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Original Article

Cytotoxic, antioxidant and antimicrobial activities of *Ipomoea carnea spp. fistulosa* (Mart. ex Choisy) D. Austin

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ABSTRACT

Cytotoxic, antioxidant and antimicrobial activities of *Ipomoea carnea spp. fistulosa* (Mart. ex Choisy) D. Austin were evaluated. The *n*-butanol fraction of the leaves showed the highest cytotoxic activity on MCF-7 and HpeG2 cell lines. The methanolic extract from the flowers showed the highest antioxidant activity (using DPPH radical scavenging test). Furthermore, the antimicrobial activity was carried out against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Penicillium chrysogenum*. The methanolic extract from the leaves and its *n*-butanol fraction showed higher indicative antibacterial activity. 2-C-methyl-D-erythritol and quercetin were isolated for the first time, in addition to *β*-sitosterol, umbelliferone, kaempferol-3-*O*-*β*-D-glucoside (astragalin) and swainsonine alkaloid.

Key Words: DPPH, MCF-7, HpeG2, 2-C-methyl-D-erythritol and swainsonine.

INTRODUCTION

Ipomoea carnea spp. fistulosa (Mart. ex Choisy) D. Austin [1] known also as *Ipomoea carnea* Jacquin [2]. It is commonly called pink morning glory or bush morning glory and it is an ornamental tree belonging to the Convolvulaceae family [3]. This plant is a native woody perennial, aggressive weed in wetlands, toxic to cattle and difficult to eradicate [4,5]. Moreover, this plant is used in folk medicine for healing wounds, skin infections and leucoderma [6], and also as a topical antiseptic and anti-rheumatic remedy [7].

Malignancy is one of the most serious diseases that damage human health in the modern world. According to the World Health Organization, 7.6 million people died of cancer in 2008 accounting for around 13% of all deaths. This number is projected to continue to rise to over 11 million in 2030 [8].

Many polyhydroxylated alkaloids, flavonoids and other compounds have been reported to show anti-cancer activity. To date, swainsonine has not been used as an anti-cancer drug, but there is a new strategy by which a low dosage of swainsonine can be used to recover the effectiveness of 5-FU or other chemotherapeutic reagents in tumors with acquired resistance [9].

The methanolic extract and the polar fractions of *I. carnea* Jacq. leaves exhibited significant antioxidant activity as they are rich in strong antioxidants, but it is also promising to say that all the fractions of this plant are potentially valuable sources of natural antioxidants and bioactive materials [10–12]. The acetone extract of *I. carnea* Jacq. leaves showed antimicrobial activity against two strains, *Proteus vulgaris* and *Salmonella typhimurium* [13].

The present study traced detailed biological assessment of variant organs extracts and fractions to discover if this plant showed a significant activity or not. Antioxidant, antimicrobial and cytotoxic activities against two different cancer cell lines types were investigated.

MATERIALS AND METHODS

For biological assays:
Antioxidant assay: 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) was purchased from (Sigma-Aldrich, Inc.,

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USA) and quercetin from Sigma Chemical Co., St. Louis, MO.

Cytotoxic assay: Breast carcinoma cell line (MCF-7) and liver carcinoma cell line (HepG2) were obtained from the American Type Culture Collection (ATTC), dimethyl sulfoxide (DMSO), trichloroacetic acid, sulforhodamine B (SRB), ethylenediaminetetraacetic acid buffer (EDTA) and acetic acid, from Sigma Chemical Co. St. Louis, MO.

Antimicrobial assay: This was performed using two different media: Nutrient agar for bacteria and Sabouraud Dextrose agar for fungi (DIFCO, USA). *Staphylococcus aureus* ATCC–6538 P, a standard strain representative of gram-positive bacteria, *Escherichia coli* ATCC–10536, a standard strain representative of gram-negative bacteria, *Candida albicans* ATCC–10231, a standard strain representative of yeast and *Penicillium chrysogenum* ATCC–11709, a standard strain representative of mould were obtained from the American Type Culture Collection (ATTC), from Sigma Chemical Co. St. Louis, MO.

Apparatus: An ELISA Processor II Microplate Reader was used in the cytotoxic assessment, while a UNICO spectrophotometer (UV/Vis) UV-2000 (UNICO Instrument CO. LTD, USA) was used in the antioxidant assay.

Institutions: The cytotoxic activity was performed in collaboration with the Egyptian National Cancer Institute, while the antimicrobial activity was performed jointly with the *Holding Company for Biological Products & Vaccines* (VACSERA).

For phytochemical studies:

^{1}H-NMR and ^{13}C-NMR spectra were recorded with a JEOL alpha 500 MHz spectrometer at 500 and 125 MHz, respectively. Chemical shifts were given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. Column chromatography (CC) was carried out on silica gel G (70─230, mesh, Merck), Alumina (Sigma-Aldrich chemical Co., USA), type WN-3, neutral and Sephadex LH-20 (20─100 µm, Sigma-Aldrich chemical Co., USA). Liquid/Liquid extraction was carried out using (Dowex 50W-X8) 20-50 u.s. mesh (Na), (BDH Chemicals, Ltd., England). TLC was carried out on silica gel plates (Kieselgel 60 F_{254}, Merck). All the reagents and solvents used for separation and purification were of analytical grade.

***Plant material*:** Leaves, flowers and seeds of *Ipomoea carnea* Jacq. were collected in September 2011 from plants growing on the borders of irrigation canals and drains in Al Qurashiyyah village, Al Santah, El-Gharbia governorate, Egypt. The plant identity was kindly verified by Prof. Kamal Hussien Shaltout, plant ecology professor at the Botany Department, Faculty of Science, Tanta University. A voucher specimen was deposited in the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Tanta University.

Extraction and Isolation: The air-dried powdered leaves and flowers (1 kg) of each, were separately extracted with MeOH by the cold maceration method untill exhaustion. The total methanolic extract was then evaporated under reduced pressure to give 196 and 171 g residue for leaves and flowers respectively. The methanolic residue was partitioned with petroleum ether (60-80^{o}C), chloroform, ethyl acetate and *n*-butanol. Different fractions were concentrated to give petroleum ether, chloroform, ethyl acetate and *n*-butanol residues in g (41.3, 36.3, 44.6, and 58.6) respectively in case of leaves. The flowers residues in g were (34.7, 32.1, 43.8 and 45.2) for petroleum ether, chloroform, ethyl acetate and *n*-butanol extracts respectively.

