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Chemical Composition and Cytotoxic Activity of the Essential Oils of *Schinus molle* Growing in Egypt

Dalia I. Hamdan, Amal A. Al-Gendy*, Assem M. El-Shazly

Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, 44519, Zagazig, Egypt.

Abstract: Essential oil (EO) of different organs of *S. molle* growing in Egypt were isolated by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). In the fruit, leaf, stem and flower EO; 45, 67, 76 and 60 compounds were identified, respectively where *α*-phellandrene (25.55%), *β*-eudesmol (10.34%), myrcene (15.28%) and *p*-cymene (25.55%), were the major compounds of these organs, respectively. *p*-Cymene was used as external standard for quantification of major oil components using GC-FID. *In vitro* cytotoxic activity was carried out using MTT assay against human colon (HCT-116), hepatocellular (HepG-2) and breast (MCF-7) carcinomas. Doxorubicin was used as positive control. Fruit oil is the most potent against HCT-116 and HepG-2, with IC_{50} values of 1.15 and 0.95 µg, respectively while flower oil is the most potent against MCF-7 (IC_{50} 0.98 µg). Authentic samples of *α*-phellandrene, myrcene and limonene exhibited high cytotoxic activity against all tested cell lines while *p*-cymene showed moderate activity. Myrcene is the most active against HCT-116, HepG-2 and MCF-7 with IC_{50} values of 1.27, 0.93 and 1.55 µg, respectively. The results suggest the selective effect of tested oils towards different types of cancer and the possible use of *S. molle* oils as anticancer drugs *in vivo*.

Keywords: Anacardiaceae, essential oils, GC-MS, MTT assay, *Schinus molle*.

1. Introduction

Essential oils (EO) have been recognized for many years as a great source of pharmaceutical agents and food additives [1]. *Schinus molle* L. (Anacardiaceae) known as Brazilian pepper and Peruvian pepper tree is originally from South America but has been introduced to the Mediterranean area and widely ornamentally planted on roadsides and gardens [2-4]. The plant plays an important role in pharmacology and pharmaceutical chemistry because of its high EO content [5-7]. In traditional cuisine, *S. molle* berries have been used as a replacement for black pepper and to prepare alcoholic drinks and beverages [8]. *S. molle* is recognized for its antimicrobial, antioxidant, anti-inflammatory, antitumor, antispasmodic, analgesic, as well as a stimulant and antidepressant activities in addition to insect repellent and negative anti-quorum sensing activities against *Chromobacterium violaceum* strain ATCC 12472 [7, 9-14]. The plant has also been used in the treatment of toothache, rheumatism, menstrual disorders, and respiratory and urinary tract infection [13, 14].

Literature review of *S. molle* is highly concerned with fruit and leaf EO composition. It also shows a wide conflict between reported data for oil composition and even for identified major compounds. This chemical variation can be attributed to genetic and/or environmental factors and to the extraction process [15]. For example, *S. molle* flowering plants samples collected from different places in Brazil showed variation in their EO composition [16-19] which in turn different from reported data for EO composition of *S. molle* growing in Mexico [20] and Argentina [9].

In Mediterranean area, another variation was reported for EO composition of *S. molle* growing in Portugal [14], Syria [4], Tunisia [3, 21-22], Turkey [2], Spain [23], and Italy [8]. Moreover, Comparative analysis of the oil separated by hydro-distillation and supercritical CO_2 extract of *S. molle* growing in Yemen [24] showed another chemical variation which in turn differed from data reported from Saudi Arabia [7] and other countries.

The volatile constituents of *S. molle* cultivated in Giza governorate, Egypt was investigated, where phellandrene and limonene are the major volatile constituents in the flower, leaf and fruit oils. Moreover, *α*-and/or *β*- pinene were the major compounds in stem oil and this is the only report for stem and flower oils [25]. Another study of leaf and fruit EO isolated from *S. molle* tree growing in Maadi area, Cairo, Egypt revealed the presence of 35 and 70 components, respectively with *α*-phellandrene, limonene, *β*-phellandrene, myrcene and α-pinene as the most abundant constituents in both plant organs [26].

The aim of this study is to investigate the EO composition of different organs of *S. molle* (fruit, leaf, stem and flower) growing in Sharkeya governate, in addition to its cytotoxic activity against human colon carcinoma (HCT-

116), human hepatocellular carcinoma (HepG-2) and human breast carcinoma (MCF-7) cell lines with their chemical composition.

2. Materials and Methods

2.1. Plant material

The fresh plant materials of *Schinus molle* L. were collected from the vicinity of Zagazig city, State of Sharkeya, Egypt in summer 2014. The samples were identified by Prof. H. Abdelbaset, Professor of Plant Taxonomy, Faculty of Science Zagazig University, Egypt and voucher specimens (SMAP-2014) were deposited in Pharmacognosy Dept., Faculty of Pharmacy, Zagazig University.

2.2. Isolation of volatile oils

Fresh plant materials (100 g each) were separately chopped and subjected to hydrodistillation in Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulphate and stored in freezer in a well sealed container prior to chemical analysis and cytotoxicity study.

2.3. Gas chromatography-mass spectrometry (GC-MS)

An Agilent 6890 gas chromatography (USA) equipped with PAS-5 ms capillary column (30 m x 0.32 mm; 0.25 µm film thickness) with splitless injector and directly coupled to an Agilent 5973 quadrupole mass spectrometer. Conditions of analysis are: injector temperature, 250°C; temperature program, 45°C isothermal for 3 min and raised to 280°C at 8°C/min, 10 min isothermal. Helium was used as carrier gas (1 mL/min). The mass spectrophotometry detector was operated in electron impact ionization mode and ionizing energy of 70 eV. The ion source temperature was 230°C. Kovats indices (RI) were calculated with respect to a set of co-injected standard hydrocarbons (C8-C24). Oil samples were dissolved in *n*-hexane (100 µL/mL) and 1 µL was injected for each sample.

