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Chemical composition and anticancer activity of *Achillea fragrantissima* (Forssk.) Sch. Bip. (Asteraceae) essential oil from Egypt

Mouchira Aly Choucry

Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt.

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The aerial parts of *Achillea fragrantissima* (Forssk.) Sch. Bip., wildly grown in Egypt, yielded 0.8 and 1.5% (v/w) of essential oils prepared by hydrodistillation (HD), or by conventional volatile solvent extraction method (preparation of the "*absolute*", SE), respectively. The volatile components of this essential oil were determined by GC–MS analyses. Twenty eight compounds were identified in the HD sample, among which caryophyllene oxide (23.50%), 1-terpinen-4-Ol (11.15%), viridiflorol and guaienol (9.84%) were the main components. Meanwhile, 21 compounds were detected in the SE sample; 1-terpinen-4-Ol (30.90%), *p*-cymen-3-Ol (21.22%) were the main components. The anticancer activity of the prepared oils was evaluated against human breast cancer cell line (MCF-7) and colon cancer cell line (HCT116). The oil prepared by hydrodistillation revealed an IC_{50} 0.51 µg/ml for MCF-7 and 0.62 µg /ml for HCT116, while that prepared by volatile solvent extraction had a value of 0.80 µg/ml for MCF-7 and 0.91 µg /ml for HCT116.

Key words: *Achillea fragrantissima,* cytotoxic and terpinen-4-ol.

INTRODUCTION

Essential oils exhibit a very interesting chemotherapeutic potential; several essential oil constituents have been described as cytotoxic agents comprising β-caryophyllene, β-elemene, δ-elemene, α-humulene, etc. (Wang *et al*., 2005; Sylvestre et al., 2006; Hou et al., 2006; Tao et al., 2006; Xiao et al., 2006).

Achillea fragrantissima (Forssk.) Sch. Bip. (*Compositae or Asteraceae*) is a broadly spread medicinal plant all over the world and has been used since early time. The genus *Achillea* (*Asteraceae*) is represented by about 115 species in the temperate regions of the Northern hemisphere, mainly in North Africa, Southeast Europe and Southwest Asia (Boulos 2002). Among this genus, *A. fragrantissima* (known by its Arabic name Qaysoom) and *Achillea santolina* (known locally by Beatheran) are represented in Egypt (Tackholm 1974). They are strongly fragrant perennial herbs and have been used by Bedouins as stomachic and anthelmintic

E-mail: mouchirachoucry@pharma.cu.edu.eg. Tel: 01001417671.

(Boulos, 1983; El-Shazly et al., 2004). Common indications for the numerous species of this genus comprise treatment of inflammation, spasms, pains, flatulence, headache, bleedings, dyspepsia and wounds. Phytochemical research of *Achillea* species have shown that many ingredients from this genus are highly bioactive (Saeidnia et al., 2011). There are various reports on the declared folk and traditional uses. *A. fragrantissima* (Forssk.) Sch. Bip.is used traditionally to treat fever (viral) and patients with chronic diseases such as arthritis and diabetes. Nevertheless, its anticancer activity has not been entirely studied yet.

Previous reports were conducted on the chemical composition of *A. fragrantissima* (Shalaby et al., 1964), antimicrobial activity of its essential oil was also reported (Barel et al., 1991). Comparison between the essential oils and extracts of *Achillea fragrantissima* (Forssk.) Sch. Bip. as well as *A. santolina* L. (*Asteraceae*) for studying their antimicrobial activity was also reported (el-Shazly et al., 2004).

Selection of the suitable method for preparation of essential oils is a tedious process and relies on several factors. Hydrodistillation is the most common and the cheapest method for isolation of essential oil from plant material, but it is well known that this process may affect the composition of the oils by isomerization, saponification or polymerization of the more labile constituents (Koedam et al., 1979).

In the present study, two different techniques were adopted for the extraction of the essential oil from the flowering aerial parts of *A. fragrantissima*. Furthermore, a comparative investigation of the prepared samples was performed to highlight that the preparation techniques have an impact on both the chemical composition and cytotoxic efficacy against human breast cancer cell line (MCF-7) and colon cancer cell line (HCT116).

