Open access • Journal Article • DOI:10.1007/S13596-018-0315-0

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Published on: 01 Sep 2018 - Oriental Pharmacy and Experimental Medicine (Springer Singapore)

Topics: DPPH and Essential oil

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Abstract

This work is devoted to the study of the chemical composition and the evaluation of the biological activities of essential oils (EOs) extracted from the flowers of *Asteriscus graveolens* Forssk. plant. The EO sample was obtained by hydrodistillation and the chemical composition analysis was performed using GC and GC/MS. The major chemical components characterizing the EO were *cis*-chrysanthenyl acetate (44.30%) and *cis*-8-acetoxychrysanthenyl acetate (33.70%). Antioxidant activity was determined using DPPH and Phosphomolybdenum tests. Although the EO presented a weak scavenging activity (420.16 mg/mL), it exhibited good reducing power using the Phosphomolybdenum assay (0.28 AAEC/mg). The most important antibacterial activity was noted for *Bacillus cereus*. The oil revealed a remarkable activity against the nine fungi species tested with percentage inhibition up to 94.12% for *Fusarium culmorum* (BTCR). More important, this work investigated for the first time the anticancer effect of this EO on two types of cancer cell lines (human liver carcinoma and Rat pheochromocytoma cell lines). The EO showed a high anticancer activity against both tumor cell lines comparing to the positive control.

Keywords *Asteriscus graveolens* Forssk · Essential oil · Chemical composition · Antioxidant activity · Antimicrobial activity · Anticancer activity

Introduction

The biological properties of natural products as essential oils (EOs) are studied to search a new drugs, antibiotics and pesticides (Buchanan et al. 2000). They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases and the preservation of food from the toxic effects of oxidants.

With the emergence of antibiotic-resistance, a reliable alternative to antibiotics is the use of EOs. These compounds are known for their antibacterial properties which have been demonstrated in vitro and in vivo (Zhiri 2006). Many works were devoted for the study of biological properties of EOs, thus, the antimicrobial activity of oils extracted from thyme; rosemary and chamomile were demonstrated (Bakkali et al. 2008; Dorman and Deans 2000; Floris et al. 1996; Marino et al. 2001). Because of the possible toxicities of the synthetic antioxidants, increasing attention has been directed toward natural antioxidants (Avlessi et al. 2004). The antioxidant activity of essential oils has been the subject of much intensive research because of their uses as preservatives in food industry even at low concentrations (Cabrera and Prieto 2010).

Fungal plant pathogens cause many damages to food production and storage. They may be at the origin of catastrophes in which large areas planted to food crops are destroyed (Strange and Scott 2005). In order to combat the losses they cause, it is necessary search remedies. Many studies have

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been devoted to the antifungal activity of EOs and revealed an important effect especially against phytopathogen fungal (Marinelli et al. 2012; Matusinsky et al. 2015; Znini et al. 2011).

Cancer belongs to a huge class of diseases, which cause more than 10% of all human deaths. Many studies showed the potential of the constituents of essential oil on several cancer cell lines (Adorjan and Buchbauer 2010).

Asteriscus graveolens Forssk (syn. *Nauplius graveolens* Forsk, *Bubonium graveolens* Forsk Maire) belongs to the *Asteraceae* family of herbs. It's very common in the North Africa. Their leaves are collected in the spring, they are prepared as infusion or decoction; the sap of the fresh leaves is used as drops for the nose and poultice for headaches. Traditionally, it is used for blennorrhagia, diabetes, diarrhea, facial neuralgia, head cold, gastralgia, pulmonary problems and sinusitis (International Union of Conservation of Nature IUCN 2005).

In the present work, we report the results of a study aimed to define chemical composition and to evaluate antioxidant, antibacterial, antifungal and for the first time the anticancer activity of *A. graveolens* EO.

Materials and methods

Plant material and essential oil extraction

The aerial part of *A. graveolens* was collected from south of Algeria, during the month of June 2013 at the flowering stage. It was identified at the National Institute of Agronomy "INA Institut National d'Agronomie". The separation of the flowers from the aerial parts was executed manually and with care. Then the air drying of the plant material was performed in the shade at room temperature for 10 days. The dried plant sample (flowers) was subjected to hydrodistillation using a Clenvenger type apparatus for 3 h. The distilled EO was dried over anhydrous sodium sulfate and stored at +4 °C until use.