2. 4. Gas chromatography flame ionization detector analysis (GC-FID)

Quantification of major components of investigated volatile oils was carried out using GC-FID analysis by Trace GC Ultra (Italy) equipped with TR-WAXMS column (30m x 0.25 mm; 0.25 µm film thickness) and splitless injector. The temperature program was 50°C isothermal for 2 min and raised to 260°C at 8°C/min, 5 min isothermal. Helium was used as carrier gas (1.5 mL/min). The injector temperature was 250°C while detector temperature was 280°C. The injection volume was 1 µL. The integration was carried out using Chrom-Card software. The identification was based upon comparison of retention time of the samples peaks and available authentics of *α*-pinene, myrcene, *α*-phellandrene, *p*-cymene and limonene. As the monoterpenes represent the major components of organ oils, *p*-cymene was used as external standard for quantification of major components. Calibration curve was carried out using serial dilution of *p*-cymene (0.00008-0.008 µg/µL). The standard exhibited high linearity with coefficient of determination (R^2) of 0.9997 at the used concentrations.

2. 5. Qualitative and quantitative analysis

The identification of the oil components was based upon comparing mass spectral data and RI with Wiley Registry of Mass Spectral Data 8th Edition, NIST Mass Spectral Library (December 2005) and the available literature [27-29]. Retention times and mass spectra were also compared with those of available authentic pure samples.

2. 6. Cytotoxic assay

EO of fruit, leaf, stem and flower (0-50 µg) were tested for cytotoxic activity against Human colon carcinoma (HCT-116), human hepatocellular carcinoma (HepG-2) and human breast carcinoma (MCF-7) cell lines. MTT assay [30-31]. Authentic samples of *α*-pinene, myrcene, *α*-phellandrene, *p*-cymene and limonene, which constitute the major components of investigated oils were tested for their cytotoxicity against the same cell lines under the same experimental conditions. Doxorubicin was used as a positive control. The optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells.

The percentage of viability = [1-(ODt/ODc)] x100%

ODt is the mean optical density of wells treated with the tested sample; ODc is the mean optical density of untreated cells.

The relation between surviving cells and EO concentration (0.39-50 µg/mL) is plotted to get the survival curve of each tumor cell line .The 50% inhibitory concentration (IC_{50}) was estimated from graphic plots of the dose response curve for each concentration using Graphpad Prism software (San Diego, CA. USA).

3. Results and Discussion

3. 1. Identification and quantification of oil components

The hydrodistillation of the fresh fruits, leaves, stems and flowers yielded 0.7, 1, 0.7 and 0.8% v/w, respectively, of pale yellow oils with a slightly pungent and pepper-like aroma. The identified constituents are listed according to the order of their elution within their chemical class in Table 1. Most of the non identified components are present as traces with relative percentage less than 0.01%. Concentrations of major EO components identified by using GC-FID analysis and calculated according to

calibration curve of *p*-cymene, are listed in Table 2 expressed as µg/mL oil. *p*-Cymene was chosen as external standard because monoterpenes represent the major identified components in all tested oils.

Altogether 123 components were identified, representing 98.90, 99.54, 99.15 and 98.49% in fruits, leaves, stems and flowers oils, respectively. In flower oil, 60 components were identified comprising 38.3% of monoterpene hydrocarbons and 41.74% of oxygenated sesquiterpenes. The oil is characterized mainly by the presence of *ρ*-cymene (25.55%), *β*-eudesmol (10.07%), elemol (9.59%), myrcene (6.13%) and *α*-pinene (5.41%) as major constituents. Additionally, 76 components were identified in the stem oil, representing 31% of monoterpene hydrocarbons and 43.82% of oxygenated sesquiterpenes with myrcene (15.28%), *β*-eudesmol (11.79%), elemol (9.79%), limonene (6.43%) and *ρ*-cymene (5.91%) as the major volatile components. Moreover, the fruit oil contained more monoterpene hydrocarbons (73.3%) where *α*-phellandrene (28.85%), limonene (22.95%) and myrcene (18.91%), are the most abundant components. For the leaf oil, 74 GC signals were observed, of which 67 were identified with *β*-eudesmol (10.34%), elemol (10.27%), *β*-bisabolenol (5.06%) and *epi*-*α*-muurolol (3.29%) as the major oxygenated sesquiterpenes (50%) and *ρ*-cymene (9.42%) as the most abundant monoterpene hydrocarbons (19.4%). Data listed in Table 2 show that fruit EO is rich in *α*-phellandrene (411µg/mL) while stem EO has significant amounts of myrcene (172 µg/mL). Additionally, *p*-cymene represents the highest concentration in leaf and flower oils (152 and 312 µg/mL, respectively). From our results, we conclude that the chemical composition of isolated essential oils from different plant parts of *S. molle* grown in Egypt showed quantitative and qualitative differences in the main components. These results are closely agree with the chemical composition of the fruit EO and different from EO of leaf, stem and flower previously reported for *S. molle* grown in Egypt [25-26].

By referring to the literature, the investigated *S. molle* EO showed a marked difference in composition, by comparison to EO from the same species collected in Tunisia [3, 21-22, 32], Portugal [14], Syria [4], Mexico [13] and Brazil [19]. Moreover, in Brazil, Pawlowski et al. showed that *α*- phellandrene, limonene, *β*-phellandrene, *β*-pinene, myrcene, *p*-cymene and *α*-pinene are the main components leaf EO, while, Simionatto et al. reported the presence of high amount of sesquiterpenes (69.1%) with *epi*-*α*-cadinol as the most abundant component (27.3%) [17-18]. On the other hand, *α*-phellandrene, limonene, *β*-phellandrene, *p*-cymene, elemol, *α*-eudesmol and *β*-eudesmol were the most abundant components in leaf EO isolated from *S. molle* L cultivated in Spain [23] and Italy [8]. Additionally, germacrene D, and *β*-caryophyllene were the main components in leaf volatile oil obtained from Turkey and Yemeni, respectively [2, 24], while *δ*-cadinene and *α*-cadinol were major in Turkey fruit oil [2]. *β*-Pinene and *α*-pinene were the major constituents in leaf EO from Costa Rica [33]. *α* -Phellandrene and sylvestrene were the major constituents in both leaf and fruit collected from Mexico [20]. Also, sabinene, (*α* and *β*)-pinene and terpinen-4-ol were major constituents in South America [9]. In Argentine, guaiol acetate, *δ*-cadinene and *γ*-caryophyllene were the major compounds identified in fresh berries oleo-resin while *γ*-caryophyllene, *γ*-muurolene and bicyclogermacrene were the majors in 1 year stored (-18°C) fruits [34]. The leaf and fruit EO form Brazillian species showed high percentage of sesquiterpene and monoterpene hydrocarbons [35]. Sabinene, limonene, germacrene D, bicyclogermacrene, and spathulenol were identified in Brazilian fruits [16]. *p*-Cymene was the major component in the oil of leaves and fruits collected from Kingdom of Saudi Arabia where *β*-pinene, *α*-terpinene and limonene were the most prominent in fruit oil [7].