The air-dried powdered seeds (340 g) were defatted with petroleum ether, then extracted with 50% aqueous EtOH to give 3 g of brown gummy residue.

The petroleum ether residue of the leaves (8 g) was saponified. The unsaponified matter (3 g) was subjected to an alumina column (120 g) and eluted with a gradient solvent system (Benzene:EtOAC = 95:5→30:70) to give 6 fractions (Fr. 1~6). Fr. 2 gave compound (1) (20 mg) and Fr. 5 (340 mg) was further refractionated using silica gel column (10 g), and eluted with solvent system (Pet. ether:CH_2Cl_2 = 50:50) to afford compound (2) (73 mg).

The ethyl acetate fraction of the flowers (1 g) was chromatographed on silica gel G column (50 g). Gradient elution was used, starting with CH_2Cl_2 and increasing the polarity with EtOAC in 5% stepwise elutions to 100% EtOAC and then increasing the polarity with MeOH in 5% stepwise increments to give 8 fractions (Fr. 1~8). Fr. 4 (43 mg) was purified by Sephadex LH-20 (100% MeOH) to give compound (3) (23 mg).

The *n*-butanol residue of the leaves (10 g) was chromatographed on silica gel G column (400 g) and gradient eluted with ($CHCl_3$:MeOH:H_2O = 9:1:0.1→6:4:1) to give 7 fractions (Fr. 1~7). Fr. 2 (200 mg) was further refractionated using a silica gel column (8 g) and isocratically eluted using a solvent system ($CHCl_3$:MeOH:H_2O = 9:1:0.1) to give five subfractions (Fr. 2-1~ Fr. 2-5). Subfraction 2-2 (34 mg) was purified by Sephadex LH-20 (100% MeOH) to give compound (4) (7 mg). Fr. 6 (972 mg) was chromatographed on

Sephadex LH-20 (100% MeOH) to give 4 subfractions (Fr. 6-1 ~ Fr. 6-4). Subfraction 6-3 (540 mg) was further refractionated and purified using silica gel column (25 g) and isocratically eluted using solvent system ($CHCl_3$:MeOH:H_2O = 6:4:0.5) to give compound (5) (324 mg).

3 g of aqueous ethanolic seeds extract was partitioned between 2% acetic acid and chloroform (200 ml). The chloroform layer was separated and the aqueous acid solution extracted twice more with chloroform (200 ml). The aqueous acidic portion was added to a 1000 ml separating funnel, with a glass wool plug above the stopcock, containing approximately 300 ml of Dowex 50W-8X ion-exchange resin. The solution and resin were mixed intermittently for 30 minutes and then the acid solution was drained into a vacuum flask under reduced pressure. The resin was washed three times with deionised distilled water (200 ml/wash) in a similar manner until it was free from acidity, and then the resin was washed three times with 200 ml of 1 M ammonium hydroxide. The combined aqueous base solution was lyophilized then redissolved in (50 ml) of 1 M ammonium hydroxide. A solution of 10% sodium hydroxide (1 ml) was added, followed by 50 ml of methanol. The basic aqueous methanol mixture was continuously extracted for 48 hours with dichloromethane (200 ml), using a liquid/liquid extractor heavier-than water. The dichloromethane solution was concentrated and then the processes of dissolving in ammonium hydroxide, methanol and sodium hydroxide as well as the liquid/liquid extraction with dichloromethane were repeated. The dichloromethane solution was evaporated to give 26 mg residue, which was recrystallised by dichloromethane / methanol mixture (96:4) to give compound (6) (20 mg).

Biological assessment of different extracts of Ipomoea carnea Jacq: Different methanolic extracts of flowers, leaves and seeds and their fractions (petroleum ether, methylene chloride, ethyl acetate and *n*-butanol) were assessed as antioxidant, antimicrobial and cytotoxic agents.

Antioxidant assay [14]**:** The antioxidant activity of different concentrations (2.5, 5, 8, 10, 20 mg/ml) of the methanolic extract of *I. carnea* Jacq. leaves, flowers and seeds were determined according to Kadri et al (2011) using the stable radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH). The degree of change in color from purple to yellow can be used as a measure of the scavenging potential of antioxidant extracts. Blank sample (ethanol without DPPH), negative control (ethanol + DPPH) and positive control, quercetin, were prepared. Each sample was measured in triplicate by using UV-Vis spectrophotometer at 517 nm and the percentage of scavenging was calculated.

Cytotoxic activity against two different cell lines [15]**:** The test was carried out according to Skehan et al. (1990) using 100 µg/ml in DMSO from the methanolic extract of each of the leaves, flowers and seeds of *I. carnea* Jacq. The extracts of the organs with the highest activities were fractionated using pet. ether, chloroform, ethyl acetate and *n*-butanol, then they were tested as a single dose (100 µg/ml). Then the most effective fraction was tested in multiple doses (5, 12.5, 25 and 50 µg/ml) using doxorubicin as a standard. The IC_{50} was determined and classified according to the procedure of Ayyad et al. (2012) procedure into very strong activity (1 to 10), strong activity (11 to 25), moderate activity (26 to 50), weak activity (51 to 100), very weak activity (101 to 200) and non cytotoxic (above 200) [16].

Antimicrobial assay [13]**:**
A) ***Antibacterial activity:*** Anti-bacterial activity was determined using 1 mg of the extracts by the paper disc diffusion method [13]. Two standard strains for gram-positive and gram-negative bacteria *Staphylococcus aureus* ATCC–6538 P and *Escherichia coli* ATCC–10536, respectively, were examined

Procedure: DMSO was used as a negative control and Streptomycin (10 µg/disc) as a positive control.
B) ***Antifungal activity*** [13]**:** The above procedure was followed using two different fungal strains: *Candida albicans* and *Penicillium chrysogenum*. Fluconazole (25 µg/disc) was used as a positive control. The classification of antibacterial and antifungal activity, according to zone diameter interpretive criteria (mm), were defined as ≥15 (susceptible), 12-14 (intermediate) and ≤11(resistant) [17].

RESULTS AND DISCUSSION

For biological studies: The obtained results revealed that the methanolic extract of the flowers displayed the strongest radical scavenging activity, while the weakest activity was observed with seeds (2.5 mg/ml) in comparison to quercetin as shown in (Table 1 and Fig. 1 and 2). The results of the cytotoxic activity of the methanolic extracts of the leaves, flowers and seeds are demonstrated in (Table 2 and Fig. 3).

