

GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *ANETHUM GRAVEOLENS* (UMBELIFERAE) ESSENTIAL OIL

Abdel Karim M.^{1*}, Osman A.¹, Amira A. E. Satti^{1,2} and Al-Hafez M.³

¹Sudan University of Science and Technology, Faculty of Science (Sudan).

²Qurayat-Jouf University, Faculty of Science and Arts, Dept. of Chemistry (Saudi Arabia).

³King Khalid University, Faculty of Science and Arts, Dept. of Chemistry (Saudi Arabia).

*Corresponding Author: Dr. Abdel Karim M.

Sudan University of Science and Technology, Faculty of Science (Sudan).

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ABSTRACT

Information of the constituents of medicinal plant is of great importance since medicinal plants are endowed with diverse phytochemicals with potential medicinal applications. This study was aimed to investigate the chemical constituents of the medicinally important *Anethum graveolens* volatile oil, and to evaluate its antimicrobial activity. 30 components were detected by GC-MS analysis. Major constituents are: D-carvone (37.80%); D-limonene (18.13%) and apiol (16.16%). The antimicrobial activity of the oil was evaluated via disc diffusion method against five standard human pathogens (Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseudomonasa aeruginosa* and the fungi *Candida albicans*). The oil showed significant activity against *Escherichia coli*, *Pseudomonasa aeruginosa* and *Bacillus subtilis*. It also showed good activity against *Staphylococcus aureus*.

KEYWORDS: Anethum graveolens, Essential oil, Constituents, Antimicrobial activity.

INTRODUCTION

Anethum graveolens (Umbeliferae) is an annual herb indigenous to southern Europe. It is now cultivated worldwide for its economic value.^[1-4]

Anethum graveolens has a long history of applications in ethnomedicine including treatment of flatulence, indigestion and convulsions. *Anethum graveolens* has antiemetic and stimulant effects and is said to increase appetite.^[5-7] It has been reported that *Anethum graveolens* possesses anti-inflammatory,^[8,9] antihyperlipidemic,^[10-12] antimicrobial,^[13-17] antinociceptive properties beside analgesic and smooth muscle relaxing effects.^[18,20] *Anethum graveolens* contains, among others, proteins (15.68%), carbohydrates (36%), fiber (14.80%), moisture (8.39%), ash(9.8%) beside polyphenols and some minerals.^[6,7,21-26]

MATERIALS AND METHODS

Plant material

Seeds of *Anethum graveolens* were purchased from the local market-Oswan, Egypt. the plant was identified and authenticated by direct comparison with a reference herbarium sample.

Instruments

A Shimadzo GC-MS-QP2010 ultra instrument with RTX-5MS column (30m, length; 0.25mm diameter; 0.25µm, thickness) was used for GC-MS analysis.

Test organisms

Anethum graveolens oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in Table (1)

Table 1: Test organisms.

Ser. No	Microorganism	Type
1	<i>Bacillus subtilis</i>	G+ve
2	<i>Staphylococcus aureus</i>	G+e
3	<i>Pseudomonas aeruginosa</i>	G-ve
4	<i>Escherichia coli</i>	G-ve
5	<i>Candida albicans</i>	fungi

Methods

Extraction of oil from *Anethum graveolens*

Hydrodistillation method was used for extraction of *Anethum graveolens* volatile oil.

Esterification of oil

A methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in 100ml methanol. A stock solution of methanolic sulphuric acid

was prepared by mixing (1ml) of concentrated sulphuric acid with (99ml) methanol. The oil (2ml) was placed in a test tube and (7ml) of methanolic sodium hydroxide were added followed by (7ml) of an alcoholic sulphuric acid. The tube was shaken vigorously for 5 minutes and left over night. (2ml) of n-hexane were added and the tube was vigorously shaken for 5 minutes The hexane layer was diluted with 5ml diethyl ether. One gram of sodium sulphate was added as drying agent. The solution was filtered and the filtrate was transferred to the GC-MS vial.

GC-MS analysis

The volatile oil from *Anethum graveolens* was analyzed by GC-MS. A Shimadzo GC-MS-QP2010 ultra instrument was used. Chromatographic conditions and oven temperature program are presented below.

Table 2: Oven Temperature Program.

Rate	Temperature (C)	hold time (min. ⁻¹)
-	50.0	0.00
7	180.0	0.00
10	300.0	0.00

Table 3: Chromatographic conditions.