From the above results, 45, 67, 76 and 60 compounds were identified in fruit, leaf, stem and flower EO of *S. molle*, respectively. *p*-Cymene, myrcene, *α*-phellandrene, and *β*-eudesmol were the major compounds in these organs respectively. Overall, the chemical composition of essential oil from *S. molle* varied considerably depending on the genetic background, origin of cultivation, season, plant parts analyzed and methods of analysis.

3. 2. Cytotoxic activity

The *in vitro* cytotoxic activity of the tested EO isolated from *S. molle* and standard doxorubicin (a broad-spectrum anticancer drug) against HCT-116, HepG-2 and MCF-7 are represented in Figures 1-3. The criteria used to categorize the activity of tested EO and authentics of pure major oils constituents (myrcene, *α*-phellandrene, *p*-cymene and limonene) against the tested cell lines based on IC_{50} values as follows: $IC_{50} \leq 20$ µg/mL = highly active, IC_{50} 21-200 µg/mL = moderately active, IC_{50} 201-500 µg/mL = weakly active and $IC_{50} > 501$ µg/mL = inactive [36]. This evaluation is also in accordance with the protocol of the American National Cancer Institute (NCI), which recommends that IC_{50} values ≤ 30 µg/mL should be considered significant for crude extracts of

Table 1: Chemical composition of essential oils isolated from *S. molle* fruit, leaf, stem and flower

Constituents		RI	Fruit	Leaf	Stem	Flower
Monoterpene hydrocarbons						
1	α-Thujene	929	-	0.07	0.04	0.05
2	α- Pinene	938	2.55	4.46	2.92	5.41
3	Camphene	953	-	0.07	0.04	0.10
4	Sabinene	973	-	0.38	-	0.34
5	β-Pinene	979	0.35	-	0.34	-
6	Myrcene	989	16.8	4.48	15.28	6.13
7	α-Phellandrene	1002	25.6	0.52	-	-
8	ρ-Mentha-1(7),8-diene	1004	-	-	-	0.28
9	α-Terpinene	1017	0.24	-	-	-
10	ρ- Cymene	1024	6.37	9.42	5.91	25.55
11	Limonene	1029	20.9	4.37	6.43	0.30
12	γ-Terpinene	1059	0.2	-	-	-
13	Terpinolene	1088	0.27	-	-	-
Total %			73.30	23.77	31	38.3
Oxygenated monoterpenes						
14	cis-Vertocitral C	1080	-	-	0.08	-
15	Camphenilone	1082	-	-	0.23	-
16	6,7-Epoxymyrcene	1092	-	-	0.23	-
17	Linalool	1096	0.39	-	-	-
18	Perillene	1103	-	0.18	0.48	0.22
19	trans-Vertocitral C	1106	-	-	-	0.05
20	cis-ρ-Menth-2-en-1-ol	1121	-	-	0.13	0.22
21	cis-ρ-Mentha-2,8-dien-1-ol	1137	-	-	0.08	0.12
22	trans-ρ-Menth-2-en-1-ol	1140	0.31	0.04	-	-
23	Camphor	1146	-	-	0.39	0.40
24	Myrcenone	1149	0.19	-	-	-
25	Karahanaenone	1159	-	0.40	-	-
26	β-Pinene oxide	1159	0.12	0.25	-	-
27	Isoborneol	1160	0.17	-	-	-
28	Chrysanthenone	1164	-	0.03	0.12	-
29	Terpinen-4-ol	1177	0.59	-	-	-
30	Cryptone	1185	-	1.43	0.89	1.08
31	cis-Pinocarveol	1184	-	-	-	0.28
32	α-Terpineol	1188	0.26	-	-	-
33	trans-ρ-Mentha-1,(7),8-dien-2-ol	1189	0.42	0.41	0.11	-
34	Citronelol	1225	0.20	-	-	-
35	cis-ρ-Mentha-1,(7),8-dien-2-ol	1230	0.15	-	-	-