The methanolic extracts of the leaves showed high cytotoxic activity compared to the methanolic extracts of the flowers and seeds in the HpeG2 cell line. While the methanolic extracts of the leaves and seeds showed convergent cytotoxic activity in

the MCF-7 cell line, in which both were higher than the methanolic extract of the flowers. Cytotoxic activities of the methanolic extract fractions of the leaves are displayed in (Table 3 and Fig. 4).

The *n*-butanol fraction showed the highest activity on both cell lines. Multi-dose cytotoxic activity of the *n*-butanol fraction of the leaves on MCF-7 and HpeG2 cell lines are shown in (Table 4 and Fig. 5, 6) respectively. It was found that the *n*-butanol fraction of the leaves reduced the viability of MCF-7 and HpeG2 cells with IC_{50} = 4.30 and 6.10 µg/ml, respectively. This fraction showed a strong activity when comparing IC_{50} with doxorubicin as shown in (Table 5, 6 and Fig. 7, 8 and 9).

The present study indicates that methanolic extract of the leaves showed higher antibacterial activity than the methanolic extracts of the flowers and seeds. The *n*-butanol fraction of the leaves exhibited indicative activity against gram-positive and gram-negative bacteria as shown in (Table 7). The methanolic extract of the seeds was the most effective against *Candida albicans*, but its effect was very weak in comparison to the positive control as shown in (Table 8).

For phytochemical studies:

Compound (1): White needle crystals. m.p. (137-139ºC), Co-TLC with an authentic *β*-sitosterol showed R_f = 0.40 using solvent system (Benzene:EtOAc = 90:10). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.65-2.24 (*m*, H-1, 2, 4, 7-9, 11, 12, 14-17, 20, 22-25, 28), 0.67 (3H, *s*, H-18), 0.78 (3H, *d*, *J* = 6.4 Hz, H-27), 0.81 (3H, *d*, *J* = 6.4 Hz, H-26), 0.82 (3H, *m*, H-29), 0.92 (3H, *d*, *J* = 6.3 Hz, H-21), 1.01 (3H, *s*, H-19), 3.52 (1H, *tdd*, *J* = 4.5, 4.2, 3.8 Hz, H-3), 5.35 (1H, *t*, *J* = 4.8 Hz, H-6).

Compound (2): White fine powder. m.p. (232ºC), ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.15 (1H, *dt*, *J* = 9.7 Hz, H-3), 6.67 (1H, *s*, H-8), 6.74 (1H, *d*, *J* = 8.6 Hz, H-6), 7.47 (1H, *d*, *J* = 8.6 Hz, H-5), 7.88 (1H, *d*, *J* = 9.5 Hz, H-4). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 102.7 (C-8), 111.8 (C-10), 111.9 (C-3), 113.6 (C-6), 130.2 (C-5), 145.0 (C-4), 156.0 (C-9), 160.9 (C-7), 161.8 (C-2).

Compound (3): Yellow amorphous powder. m.p. (176-178ºC), ^{1}H-NMR (500 MHz, Pyridine-d_5) δ: 3.90-4.20 (6H, *m*, glc H-2, H-3, H-4, H-5, H_2-6), 5.86 (1H, *d*, *J* = 7.45 Hz, glc H-1), 6.62 (1H, *d*, *J* = 2.1 Hz, H-6), 6.65 (1H, *d*, *J* = 2.1 Hz, H-8), 7.24 (2H, *d*, *J* = 8.6 Hz, H-3′, 5′), 8.26 (2H, *d*, *J* = 8.6 Hz, H-2′, 6′),. ^{13}C-NMR (125 MHz, Pyridine-d_5) δ: 93.6 (C-8), 98.4 (C-6), 103.6 (C-10), 114.9 (C-3′, C-5′), 120.5 (C-1′), 130.6 (C-2′, 6′), 133.5 (C-3), 156.3 (C-9, 2), 159.4 (C-4′), 160.7 (C-5), 164.9 (C-7), 177.2 (C-4); (glc C-1–6) δ: 102.2, 74.2, 77.1, 69.6, 76.3 , 60.7.

Compound (4): Yellow amorphous powder. m.p. (316-318ºC), Co-TLC with an authentic quercetin showed R_f = 0.72 using solvent system (CH_2Cl_2:MeOH:H_2O = 9:1:0.1), ^{1}H-NMR (500 MHz, Pyridine-d_5) δ: 6.71 (1H, *d*, *J* = 1.7 Hz, H-6), 6.76 (1H, *d*, *J* = 1.7 Hz, H-8), 7.39 (1H, *d*, *J* = 8.6 Hz, H-5′), 7.96 (1H, *d*, *J* = 8.0 Hz, H-6′), 8.45 (1H, *d*, *J* = 1.7 Hz, H-2′).

Compound (5): Light yellow powder. m.p. (82-83ºC), ^{1}H-NMR (500 MHz, D_2O) δ: 1.07 (3H, *s*, CH_3-2), 3.46 (1H, *d*, *J* = 11.5 Hz, H-1), 3.54 (1H, *dd*, *J* = 8.6, 11.5 Hz, H-4), 3.54 (1H, *d*, *J* = 11.5 Hz, H-1), 3.61 (1H, *dd*, *J* = 2.8, 8.6 Hz, H-3), 3.84 (1H, *dd*, *J* = 2.3, 11.5 Hz, H-4). ^{13}C-NMR (125 MHz, D_2O) δ: 18.5 ($\underline{C}H_3$-2), 62.1 (C-4), 66.4 (C-1), 74.2 (C-2), 75.0 (C-3).

Compound (6): White crystals. m.p. (144-145ºC), ^{1}H-NMR (500 MHz, D_2O) δ: 1.32 (1H, *m*, H-7*α*), 1.59 (1H, *m*, H-6*β*), 1.81 (1H, *m*, H-6*α*), 2.07 (1H, *dd*, *J* = 9.2, 3.4 Hz, H-5*β*), 2.39 (1H, *m*, H-8a), 2.96 (1H, *m*, H-7*β*), 3.11 (1H, *dd*, *J* = 12.2, 10.6 Hz, H-5*β*), 3.68 (1H, *m*, H-5*α*), 3.76 (1H, *m*, H-3*α*), 3.86 (1H, *ddd*, *J* = 9.7, 5.8 Hz, H-8), 4.32 (1H, *m*, H-2), 4.43 (1H, *m*, H-1). ^{13}C-NMR (125 MHz, D_2O) δ: 23.7 (C-6), 32.4 (C-7), 52.5 (C-5), 60.8 (C-3), 67.1 (C-8), 69.0 (C-2), 69.5 (C-1), 75.2 (C-8a).

