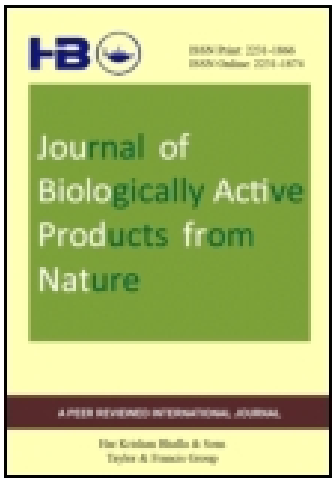

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GC-MS profile and cytotoxicity of the hydrodistilled and extracted volatiles of the buds and flowers of Spathodea campanulata P. Beauv.

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GC-MS profile and cytotoxicity of the hydrodistilled and extracted volatiles of the buds and flowers of *Spathodea campanulata* P. Beauv.

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Abstract: Volatiles of *Spathodea campanulata* buds and expanded flowers were isolated by hydrodistillation / solvent extraction and direct solvent extraction. Products analysed included: primary and recovered oils (PO/RO) derived from distillates, chloroform extractives (CE) of the non-distilled fractions, and hexane concretes (HC). Number of components identified by GC-MS varied from 35-60 (88.56 - 98.17 % of the total composition). PO/RO showed close amounts of hydrocarbons and oxygenated compounds; yet, the latter prevailed in CE and HC samples. Hydrocarbon fraction was the least in buds CE (15.36 %) and greatest in flowers PO/RO (48.04 %); meanwhile, oxygenated constituents dominated flowers HC (74.04 %). Chief hydrocarbons were: alkanes and alkenes (flowers CE, 32.46 %; major phytane 16.41 %), and terpenoids (PO/RO: buds 22.44 %, major α-pinene 9.34 % *vs*. flowers 19.91 %, major β-selinene 8.51 %). Oxygenated constituents included: carbonyl compounds (flowers CE 31.83 %; major 6-benzofurancarboxyaldehyde 27.75 %); acids (HC: flowers 52.16 %, major hexadecanoic acid 32.47 % *vs*. buds 51.64 %, major 9, 12-octadecadienoic acid 30.19 %); esters and lactones (buds CE, 66.24 %; major 1, 2-benzenedicarboxylic acid diisooctyl ester, 38.99 %; and PO/RO, buds 31.52 % *vs*. flowers 28.01 %). The volatiles exhibited variable cytotoxic activities against (MCF7) and (HCT116) cell lines. Buds HC demonstrated the lowest IC_{50} (4.2 μg/ml). In conclusion, the stage of development and isolation techniques obviously influenced yield, composition and bioactivity.

Key words: *Spathodea campanulata*, floral volatiles, isolation techniques, stage of development, GC-MS, cytotoxicity.

Introduction

Spathodea is a monotypic Bignoniaceous genus [1]. The single species *Spathodea campanulata* is commonly known as Fountain or African tulip tree; it is native to equatorial Africa and is planted extensively as ornamental throughout the tropics for its very showy reddish orange or crimson (rarely yellow) flowers [2] . The flower buds are brown, horn-shaped, and filled with sap that squirts out on squeezing [1]. The plant name "*Spathodea*" refers to the shape of the calyx from Greek "spathe-like". The tree is medium sized with large pinnate foliage and large showy orange or scarlet, curved flattened, tulip-like flowers arranged in terminal panicles or racemes [3] above the foliage at the top of the trees.

Most traditional medicinal uses [4-6] and scientifically-based bioactivities [6,7-18,] as well as, phytochemical studies [17,18,19-24] were ascribed to the stem barks and leaves. However, relatively little information could be traced concerning those of the flowers. These were reported to be used as diuretic and anti-inflammatory [25] and the flower extract for silk dyeing [26]. In addition, the insect mortality [27], anti-solar [28], antioxidant [29,30] and antimicrobial [25,31, 32] effects of the flowers were evaluated.