Table 1. continued

36	Neral	1238	0.40	-	-	-
37	(E)-Ocimenone	1238	-	0.23	-	-
38	Cumin aldehyde	1241	-	0.26	0.24	0.69
39	cis-Pulegol	1229	-	0.59	0.34	-
40	Piperitone	1252	0.03	-	-	-
41	cis-Piperitone epoxide	1254	-	-	0.29	0.46
42	trans-Piperitone epoxide	1256	-	0.50	1.82	2.98
43	Perilla aldehyde	1271	-	2.89	0.06	0.21
44	Citronellylformate	1273	-	0.21	0.07	-
45	α- Terpinen-7-al	1285	-	0.20	1.65	2.04
46	(3Z,6Z,9Z)-Tetradecatriene	1289	-	2.66	-	-
47	Thymol	1290	0.42	-	-	-
48	ρ- Cymen-7-ol	1290	-	0.46	0.57	0.27
49	γ-Terpinen-7-al	1291	-	2.84	1.09	1.71
50	Trans-(E)-Jasmonol	1324	-	2.37	1.25	1.95
51	Piperitenone	1343	-	0.23	-	-
52	neoiso-Carvomenthyl acetate	1350	-	0.06	-	-
53	Citronellyl acetate	1352	-	0.32	-	0.25
54	4aα,7α, 7aα-Nepetalactone	1360	-	0.14	0.15	0.40
55	Carvacrol acetate	1370	-	-	-	0.30
Total %			3.65	16.7	10.27	13.63
	Sesquiterpene hydrocarbons					
56	α-Cubebene	1348	-	0.18	0.24	-
57	α-Copaene	1376	0.02	0.32	0.28	-
58	β-Cubebene	1388	-	-	0.02	0.10
59	β- Elemene	1390	0.22	1.64	1.49	0.71
60	α- Gurjunene	1409	0.17	-	0.48	0.23
61	β-Funbrene	1414	-	-	1.20	0.85
62	(E)-Caryophyllene	1419	0.66	-	-	1.05
63	γ- Elemene	1436	-	-	2.59	0.24
64	Aromadendrene	1441	-	-	0.14	-
65	α-Humulene	1454	0.18	0.68	0.46	-
66	allo-Aromadendrene	1460	-	-	0.23	0.11
67	γ- Muurolene	1479	0.12	0.74	0.55	0.12
68	α-Amorphene	1484	0.19	-	-	-
69	Germacrene D	1485	0.69	-	-	-
70	β-Selinene	1490	-	0.38	1.27	0.24
71	α-Selinene	1498	-	-	1.90	-
72	α- Muurolene	1500	0.79	1.73	-	0.59
73	γ- Cadinene	1520	-	0.71	1.10	0.39

Table 1. continued

74	δ- Cadinene	1523	3.53	1.83	-	0.12
75	α- Cadinene	0.21	-	0.21	0.08	-
76	Germacrene B	-	-	-	1.71	-
77	Cembrene	-	-	-	0.08	0.07
Total %			6.57	8.42	13.82	4.82
	Oxygenated sesquterpenes					
78	Elemol	1549	2.10	10.27	9.79	9.59
79	Palustrol	1568	-	0.19	0.19	0.04
80	Germacrene D-4-ol	1575	0.35	-	-	-
81	Spathulenol	1578	-	3.56	1.15	1.68
82	Caryophyllene oxide	1583	0.12	-	-	-
83	Viridiflorol	1592	0.21	0.38	0.58	0.54
84	Ledol	1602	0.15	0.49	0.27	0.27
85	10-epi-γ-Eudesmol	1623	-	4.30	4.28	0.33
86	γ-Eudesmol	1632	0.79	-	-	4.24
87	epi-α-Muurolol	1642	2.52	3.29	2.05	1.89
88	β-Eudesmol	1650	-	10.34	11.79	10.07
89	α-Cadinol	1654	5.14	-	-	-
90	trans-Calamenen-10-ol	1669	-	0.85	1.01	0.43
91	Guaia-3,10(14)-dien-11-ol	1677	-	0.56	0.27	0.76
92	Elemol acetate	1680	-	0.67	0.37	-
93	Germacra-4(15),5,10(14)-trien-1- α-ol	1685	-	0.84	1.64	-
94	Shyobunol	1689	0.83	-	-	-
95	(Z)-Apritone	1689	-	-	-	0.75
96	Amorpha-4,9-dien-2-ol	1700	0.19	-	-	2.03
97	Nootkatol	1715	0.17	-	-	3.30
98	(2Z,6E)-Farnesol	1724	-	-	-	0.22
99	Oplopanone	1740	-	1.07	0.24	-
100	γ-Costol	1746	-	-	0.51	-
101	δ-α-11-Elemodiol	1747	-	-	-	0.09
102	α-Costol	1774	-	2.12	2.64	2.55
103	Hinesol acetate	1784	-	-	0.88	-
104	(Z)-Nerolidylisobutyrate	1784	-	0.22	-	-
105	β-Bisabolenol	1789	-	5.06	1.35	-
106	8-α-Acetoxyelemol	1793	-	1.61	0.55	-
107	Eudesm-11-en-4-α-6-α-diol	1808	-	0.3	0.24	-
108	α-Chenopodiol	1855	-	0.53	0.62	1.10
109	β-Chenopodiol-6-acetate	1890	-	-	-	1.69
110	Kudtdiol	1912	-	1.14	0.86	-

Table 1. continued

111	11,12,dihydroxy-Valencene	1914	-	2.04	1.46	-
112	Carissone	1927	-	0.13	0.04	0.10
113	Cembrene	1938	0.32	-	0.08	0.07
114	α-Chenopodiol-6-acetate	1966	-	0.24	0.24	-
115	Abieta-8,12-diene	2022	-	-	0.12	-
116	(E,E)-Geranyl linalool	2027	-	-	0.49	-
117	Abienol	2149	-	-	0.11	-
Total %			12.9	50.2	43.82	41.74
		Others				
118	Benzyl alcohol	1031	-	0.13	-	-
119	Methyl octanoate	1127	2.51	-	-	-
120	n-Docosane	2200	-	-	0.04	-
121	n-Tricosane	2300	-	0.17	-	-
122	n-Tetracosane	2400	-	-	0.07	-
123	n-Pentacosane	2500	-	0.15	0.13	-
Total %			2.51	0.45	2.51	0.45
% of total identified compounds			98.9	99.54	99.15	98.49
Number of identified compounds			45	67	76	60

All the components were identified by GC-MS and RI
Order of elution and percentage of components are given on fused silica column PAS-5ms.

Table 2: Quantification of major components of essential oils isolated from *S. molle* fruit, leaf, stem and flower

Compounds	Concentration (µg/mL)			
	Fruit	Leaf	Stem	Flower
α- Pinene	10.20	13.62	7.80	10.80
Myrcene	98.00	51.60	172.00	49.20
α-Phellandrene	411.00	-	-	-
p-Cymene	131.00	152.00	120.60	312.00
Limonene	164.50	112.20	81.20	14.00

plant origin as well as IC_{50} values ≤ 4 µg/mL for pure substances [37].