Identification of the compounds isolated in **(Fig. 10)** was based on comparison of our ^{1}H-NMR and ^{13}C-NMR data with those reported in literature, and their structures were elucidated as *β*-sitosterol (1) [18], umbelliferone (2) [19], kaempferol-3-*O*-*β*-D-glucoside (astragalin) (3) [20–22], quercetin (4) [23,24], 2-C-methyl-D-erythritol (5) [25] and swainsonine alkaloid (6) [26–28]. 2-C-methyl-D-erythritol and quercetin were isolated for the first time from *I. carnea* Jacq.

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Table 1: Free radical scavenging activity

Sample	Concentration in assay (mg/ml)	% Scavenging of DPPH ± S.E.
Leaves methanolic extract	20	96.79 ± 0.15
	10	92.95 ± 0.04
	8	79.82 ± 0.14
	5	62.09 ± 0.02
	2.5	43.00 ± 0.03
Flowers methanolic extract	20	95.62 ± 0.06
	10	91.02 ± 0.04
	8	81.59 ± 0.05
	5	73.05 ± 0.04
	2.5	67.45 ± 0.06
Seeds methanolic extract	20	72.11 ± 0.04
	10	64.60 ± 0.04
	8	37.23 ± 0.03
	5	22.85 ± 0.03
	2.5	20.62 ± 0.04
Quercetin	2.5	100

Table 2: Single dose cytotoxic activity of *I. carnea* Jacq. methanolic extracts of leaves, flowers and seeds

	% Inhibition ± S.E.	
Organ	***HpeG2 cell line***	***MCF-7 cell line***
Leaf	80.74 ± 0.77	79.06 ± 1.06
Flower	55.91 ± 0.95	66.41 ± 1.04
Seed	76.06 ± 0.96	74.23 ± 2.13

Table 3: Single dose cytotoxic activity of fractions of methanolic extract of the leaves

Sample (Leaf fraction)	***% Inhibition ± S.E.***	
	HpeG2 cell line	***MCF-7 cell line***
Pet. ether fraction	69.90 ± 0.42	69.55 ± 0.22
Chloroformic fraction	54.48 ± 0.19	57.50 ± 0.14
Ethyl acetate fraction	66.52 ± 0.19	62.26 ± 0.08
n-Butanol fraction	74.65 ± 0.10	76.80 ± 0.09

Table 4: M**ulti-dose cytotoxic activity of *n*-butanol fraction of the leaves on MCF-7 and HpeG2 cell lines**

Sample conc. (µg/ml)	***MCF-7 cell line***	***HpeG2 cell line***
	Mean ± S.E	***Mean ± S.E***
0.00	1.000 ± 0.016	1.000 ± 0.059
5.00	0.438 ± 0.037	0.521 ± 0.016
12.50	0.280 ± 0.036	0.324 ± 0.036
25.00	0.166 ± 0.014	0.216 ± 0.015
50.00	0.254 ± 0.029	0.202 ± 0.018

Table 5: Multi-dose cytotoxic activity of doxorubicin on MCF-7 and HpeG2 cell lines

Sample conc. (µg/ml)	***MCF-7 cell line***	***HpeG2 cell line***
	Mean ± S.E	***Mean ± S.E***
0.00	1.000 ± 0.014	1.000 ± 0.056
5.00	0.400 ± 0.032	0.463 ± 0.013
12.50	0.440 ± 0.035	0.427 ± 0.021
25.00	0.377 ± 0.029	0.439 ± 0.025
50.00	0.405 ± 0.031	0.444 ± 0.029

Table 6: Comparison between the IC_{50} of *n*-butanol fraction and doxorubicin on both MCF-7 and HpeG2 cell lines

Sample	IC_{50} µg/ml	
	MCF-7 cell line	HpeG2 cell line
n-Butanol	4.30	6.10
Doxorubicin	4.13	4.37

Table 7: Antibacterial activity of total methanolic extracts and fractions of *I. carnea* Jacq. against *Escherichia coli* and *Staphylococcus aurues*

Sample	*Diameter of inhibition zone (mm)*[a]	
	Escherichia coli	*Staphylococcus aurues*
-ve control	-	-
Leaves methanolic extract	17	19
Flowers methanolic extract	8	-
Seeds methanolic extract	-	13
Leaves Pet. ether fraction	-	-
Leaves Chloroform fraction	-	-
Leaves Ethyl acetate fraction	9	-
Leaves *n*-Butanol fraction	21	32
Flowers Pet. ether fraction	-	-
Flowers Chloroform fraction	-	-
Flowers Ethyl acetate fraction	12	-
Flowers *n*-Butanol fraction	8	9
+ve control	15	15

- = no activity.

(a) Zone of inhibition including the diameter of filter paper disc (7 mm).

Table 8: Antifungal activity of methanolic extracts and fractions of *I. carnea* Jacq. against *Candida albicans* and *Penicillium chrysogenum*

Sample	*Diameter of inhibition zone (mm)*[a]	
	Candida albicans	*Penicillium chrysogenum*
-ve control	-	-
Leaves methanolic extract	-	-
Flowser methanolic extract	9	-
Seeds methanolic extract	13	-
Leaves Pet. ether fraction	-	-
Leaves Chloroform fraction	-	-
Leaves Ethyl acetate fraction	-	-
Leaves *n*-Butanol fraction	11	-
Flowers Pet. ether fraction	-	-
Flowers Chloroform fraction	10	-
Flowers Ethyl acetate fraction	-	-
Flowers *n*-Butanol fraction	-	-
+ve control	20	18

- = no activity.

(a) Zone of inhibition including the diameter of filter paper disc (7 mm).

