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Research Article

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Chemical composition and antifungal activity of essential oil of *Anethum graveolens* L. from South-western Algeria (Bechar)

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ABSTRACT

The essential oil of Anethum graveolens L. seeds growing wild in South-western Algeria (Bechar) was obtained by hydrodistillation and investigated by GC and GC/MS.Fifteen compounds were identified, which comprised 98.93% of the total constituents. The main compounds were apiol (32.78%) and carvone (31.04%), followed bylimonene (21.26%); piperitone (6.11%) and α-phellandrene (3.28%)amounted to 94.47% of the oil. The antifungal properties of the selected oil were tested against seven fungi. The results of direct contact method showed that the oil was active against mycelial growth of fungal. All strains were inhibited at minimum inhibitory concentrations (MICs) as from 1/500 v/v, except Aspergillus niger at MIC (1/180 v/v). Alternaria alternata was most sensitive, being inhibited at MIC as weak as 1/6500 v/v. In the other hand, a concentration of 1/370 v/v was sufficient to inhibit the sporulation and spores' germination.

Keywords: *Anethum graveolens* L., GC-MS, Essential oil, Antifungal activity.

INTRODUCTION

Plant used for traditional medicine contains a wide range of substances that can be used to treat chronic as well as infectious diseases[1].In recent years there has been an increasing interest in the use of natural substances, and some questions concerning the safety of synthetic compounds have encouraged more detailed studies of plant resources.Essential oils, odors and volatile products of plant secondary metabolism, have a wide application in folk medicine as well as in fragrance industries[2],they have been used by several workers for controlling fungi, bacteria, viruses and insect pests. The main reasons for using essential oils as antifungal agents is their natural origin and low chance of pathogens developing resistance[3].The plant used in this study, *Anethum graveolens* L. or dill, belonging to Apiaceae (Umbelliferae) family, was selected based on its traditional use in Algerian medicine. It is an annual aromatic herb known for culinary and medicinal use since ancient times,growing in the Mediterranean region, but it is native to southwest Asia or southeast Europe, is widely used for flavoring foods and beverages due to its pleasant spicy aroma, and for the treatment of many pathological conditions such as disease of the uterus and cervical ectropio[4]. Its pharmacological properties, such as its antibacterial activity, as well as antihyperlipidemic and antihypercholesterolemic effects[5],antimycobacterial, antioxidant, cancer

chemopreventive, antidiabetic, and anti-inflammatory[6] have been reported. Dill has been also used traditionally in Algeria for gastrointestinal ailments such as flatulence, indigestion, stomachache colic and to tract intestinal gas.

In the course of a study aimed at phytochemical screening of the South-western Algeria flora, we wish to report here the study of the antifungal activity and chemical composition of essential oil of *A. graveolens* L.Seeds.

EXPERIMENTAL SECTION

Plant material

The seed parts of *A. graveolens* L. plant were harvested from the Igli Region of Bechar Department (South-West of Algeria), distanced from Algiers of 999 km. A voucher specimen (LB/13/UB-005) of the plant was deposited at the herbarium of laboratory of botany at the biology Institute, University Tahri Mohamed Bechar.

Essential oil isolation

The seeds of *A. graveolens* L. were submitted for 4 h to hydrodistillation using a Clevenger-type apparatus according to the European Pharmacopoeia method [7].The essential oils were dried over anhydrous sodium sulphate and then stored in sealed glass vials at 4 to 5°C prior to analysis.

Gas chromatography and Mass spectroscopy analysis

A Hewlett-Packard HP6890 gas chromatograph equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, J&W Scientific) and a FID detector regulated with 260°C and supplied with a mixture of gas H_2/Air and a Split-splitless injector regulated with 240°C were used for the percentage determination of oil components. The mode of injection is Split (split ratio: 1/50, flow: 66 ml/minutes). The gas used is the nitrogen with a flow rate of 1.7 ml/minutes. The temperature of the column is programmed from 60 to 325°C at a rate of 4°C/minutes. The apparatus is controlled by a computing system of the type « HP ChemStation », managing the operation of the apparatus and allowing following the evolution of the chromatographic analysis. The Volume injected is 1 µl. Retention indices were calculated using the retention time of n-alkanes that were injected after the oil at the same chromatographic conditions. Identification of the oil components was based on their indices of Kováts (IK) and by comparison of their mass spectral fragmentation patterns with those reported in the literature [8], and mass spectra, obtained from the GC–MS analysis on a Hewlett-Packard HP6890/HP5973 instrument equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, J&W Scientific) was used with helium as carrier gas at a flow rate of 2 ml/minutes. The GC analytical parameters were the same as those listed above, and the mass spectrometry was run in the electron impact (EI) at 70 eV.