The results show that HCT-116 and HepG-2 are more sensitive to fruit oil with IC_{50} 1.15 and 0.95 µg/mL, respectively (Figure 4) which is most probably attributed to the presence of high α-phellendrene content (25.6%) as well as limonene (20.9%) and myrecene (16.9%), that is confirmed by potential activity of these components as illustrated by Figures 5-6 and low IC_{50} values (Figure 8). Doxorubicin exhibited a stronger effect with IC_{50} 0.47 µg/mL (Figure 4). Fruit oil shows also potential activity against MCF-7 which is further confirmed by its IC_{50} values (2.31µg), and its main components α-phellendrene (2.63 µg), limonene (2.16 µg) and myrecene (1.55 µg) as shown in Figures 3-4, 7-8.

Cell viability percentage of tested authentics of pure oil components shown in Figures 5-7 as well as their IC_{50} values (Figure 8)

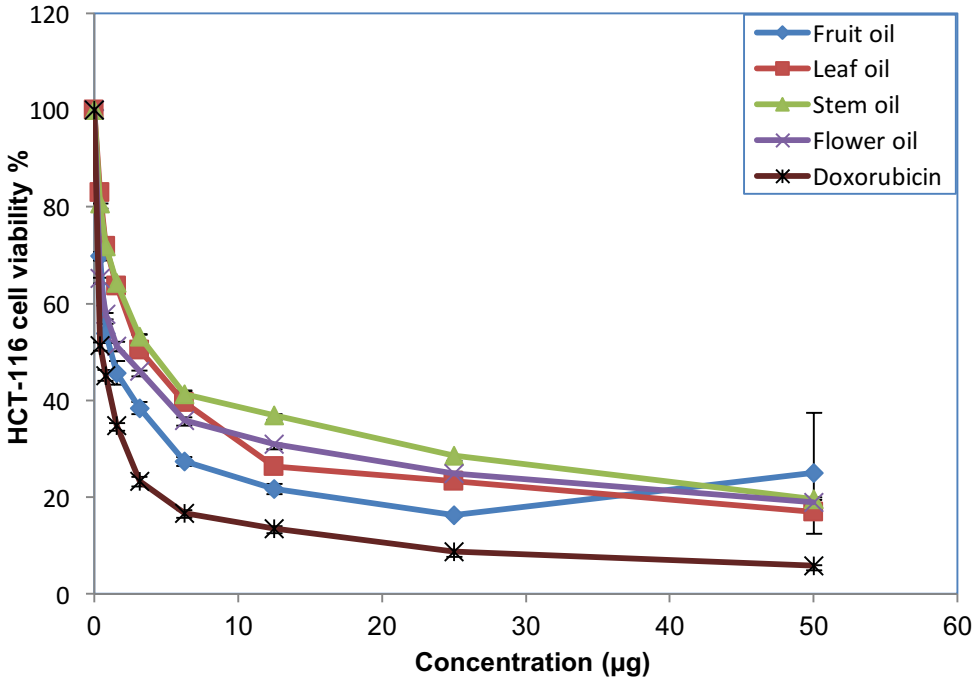

Figure 1. Cytotoxic activity of *S. molle* essential oils isolated from fruit, leaf, flower and stem against human colon carcinoma cell line (HCT-116).

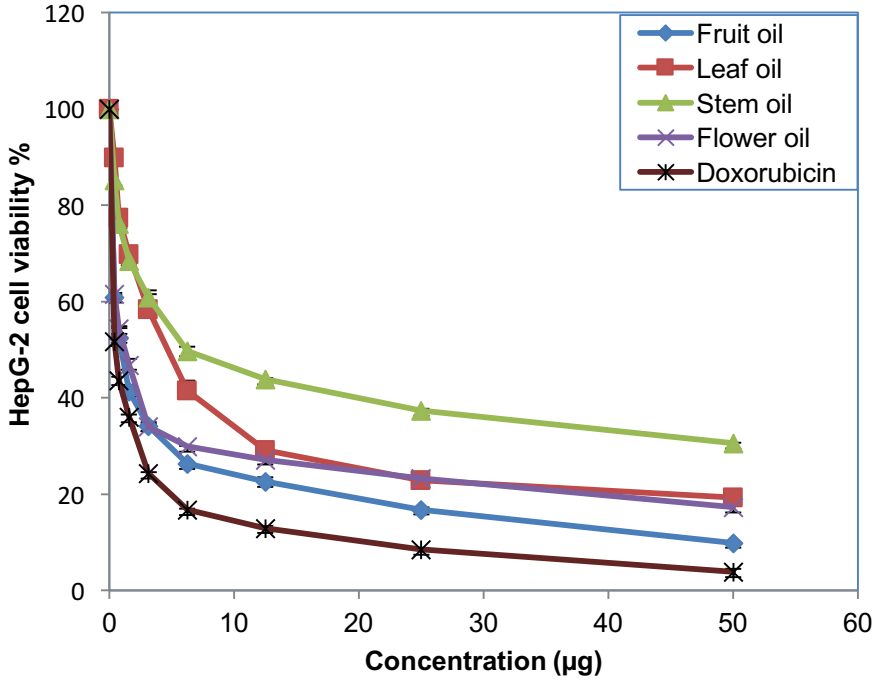

Figure 2. Cytotoxic activity of *S. molle* essential oils isolated from fruit, leaf, flower and stem against human hepatocellular carcinoma cell line (HepG-2).

