standard cell line and given arbitrary value of 1.0. Thus, the relative potency of *β-sitosterol-β-D-glucoside* extracted from *J. brandegeana* in against breast carcinoma, hepatocellular carcinoma, colon carcinoma and prostate carcinoma were 1.20, 1.49, 2.76 and 4.17 times more effective than the effect of *β-sitosterol-β-D-glucoside* against larynx carcinoma and/or cervical carcinoma cell lines respectively. (Table 5 & Figure 6).

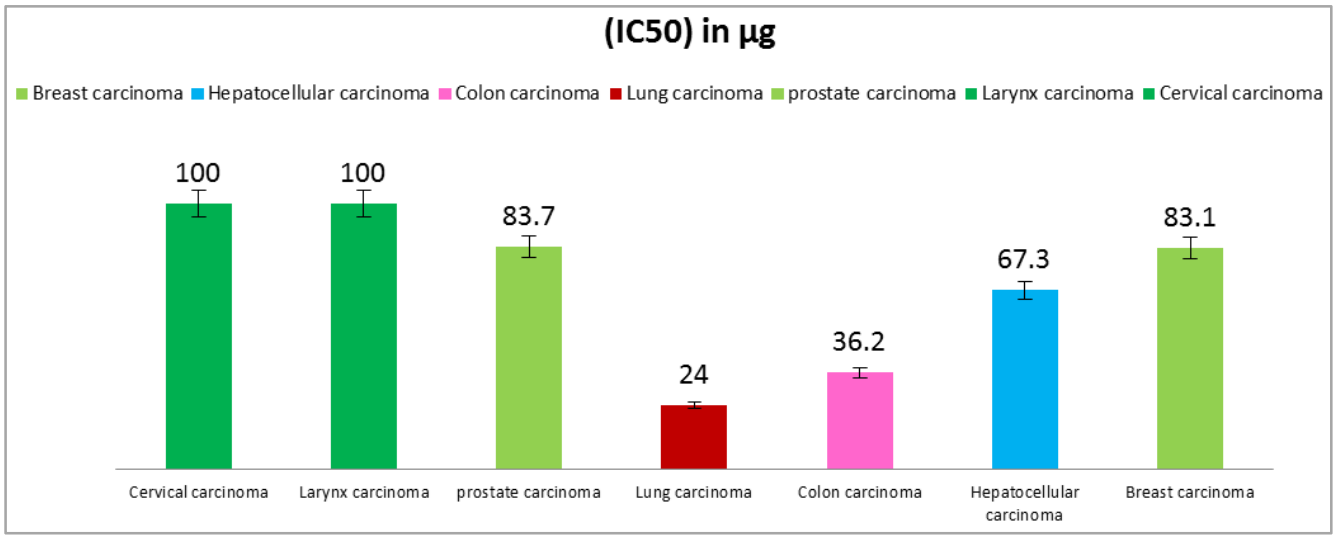


Figure 4. *IC_{50} values of β-sitosterol-β-D-glucoside from J. brandegeana leaves methanolic extract on different types of carcinoma cell lines.*