Antifungal activity

Fungal strains

Seven fungal (*Aspergillus niger* MTTC2425, *Aspergillus flavus* MTTC 2799, *Aspergillus ochraceus* CECT 2092, *Penicillium expansum*MTTC 1344, *Fusarium oxysporum f.sp. albedinis*, *Alternaria alternata* MTCC 2724and *Cladosporium species* MTCC 1003) were used to evaluate the Antifungal properties of the essential oil. They belong to the fungus collection of biology laboratory of Bechar University. They are cultivated on nutritive medium PDA (*potato dextrose agar*) during seven days in the darkness and at room temperature 25°C. To prepare spore suspension, the fungi grown spores were suspended in 0.85% (w/v) sodium chloride to prepare the homogeneous spore suspension [9], using Malassez hematimeter in order to obtain the concentration of 10^5spore/ml.

Evaluation of the radial growth on solid medium

The minimum inhibitory concentrations (MIC) of essential oil were measured according to the contact direct method. Because of none-miscibility of essential oil with water and thus the culture medium, emulsification was performed with a 0.2% agar solution [10]. Dilutions of 1/10, 1/15, 1/37, 1/150 and 1/750 were prepared in the agar solution. In the test tubes containing each one 13,5 ml of solid medium PDA, autoclaved for 20 minutes at 121°C and cooled to 45°C, aseptically added 1.5 ml of each dilution in order to obtain final concentrations 1/100, 1/150, 1/370, 1/1500 and 1/7500 (V/V).Controls containing culture medium and 0.2% agar solution alone are also prepared. After agitation, the selected solutions were transferred into a Petri plates which were inoculated with sporal solution, the incubation was carried out at 25°C for 7 days [11]. The antifungal index is calculated by the following formula: PI= [(DT- D) / DT] ×100 %. D is mean diameter of mycelial growth of the test and DT is mean diameter of mycelial growth in control [12].

Evaluation of the sporulation

From the dishes used for the evaluation of the mycelial growth on solid medium, incubated at 25°C for 10 day for each strain at various concentrations were taken four washers 5mm diameter and transposed into tubes containing 1ml of sterile distilled water. Fungal suspension is then agitated using a vortex mixer to release the spores of the conidiophores. One counts the total number of spores using a Malassez cell at a rate of 10 counts per suspension. The values are expressed as number of spores per unit area (mm^2) [13]. The percentage inhibition of sporulation is determined by the following formula: $I_S = [(N_0 – Nc) / N_0] \times 100$ %. N_0 is mean number of the spores estimated in control and Nc is mean number of the spores estimated in the presence of essential oil.

Evaluation of germination

The sporal suspension collected was adjusted to 10^5 spores/ml of distilled water using a Malassez cell. 0.1 ml of the suspension sporal was plated on petri dishes containing PDA medium to which oils are incorporated at the same concentrations as above with three repetitions performed simultaneously by concentration. Counting spores germinated or not was performed on a total of 200 spores after 18 hours of incubation at 25°C in the dark. A spore was considered germinated if length of the germ tube is higher than its smaller diameter [14]. The percentage inhibition of germination is determined by the following formula:$Ig = (N_0- Nc) / N_0 \times 100\%$. N_0 is mean number of germinated spores in control and Nc is mean number of germinated spores in the presence of essential oil.

RESULTS AND DISCUSSION

The physicochemical analysis showed an essence light yellow with a spicy smell (aniseed).Oil yield of the plant was determined as 2.10% (w/w),is low compared with that most usually obtained from dill seeds of china (3.5%) [5] and Uzbekistan (4.2%) [15]; however, it is superior with those reported in the literature for the oil dill seeds of Turkey (0.23%) [6].