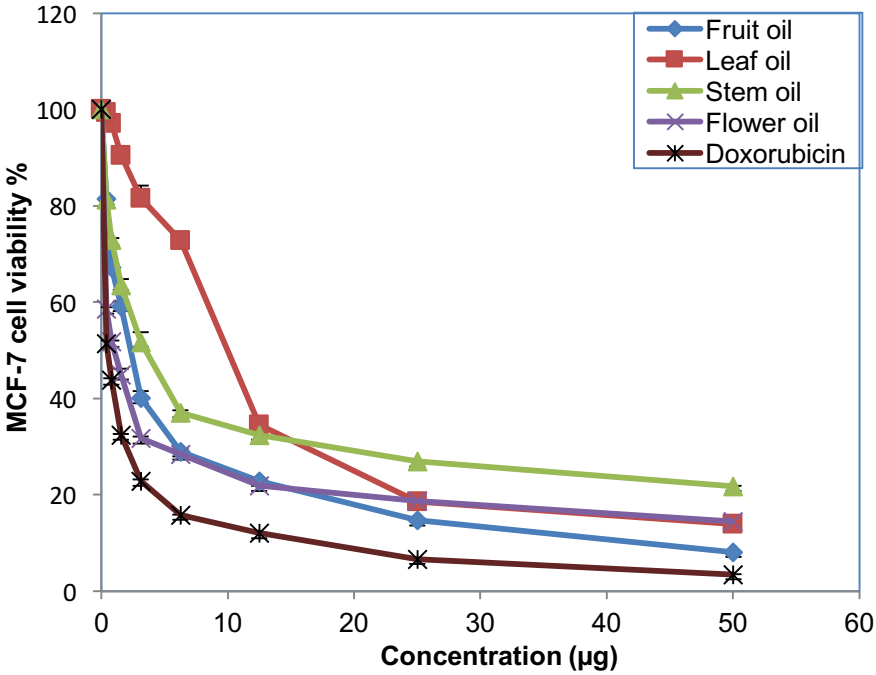

Figure 3. Cytotoxic activity of *S. molle* essential oils isolated from fruit, leaf, flower and stem against human breast carcinoma cell line (MCF-7).

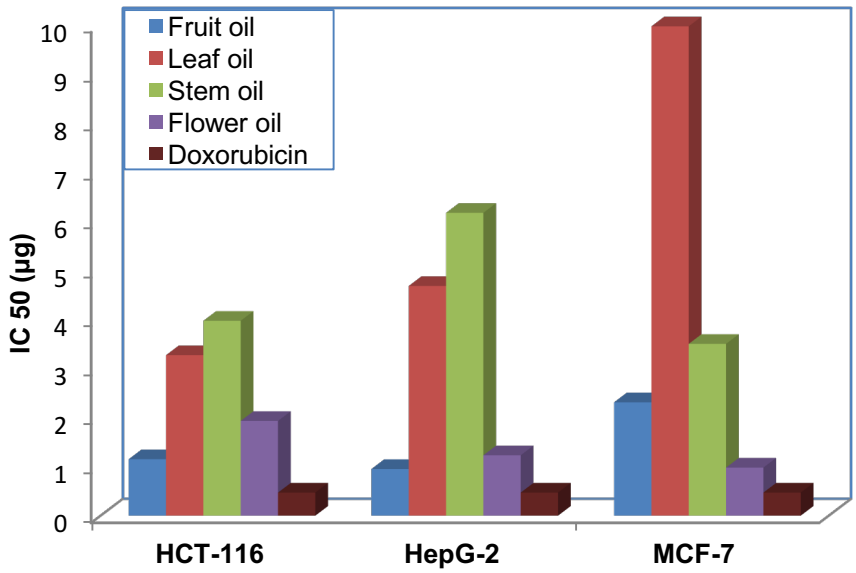

Figure 4. IC_{50} of *S. molle* essential oils isolated from fruit, leaf, stem and flower against tested cell lines.

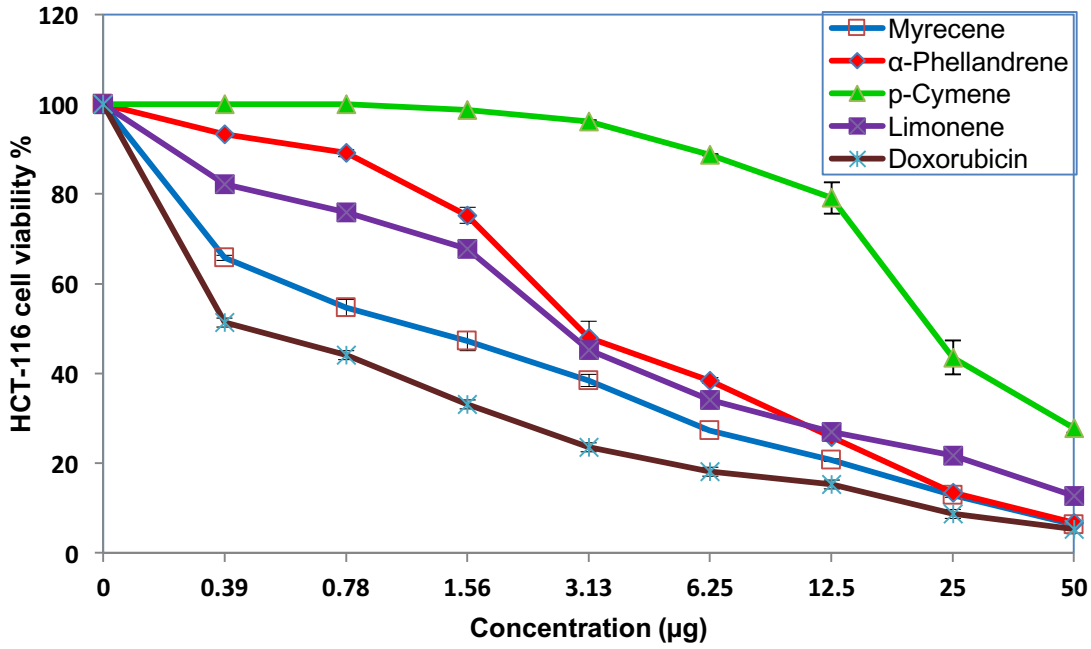

Figure 5. Cytotoxic activity of tested authentic essential oils components against human colon carcinoma cell line (HCT-116).