MATERIALS AND METHODS

Plant material

Fresh aerial parts of *A. fragrantissima* (Forssk.) Sch. Bip. (*Asteraceae*) were collected from North coast of Alexandria, Egypt, throughout spring 2008. The plant was authenticated by the Department of Botany, Faculty of Science, Cairo University, Giza, Egypt. The voucher specimen (AF-2008-51) is stored at the museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

Preparation of the essential oils

Fresh flowering aerial parts of *A. fragrantissima* were distilled in a modified Clevenger apparatus using distilled water for approximately 3 h. Another aliquot was prepared by the conventional volatile solvent extraction method (preparation of the floral absolute) (SE) (1 kg for each). The oil prepared in each case passed over anhydrous sodium sulfate to be dried and was stored at −20°C until further analysis.

Analysis of the obtained oils

The oils were subjected to GC mass analysis using an Agilent GC-MS system, model 6890, equipped with an Agilent mass spectroscopic detector (MSD), model 5937. Analysis was performed on a 30 m long, cross-linked 5% phenyl polysiloxane (HP-5MS, Hewlett Packard, USA) fused-silica column (i.d. 0.25 mm, film thickness 0.25 µm) and the gas chromotograph was operated under the following conditions: The initial temperature was 80°C, and kept isothermal for 3 min, then increased to 260°C at 8°C/min. The end temperature also kept isothermal for 15 min. The ion source temperature and the quadrupole temperature was 230 and 150°C, respectively. Helium gas was adjusted at a flow rate of 0.1 ml/min. The HP quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV, and scan range from 40 to 500 *m/z* at 3.62/scan. Volatile components were built on a Wiley MS Databases Library (6^{th} ed), the components of oil were identified; using AMDIS software (www.amdis.net); by their retention indices relative to Adams (2004) and mass spectra matching to Environmental Protection Agency/National Institutes of Health (EPA/NIH). Authentic standards when available with data reported in the published literature were also used for additional identification.

Cytotoxicity assay

The potential cytotoxicity of the prepared essential oils by the two methods of the aerial parts of *Achillea fragrantissima* (Forssk.) Sch. – Bip. (Family: Compositae) were tested using the method discussed by Skehan et al. (1990) on two human cell lines (HCT116): colon cancer cell line and MCF-7 (breast cancer cell line). The cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the samples to let attachment of cells to the wall of the plate. Sample under test with different concentrations (0, 1, 2.5, 5 and 10 µg/ml in DMSO) were added to the cell monolayer. Triplicate wells were arranged for each individual dose. The sample under test was incubated with monolayer cells for 48 h at 37°C, in an atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with sulphorhodamine B stain. Attached stain was recovered with Tris EDTA buffer while, excess stain was washed with acetic acid. An ELISA reader was used to measure color intensity. The relation between surviving fraction and the extract concentration is plotted to get the survival curve of each tumor cell line after the specified sample. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specified sample. Similarly, the IC_{50} (dose of the extract which reduces survival to 50%) and IC_{10} (dose of the extract which reduces survival to 10%) for each extract were calculated using GraphPad Prism software, unpaired t test was used for testing the significance, and presented in Table 2.

RESULTS AND DISCUSSION

The method of preparation of the essential oil affect the yield of the oil as 0.8 and 1.5% (v/w) of essential oil was obtained from hydrodistillation (HD) and volatile solvent extraction (SE) samples, respectively, also the color (yellow, and dark yellow, respectively) and specific gravity of the samples had also been affected (0.8501, and 0.8641 of HD and SE samples, respectively). Qualitative and quantitative differences were noticed as regards the method of extraction applied as represented by the results shown in Table 1. Results of GC-MS

Table 1. Composition of the essential oil of the aerial parts of *A. fragrantissima* (Forssk.) Sch. Bip prepared by hydrodistillation (HD) and volatile solvent extraction (SE)