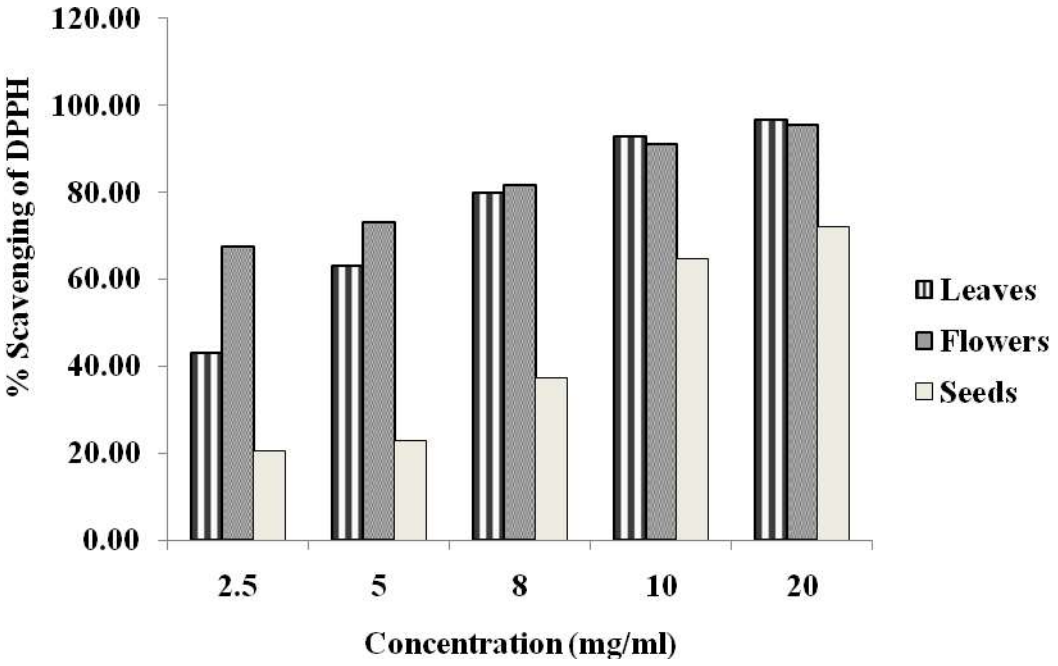

Fig. 1. Free radical scavenging activity of methanolic extracts of different organs

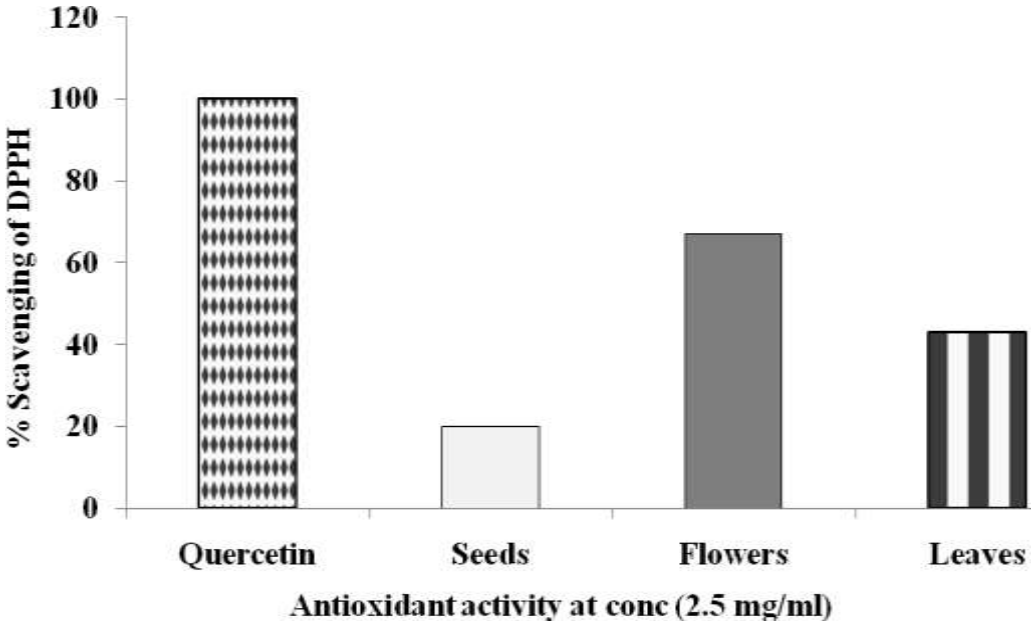

Fig. 2. Scavenging activity of methanolic extracts of different organs at conc. (2.5 mg/ml) and quercetin

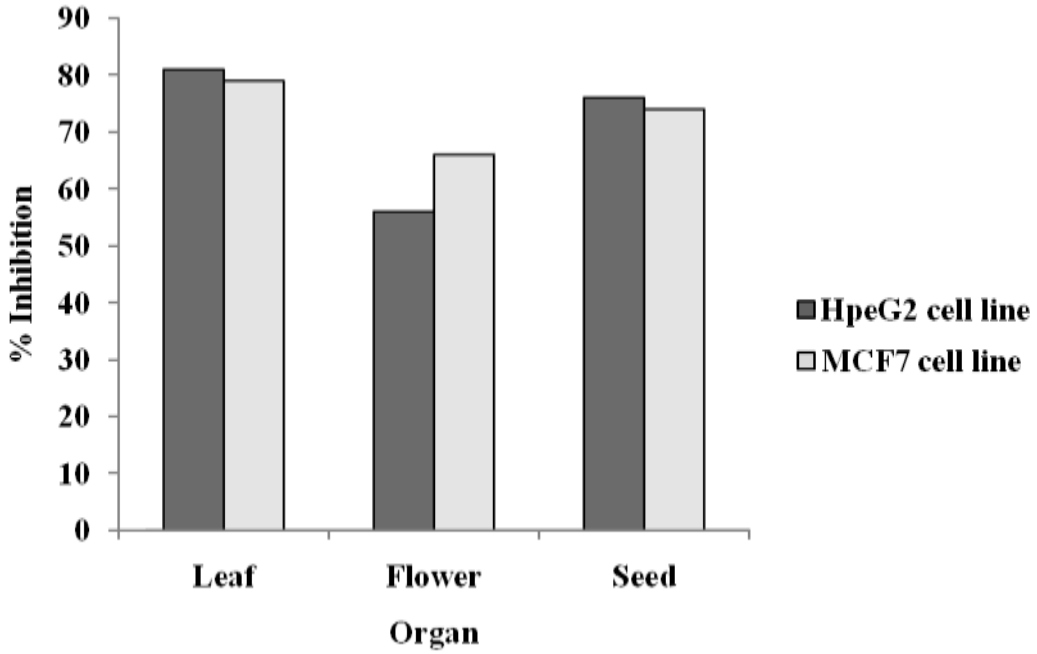

Fig. 3. Single dose cytotoxic activity of methanolic extracts of the leaves, flowers and seeds