Among constituents reported from the flowers

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were: anthocyanins [33,34] and triterpenoids and steroids in the floral nectar [35]. Recently, the ethanolic extract of the flower was analyzed by GC-MS [36] and the phytochemical study was carried out [31,37,38]. In another publication, we reported the isolation and identification of phytol, α-methylcinnamaldehyde, β-sitosterol-3-acetate, naringenin, catechin-3-O-α-rhamnopyranoside and 5,6,4'trihydroxy flavonol-7-O-α-rhamnopyranoside from the ethanol extract of the flower [32].

The lack of published data concerning the composition and cytotoxity of the floral volatiles of either the exotic or local plants stimulated the performance of this work. The influence of stage of development and isolation techniques on the chemical profile and bioactivity were investigated.

Material and methods

Plant material

Inflorescences of *Spathodea campanulata* P. Beauv. were collected, from trees cultivated in the National Egyptian Zoo-Garden (Giza, Egypt) during the flowering season (June-September, 2009-2011). The plant was kindly authenticated by Mrs. Therese Labib (Herbarium Section, Orman Garden, Giza, Egypt) and its identity was confirmed by Dr. Hasnaa Ahmed Hosny, Professor of Plant Taxonomy (Department of Botany, Faculty of Science, Cairo University). Voucher specimens (# 013.04.30) were kept at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt. Buds and expanded flowers were separated and individually used while fresh.

Isolation procedures

Hydrodistillation/Solvent extraction

Fresh samples of buds and expanded flowers (2 Kg, each) were, separately, hydrodistilled in a Clavenger-type apparatus by applying the method of Rajeswara *et al.*[39]. The 'primary oils' (PO) were separated from the distillate and dried over anhydrous sodium sulphate. The yellow aqueous condensate was then collected and exhaustively extracted with hexane to trap any dissolved volatiles; the fragrant hexane extract obtained was subjected to distillation under reduced pressure at a temperature not exceeding 40°C, for 1 h, to yield the 'recovered oils' (RO) which, after drying (anhydrous sodium sulphate), were mixed with their corresponding PO to yield the overall hydrodistilled oily volatiles (PO/RO). Moreover, the aromatic-smelling components of the non-distilled water were obtained as chloroform extractives (CE). This was performed, after removal of the plant material *via* filtration, by repeated liquid-liquid extraction followed by complete evaporation of the solvent and drying.

Direct solvent extraction

Fresh samples of buds and expanded flowers (1 Kg, each) were separately extracted by cold percolation with hexane till exhaustion. The solvent, in each case, was completely distilled under reduced pressure at a temperature not exceeding 40°C to yield the hexane concrete (HC).

The percentage yields (w/w) of the volatiles PO, RO, CE, and HC were calculated on fresh weight basis and listed in Table 1. The samples were then stored, in sealed glass vials, at -4°C for analysis.

GC-MS analysis

The chemical profiles of the isolated products *viz.*, PO/RO, CE, and HC of both buds and expanded flowers were investigated by GC-MS. The analysis was performed on Agilent 6890 gas chromatograph equipped with Agilent mass spectrophotometric detector, with a direct capillary interface and fused silica capillary column HP-5 MS (30 m x 320 μm x 0.25 μm film thickness). Helium was used as carrier gas at approximately 1 ml/min., pulsed splitless mode. The solvent delay was 3 minutes and the injection size was 1 μl. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 eV and scanning being from m/z 50 to 500. The ion source temperature was 230°C and the quadrupole temperature 150°C. The electron multiplier voltage (EM voltage) was maintained at 1050 V above auto tune. The instrument was manually tuned using perfluorotributylamine (PFTBA). The GC temperature program was started at 70°C (3

min.) then elevated to 260°C at a rate of 8°C/ min. the injector and detector temperatures were set at 250 and 280°C, respectively.

Identification of the components was achieved by library search database, Wiley Mass Spectral Database (Wiley 7 Nist 05 Lib. and W8N08 Lib.) and by comparing their retention times, retention indices and mass fragmentation patterns with those of the available references, as well as, published data [40-44]. The percentage composition was determined by computerized peak area measurements. The various types of non-oxygenated and oxygenated constituents detected in the different samples were comparatively displayed in Tables (2 and 3).