S/N	Rt	The compound	The distilled sample	The sample prepared by volatile solvent extraction	M^+	Base peak
1	8.78	Sabinene	-	2.81	136	93
2	9.17	β-Myrecene	-	1.74	136	93
3	9.36	2,5,5-Trimethyl-3,6-heptadien-2-Ol (Yomogi alcohol)	6.43	-	154	59
4	9.71	α-Terpinene	-	1.02	136	121
5	9.89	O-Cymene	-	0.36	136	93
6	9.96	dl-Limonene	-	1.11	136	93
7	10.14	2-Methyl-4,6-octadien-2-Ol	1.81	-	154	59
8	10.58	γ-Terpinene	1.22	3.71	136	93
9	10.80	*Cis-β*-Terpineol	-	1.41	136	121
10	11.10	Artemesia alcohol	2.08	-	154	85
11	11.19	α-Terpinolene	-	1.21	154	71
12	11.42	Linalol	1.81	1.31	154	71
13	11.54	α-Thujone	3.72	-	154	59
14	11.88	3-Terpinen-1-Ol	-	1.35	154	139
15	12.24	Terpinen-1-Ol	1.56	1.02	154	92
16	12.94	1-Terpinen-4-Ol	11.15	30.90	154	71
17	13.21	α-Terpineol	0.82	1.26	154	59
18	13.57	1,1-dioxy-tetrahydro-Thiophene	-	0.90	120	56
19	15.01	*p*-Cymen-3-Ol	8.21	21.22	150	135
20	15.19	*p*-Cymen-2-Ol	-	1.55	150	135
21	15.57	Myrteny acetate	0.61	-	175	91
22	16.53	Clovene	3.22	-	204	189
23	17.10	β-Caryophyllene	1.29	-	204	93
24	18.16	α-Bisabolene	1.26	-	204	93
25	18.44	β-Bisabolene	6.88	1.07	204	69
26	19.23	Δ8-Dehydro-Bisablen-12-Ol	0.80	-	220	79
27	19.28	Dendrolasin	1.28	-	218	69
28	19.72	Caryophyllene oxide	23.50	0.52	207	79
29	20.08	Methyl Davanafuran	1.42	-	220	109
30	20.41	*Caryophylla-4(12),8(13)-dien-5-β-Ol*	0.37	-	220	136
31	20.46	Guaiol	0.37	-	222	59
32	20.58	Germacrene-D-4-Ol	0.95	-	222	81
33	20.65	Veridiflorol	9.84	1.31	222	109
34	20.70	Guaienol	9.84	-	220	55
35	20.292	2-Isopentyl oxy,5-methoxy-*p*-cymene	2.44	-	248	179
36	22.25	2-Pentyl decanone-6,10,14-trimethyl	0.83	-	250	58
37	22.43	Isophytol	5.29	6.9	296	71
38	22.60	Phytol	1.01	-	296	71
39	26.97	*trans*-Caryophyllene	-	8.67		

analysis of the essential oils prepared from the aerial parts of *A. fragrantissima* samples was recorded (Table 1), 28 compounds were identified constituting 98.34% of the sample prepared by HD, while 21 compounds were identified in the sample prepared by SE method, constituting 91.04 %. The major components in the sample prepared by HD were caryophyllene oxide (23.50%) and 1-terpinen-4-Ol (11.15), while the major components in the sample prepared by SE were 1-terpinen-4-Ol (30.90), *p*-cymen-3-Ol (21.22%). The

Table 2. *In vitro* cytotoxicity of the essential oils of *A. fragrantissima* (Forssk.) Sch. Bip.