The essential oil of*A. graveolens* L.was analysed by chromatography (GC) and gas chromatography-mass spectral (GC-MS).15 compounds, representing 98.93% of the total essential oil content of*A. graveolens* L.,were identified (Table 1).The most prominent components were apiol (32.78%) and carvone (31.04%), followed bylimonene (21.26%),piperitone (6.11%) and α-phellandrene (3.28%). They constituted 94.47% of total oil.According to Small [16], the essential oil of dill consists of over 10 different aromatic compounds; carvone, limonene and α-phellandrene constitute approximately 90% of total compounds. Attokaran [17] reported that, the main compound of essential oil from seeds of dill is the carvone with a rate of 30 to 60%.Although highly heterogeneous, qualitatively the data appear to be in agreement with those reported for the essential oil composition of *A. graveolens* L. from China, Iran andIndiaarerich incarvone, limonene and dill apiole [5, 17,18].Other chemotypes such as oilsfrom the Uzbekistan and Romaniaare characterized by a high quantity of carvone, limonene and trans-dihydrocarvone [19, 20]. However, our essential oil differs from those reported by Vera and Chane-Ming [21] for the Icelandic oil and Orhan and al., [6] for the oil of Turkey,they arerich inα-phellandrene.

Table 1. Chemical composition ofvolatile oil of *A. graveolens* L.

N°	IK	Composés	SsAire %	N°	IK*	Composés	SsAire %
1	937	1R-α-pinène	0.54	9	1139	L-camphor	0.53
2	976	sabinene	0.20	10	1184	Dill ether	0.67
3	991	myrcene	0.19	11	1193	dihydrocarvone	0.30
4	1001	2-carene	0.22	12	1200	E-dihydrocarvone	1.18
5	1005	**α-phellandrene**	**3.28**	13	1242	**carvone**	**31.04**
6	1026	p-cymène	0.51	14	1252	**piperitone**	**6.11**
7	1031	**limonene**	**21.26**	15	1680	**apiol**	**32.78**
8	1076	o-Isopropenyltoluene	0.12	**Total identified**			98,93 %

*Note: * Kováts indices calculated on DB5 column with reference to n-alkanes injected after the oil at the same chromatographic conditions.*

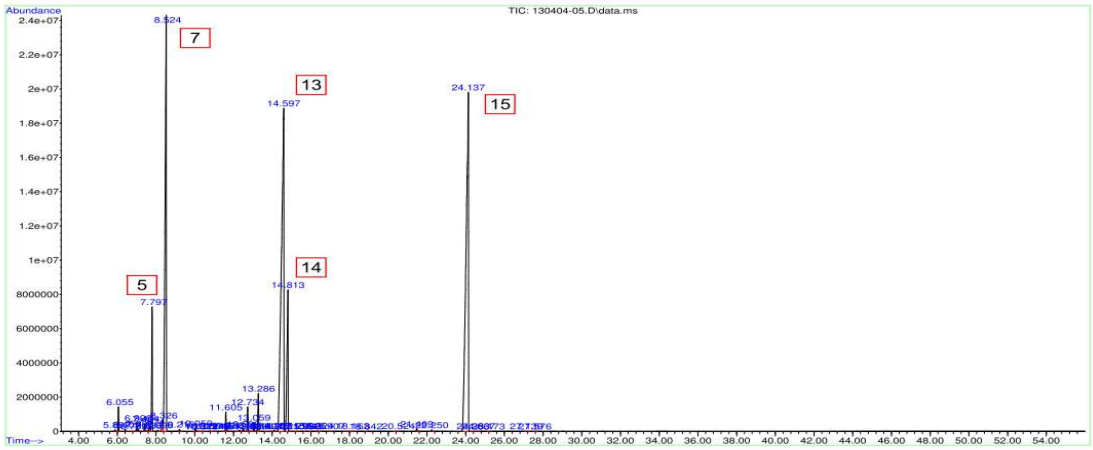

Figure 1. GC-MS analysis of*A. graveolens* L.essential oil (the numbers of the peaks can be identified in table 1)

The essential oil of *A. graveolens* L. pronounced antifungal efficacy against all tested fungi (Table 2). For fungal mycelial growth,all strains were inhibited at MICs as from 1/500 v/v, except *A. niger* at MIC 1/180 v/v. *Alternaria alternata* was most sensitive, being inhibited at MIC as weak as 1/6500 v/v.However, concentration of 1/370 v/v was found fungicide for all tested strains, except *A. niger* at 1/150 v/v. On the other hand, the essential oil showed, *in vitro*, an important antifungal activityagainst germination and sporulation spores'. All fungal strainswere inhibited at concentrations as weak as 1/370 v/v. *F. oxysporum f.sp. albedinis* and *Alternaria alternata*were most sensitive, being inhibited as from 1/1500 v/v,*A. flavus* also was inhibited to sporulate at this concentration.