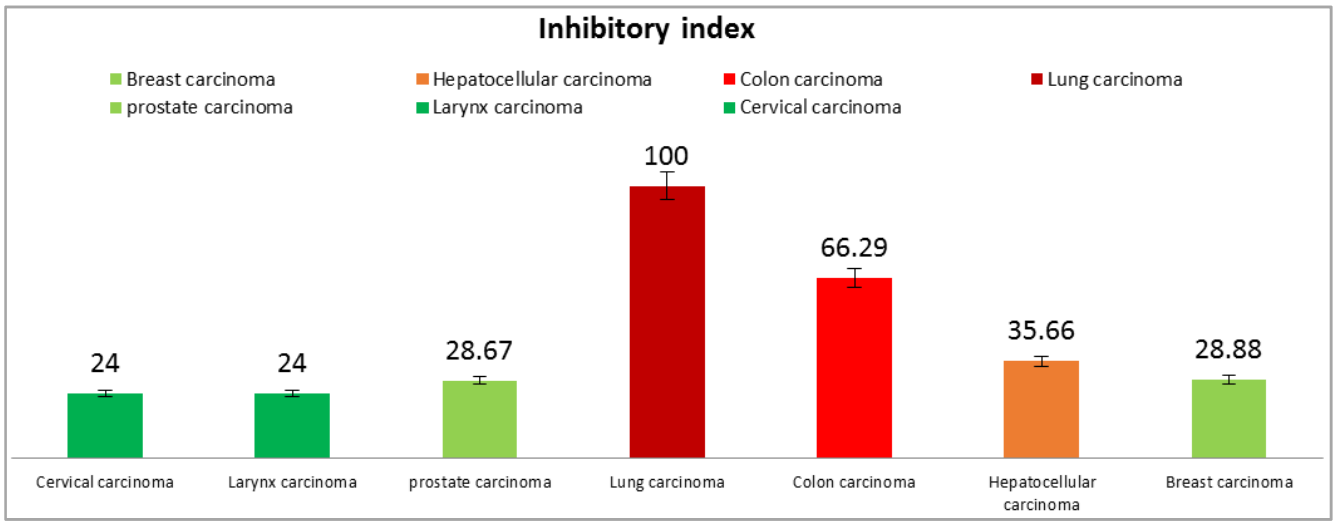


Figure 5. *Inhibitory indices for different types of carcinoma cell lines exposed to β-sitosterol-β-D-glucoside from J. brandegeana leaves methanolic extract.*

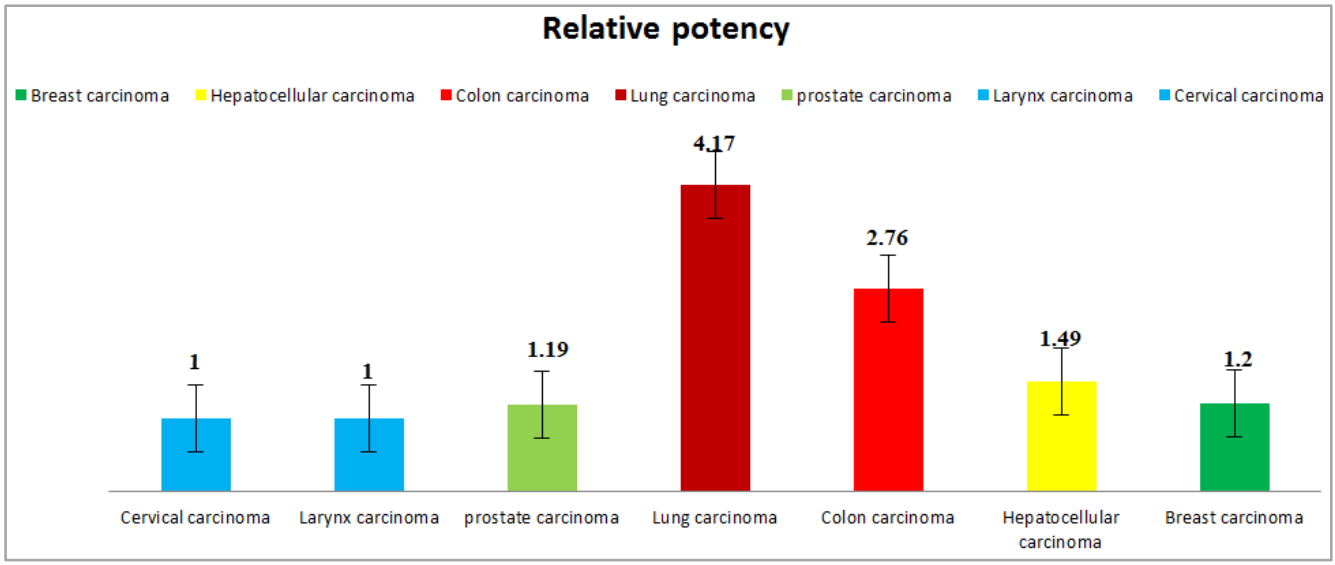


Figure 6. *Relative potency levels in different types of carcinoma cell lines exposed to β-sitosterol-β-D-glucoside from J. brandegeana leaves methanolic extract.*

Table 5. *Inhibitory index and relative potency levels values based on IC_{50} for different types of carcinoma cell lines exposed to β-sitosterol-β-D-glucoside from J. brandegeana leaves methanolic extract.*

Cell line name	(IC50) in µg	Inhibitory index	Relative potency levels
Breast carcinoma	83.1	28.88	1.20
Hepatocellular carcinoma	67.3	35.66	1.49
Colon carcinoma	36.2	66.29	2.76
Lung carcinoma	24.0	100.0	4.17
Prostate carcinoma	83.7	28.67	1.19
Larynx carcinoma	100	24.00	1.0
Cervical carcinoma	100	24.00	1.0