Column oven temperature	50.0 ^o C
Injection temperature	300.00 ^o C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	100.0 KPa
Total flow	50.0 ml/min
Column flow	1.69 ml/min
Linear velocity	44.7 cm/sec
Purge flow	3.0 ml/min
Split ratio	-1.0

Testing of antimicrobial susceptibility

Bacterial culture was performed on Mueller-Hinton agar, while fungal cultures were maintained on Sabouraud

dextrose agar. The paper disc diffusion method was used to screen the antimicrobial activity of the oil. The experiment was carried out according to.^[27] Bacterial suspension was diluted with sterile physiological solution to 10^8 cfu/ml (Turbidity=McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1.6mm in diameter) were placed on the surface of the MHA and soaked with 100mg/ml of test sample. The inoculated plates were incubated at 37^oC for 24 hours in the inverted position. The diameters (mm) of the inhibition zones were recorded as average of two replicates.

RESULTS AND DISCUSSION

GC-MS Analysis of *Anethum graveolens* volatile oil

GC-MS Analysis of *Anethum graveolens* volatile oil was performed. The analysis revealed the presence of 30 components (Table 4). The typical total chromatograms (TIC) is shown in Fig 1.

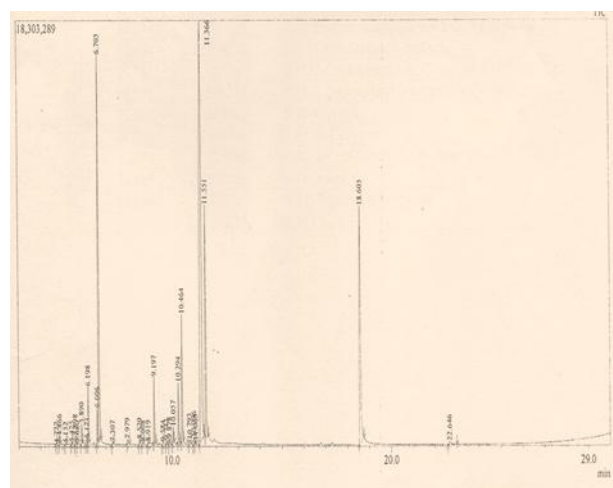


Fig. 1: Typical total ion chromatograms (TIC).

Table 4: Constituents of *Anethum graveolens* oil.

No.	Name	Ret. Time	Area%
1.	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	4.727	0.02
2.	.alpha.-Pinene	4.866	0.25
3.	Camphene	5.152	0.03
4.	3-Carene	5.425	0.06
5.	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	5.598	0.25
6.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	5.680	0.05
7.	.beta.-Myrcene	5.890	0.64
8.	(+)-2-Carene	6.121	0.18
9.	.alpha.-Phellandrene	6.198	1.89
10.	p-Cymene	6.606	1.11
11.	D-Limonene	6.703	18.13
12.	.gamma.-Terpinene	7.307	0.08
13.	o-Isopropenyltoluene	7.979	0.21
14.	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis-	8.529	0.17
15.	trans-p-Mentha-2,8-dienol	8.668	0.11
16.	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-	8.919	0.06

17.	(+)-2-Bornanone	9.197	2.39
18.	Santolina alcohol	9.584	0.06
19.	1-(1,2,3-Trimethyl-cyclopent-2-enyl)-ethanone	9.743	0.17
20.	Terpinen-4-ol	9.878	0.23
21.	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	10.057	0.57
22.	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-	10.294	2.20
23.	Cyclodecene, 1-methyl-	10.464	5.01
24.	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-	10.793	0.17
25.	1-Isopropenyl-3-propenylcyclopentane	10.996	0.24
26.	Carveol	11.063	0.10
27.	D-Carvone	11.366	37.80
28.	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	11.551	11.32
29.	Apiol	18.605	16.16
30.	Tolclofos-methyl	22.646	0.34

Major constituents of the oil are:

- (i) D-Carvone (37.80%).
- (ii) D-Limonene (18.13%).
- (iii) Apiol (16.16 %).

The mass spectrum of D-carvone is shown in Fig. 2. The molecular ion $M^+ [C_{10}H_{14}O]^+$ appeared at

m/z 150 (RT. 11.366). The mass spectrum of D-limonene is displayed in Fig. 3. The signal at m/z 136 corresponds the molecular ion $M^+[C_{10}H_{16}]^+$. Fig. 4 shows the mass spectrum of apiol. The signal which appeared at retention time 18.605 (m/z 222) corresponds the molecular ion $M^+[C_{12}H_{14}O_4]^+$.