Evaluation of the cytotoxic potential

The cytotoxic potential of all samples (PO/RO, CE and HC) was *in-vitro* assessed against two tumor cell lines *viz*., breast carcinoma cell line (MCF7), and colon carcinoma cell line (HCT116). Cytotoxic investigation was carried out at the Cancer Biology Department of National Cancer Institute (Cairo, Egypt), adopting sulforhodamine B stain (SRB) assay [45]. The volatiles and different extracts were tested for their cytotoxicity at different concentrations in DMSO (0-25 µg/ml), against human tumor cell lines. The results were compared to those of the standard doxorubicin (10 mg, Adriamycin hydrochloride, in 5 ml IV injection, Pharmacia, Italy) at the same concentrations. The surviving fractions, expressed as percent, are recorded in Table 4. The dose which reduces cell viability of each cell line to 50 % (IC_{50}) was deduced for the individual samples and the values were presented in Figure 1.

Results and Discussion

The slightly fragrant products isolated by the two processes from the buds and expanded flowers of *Spathodea campanulata* P. Beauv. were obtained as viscous liquids (PO/RO) and semisolid residues (CE and HC). They slightly differ in color from pale (PO/RO) to dark (CE and HC) yellow. The total yield of the hydro-distillation / solvent extraction products (PO/RO and CE) exceeded those obtained by direct solvent extraction (HC) reaching about 1.6 times in the buds and 1.3 times in the flowers (as depicted in Table 1).

Results obtained by GC-MS analysis of the diverse samples revealed relevant qualitative and quantitative variability probably due to difference in both the stage of flower development and isolation techniques. The components were categorized according to their chemical nature and listed, together with their identification criteria and relative percentages, in Tables (2 and 3).

The PO/RO samples showed approximately similar amounts of hydrocarbons and oxygenated constituents; nevertheless, in the CE and HC samples the oxygenated components prevailed. The lowest percentage of hydrocarbons was recorded for the CE of the buds (15.36) and the highest in the PO/RO of the flowers (48.04). Meanwhile, the highest percentage of oxygenated constituents (74.04 and 73.2 %) was found in the HC of the flowers and CE of the buds, respectively, and the lowest percentage recorded for its PO/RO (49.21 %).

A series of alkanes (Table 2), C9-C29, mostly with straight chain carbon skeleton, was detected; 15 of which were common in all the samples (C12, C13, and C16-C29). A single alkene, eicosene <1-> was identified in the CE samples. The highest amount of alkanes and alkenes was in the CE of the flower (32.46 %) with phytane as major (16.41 %) and were the least in the HC of the buds (12.49 %). Terpenoid hydrocarbons (Table 2) were detected in appreciable amounts in the PO/RO samples (buds 22.44 and flowers 19.91 %); monoterpenoids prevailed in the buds (16.25 %, with major α-pinene 9.34 %) and were absent in the flowers in which, only sesquiterpenoids were detected being dominated by β-selinene (8.51 %). The diterpenoid abietatriene was detected in traces in the PO/RO of the buds. Aromatic hydrocarbons were minor, with a total amount reaching from 0.22-3.90 % in the different samples and are present mainly in the CE and HC samples.

Diverse types of oxygenated constituents were identified (Table 3): carbonyl compounds dominated the CE of the flowers (31.83 %), acids the HC samples (flowers 52.16 %, buds 51.64 %) and esters and lactones the CE of the buds

Table 1. Percentage yield of the volatiles of the buds and expanded flowers of *Spathodea campanulata* P. Beauv.

