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Research Article

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GC-MS Studies and Antioxidant Activity of *Apium graveolens* Grown in Saudi Arabia

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Abstract *Apium grveolens* L. is an annual or perennial plant in the family Apiaceae. Phytochemical studies indicated the presence of many phytochemicals including alkaloids, steroids and flavonoids. The seeds, leaves and essential oils are all used in ethnomedicine. *Apium grveolens* can reduce the risk of cardiovascular and liver diseases. It can also treat jaundice and gout and can protect against urinary tract obstruction. In this study *Apium grveolens* oil was studied by GC-MS. A total of 45 constituents were identified. Major constituents are: 9-octadecenoic acid methyl ester (56.04 %); 9,12-octadecadienoic acid methyl ester (13.47%); hexadecanoic acid methyl ester (12.05%); methyl stearate (4.69%). The oil was assessed for antimicrobial activity. At a concentration of 100mg/ml the oil showed partial activity against *Escherichia coli*, *Staphylococcus aureus* and the fungal species *Candida albicans.*

Keywords *Apium grveolens*, Oil, GC-MS Analysis, Antimicrobial Activity

Introduction

Humans used medicinal plants for fighting diseases since time immemorial. Herbal medicine is affordable and cost-effective [1-2]. Recently research focused on the role of plant secondary metabolites in treating various human disorders [3-5]. Pharmcological studies indicated positive effect of medicinal plants on liver disorder, anemia, renal problems, hormone disorders, infertility, inflammation, skin infections and other human disorders [6-10].

Apium grveolens L. is an annual or perennial plant in the family Apiaceae. It grows along tropical and subtropical Africa and Asia and throughout Europe [11]. Phytochemical studies indicated the presence of many phytochemicals including alkaloids, steroids and flavonoids [12]. The plant also contains [13] carbohydrates beside vitamins A and C. The seeds, leaves and essential oils are all used in ethnomedicine. Studies showed that *Apium grveolens* can reduce the risk of cardiovascular and liver diseases. It can also treat jaundice and gout and can protect against urinary tract obstruction [14-17].

Leaves can increase spermatogenesis and imrovee fertility [18-20]. *Apium grveolens* is hypoglycemic, hypotensive and heart tonic [7, 21-22]. *In vivo* studies indicated that *Apium grveolens* possesses antifungal and anti-inflammatory properties [13,23]. The antibacterial effect of *Apium grveolens* essential oil has been documented [24]. The plant is used traditionally against asthma, skin infections, asthenopia, bronchitis, vomiting, fever and tumors [16, 25-26].

Materials

Plant Material

Apium grveolens seeds were purchased from the local market Rhyadh-Saudi Arabia. The plant was authenticated by direct comparison with a herbarium sample. The seeds were shade – dried at room temperature and powdered

GC-MS Analysis

GC-MS analysis was conducted on a Shimadzo GC-MS-QP2010 Ultra instrument with RTX-5MS column (30m, length; 0.25 mm diameter; 0.25 µm, thickness)**.**

Test organisms

The oil from *Apium graveolens* seeds was screened for antimicrobial activity using the standard microorganisms shown in Table 1.

Table 1: Test organisms

S. No.	Micro organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
5	*Candida albicans*	fungi

Methods

Extraction of oil

Powdered shade-dried seeds of *Apium graveolens* (300g) were exhaustively extracted with n-hexane at room temperature. The solvent was removed under reduced pressure to give the oil. The oil was esterified as follows: the oil (2 ml) was placed in a test tube and 7ml of alcoholic sodium hydroxide were added followed by 7ml of alcoholic sulphuric acid. The tube was stoppered and shaked vigorously for five minutes and then left overnight. Then (2ml) of supersaturated sodium chloride were added followed by (2 ml) of normal hexane and the tube was vigorously shaked for five minutes. The hexane layer was then separated. (5 µl) of the hexane extract were mixed with 5ml diethyl ether. The solution was filtered and the filtrate (1 µl) was injected in the GC-MS vial.