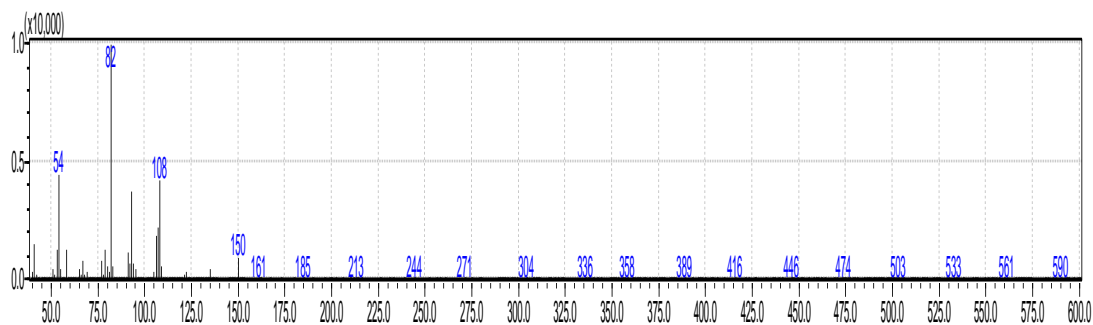
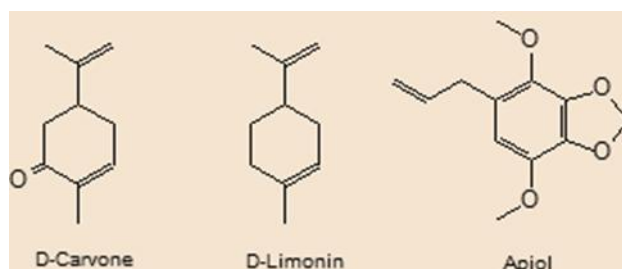


Fig. 2: Mass spectrum of D-carvone.

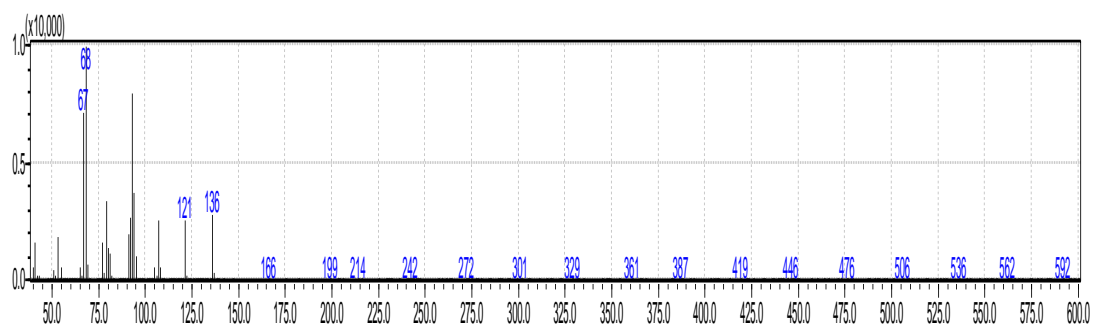


Fig. 3: Mass spectrum of D-limonene.

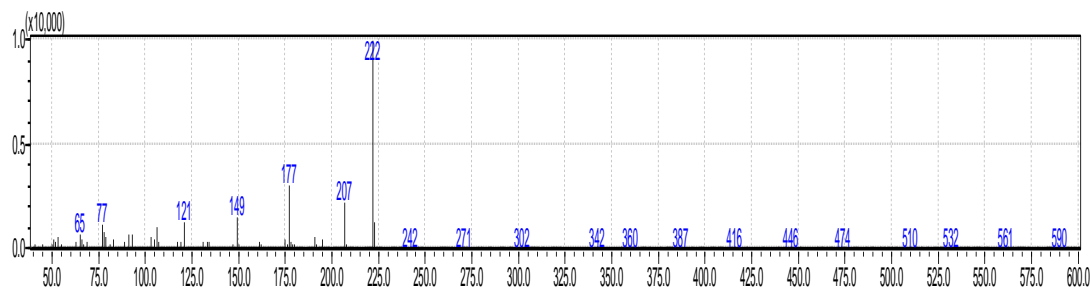


Fig. 4: Mass spectrum of apiol.

Antimicrobial assay

The oil was screened for antimicrobial activity against five standard human pathogens, the average of the diameters of the growth of inhibition zones are depicted in Table (5). The results were expressed in terms of the diameter of the inhabitation zone:

<9 mm inactive

9-12 mm partially active

13-18 mm active

>18 mm very active

Ampicilin, gentamicin and clotrimazole were used as positive control.

Table 5: Inhibition zones of oil.

Sample	Sa	Bs	Ec	Ps	Ca.
Oil (100mg/ml)	17	21	20	20	14
Ampicilin (40mg/ml)	30	15	--	--	--
Gentamicin (40mg/ml)	19	25	22	21	--
Clotrimazole (30mg/ml)	--	--	--	--	38

Ec.: *Escherichs coli*.

Ps.: *Pseudomonas aeruginosa*.

Sa.: *Staphylococcus aureus*.

Bs.: *Bacillus subtilis*.

Ca.: *Candida albicans*.

The oil showed significant activity against *Escherichia coli*, *Pseudomonasa aeruginosa* and *Bacillus subtilis*, while it showed a good activity against *staphylococcus aureus*.

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