Plant material	Isolated products (% yield, g/100g fresh weight)				
	Hydrodistillation/ Solvent extraction				Solvent extraction
	PO	RO	CE	Total	HC
Buds	0.17	0.27	0.26	0.70	0.44
Expanded flowers	0.23	0.31	0.24	0.78	0.61

PO: primary oil
RO: recovered oil
CE: chloroform extractive
HC: hexane concrete

(66.24 %) as well as the PO/RO samples (buds 31.52 %, flowers 28.01 %). Alcohols were detected in lower amounts in all the samples (0.28-7.8 %) and phenols (0.49-3.22 %) in all except HC of the flowers. The CE samples appeared free from acids, ethers and oxides. Constituents detected in appreciable amounts were: 1,2 benzenedicarboxylic acid diisooctyl ester (38.99 %, CE of buds), hexadecanoic and 9, 12-octadecadienoic (linoleic) acids (32.47 and 30.19 %, HC of flowers and buds, respectively) along with 6-benzofurancarboxyaldehyde (27.75 %, CE of flowers). In addition, the profile of all samples was characterized by the presence of damascone <β-3 hydroxy-> (0.13 -0.89 %), a norisoprenoid derivative from the rose ketones recognized as important fragrant ingredients in perfumery even at low concentration [46].

Besides, a number of non-oxygenated and oxygenated hydroazulenes were identified in the PO/RO samples reaching upto 11.87 % in the flowers with aromadandrene (5.11 %) and longifolol (2.88 %) as major. Meanwhile, their percentage was lower in the buds (6.02 %), in which longifolene (2.19 %) dominated the hydrocarbon fraction and longifolol (1.07 %) was the major alcohol of the group.

Notably, only few of the aforementioned components were previously identified by GC-MS in the petroleum ether, methanolic [37] and ethanolic [36] extracts of the flowers collected from plants growing abroad; the detected constituents included heneicosane, tricosane, hexadecanoic acid methyl ester and 1,2- benzene dicarboxylic acid diisooctyl ester and oleic acid in the petroleum ether extract and an azulene derivative in methanol extract [37] as well as, hexadecanoic acid, oleic acid and 1, 2 benzene bicarboxylic acid diisooctyl ester in the ethanolic extract [36]. Nevertheless, constituents like levodopa, bis (2-ethylhexyl phthalate) (petroleum ether extract), thujopsene (methanol extract) [37] and butane, 1,1 diethoxy-3-methyl- (ethanolic extract) [36] could not be identified in any of the samples derived from the local plant but instead 9,12 octadecadienoic (linoleic) acid and 6-benzofuran carboxy-aldehyde, tetradecanoic acid, isopropyl ester and 9,12,15 octadecatrienoic acid (Z,Z,Z), methyl ester (Methyl linolenate) were identified.

Evaluation of the Cytotoxic Potential

Under the experimental conditions adopted and from data displayed in Table (4) and represented in Figure (1), it could be concluded that the floral volatiles of the buds and expanded flowers of *Spathodea campanulata* P. Beauv. exhibited variable antitumor activities against the two tested cell lines viz., Mammary Carcinoma (MCF7) and Human Colon Carcinoma (HCT116) cell lines.

In this respect, the hexane concrete (HC) of the buds of *Spathodea campanulata* P. Beauv. demonstrated the lowest IC_{50} when tested against MCF7 cells (4.2 µg/ml). Concerning the effects of the tested samples on HCT116, the hexane concrete HC of the flower appeared the most active followed by the CE of the buds (IC_{50}, 15.6 and 19.5 µg/ml, respectively) being much less in activity compared to Doxorubicin (IC_{50}. 0.7 µg/

Table 2. Non-oxygenated compounds identified by GC-MS in the distilled primary and recovered oils (PO/RO), chloroform extractives (CE) and hexane concretes (HC) of the buds and expanded flowers of *Spathodea campanulata* P. Beauv.