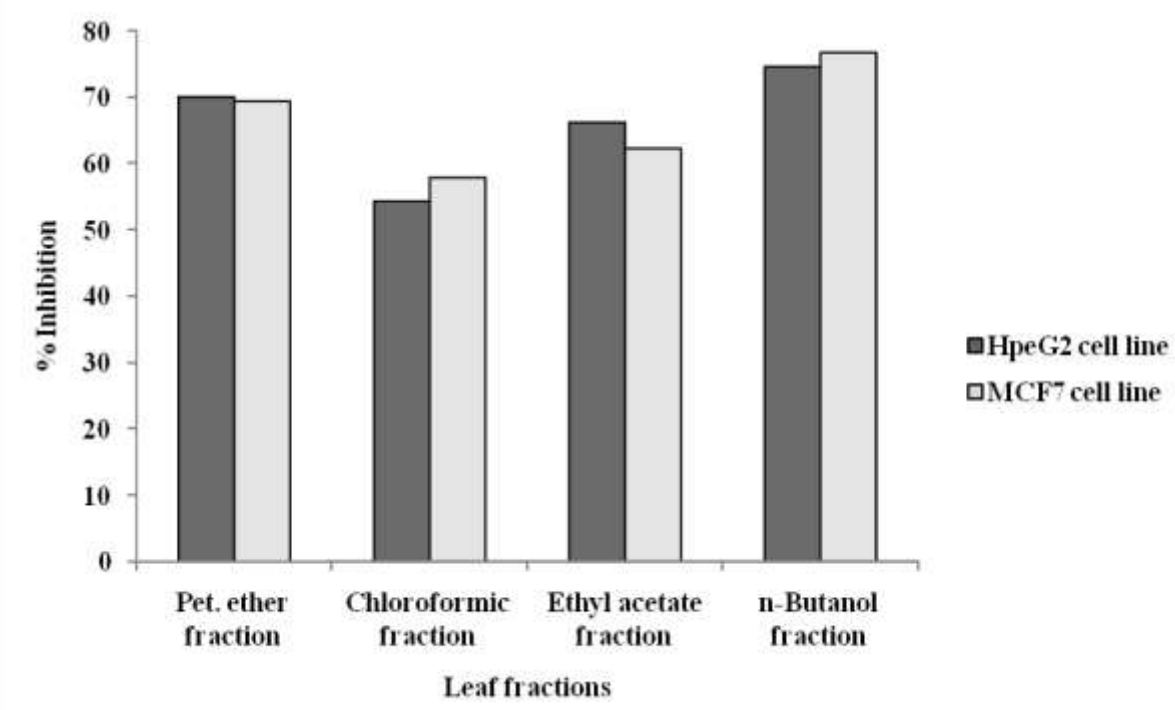

Fig. 4. Single dose cytotoxic activity of methanolic extract fractions of the leaves

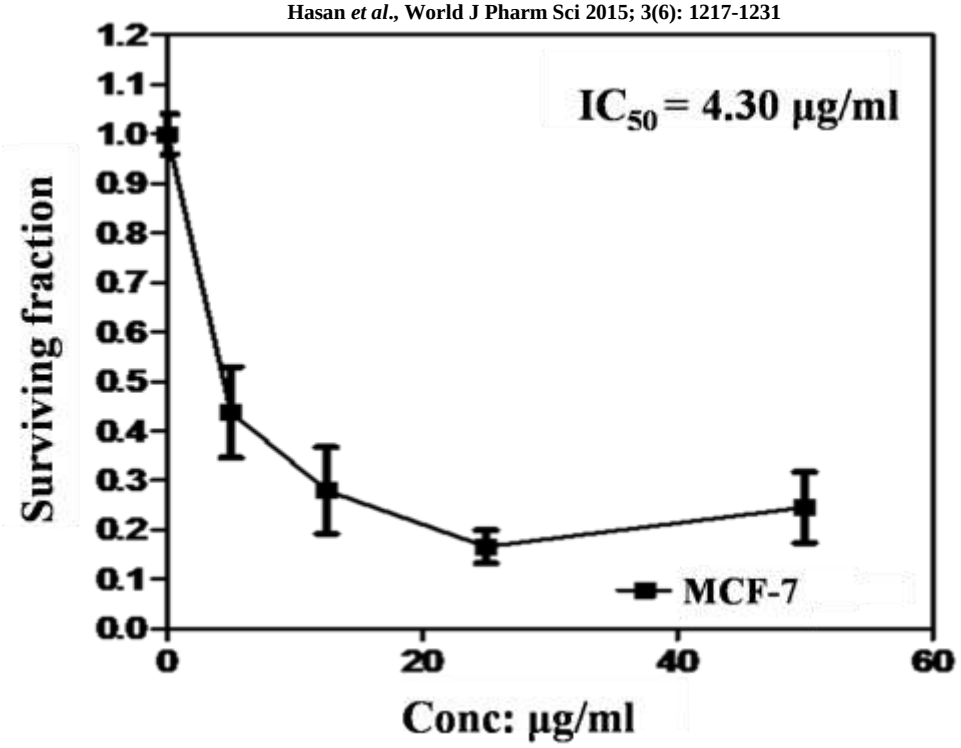

Fig. 5. Multi-dose cytotoxic activity of the leaves *n*-butanol fraction on MCF-7 cell line