Gas chromatography and GC/MS analysis

An Agilent technologies 7890A gas chromatograph (GC) equipped with a flame ionization detector (FID) was used for compound separations with a HP5 capillary column (30 m × 0.32 mm, film thickness 0.40 µm). The oven temperature program was as follows: 2 min at 80 °C, from 80 to 200 °C at 5 °C/min, then 5 min at 200 °C, then from 200 to 260 °C at 20 °C/min, then 5 min at 260 °C. Detector and inlet temperatures were 280 °C. Hydrogen was used as carrier gas at a constant flow of 1 mL/min with a split ratio 1:70. A volume of 1 µL of EO diluted in dichloromethane was injected manually. The retention indices of constituents were calculated relative to a series of *n*-alkane standards *n*-C_8-C_{26}.

The GC/MS analysis of the EO was carried out on an Agilent HP-6890 coupled to 5973 mass spectrometer and equipped with UB-WAX capillary column (30 m × 0.25 mm, film thickness 0.25 µm) and quadrupole detector (70 eV). The carrier gas flow (Helium) was fixed at 1 mL/min. The transfer line and injector temperatures were fixed at 220 and 250 °C, respectively. 1 µL of diluted EO in ethanol solution (1:100, v/v) was injected manually in a splitless mode.

Identification of components was based on comparison of their mass spectra with those of WILEY and NIST Libraries as well as on comparison of their retention indices with literature.

Antioxidant activity

DPPH assay

In the presence of a proton donor substance, the free radical DPPH takes the non-radical form and loses its purple color (Molyneux 2004). One milliliter of EO dilution in ethanol solvent was added to 1 mL of 200 µM DPPH solution prepared in ethanol solvent. After incubation for 30 min in a dark, the absorbance was measured at 517 nm against a blank (ethanol). Decreasing the absorbance of DPPH indicates an increase in DPPH radical scavenging activity, which is calculated according to the equation:

$$I\% = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Control was prepared by a mixture of 1 mL of DPPH solution and 1 mL of ethanol. The sample concentration providing 50% of radical scavenging activity (IC_{50}) was determined through the curves obtained. The lower IC_{50} indicates higher radical scavenging activity and vice versa. Ascorbic acid and α-tocopherol were used as standards.

Phosphomolybdenum assay

The reducing power of EO was measured according to the method of Prieto et al. (1999). The principle is based on the reduction of molybdate VI to molybdate V in presence of reducing substance, then the molybdate V form a green-colored complex and the absorbance is measured at 695 nm. An aliquot of 0.2 mL of EO solution in ethanol was added to 2 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were sealed and incubated in water bath at 70 °C for 90 min. After the incubation period, and when the samples

had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank containing 2 mL of reagent solution and 0.2 mL of ethanol. The antioxidant capacity was expressed as equivalents of ascorbic acid AAEC.

Antibacterial activity

Bacterial strains

For the determination of the antibacterial activity of the EO, we used standard and isolated strains of the following Gram-positive bacteria: *Bacillus cereus* ATCC 12778, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, MRSA ATCC 43300. The EO was also tested on Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* (isolate). The microorganisms were obtained from the culture collection of the "Regional Veterinary Laboratory" and the "Medical Analysis Laboratory of the hospital of Laghouat".

Disc diffusion method

This method was employed to evaluate the antibacterial activity (Bekhechi et al. 2008). All bacterial strains were first grown on nutrient agar plates at 37 °C for 18–24 h, prior to inoculation into the nutrient broth. One hundred micro-liter of bacterial suspension containing 10^8 cell/mL was spread onto Mueller–Hinton culture medium. A sterile filter disc (diameter 6 mm) impregnated with 10 µL of the diluted EO in 10% DMSO (1/4), was placed onto the plate culture. The Petri dishes were stored at 4 °C for 2 h and then incubated for 24 h. The diameters (mm) of the inhibition zones were measured. Each experiment was done in triplicate.

Antifungal activity

Strains of *Fusarium culmorum* (124, 319) and *Fusarium graminearum* (156, 91) was kindly provided by the MycSa-INRA of Bordeaux. The strains of *F. culmorum* T7 and BTCR by Bentouati Sihem mycotheque and *Fusarium oxysporum* f. sp *pisi* was obtained from Laboratoiry of mycology, agronomy department of Blida. *Fusarium oxysporum* f. sp. *Albedinis* and *Fusarium oxysporum* f. sp *lycopersici* was provided by the "Regional Station of Plant Protection of Ghardaïa" in Algeria.