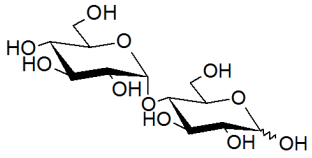
4. Discussion

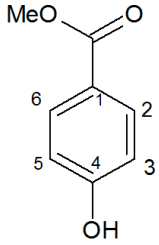
4.1. Compounds

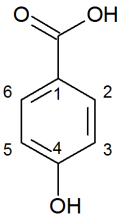
A total of six compounds were isolated from the desalted and defatted 70% methanol extract of *J. brandegeana* leaves through consecutive column chromatographic separations. On the basis of chemical and physicochemical analyses as well as their comparison with published data their structures were identified as maltose [12, 13], methyl-4-hydroxybenzoate [30], p-hydroxybezoic acid [14], Quercetin 3-*O*-β-D-$^4C_1$-galactopyranoside [15], along with Lupeol [16]. The major isolated pure compound 5 *β-sitosterol-β-D-glucoside* were in agreement with those indicated by Peshin, T. *et al.* [17]; white crystals (200 mg). The IR spectrum showed an absorption band at 3450 cm^{-1} due to the presence

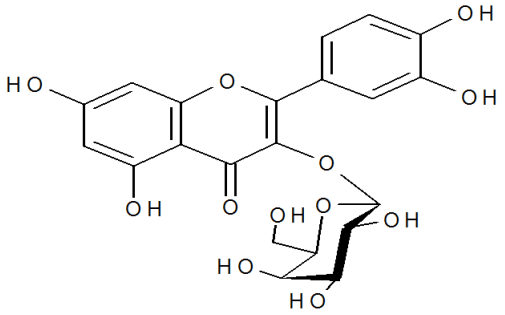
of –OH stretching. The bands at 1630 cm^{-1} was suggestive of a >C=C< moiety. The ^{1}H- NMR spectrum (Figure 1) showed downfield signal integrated for one proton (1H) intensity at δ_H 5.34 ppm, indicative of olefinic proton (H-6). The spectrum had a multiplet at δ_H 3.4 ppm indicative of an oxymethine proton (H-3). The spectrum showed the presence of six methyl protons at δ_H 0.69 (H-18), 1.00 (H- 19), 0.82 (H-27), 0.84 (H-26), 0.86 (H-21) and 0.82 (H-29) respectively. In addition to 1H signal was assigned at δ_H4.11 (d, *J*=7.8 Hz) of β- glucosyl. ^{13}C- NMR (Figure 2) spectrum revealed the presence of 29 carbons suggestive of a steroidal compound. The signals at δ_C 140.5 (C-5), 41.91 (C-13) and 36.27 (C-10) were assigned to three quaternary carbons. The signal at δ_C 76.99 ppm was for oxymethine carbon (C-3). One olefinic carbon at δ_C 140.51 and 121.29 ppm were for C-5 and C-6; respectively and only one anomeric carbon of glucose moiety at 100.82 ppm. All other data illustrated in Table 2 and Figures 1 and 2.

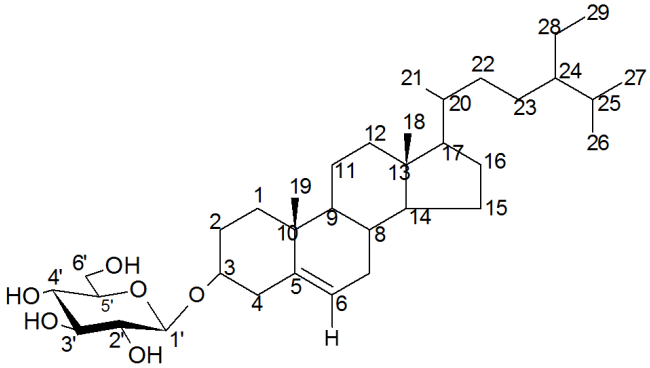
As recorded by Pavia, D. L. *et al.* [18] who reported that the absorption bands in IR at 1095 cm^{-1} was indicative of the presence of –C-O-C- structural feature in the compound. Also, Finar, I. L. [28] indicated that the absorption bands at 920 cm^{-1} supported its steroidal nature. So, the compound was identified as β-sitosterol-β-D-glucoside based on IR, 1H-NMR and 13C- NMR spectral data are in a close agreement with the reported values [19]. This is the first time for isolation of this compound from *Justicia brandegeana* leaves but previously isolated from *J. gendarussa* [20, 21].