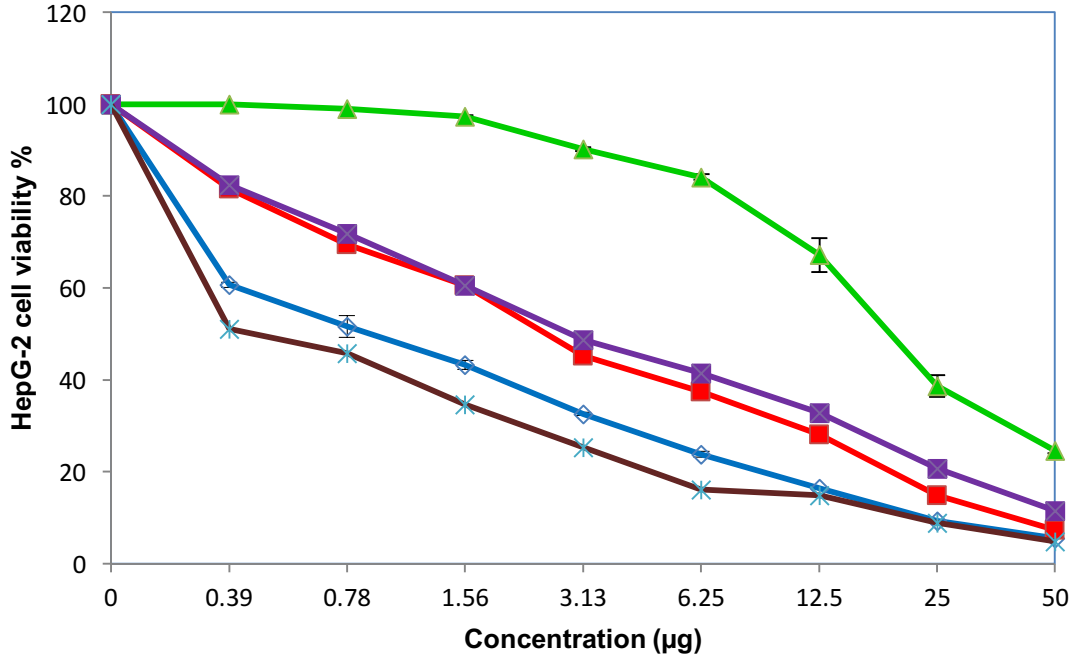

Figure 6. Cytotoxic activity of tested authentic essential oils components against human hepatocellular carcinoma cell line (HepG-2).

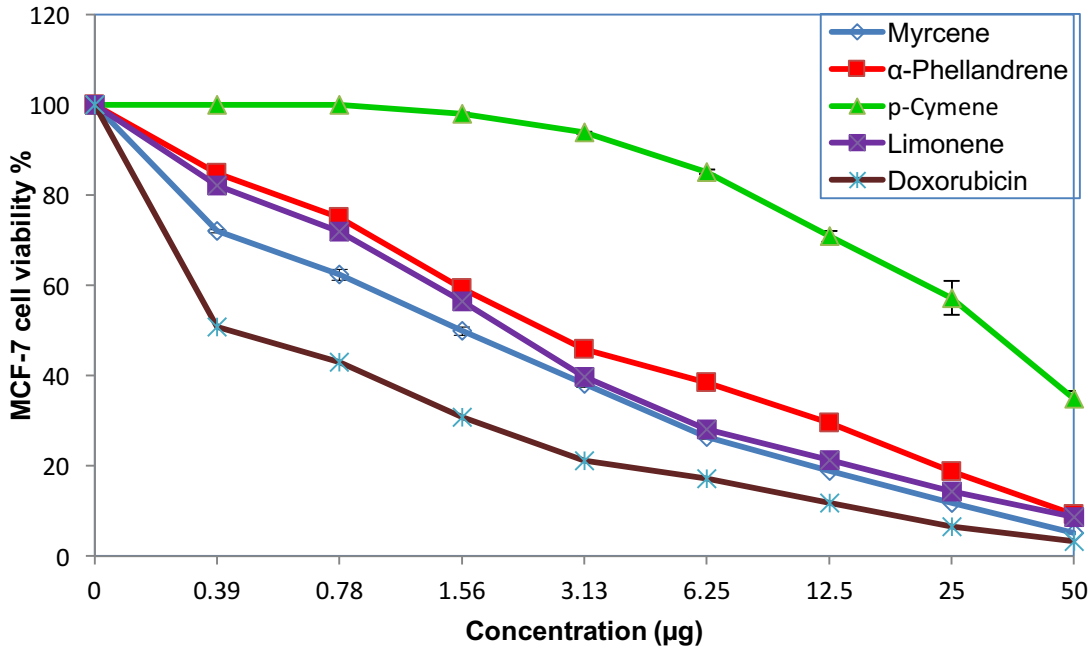

Figure 7. Cytotoxic activity of tested authentic essential oils components against human breast carcinoma cell line (MCF-7).