Antifungal activity was studied by using contact assay. The EO dilutions in 2% agar solution was added to the molten PDA medium and poured into Petri dishes (El Ajjouri et al. 2008). The control plate contains PDA added 2% w agar solution. A 6 mm diameter disk of the fungal species was cut from a 1-week-old culture on PDA plates, and then the mycelia surface of the disk was placed upside down on the center of dish.

The incubation was performed at 25 °C for 7 days. The Extension Diameter (millimeters) of hyphae from the center to the side of the dish was measured and the inhibition percentage was calculated as follows (Aoudou et al. 2010):

$$I(\%) = \left(1 - \frac{D_0}{D_I}\right) \times 100$$

where D_0 is an average of 3 replicates of hyphal extension (mm) of controls and D_I is an average of 3 replicates of hyphal extension (mm) of plates treated with EO.

Anticancer activity

Cell lines HePG2 (human liver carcinoma) and PC12 (Rat pheochromocytoma cells) were maintained at 37 °C in a 5% CO_2 atmosphere. Both cell lines were grown in a medium containing 5 mL of RPMI-1640 supplemented with 10% fetal bovine serum (FBS).The effect of the EO on cell viability of the two cancer cell lines was determined by 3 [4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium-bromide (MTT) assay (Mosmann 1983). The growing cells were plated in 96-well microplates at a density of 5×10^3 cells per well in 100 µL of culture medium and allowed to adhere for 24 h before treatment. Increasing concentrations of EO were then added (100 µL/well). The cells were incubated 48 h in the presence and absence of essential oil. 5-fluorouracil (6.25–100 µg/mL) was used as positive control. 20 µL of MTT (5 mg/mL of PBS) were added to each well, and the cells were incubated for a further 4 h. After removal of the medium, 200 µL DMSO was added to each well. The absorbance was recorded on a micro plate reader at the wavelength of 490 nm. The effect of the essential oil on cell growth inhibition was assessed as percentage cell growth inhibition. This test was replicated five times.

Results and discussion

Essential oil composition

Hydrodistillation of *A. graveolens* flowers yielded 1.16 ± 0.2 mL of yellow essential oil upon 100 g of dry matter.

Forty-two compounds were identified by GC and GC/MS (Table 1). This analysis showed that the EO contains *cis*-chrysanthenyl acetate (44.30%), *cis*-8-acetoxychrysanthenyl acetate (33.70%) and τ-muurolol (6.51%) as major components. The nature of *cis*-chrysanthenyl acetate and *cis*-8-acetoxychrysanthenyl acetate was consistent with Cristofari et al.'s study (2012), where the major components

Table 1 Chemical composition of *A. graveolens* (Forssk) flowers essential oil

	Compound	LRI[a]	Peak area (%)
01	*α*-Pinene	926	0.75
02	Camphene	946	0.19
03	Sabinene	974	0.18
04	*β*-Pinene	982	0.04
05	*β*-Myrcene	991	0.34
06	*p*-Cymene	1000	0.04
07	*α*-Phellandrene	1010	0.03
08	Limonene	1031	0.09
09	*α*-Terpinolene	1089	0.03
10	Linalool	1097	0.06
11	*α*-Thujone	1101	0.04
12	*β*-Ocimene	1144	0.03
13	*cis*-Chrysantenol	1148	0.11
14	Menthol	1165	0.36
15	Borneol	1171	0.04
16	*trans*-Isopulegone	1183	0.06
17	Myrtenal	1208	0.05
18	*cis*-Chrysanthenyl acetate[b]	1270	**44.30**
19	Bornyle acetate	1293	0.1
20	NI	1315	0.14
21	NI	1339	0.03
22	Eugenol	1360	0.06
23	*iso*-Eugenol	1455	0.03
24	*α*-Caryophyllene	1464	0.08
25	Aromadendrene	1471	0.26
26	*γ*-Muurolene	1485	0.07
27	Germacrene D	1491	0.18
28	*β*-Selinene	1494	0.05
29	NI	1497	0.01
30	Bicyclogermacrene	1507	0.06
31	*β*-Bisabolene	1515	0.07
32	*γ*-Cadinene	1523	0.45
33	*δ*-Cadinene	1531	0.33
34	Nerolidol	1538	0.77
35	Kessane	1545	0.05
36	Calamenene	1550	0.07
37	NI	1554	0.06
38	3,5,5-Trimethyl-hexahydro-pentalen-1-one	1557	0.06
39	Eudesma-3,7(11)-diene	1568	0.13
40	NI	1574	1.07
41	NI	1586	0.13
42	*cis*-8-acetoxychrysanthenyl acetate[b]	1599	**33.70**
43	Methoxy eugenol	1612	0.07
44	NI	1620	0.57
45	NI	1625	0.08
46	NI	1638	0.61
47	NI	1646	0.13
48	*τ*-Muurolol	1652	**6.51**
49	*α*-Eudesmol	1656	0.08
50	*α*-Cadinol	1665	0.24
51	*α*-Bisabolone oxide	1696	1.59
52	Bisabolone	1756	3.61
	Total identified		95.36