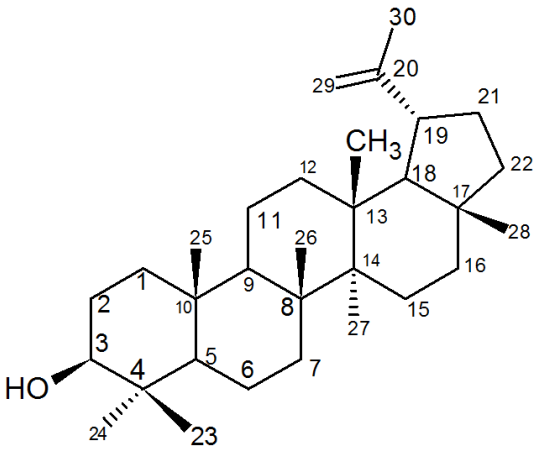
4-O-(α-D-glucopyranosyl)- β-D-glucopyranose  (1)

methyl-4-hydroxybenzoate  (2)

p-hydroxybenzoic acid  (3)

Quercetin 3-O-β-D-4C1-galactopyranoside  (4)

β-Sitosterol-β-D-glucoside  (5)

Lupeol  (6)

4.2. Anticancer Activity

According to cytotoxicity or growth inhibition results of the different type of cancer by *β-sitosterol-β-D-glucoside*, it was found that *β-sitosterol-β-D-glucoside* highly toxic to Lung carcinoma followed by Colon carcinoma, Hepatocellular carcinoma, Breast carcinoma, Larynx carcinoma, while the Cervical carcinoma is the least affected one.

Regarding, the viability of the different carcinoma cell lines exposed to the serial concentration of *β-sitosterol-β-D-glucoside* extract, the results indicated that there is inverse relationship between concentration used and viability of the different carcinoma cells. Also, it was found that Cervical carcinoma was the most resistant to *β-sitosterol-β-D-glucoside* followed by larynx carcinoma, Breast carcinoma, Hepatocellular carcinoma, Colon carcinoma, whereas, Lung carcinoma is the most susceptible one. When comparing among the different carcinoma types according to potency level, it can be arranged descendingly according number of folds, as following Lung carcinoma, Colon carcinoma, Hepatocellular carcinoma, Breast carcinoma, Prostate carcinoma, Larynx carcinoma and/or Cervical carcinoma. These results are going in the same line with that of Chariandy, C. M. *et al.* [22] who concluded that the ethanolic extract of *J. neesii* have anticancer effect. Moreover, Subbaraju, G. V. *et al.* [31] suggested that ethanolic extract of *J. glauca* have cytotoxicity effect. Also, Konoshima, T. *et al.* [23] & Kavitha, J. *et al.* [24] mentioned that methanolic extract of *J. purpurea* has antitumor effect on mouse, skin, and pulmonary carcinogenesis. In recent years, extensive phytochemical and pharmacological studies have further confirmed that Taiwanin E methyl ether (TEME), a kind of arylnaphthalene lignan, was first isolated from *J. procumbens* in 1996, is a potential antitumor active component in *J. procumbens* [25]. TEME has shown some anticancer activities on many kinds of cancer cells with *in vitro* methyl thiazolyl tetrazoliym assay metabolic rate screening in laboratory, including human colon cancer cell (HT-29), human liver cancer cell (Bel-7402) and human colonic gland cancer cell (HT-29) in preliminary assay. In addition, further research has indicated that TEME shows some tumor-inhibiting effects in S180 tumor-bearing rats and H22 transplanted rats. Owing to its potential antitumor effects, TEME is being further investigated as a new drug-development target [1].

J. procumbers in Chinese folklores is used as an herbal remedy for treatment of cancer. The methanol extract of the whole plant showed significant inhibitory activity *in-vivo* against P-388 lymphocytic leukemia growth in BDF, male mice (at 50 mg/kg/day i.p.) as well as *in-vitro* in 9-KB (human nasopharyngeal carcinoma) cell culture assay [25]. Justicidin B isolated from ethanolic extract of *J. pectoralis* was screened for cytotoxicity *in-vitro* and found to be active in P-388 (9 PS) ED; 50 3.3 pg /ml and in NSCLCN6 IC50 28pg/ml. Elenoside isolated from *J. hyssopifolia* was found to be cytotoxic to various leukemia cell lines at a concentration of 10"4 M (79-97% growth inhibition). Some activity was also observed at 10'4 M against specific melanoma cell lines, CNS cancer cell line, a renal cancer cell line and colon cancer cell line. Cytotoxicity of various compounds isolated from *J. ciliata* were studied against several cancer cell types using MTT assay. J-A, cilinaphthalide A and Taiwanin E methyl ether exhibited significant *in- vitro* cytotoxicity. *J. micrantha* showed a weak cytotoxic effect (*in vitro*) against human nasopharyngeal carcinoma and human colorectal adenocarcinoma. Ethanolic extract exhibited anticancer activity against P-388 lymphocytic leukaemia in mice [26, 27].