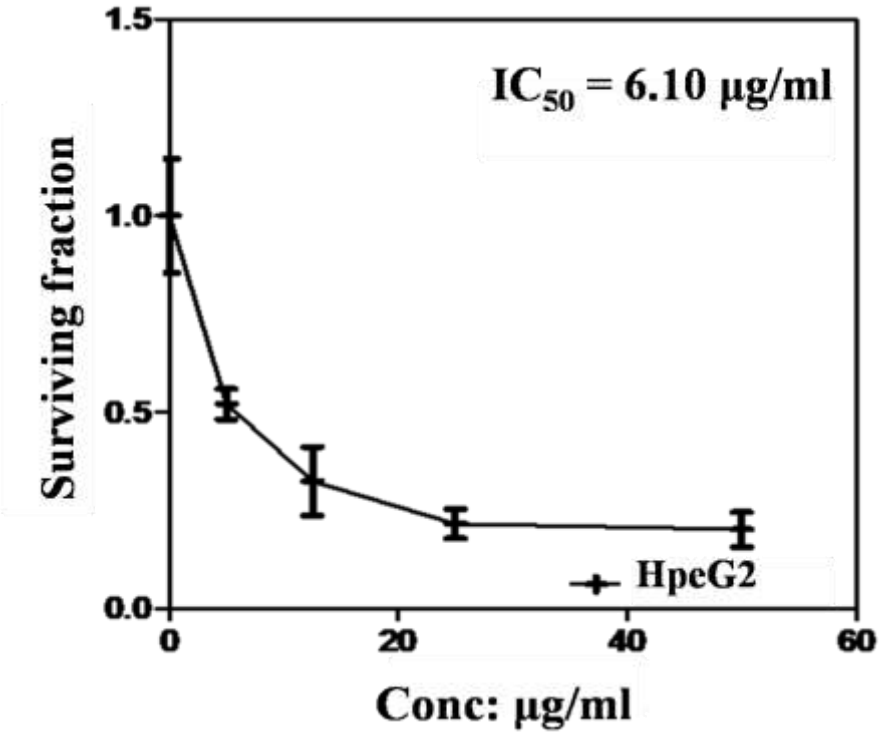

Fig. 6. Multi-dose cytotoxic activity of the leaves *n*-butanol fraction on HpeG2 cell line