Bold values represent the main identified chemical components

NI Not Identified

[a]Retention indices on HP-5 column

[b]Identified by comparison of obtained mass spectra with those literature (Obtained by Cristofari et al. 2012)

reported were *cis*-8-acetoxychrysanthenyl acetate (48.5%) as a new monoterpenic compound, *cis*-chrysanthenyl acetate (13.4%), τ-cadinol (11.4%) and 6-oxocyclonerolidol (7.8%). Cheriti et al. (2007) have shown that the chemical composition of flowers essential oil of *Bubonium graveolens* (Forssk.) (Collected from south-western Algeria) was dominated by 2,6-dimethyl-1,6-heptadien-4-yl acetate (19.4%), *trans*-chrysanthenyl acetate (18.7%), 1,8 cineole (16.5%) and *δ*-cadinol (13.9%). Another study on *A. graveolens* ssp. *odorus* revealed that the major components of the aerial parts EO of this plant were 6-oxocyclonerolidol (30.72%), *epi*-α-cadinol (14.5%) and *α*-bisabolone oxide (3.56%) (Alilou et al. 2014). It was found that the chemical composition of the EO of *A. graveolens* (Forssk.) from this study was quiet different from those of previous works cited above. These differences in the chemical composition could depend on climatic conditions and chemotypes.

Antioxidant activity

DPPH assay

The antioxidant capacity of the EO was determined by comparison to the activity of known antioxidants: ascorbic acid and *α*-tocopherol. The assessed EO was able to reduce the stable, purple colored radical DPPH to the yellow-colored DPPH-H reaching 50% of reduction with an IC_{50} of 420.16 mg/mL. Comparison of the IC_{50} of investigated EO with those obtained by ascorbic acid and *α*-tocopherol showed that this EO expressed very weak activity compared to the standards (Table 2). The weak DPPH radical scavenging activity of this oil could be attributed to the absence of active components. When Alilou et al. (2014) studied the antioxidant activity of *A. graveolens* ssp. *odorus* EO using DPPH test, they found an important activity expressed as IC_{50} value of 0.2498 mg/mL, except they used the whole aerial part of this plant which certainly should make the difference in this comparison.

Table 2 Antioxidant activity of *A. graveolens* (Forssk) essential oils (flowers part)

Sample	DPPH assay	Phosphomolybdenum assay
	IC_{50} (mg/mL)	(AAEC)
Asteriscus graveolens	420.16 ± 0.01	0.28 ± 0.01
Ascorbic acid	0.0077 ± 0.0001	–
α-tocopherol	0.020 ± 0.001	0.57 ± 0.02

Table 3 Antibacterial activity of *A. graveolens* (Forssk) flowers essential oil

Bacterial strains	Diameters of inhibition zones (mm)
1/4	
Escherichia coli	9.67 ± 0.58
Klebsiella pneumonia	10.33 ± 0.58
Salmonella typhi	9.00 ± 0.00
Pseudomonas aeruginosa	10.00 ± 0.00
Bacillus cereus	12.5 ± 0.50
Enterococcus faecalis	10.00 ± 0.00
MRSA	9.33 ± 0.58
Staphylococcus aureus	9.00 ± 0.00

Phosphomolybdenum assay

The results for Phosphomolybdenum assay were presented as equivalents of ascorbic acid per milligram. Higher values indicate better antioxidant activity. The EO showed important value relative to ascorbic acid (Table 2). The activity of the EO of *A. graveolens* represented about half (roughly) of α-tocopherol activity. Vidic et al. (2016) used this method to evaluate the antioxidant activity of some Asteraceae species (*Achillea millefolium* L., *Arnica Montana* L., *Artemisia absinthium* L., and *Artemisia annua* L.) and the highest antioxidant activity using the Phosphomolybdenum assay was obtained for *Arnica Montana* L. (55.69 mg AAEC/g).