5. Conclusion

Generally, six metabolite compounds were isolated and identified from leaves extract of *Justicia brandegeana.* As well as their structure was elucidated based on different spectroscopic analysis. In addition, anti-tumor activity of *β-sitosterol-β-D-glucoside* in different cell lines was evaluated. Thus, *β-sitosterol-β-D-glucoside* is a promising anti-cancer drug specifically against lung cancer after determining the safe dose for human use.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

S. A., A. E, and I. M. conceived and designed the experiments; A. E., I. M., S. A., A. A. performed plant extraction, purification, and analysis. A. E., S. A., A. A., I. M., A. H. and K. S. performed data analysis and paper writing.

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References

[1] Geone M. C., Antônio F. de C. A., 2012. Chemical constituents and biological activities of species of *Justicia* - a review Revista Brasileira de Farmacognosia Brazilian, *Journal of Pharmacognosy* 22 (1): 220-238.

[2] Maria, R. J., Angel, J. A., Luis, A. S., Candy, C., Luis, Á. G., Fabiola, D., Sandra, P. M., Concepción, G., Marisela, R. G., Maricela, G., Eduardo, E., Rocio, Z., Pedro, M., Alejandro, G., 2011. Antimicrobial and Cytotoxic Effects of Mexican Medicinal Plants. *Natural Product Communications,* 6 (12), 1925-1928.

[3] Alonso-Castro, A. J., Villarreal, M. L., Salazar-Olivo, L. A., Gomez Sanchez, M., Dominguez, F., Garcia-Carranca, A., 2011. Mexican medicinal plants used for cancer treatment: pharmacological, phytochemical and ethnobotanical studies. *J. Ethnopharmacol* 133: 945-972.

[4] Hernando Herrera-Mata, Alfredo Rosas-Romero and Oscar C. V., 2008. Biological activity of "Sanguinaria" (*Justicia secunda*) extracts. Pharmaceutical Biology. 40 (3): 206-212.

[5] García-Carrancá, A., 2011. Antimicrobial and cytotoxic effects of Mexican medicinal plants. *Natural Product Communications*, 6 (12): 1925-1928.

[6] Kumar, S. R., C. Venkateshwar, G., Samuel, Gangadhar, R. S., 2013. Phytochemical screening of some compounds from leaf extracts of *Holoptelea integrifolia* (Planch.) and *Celestrus emarginata* (Grah.) used by Gondu tribes at A dilabad district, Andhra Pradesh, India. *International Journal of Engineering Science Invention*, (2), 65–70.

[7] Ibrahim, M. T., Shafei, A. A., Mahrous, F., 2017. Phytochemical and Biological Studies of Natural Egyptian Recipe with Anticancer Effect. *IOSR Journal of Pharmacy and Biological Sciences,* 12 (1) 29-39.

[8] Wang, H., Khor, T. O., Shu, L., Su, Z. Y., Fuentes, F., Lee, J. H., Kong A. N., 2012. Plants vs. cancer: A review on natural phytochemicals in preventing and treating cancers and their drug ability. Anti-cancer Agents Med. Chem., 12 (10) 1281-1305.

[9] Alasmary FA, Awaad AS, Kamal M, Alqasoumi SI, Zain ME. Antitumor activity of extract and isolated compounds from *Drechslera rostrata* and *Eurotium tonophilum*. *Saudi Pharm J*. 26 (2): 279- 285. https://doi.org/10.1016/j.jsps.2017.11.011

[10] Elaasser, M. M., Abdel-Aziz, M. M., El-Kassas, R. A., 2011. Antioxidant, antimicrobial, antiviral and antitumor activities of pyranone derivative obtained from *Aspergillus candidus*. *J. Microbiol Biotech Res*. 1, 5-17.

[11] Gómez-Sánchez, M., Goma, S. M, Rihadh, S. M., Mohammoud, E. A., Elaasser, M. M., 2015. Synthesis and anticancer activities of thiazoles, 1,3-Thiazines and Thiazolidine using Chitosan-Grafted-Poly (vinylpyridine) 91 (6): 1227-1243.