Constituents of the oil

The studied oil was analyzed by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument. Helium was used as carrier gas. Chromatographic conditions are presented below:

- Oven temperature program

Rate: ---; Tempt. , 150.0 ºC; Hold time ($min.^{-1}$), 1.00

Rate: 4.00; Tempt. , 300.0 ºC; Hold time ($min.^{-1}$), 0.00

-Chromatographic conditions

Column oven temperature	150.0 °C
Injection temperature	300.0 °C
Rate	4./min.
Injection mode	Split
Flow control mode	Linear velosity
Pressure	139.3 KPa.
Total flow	50.o ml/min.
Column flow	1.54 ml/sec.
Linear velocity	47.2 cm/sec.
Purge flow	3.0 ml/min.
Split ratio	- 1.0

Antimicrobial assay

Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungus respectively. The media were prepared according to manufacture instructions.

Cultures ($5.0x10^7$ cfu/ml) were streaked on the surface of the solid medium contained in Petri dishes. Filter paper discs (Oxid, 6mm) were placed on the surface of the inoculated agar and then impregnated with 100 mg/ml of test sample. For bacteria the plates were incubated at 37 ^oC for 24 h., while for fungi the plates were incubated at 25 ^oC for 3 days. The assay was carried out in duplicates and the diameters of inhibition zone were measured and averaged. Ampicilin, gentamicin and clotrimazole were used as positive control and DMSO as negative control.

Results and Discussion

GC-MS analysis of *Apium graveolens* oil was conducted and the identification of the constituents was accomplished by retention times and MS fragmentation pattern.

Constituents of Oil

The GC-MS spectrum of the studied oil showed the presence of 45 constituents (Table 2)).The typical total ion chromatograms (TIC) is shown in Fig. (1).

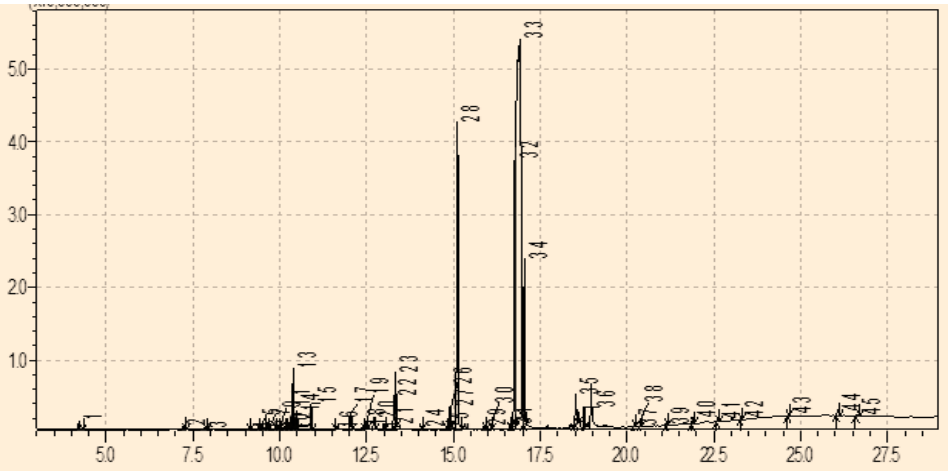

Figure 1: Total ion chromatograms

The major constituents of the oil are:

i) 9-Octadecenoic acid methyl ester (56.04%)
ii) 9, 12-Octadecadienoic acid methyl ester (13.47%)
iii) Hexadecanoic acid methyl ester (12.05%)
iv) Methyl stearate (4.69%)