Antibacterial activity

The inhibitory effect of *A. graveolens* essential oil on the growth of eight bacterial strains was tested. The EO showed various degrees of antibacterial activity depending on the bacterial strain. As showed in Table 3 the most important activity was observed on *B. cereus* with inhibition diameter of 12.5 ± 0.50 mm. For the other bacterial strains, the inhibition zone was ranging from 9 to 10.33 mm (Table 3). According to De Billerbeck (2007) the activity of *A. graveolens* EO is intermediate. The antibacterial activity of leaves EO of *Bubonium graveolens* was significant against *E. Coli*, *E. faecalis* and *S. aureus,* especially against *K. pneumonia* and *P. aeruginosa* comparing with commercial antibiotics (Melekmi et al. 2006).

Antifungal activity

Table 4 shows the mean radial growth of the fungus species on solid medium containing *A. graveolens* EO. The mycelial growth diameter of each species was measured during 6 days of incubation. The *A. graveolens* EO exhibited antifungal activity against the phytopathogenic fungi (Table 4). The percentage inhibition of mycelial growth increased with increasing concentration of EO for all strains tested. The most important activity was noted against *F. culmorum* BTCR with an inhibition growth of 94.12% corresponding to 20 µL/mL of EO. The inhibitory concentration of 50% of growth (IC_{50}) for the species varied between 0.5 and 1.0 µL/mL. This activity could be due to the major component of this EO (*cis*-chrysanthenyl acetate and

Table 4 Effect of *A. graveolens* flowers essential oil on Mycelial growth of FOA, FOL, FOP, FG91, FG156, FC124, FC319, T7 and BTCR

	Percentage inhibition of growth (%)					
	0.5 µL/mL	1 µL/mL	2 µL/mL	4 µL/mL	10 µL/mL	20 µL/mL
Fusariumo.f. sp. *albedinis*	59.91	62.00	65.23	78.00	80.89	84.25
Fusariumo.f. sp. *lycopercisi*	43.79	55.64	69.60	83.48	85.42	87.18
Fusariumo.f. sp. *pisi*	27.86	48.57	60.48	72.62	78.57	81.71
Fusarium graminearum 91	32.77	63.87	81.51	86.97	91.60	**92.86**
Fusarium graminearum 156	49.13	77.39	82.61	85.22	90.00	**92.17**
Fusarium culmorum 124	58.67	71.69	76.91	79.33	81.73	84.67
Fusarium culmorum 319	51.63	65.69	77.15	86.18	91.22	**92.68**
Fusarium culmorum T7	53.25	60.57	71.54	80.49	86.59	**91.46**
Fusarium culmorum BTCR	39.22	56.86	82.35	84.71	92.94	**94.12**

Bold values indicate the highest inhibition percentage values recorded

The radial mycelial growth was measured 6 days after inoculation (the data reported are the percentage inhibition %)

cis-8-acetoxychrysanthenyl acetate) or to a synergic effect of some constituents. Strains of *F. graminearum* and *F. culmorum* were susceptible to Tea tree EO (Terzi et al. 2007). Comparing to the study of Cakir et al. (2005), the EO of *Hypericum linarioides* had a weak activity on *F. culmorum* with inhibition percentage of 13.6% at 5 mg/mL. In another study the *Elettaria cardamomum* EO had an important activity against *F. graminearum* ($90 \pm 1.3\%$ at 3000 ppm) using the inverted Petri dish method (Singh et al. 2008).

The EO of *A. graveolens* aerial parts have a strong activity against *Alternaria* spp. and moderate activity against *penicillium expansum* with inhibition rate of 100% and 74.63%, respectively at 0.2% (v/v) of the oil (Znini et al. 2011). The EO of *Schinus* spp. and *Thyme* have also a strong activity against *F. graminearum* (Sampietro et al. 2014).