KI	RT	Compounds	Relative percentage (%)					
			PO/RO		CE		HC	
			Bud	Flower	Bud	Flower	Bud	Flower
		Alkanes						
900	5.58	Nonane	-	-	-	-	0.11	0.59
1100	9.93	Undecane	0.34	0.10	-	-	0.54	0.25
1160	11.18	Undecane, 2-methyl-	-	-	-	-	0.54	1.00
1170	11.34	Undecane, 3- methyl	-	0.67	-	-	-	1.66
1200	11.92	Dodecane	0.31	1.01	0.20	0.24	0.65	1.29
1253	12.52	Dodecane, 6- methyl	-	-	-	-	0.18	-
1300	14.52	Tridecane	0.10	0.41	0.22	0.45	0.33	0.22
1600	18.54	Hexadecane	0.34	0.63	0.65	0.28	0.07	0.44
1642	19.39	Undecane,1-cyclopentyl	-	-	-	0.28	-	-
1700	20.13	Heptadecane	0.10	0.39	1.71	0.61	0.37	0.56
1800	21.31	Octadecane	0.87	0.07	1.20	0.38	0.48	1.03
1808	21.44	Hexadecane, 2,6,10,14-tetramethyl- [Phytane]	-	0.39	1.74	16.41	-	-
1900	22.96	Nonadecane	0.66	0.70	0.60	0.36	0.19	1.00
1995	23.49	1-Eicosene	-	-	0.61	0.51	-	-
2000	23.44	Eicosane	0.11	0.32	1.01	0.82	-	-
2100	25.2	Heneicosane	1.04	1.12	1.71	2.93	1.00	0.68
2200	26.13	Docosane	1.16	8.49	1.10	2.02	0.07	0.91
2300	27.28	Tricosane	5.63	6.24	0.91	1.22	3.15	2.01
2400	28.23	Tetracosane	1.50	1.48	0.15	2.58	1.56	1.17
2500	29.23	Pentacosane	3.50	4.41	0.75	0.97	1.00	1.04
2600	30.2	Hexacosane	0.81	0.25	0.39	0.75	0.09	0.17
2700	31.13	Heptacosane	1.63	0.87	0.37	0.63	0.78	1.40
2800	31.97	Octacosane	0.47	0.08	0.37	0.38	0.45	0.27
2900	32.98	Nonacosane	0.72	0.28	0.20	0.66	0.93	0.72
Total % of alkanes and alkenes			19.29	27.91	13.89	32.48	12.49	16.41

table 2. (continued).

KI	RT	Compounds	Relative percentage (%)					
			PO/RO		CE		HC	
			Bud	Flower	Bud	Flower	Bud	Flower
		Monoterpenoids						
939	6.34	α-Pinene	9.34	-	-	-	2.62	-
954	6.69	Camphene	0.63	-	-	-	0.06	-
979	7.31	β-Pinene	5.81	-	-	-	0.97	-
991	7.65	β-Myrcene	-	-	-	-	3.04	-
1003	7.94	α-Phellandrene	-	-	-	-	0.35	-
1029	8.49	Limonene	0.47	-	-	-	3.76	-
1030	8.63	β-Phellandrene	-	-	-	-	0.50	-
1108	11.55	*p*-mentha-1, 3, 8-triene	-	-	0.23	0.17	-	-
Total of monoterpenoid hydrocarbons			16.25	0	0.23	0.17	11.3	0.0
		Sequiterpenoids						
1377	15.14	α-Copaene	0.19	-	-	-	0.13	-
1389	15.48	β-Elemene	0.59	3.14	-	-	0.76	1.21
1408	16.17	Longifolene [AZ]	2.19	0.07	-	-	0.07	-
1412	16.52	α-Cedrene [AZ]	0.38	0.25	-	-	0.07	-
1440	16.58	α-Guaiene [AZ]	1.41	1.17	-	-	0.15	-
1441	16.61	Aromadendrene [AZ]	0.31	5.11	-	-	-	-
1490	17.51	b-Selinene	1.03	8.51	-	-	0.19	2.61
1498	17.53	Viridiflorene[AZ]	-	0.45	-	-	-	-
1529	17.64	*trans*-Calamenene	-	0.66	-	-	-	-
1677	19.96	Cadalene	-	0.55	-	-	-	-
Total of sesquiterpenoid hydrocarbons			6.10	19.91	0	0	1.37	3.82
		Diterpenoids						
2057	24.86	Abietatriene	0.09	-	-	-	-	-
Total of diterpenoid hydrocarbons			0.09	0	0	0	0	0

table 2. (continued).