The extract	IC 50 (µg)[a]	
	Breast cancer cell line (MCF-7)	Colon cancer cell line (HCT116)
The oil prepared by distillation	0.51	0.62
The oil prepared by volatile solvent extraction	0.80*	0.91*

*Significantly different at $P < 0.05$; [a] S.E. is less than 0.1.

percentage of oxygenated monoterpenes (Table 1) was higher in the sample prepared by SE (61.23%) than in the sample prepared by HD (38.83%). Nevertheless, the percentage of oxygenated sesquiterpene was higher in the sample prepared by HD than that in the sample prepared by SE (44.30 and 2.42%, respectively). On the other hand, the percentage of sequiterpene hydrocarbons was higher in the HD sample than in the sample prepared by SE (13.02 and 9.74 %, respectively), while the monoterpene hydrocarbons was higher in the sample prepared by SE (10.75 %) than that in the sample prepared by HD (1.22%).

From the above results, it can be concluded that the method of preparation of volatile oil can affect both the percentage of the oil and also the percentages of the constituents and chemical classes.

Recently, natural products aided to run a base for many of the pharmaceutical agents used in cancer treatment (Pietras and Weinberg, 2005). Chemotherapeutic drugs develop day by day, dangers of life threatening host toxicity. Researches, hence, proceed to progress drugs, which selectively destroy only tumor cells. Therefore, cytotoxic activity of the two prepared samples was tested on two human cancer cell lines: colon cancer cell line (HCT116), and breast cancer cell line (MCF7) (Table 2). The two samples showed cytotoxic activity but the HD sample was more potent than the SE sample as it showed the lowest IC_{50} values (0.51 and 0.62 µg/ml) against the breast, and colon cancer cell line, respectively by using GraphPad Prism software, unpaired t test and P values <0.05 were considered significant. This evidence is probably attributed to the high content of β-caryophyllene oxide present in the HD sample which has been proven to possess potent cytotoxic activity against HepG2, AGS, HeLa, SNU-1 and SNU-16 cells (Neung et al., 2011). Meanwhile, iso-caryophyllene cytotoxicity was induced by lipid peroxidation, membrane permeabilization in L-929 cells and cell shrinking. Lipid oxidation could be initiated by oxidized iso-caryophyllene derivatives (Jean et al., 2013).

Caryophyllene oxide was previously reported to inhibit mitochondrial electron transport chain through direct complex I inhibition (Monzote et al., 2009). Similarly, it is possible that iso-caryophyllene blocks mitochondrial electron transport chain generating ROS such as superoxide anion and hydrogen peroxide (Fariss et al., 2005).

Antioxidants and anticancer properties of *A. alexandri-regis* herbal extracts have been studied before. The chloroform and ethyl acetate extracts exhibited a strong anticancer effect against both HeLa and K562 cancer cell lines, with an IC_{50} value of 25.92 ± 4.96 µg/ml for HeLa cancer cells and lower cytotoxicity effect against K562 leukemia cells, 48.59 ± 18.31 µg/ml.

Meanwhile, the methanol extract was found to possess a moderate cytotoxic *in vitro* activity against HeLa and K562 cells (Kundakovic et al., 2005). Anti-proliferative activity of the aqueous and hydro-alcoholic extracts of *A. fragrantissima* was previously investigated using the MTT assay. The cytotoxic activity using an extract in a concentration up to 200 µg/ml, did not retain activity against the MCF-7 cells (Hana et al., 2014).

Exclusively, the anti-proliferative potential effect of *A. fragrantissima* extracts has not been studied yet. A recent study described cytotoxic effect of *A. fragrantissima* aqueous extract on HepG2 human hepatocellular carcinoma cells (Thoppil et al., 2013).

Conclusion

The cytotoxic activity of *Achillea* essential oil may be ascribed to the synergistic effect of all the constituents. The results of the present study indicate the potent cytotoxic activity of the essential oil prepared either by HD or SE from the aerial parts of *A. fragrantissima* and its major components: caryophyllene oxide, 1-terpinene-4-Ol, veridiflorol and guaienol against breast cancer cells (MCF7) and colon (HCT116). Although, the actual mechanism by which they displayed antitumor activity is not known, it may be due to their interference with cell growth. This will be the issue of upcoming researches. The results of this study may lead to the improvement of the convention of this plant in a substitute cytotoxic treatment.

Conflict of Interests

The authors have not declared any conflict of interests.

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