Anticancer activity

The results for cell growth inhibition by *A. graveolens* EO against Rat pheochromocytoma (PC12) and human liver carcinoma (HepG2) cell lines for various concentrations are shown in Figs. 1 and 2, respectively. This experiment shows that the EO of *A. graveolens* has an important activity against PC12 cell line comparing to the positive control. The activity against HepG2 cell line was less important at low concentrations but at 100 µg/mL, 82.98% of cells growth was inhibited. This activity could be due to their major components (*cis*-chrysanthenyl acetate and *cis*-8-acetoxychrysanthenyl acetate) or to a synergic with other components. Many species of *Asteraceae* (*Matricaria chamomilla* L., *Artemisia desertorum* L., *Artemisia chamaemelifolia* Vill.) are known for their anticancer activity (regarding their EOs) (Lesgards et al. 2014).

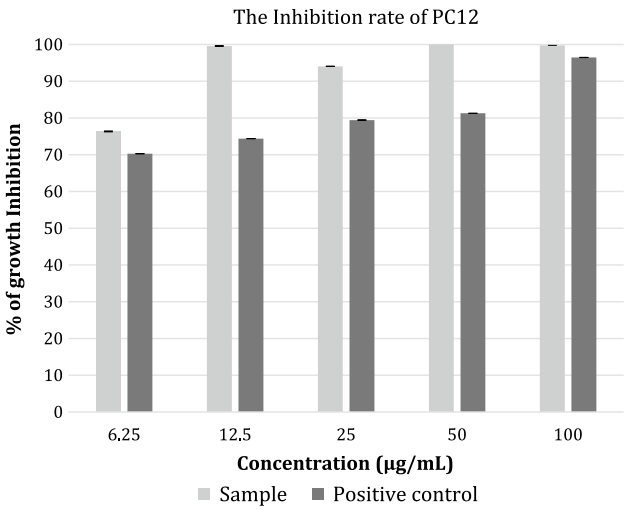

Fig. 1 Anticancer activity of *A. graveolens* flowers essential oil against PC12 cell lines

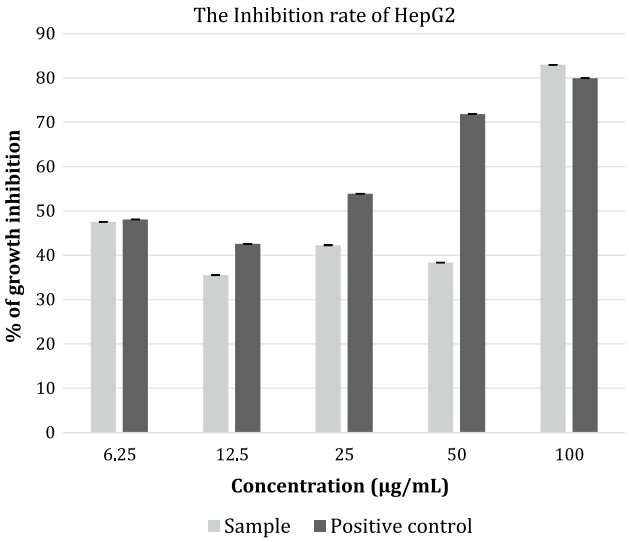

Fig. 2 Anticancer activity of *A. graveolens* flowers essential oil against HepG2 cell lines

The concentrations providing 50% of cell growth inhibition were less than 6.26 µg/mL for the PC12 cell line and more than 50 µg/mL for the HepG2. The results obtained by Wang et al. (2012) showed that the human hepatocellular liver carcinoma cell line (Bel-7402) were the most resistant to *Rosmarinus officinalis* L. EO and its major components among the tested cancer cell lines; however EO from *Artemisia indica* (Asteraceae) have a strong toxic effect on liver cancer cell lines HepG2 (Rashid et al. 2013).

Conclusion

The chemical composition of *A. graveolens* (Forssk) flowers essential oil was determined. The results showed two major components: *cis*-chrysanthenyl acetate and the *cis*-8-acetoxychrysanthenyl acetate. Moreover, we found for the first time some interesting antifungal and anticancer activities suggesting the presence of very active components in this EO. Further studies to elucidate the mechanisms of action, and the possible compounds involved in these activities will be undertaken.

Acknowledgements We thank Ms Sihem Touati for her help as well as the'Unité de Recherche Mycologie et Sécurité des Aliments' MycSa of Bordeaux for their kind help and suggestions.

Compliance with ethical standards

Ethical statement The study was conducted following the approval by the Institutional Animal Ethical Committee of Amar Télidji University, Laghouat, Algeria.

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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