KI	RT	Compounds	Relative percentage (%)					
			PO/RO		CE		HC	
			Bud	Flower	Bud	Flower	Bud	Flower
		Aromatic hydrocarbons						
994	7.18	1,3,5 Trimethyl benzene [Mesitylene]	-	-	-	-	0.11	1.47
1025	8.44	p-Cymene	0.25	0.22	-	-	0.76	0.65
1050	8.99	1,2 -Diethyl benzene	-	-	-	-	0.49	1.61
1305	14.65	Naphthalene, 1-methyl	-	-	-	-	0.17	0.17
1407	15.82	Naphthalene, 2,7-dimethyl	-	-	0.32	0.17	-	-
1493	17.15	Acenaphthylene	-	-	0.92	1.52	-	-
Total of aromatic hydrocarbons			0.25	0.22	1.24	1.69	1.53	3.90
Total identified non-oxygenated compounds			41.98	48.04	15.36	34.34	26.69	24.13
No. of identified non-oxygenated compounds			30	29	21	22	36	25

[AZ]: Azulene derivative

Table 3. Oxygenated components identified by GC/MS in the primary and recovered distilled oils (PO/RO), chloroform extractives (CE) and hexane concretes (HC) of the buds and expanded flowers of *Spathodea campanulata* P. Beauv.

KI	RT	Compounds	Relative percentage (%)					
			PO/RO		CE		HC	
			Bud	Flower	Bud	Flower	Bud	Flower
		Alcohols						
1032	10.03	Benzyl alcohol	0.50	0.29	0.45	0.28	-	-
1145	11.02	*trans*-Verbenol	0.56	-	-	-	-	-
1169	11.25	Borneol	0.50	-	-	-	0.30	-
1189	11.62	*trans*-Isocarveol	0.34	-	-	-	0.48	-
1533	18.18	*cis*-Nerolidol	-	0.87	-	-	-	-
1563	18.35	*trans*-Nerolidol	-	0.18	-	-	-	-
1590	18.72	Viridiflorol [AZ]	-	0.73	-	-	-	-

table 3. (continued).

KI	RT	Compounds	Relative percentage (%)					
			PO/RO		CE		HC	
			Bud	Flower	Bud	Flower	Bud	Flower
1601	19.06	Guaiol[AZ]	-	0.66	-	-	-	-
1640	19.28	Isospathulenol [AZ]	0.66	0.55	-	-	-	-
1654	19.68	α-Cadinol	0.81	1.42	-	-	0.17	0.31
1715	20.25	Longifolol [AZ]	1.07	2.88	-	-	-	-
2096	25.34	Phytol (Z)	0.28	-	-	-	4.36	-
	Total of alcohols		4.72	7.58	0.45	0.28	5.31	0.31
		Carbonyl compounds						
1146	11.04	Camphor	1.13	-	-	-	-	-
1289	13.81	2,4,6- Octatrienal	-	-	-	0.66	-	-
1291	14.21	γ-Terpinen-7-al	0.69	-	-	-	-	-
1512	14.82	α-methyl-Cinnamic aldehyde	-	-	0.60	3.97	-	-
1355	16.52	6-Benzofurancarboxyaldehyde	-	-	0.35	25.75	-	-
1455	16.77	Geranyl acetone	-	0.73	-	-	-	0.16
1626	19.24	β-3-hydroxy-Damscone	0.81	0.42	0.77	0.89	0.13	0.41
1715	20.27	Pentadecanal	-	0.41	-	0.26	-	0.13
1830	22.00	2 pentadecanone, 6,10,14 trimethyl [Hexahydro farnesyl acetone]	0.72	1.65	2.31	0.30	0.15	0.35
1913	23.02	Farensyl acetone <(5E,9E)>	0.25	2.90	-	-	-	-
2888	32.71	Cyclopropaneoctanal, 2-octyl	-	-	-	-	0.20	-
	Total of carbonyl compounds		3.6	6.11	4.03	31.83	0.48	1.05
		Acids						
1965	23.96	Hexadecanoic acid	0.16	5.74	-	-	20.33	32.47
2152	25.65	9,12-Octadecadienoic, (E,E) acid [linoleic acid]	3.41	1.16	-	-	30.19	18.69
2170	25.72	9-Octadecenoic , (E) acid [oleic acid]	-	-	-	-	1.12	1.00
	Total of acids		3.57	6.9	0	0	51.64	52.16
		Ethers and oxides						
1031	8.67	1,8-Cineole	0.31	-	-	-	-	-
1583	18.61	Caryophyllene oxide	4.50	0.12	-	-	-	-
1753	20.59	Oxirane hexadecyl	-	-	-	-	0.07	-