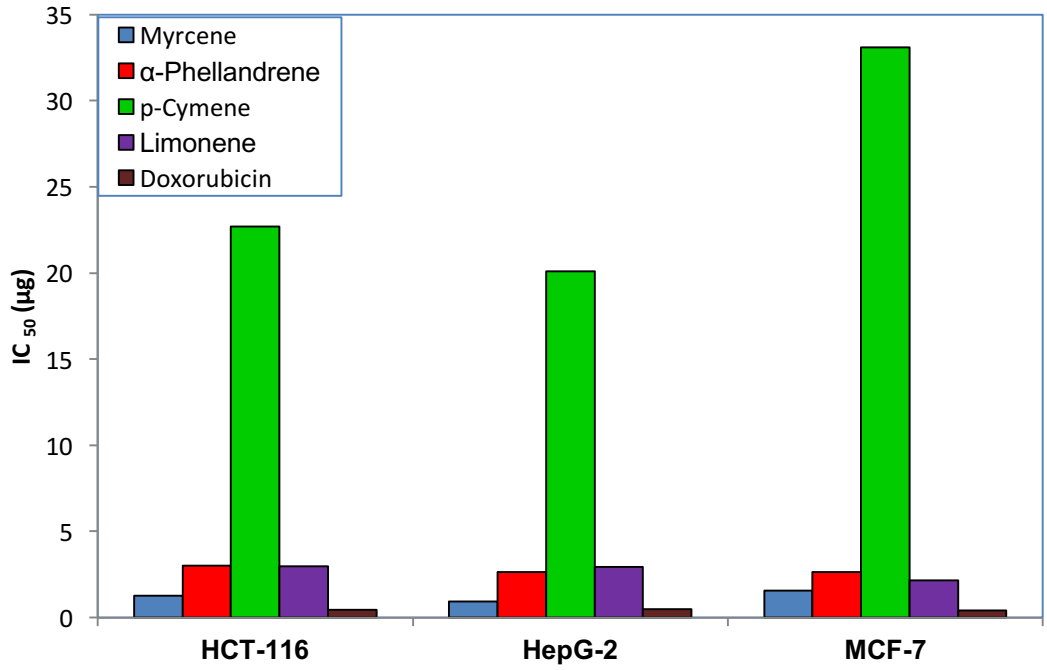

Figure 8. IC_{50} of tested authentic essential oils components against tested cell lines.

confirmed that myrcene has the highest activity against HCT-116, HepG-2 and MCF-7 cell lines with IC_{50} 1.27, 0.93 and 1.55 µg, respectively. Additionally, limonene (IC_{50} 2.97, 2.95 and 2.16 µg) and *α*-phellandrene (IC_{50} 3, 2.63 and 2.63 µg) showed potential activity while *p*-cymene (IC_{50} 22.7, 20.1 and 33.1 µg) showed the least activity compared with other tested authentics against the tested cell lines respectively. *α*-

Phellandrene exhibited equal inhibitory effect on HCT-116 and MCF-7.

According to guidelines stated by Srisawat et al. [36], *p*-cymene exhibited moderate cytotoxicity against all tested cell lines while according to Geran et al. [37], *p*-cymene has significant activities against HCT-116 and HepG-2. The cytotoxic effect of *p*-cymene is attributed to its effect on mitochondria by changing the mitochondrial proton motive force and ATP synthesis capacity [38].

p-Cymene represents the major compound in flower oil (Table 1, 2). Although *p*-cymene exhibited the least activity against tested cell lines, the flower oil showed pronounced activity against MCF-7. It was reported that *β*-eudesmol (major compound of flower oil) had no cytotoxicity against MCF-7 cell line [39]. Thus, the activity of the flower oil is probably attributed to *α*-pinene, myrecene and elemol in addition to other minor components, which could exert additive or synergistic cytotoxic effects [40].

During our study, leaf and stem oils of *S. molle* exhibited significant activities against tested cell lines with variable degrees which is attributed to the presence of *α*-pinene, myrcene and limonene in reasonable concentrations (Table 1-2). Unfortunately, we could not evaluate the cytotoxicity of *β*-eudesmol and elemol sesquiterpenes due to their unavailability.

EO of *S. molle* leaves grown in Costa Rica showed cytotoxic effects in several cell lines including HepG-2 by a mechanism related to apoptosis [41]. In a recent study dealing with cytotoxic activity of the EO of some medicinal plants, it was found that plants containing oil constituents as *α*-thujene, *α*- and *β*-pinene, camphene, myrcene, *α*-phellandrene, limonene, linalool, *E*-caryophyllene, and germacrene-D, which are available in EO of *S. molle* different organs exhibited promising cytotoxic activity against different cell lines including MCF-7 and HepG-2 [42].

In a previous report for cytotoxic activity of *S. molle* growing in Egypt, the leaf oil showed a significant activity against Ehrlich ascites carcinoma cell line while the fruit oil exhibited a significant inhibitory activity on the viability of brain cancer cell line (U-251) and MCF-7 [26].

Methanolic extract of *S. molle* growing in Argentine showed cytotoxic activity against HepG-2 with IC_{50} 50 µg/mL [43]. Upon comparison with our results, it is clear that the methonolic extract is less potent than the oils isolated from different organs as indicated by the values of IC_{50} for the oils separated from fruit (0.95 µg), flower (1.23 µg), leaf (4.68 µg) and stem (6.17 µg).

EO of *S. molle* berries growing in Tunisia showed a weak cytotoxic activity against MCF-7 compared to the used standard tamoxifen. The major identified components were significantly different from our reported data for fruit EO composition [44].

Monodora myristica volatile oil rich in *α*-plellandrene exerted cytotoxic activity against MCF-7 cell line [45]. It was reported that *α*-phellandrene altered gene expression in mouse leukemia *in vitro*. It induced DNA damage, condensation in a concentration-dependent manner and cell death [46-47]. Limonene showed a strong dose-dependent effect on the inhibition of HepG-2 by using MTT assay [40]. Moreover, it was cytotoxic (IC_{50} = 74.7 µg/mL) against MCF-7 [48].

The essential oil of *Angelica decursiva* rich with *α*-pinene and tested authentic *α*-pinene showed significant activity against MCF-7 where the activity of the oil exceeded the pure *α*-pinene [49]. *α*-Pinene could induce the cell death of HepG-2 cells possibly, by modulating oxidative stress-related signaling pathways [50].

The difference between reported data for cytotoxic activity is mainly attributed to difference in oil composition due to ecological variation. It would be interesting to elucidate that different components of the oil could have potential antitumor effects, either alone or in combination [41].

Conclusion

α-Phellandrene, *β*-eudesmol, myrcene and *p*-cymene, were the major compounds of the fruit, leaf, stem and flower EO of *S. molle*, respectively. This study represents the first report for cytotoxic activity of flower and stem oils. The fruit oil showed the most potent cytotoxic activity against HCT-116 and HepG-2 cell lines while the flower oil is the most potent against MCF-7. The results suggest the selective effect of tested oils towards different types of cancer and the possible use of *S. molle* oils as anticancer drugs *in vivo*.

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