The mass spectrum of 9-octadecenoic acid methyl ester is shown in Fig. 2. The peak at m/z 296, which appeared at R.T. 16.933 in total ion chromatogram, corresponds the molecular ion: $M^+[C_{19}H_{36}O_2]^+$. The signal at m/z 266 is due to loss of a methoxyl. The mass spectrum of 9,12-octadecadienoic acid methyl ester is depicted in Fig. 3. The signal which was observed at m/z 294 (R.T. 16.774) is due to $M^+[C_{19}H_{34}O_2]^+$, while the signal at m/z 263 corresponds to loss of a methoxyl. Fig. 4 shows the mass spectrum of hexadecanoic acid methyl ester. The peak m/z 270 (R.T. 15.119) was detected in the spectrum. It corresponds $M^+[C_{17}H_{34}O_2]^+$. The peak at m/z239 is due to loss of a methoxyl. The EI mass spectrum of methyl stearate is displayed in Fig. 5. The peak at m/z 298 (R.T. 17.042) is due to $M^+[C_{19}H_{38}O_2]^+$, while the signal at m/z 267 corresponds to loss of a methoxyl group.

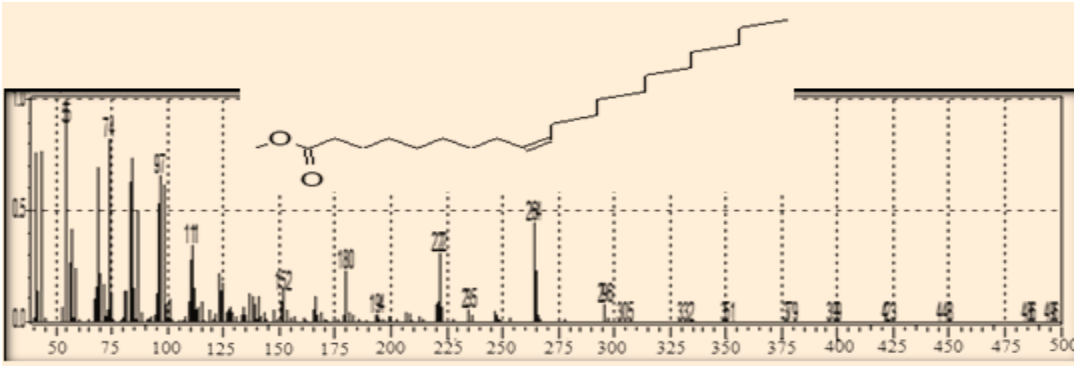

Figure 2: Mass spectrum of 9-octadecenoic acid methyl ester

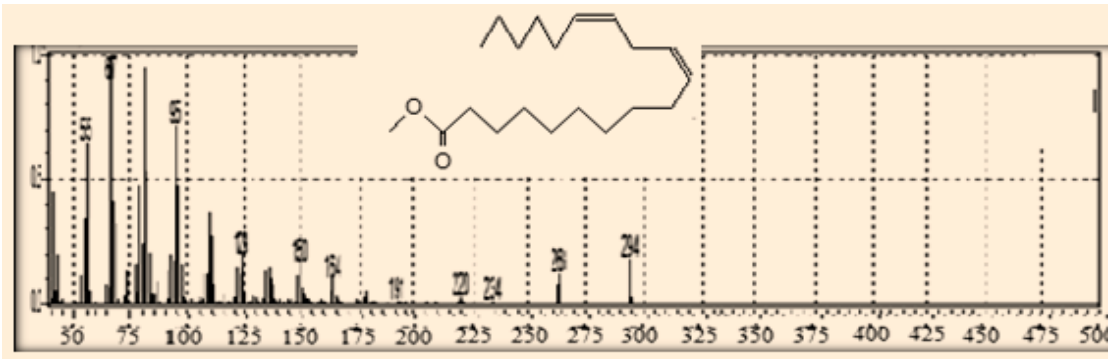

Figure 3: Mass spectrum of 9,12-octadecadienoic acid methyl ester

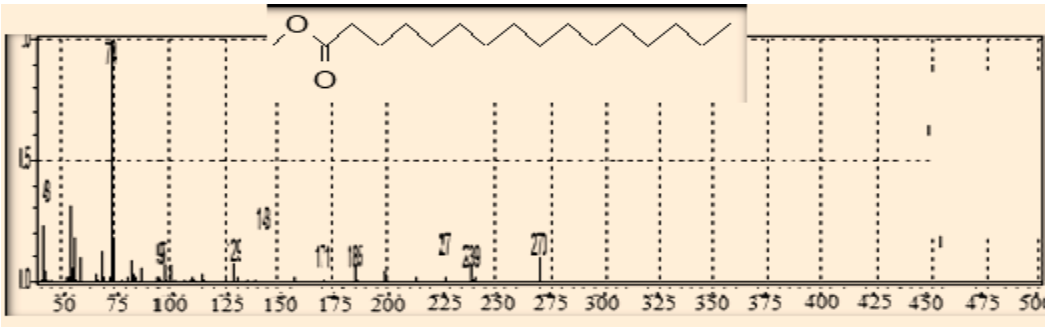

Figure 4: Mass spectrum of hexadecanoic acid methyl ester

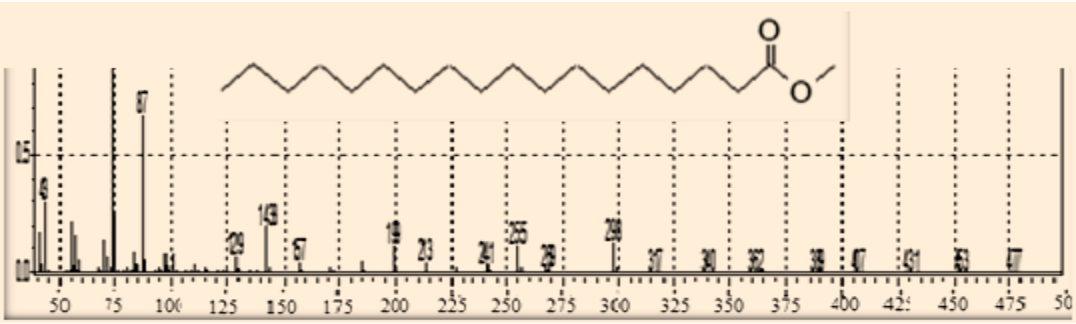

Figure 5: Mass spectrum of methyl stearate