table 3. (continued).

KI	RT	Compounds	Relative percentage (%)					
			PO/RO		CE		HC	
			Bud	Flower	Bud	Flower	Bud	Flower
3066	35.04	12-Oxa-bicyclo (9.1.0)-dodecane	-	-	-	-	0.07	-
	Total of ethers and oxides		4.81	0.12	0	0	0.	14 0
		Esters and lactones						
1289	13.67	Bornyl acetate	3.94	-	-	-	0.24	-
1349	14.88	Terpinyl acetate	0.41	-	-	-	-	-
1479	18.01	2(4H)-Benzofuranone, 5,6,7,7-a-tetrahydro-4,4,7a-trimethyl	-	-	-	0.50	-	0.80
1812	21.45	Tetradecanoic acid, isopropyl ester	0.13	9.29	1.00	3.32	0.12	4.79
1818	22.08	Farnesyl acetate	1.13	0.78	-	-	-	-
1826	22.47	Phthalic acid, diisobutyl ester	0.06	0.71	2.10	0.28	-	-
1913	23.06	Hexadecanoic acid, methyl ester	1.22	3.26	-	-	1.14	4.34
1940	23.72	Phthalic acid, dibutyl ester	2.69	3.25	20.77	2.09	-	-
1992	24.27	Hexadecanoic acid, ethyl ester	1.50	0.76	-	-	-	-
2078	25.21	9,12,15-Octadecatrienoic acid (Z,Z,Z), methyl ester [Methyl linolenate]	10.54	0.59	-	-	5.94	8.09
2253	27.02	Tributyl acetyl citrate	4.12	0.96	3.38	3.57	-	-
2540	29.91	1,2 benzene bicarboxylic acid, diisooctyl ester	5.78	8.41	38.99	13.98	3.11	2.50
	Total of esters and lactones		31.52	28.01	66.24	23.74	10.55	20.52
		Phenols						
1191	11.84	2-allyl-Phenol	-	-	0.33	0	0.17	-
1229	13.16	p-Vinylphenol	-	-	1.51	0.78	-	-
1289	13.93	Thymol	0.28	0.49	0.37	1.33	-	-
1309	14.42	p-Vinylguaiacol	-	-	0.27	1.11	-	-
1359	15.03	Eugenol	0.85	-	-	-	2.29	-
	Total of phenols		1.13	0.49	2.48	3.22	2.46	0
	Total of identified oxygenated compounds		49.35	49.21	73.20	59.07	70.58	74.04
	No. of identified oxygenated compounds		30	26	14	16	19	13
	Total of identified compounds		91.33	97.25	88.56	93.41	97.27	98.17
	Total No. of identified compounds		60	55	35	38	55	38

[AZ]: Azulene derivative

ml (MCF7) and 0.69 µg/ml (HCT116). On the other hand, both PO/RO of buds and flower showed relatively higher IC_{50} against MCF7 (20.6 and 21.1 µg/ml, respectively) and HCT116 (23, 20.6 µg/ml, respectively).