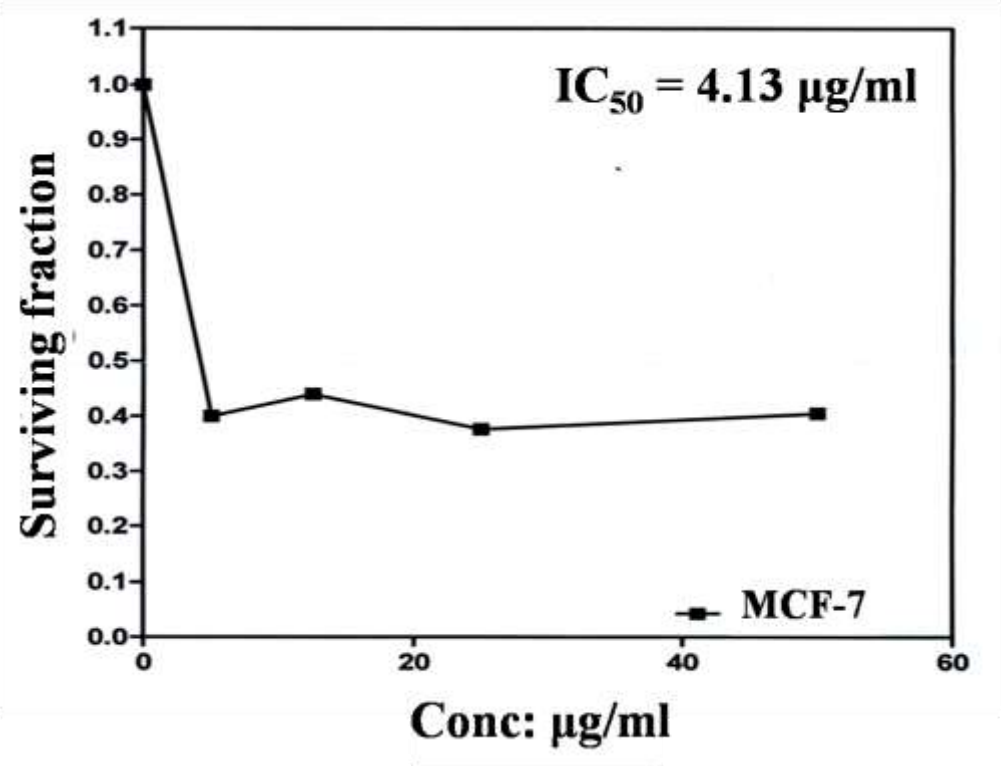

Fig. 7. Multi-dose cytotoxic activity of doxorubicin on MCF-7 cell line

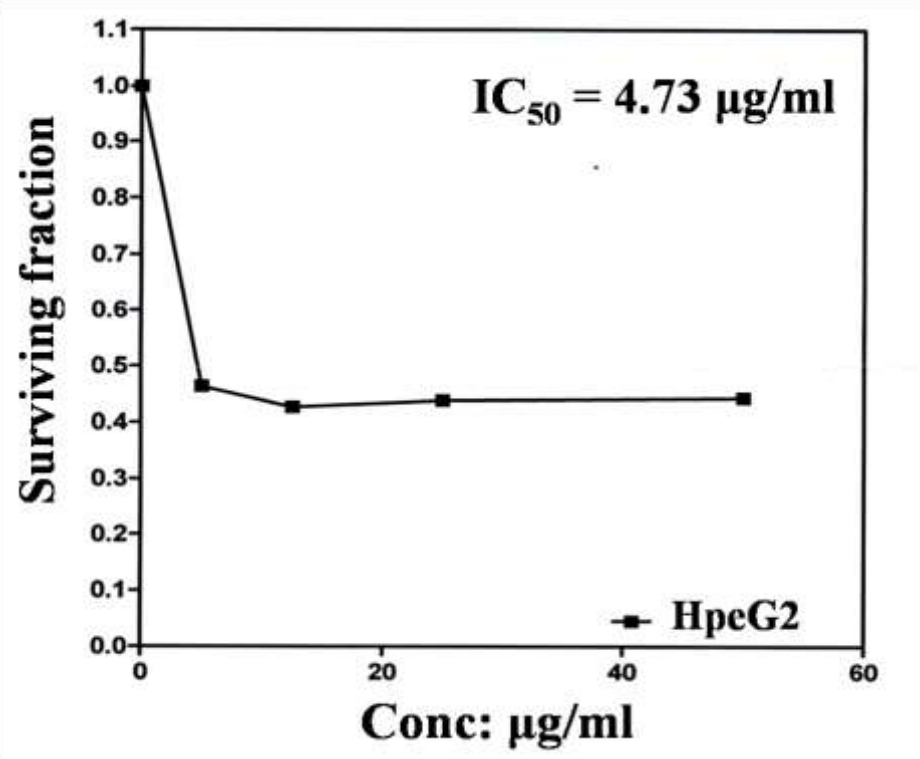

Fig. 8. Multi-dose cytotoxic activity of doxorubicin on HpeG2 cell line

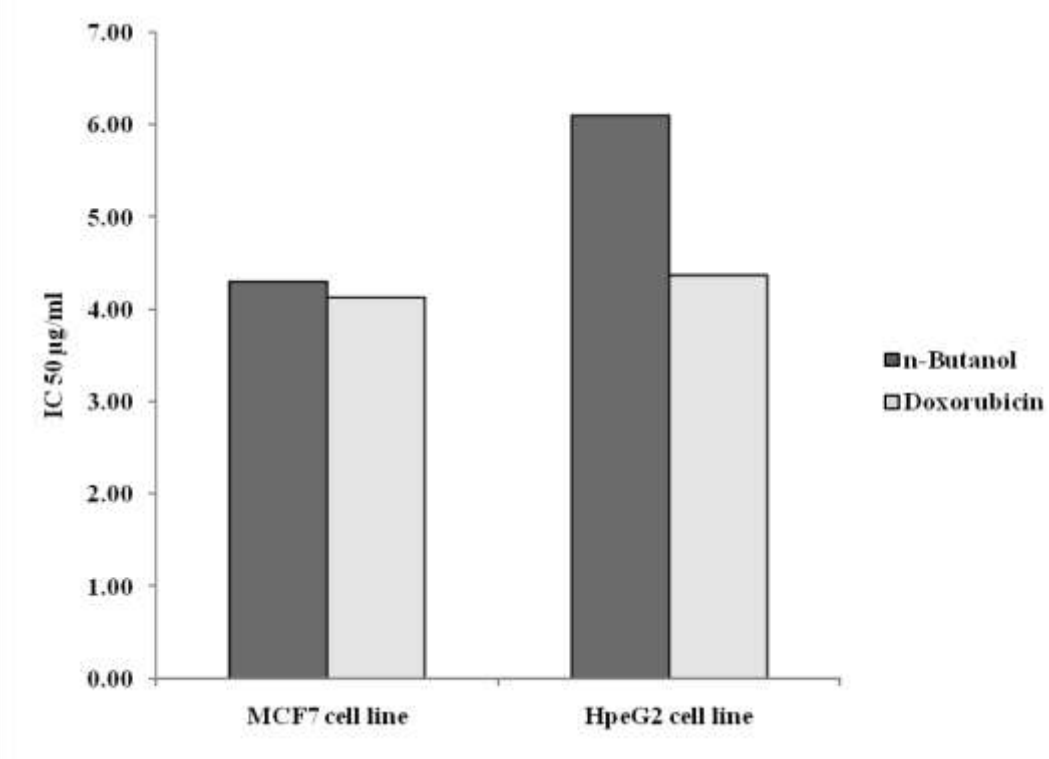

Fig. 9. IC_{50} of *n*-butanol fraction and doxorubicin

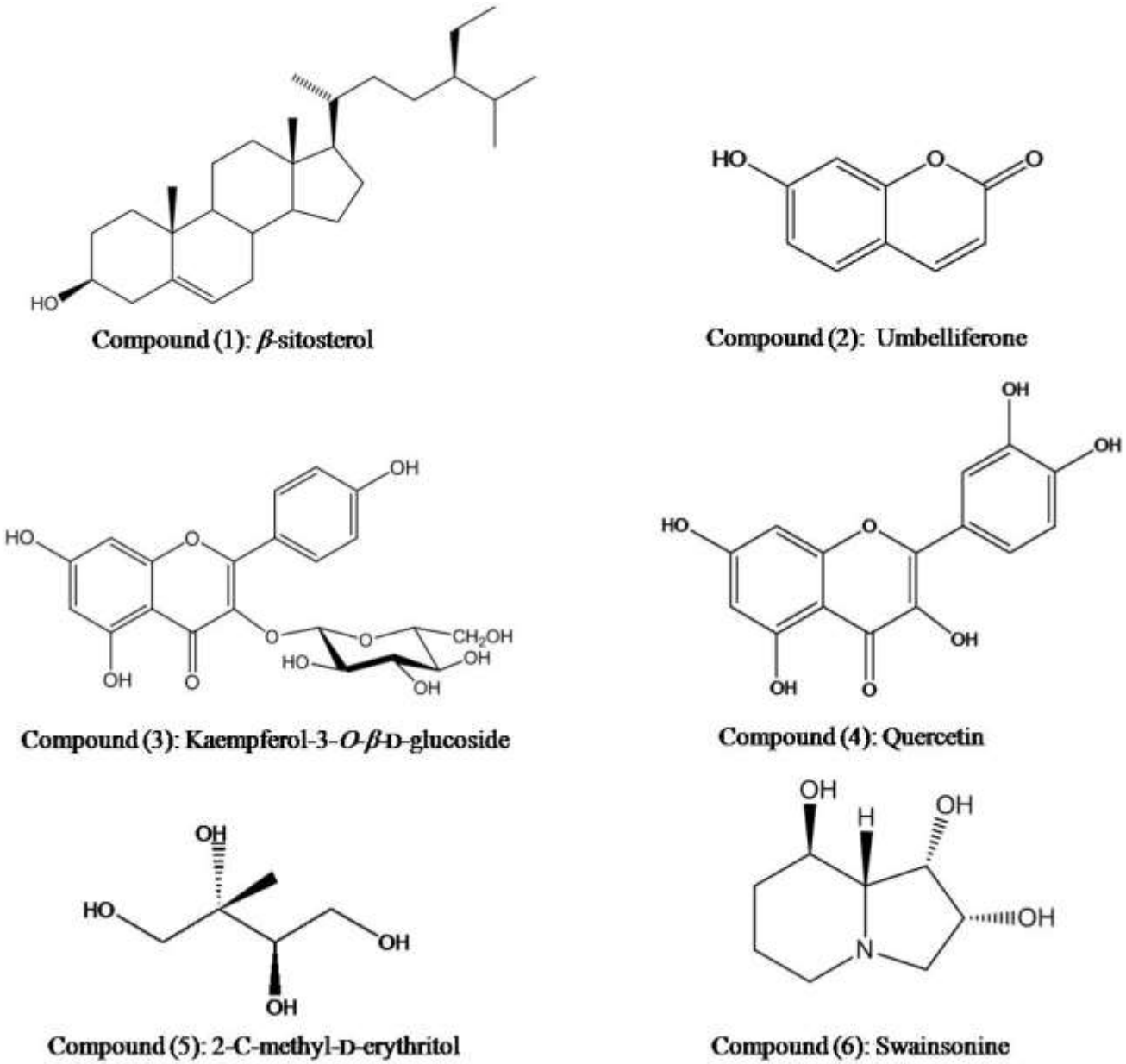

Fig. 10. Structures of compounds (1–6) isolated from *Ipomoea carnea* Jacq.

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