[12] William, A. B. 2006. NMR Spectroscopy in the study of carbohydrates: characterizing the structural complexity school of molecular and microbial biosciences, University of Sydney, New South Wales Australia.

[13] Geoffrey, F., Antony, B., Bruce, S., 2009. Chemistry, Biochemistry, and Biology of 1-3 Beta Glucans and Related Polysaccharides. Chapter 3.1 - Plant and Microbial Enzymes Involved in the Depolymerization of (1,3)-β-d-Glucans and Related Polysaccharides. 119-170.

[14] Amelia, T. H., Melo, A. A., Paulo, R. M. H., Ene L. L., 1996. *Ervatamia coronaria*: Chemical constituents and some pharmacological activities. *J. Ethnopharmacology*. 50 (1): 1925.

[15] Agrawal, P. K., 1989. ^{13}C NMR of flavonoids, Chapter 6, Elsevier, New York. Alonso-Castro, A. J., Villarreal, M. L., Salazar-Olivo, L. A., Gomez Sanchez, M., Dominguez, F., Garcia-Carranca, A., 2011. Mexican medicinal plants used for cancer treatment: pharmacological, phytochemical and ethnobotanical studies. *J. Ethnopharmacol* 133: 945-972.

[16] Jamal, A. K., Yaacob, W. A., Din. L. B., 2008. A chemical study on Phyllanthus reticulates, *Journal of Physical Science,* 19 (2), pp. 45-50.

[17] Peshin, T.; Kar, H., 2017. Isolation and Characterization of _-Sitosterol-3-O-_-D-glucoside from the Extract of the Flowers of Viola odorata. Br. J. Pharm. Res. 16, 1–8.

[18] Pavia, D. L., Gary, M.; Lampman, G. and Kriz, S., 2001. Introduction to spectroscopy 3rd ed., 58.

[19] Kolak, U., Topcu, G., Birteksoz, S., Otuk, G. and Ulubelen, A., 2005. Terpenoids and steroids from the roots of *Salvia blepharochlaena*, *Turk. J. Chem*., 29: 177-186.

[20] Bachheti, R. K. P., Pandey, D., Archana, J., Vikas, R., 2011. Chemical analysis of aerial parts of *Justicia gendarussa*. *Int. J. Chem Tech. Res.,* 3 (1): 244-247.

[21] Kavitha, K., Sridevi -Sangeetha, K. S., Sujatha, K., Umamaheswari, S., 2014. Phytochemical and pharmacological profile of *Justicia gendarussa* Burm f. – review. *Journal of Pharmacy Research.,* 8 (7) 990-997.

[22] Chariandy, C. M., Seaforth, C. E., Phelps, R. H., Pollard, G. V., Khambay, B. P. S., 1999. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. *J Ethnopharmacol* 64: 265-270.

[23] Konoshima, T., Atta-Ur-Rahman, T., 2000. Anti-tumor-promoting activities (cancer chemo preventive activities) of natural products. *Stud Nat Prod Chem* 24: 215-267.

[24] Kavitha, J., Gopalaiah, K., Rajasekhar, D. G, Subbaraju, V. 2003. Juspurpurin, an unusual secolignan glycoside from *Justicia purpurea. J Nat Prod. 66*: 1113-1115.

[25] Fukamiya, N., Lee, K. H., 1986. Two cytotoxic principles from *Justicia procumbens. J. Nat. Prod*., 49 (2), 348-350.

[26] Rao, C. B., Suseela, K., Rao, P. V. S., Krishna, P. G., Raju, G. V. S., 1984. Chemical examination of some Indian medicinal plants. *Indian. J. Chem.,* 23B (8) 787-788.

[27] James, L. C., 1998. Antiviral activity of lignans. J. Nat. Prod., 61, 1447-1451.

[28] Finar, I. L., 1975. Organic chemistry, Stereochemistry and the chemistry of Natural products, 5th ed., 2. 696.

[29] Liebermann, C., Burchard, H., 1980. Chem. Zentr. (61) 1.

[30] Solihah, S., Ikram, M. S., 2013. Phenolic compounds from the fruits of *Orania sylvicola* (Sebatian Fenol daripada Buah *Orania sylvicola*). *The Malaysian Journal of Analytical Sciences*, 17, (2) 276-280.

[31] Subbaraju, G. V., Kavitha, J. D. R., Rajasekhar, D., Jimenez, J. I., 2004. Jusbetonin, the first indolo [3,2-b] quinoline alkaloid glycoside, from *Justicia betonica*. *J Nat Prod.* 67: 461-462.