Table 2. Antifungal activity of essential oil from *A. graveolens* L. expressed in (%) with MIC (v/v)

		***Aspergillus flavus*MTTC 2799**	***Aspergillus ochraceus*CECT 2092**	***Aspergillus niger* MTTC 2425**	***Fusarium oxysporum f.sp. albedinis***	***Penicillium expansum*MTTC 1344**	***Alternaria alternata* MTCC 2724**	***Cladosporium species* MTCC 1003**
Control	**a**	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0
	c	0	0	0	0	0	0	0
1/7500 (v/v)	**a**	8.39	11.62	11.94	36.36	36.53	58.82	23.80
	b	50	22.07	31.57	44.44	20	29.16	51.25
	c	73.91	77	70.30	88.50	81.05	92.85	89.09
1/1500 (v/v)	**a**	51.56	23,25	30.91	45.45	42.30	100	50.33
	b	100	42.85	78.97	100	42	100	61.25
	c	91.30	92	87.28	100	94.73	100	94.44
1/370 (v/v)	**a**	100 (f)	100 (f)	77.61	100 (f)	100 (f)	100 (f)	100 (f)
	b	100	100	100	100	100	100	100
	c	100	100	100	100	100	100	100
1/150 (v/v)	**a**	100	100	100 (f)	100	100	100	100
	b	100	100	100	100	100	100	100
	c	100	100	100	100	100	100	100
1/100 (v/v)	**a**	100	100	100	100	100	100	100
	b	100	100	100	100	100	100	100
	c	100	100	100	100	100	100	100
MIC (v/v)		1/550	1/550	1/180	1/750	1/500	1/6500	1/600

Notes: a, radial growth; b, sporulation; c, germination; f, fungicidal effect.

A previous study reported that dill seed oil is found to be highly effective for controlling the growth of *P. citrinum* and *A. niger* by poison food techniques. Moreover, the volatile oil completely inhibited the growth of *Fusarium graminearum* at 6 µl dose[22].In others studies, the MICs of this oil for four tested fungi (*A. flavus*, *A. oryzae*, *A. niger* and *Alternaria alternata*) was found to be 2 µl/ml [5].

The antifungal activity of this essential oil might be due to their major's contents of esters (apiol) and terpene ketone (carvone) compounds, because the hydrocarbon monoterpenes such as limonene (21.26%) recognized for their low antimicrobial activity [11].According Akono and al., [23], the biological activity of an essential oil is related to its

chemical composition, structure and functional groups of their constituents (alcohols, phenols, ethers, aldehydes and terpene ketone compounds), knowing that the proportions of the major compounds play a key role [24]. Also,Soković and al., [25],have shown thatketones, aldehydes and alcohols are active, exhibit strong antimicrobial activity, but with differing specificity and levels of activity, which is related to the present functional group, but also associated with hydrogen-bounding parameters in all cases. The presence of an oxygen function (such as carvone) in the framework increases the antimicrobial properties of terpenoids. The active antimicrobial compounds of essential oils are generally terpene. The mechanism of this class of compounds is not fully understood but is speculated to involve membrane disruption by lipophilic compounds [5].Indeed, dill oil was previously found to have a strong acetylcholinesterase-butyrylcholinesterase inhibiting property[6]. Tian and al., [4] reported that the plasma membrane and the mitochondria are the antifungal targets of dill oil.

Finally, previous studies confirm thatessential oils have multiple functions, inhibit spore germination, elongation of the mycelium and sporulation [26].

CONCLUSION

From these results, it appeared that theessential oil of *A. graveolens* L. seedsexhibited high antifungal activity according to the amount of esters and terpene ketone compounds. Thisoil can be used asantifungal therapeutic agent and may be suggested as alternative fungicide.

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