Results obtained during assessment of the cytotoxic potential of the hexane concrete (HC) of the buds of *Spathodea campanulata* P. Beauv. are in accordance with formerly reported data related to eugenol [47], phytol [48], limonene [49], in addition to the cancer preventive activity attributed to 9,12-octadecadienoic and 9, 12, 15-octadecatrienic acids methyl esters [48]. The present study was the first concerning the cytotoxic

Table 4. Cytotoxic effect (expressed as % cell viability) of the volatiles of the buds (B) and expanded flowers (Fl) of *Spathodea campanulata* P. Beauv. on human mammary carcinoma (MCF7) and colon carcinoma (HCT116) cell lines

Dose µg/ml	% Cell viability MCF7						
	PO/RO		CE		HC		Dox
	B	Fl	B	Fl	B	Fl	
0	100	100	100	100	100	100	100
5	67	74	67	82	41	63	45
12.5	54	55	61	70	17	57	31
25	48	47	46	46	21	50	8
	% Cell viability HCT116						
0	100	100	100	100	100	100	100
5	82	76	98	98	87	81	42
12.5	67	72	67	76	62	56	29
25	46	37	36	51	48	29	10

PO: primary oil; RO: recovered oil; CE: chloroform extractive; HC: hexane concrete; Dox: doxorubicin

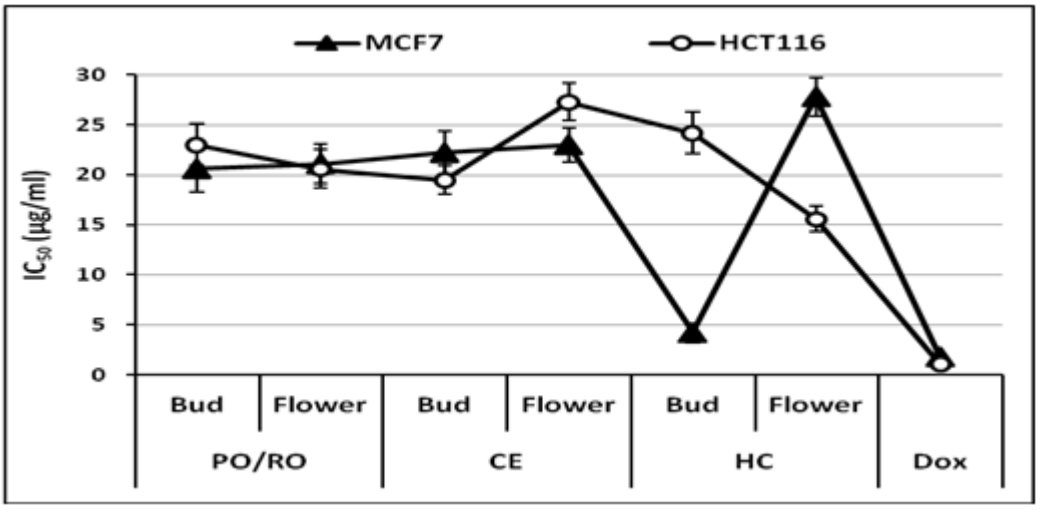

Figure 1. IC_{50} µg/ml of volatile constituents in the expanded flowers and buds of *Spathodea campanulata* P. Beauv. on human mammary carcinoma (MCF7) and colon carcinoma (HCT116) cell lines compared to Doxorubicin (Dox)

activity of *Spathodea campanulata* P. Beauv. on tumor cell lines.

In conclusion, the qualitative and/or quantitative variability among the GC-MS profiles of the investigated samples obviously reflects the influence of both the stage of development and isolation procedures on the yield and composition of the volatiles of the flowers of *Spathodea campanulata* P. Beauv. Hydro-distillation /solvent extraction appeared more efficient yielding products in higher amounts with a greater number of components especially terpenoids (PO/RO); while hexane concretes were characterized by a high percentile of oxygenated constituents especially acids and esters. Morever, the occurrence of the latter types of constituents in these extracts could be responsible of their high cytotoxic potential.

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