Table 2: Constituents of the oil

No.	Name	Ret. Time	Area%
1.	D-Limonene	4.214	0.34
2.	(-)-Carvone	7.205	0.29
3.	Anethole	7.813	0.25
4.	1-Hepten-4-ol, 4-propyl-	9.109	0.07
5.	6-Heptene-2,4-diol	9.393	0.02
6.	Caryophyllene	9.524	0.17
7.	Pentane, 2,2'-[ethylidenebis(oxy)]bis-	9.649	0.01
8.	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	9.870	0.05
9.	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	9.960	0.01
10.	4,5-di-epi-aristolochene	10.153	0.01
11.	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	10.208	0.01
12.	Alloaromadendrene	10.254	0.05
13.	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	10.383	1.79
14.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	10.483	0.52
15.	Ledol	10.902	0.75
16.	Caryophyllene oxide	11.591	0.05
17.	Apiol	12.027	0.59
18.	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	12.392	0.25
19.	butylphthalide	12.469	0.14
20.	1-Propanone, 1-(3-cyclohexen-1-yl)-2,2-dimethyl-	12.711	0.56
21.	Methyl tetradecanoate	13.000	0.16
22.	1,3-Benzenediamine	13.282	1.25
23.	2-(3-Hydroxybutyl)cyclooctanone	13.335	1.74
24.	Pentadecanoic acid, methyl ester	14.078	0.13
25.	7,10-Hexadecadienoic acid, methyl ester	14.800	0.02
26.	7-Hexadecenoic acid, methyl ester, (Z)-	14.885	0.28
27.	9-Hexadecenoic acid, methyl ester, (Z)-	14.901	0.59
28.	Hexadecanoic acid, methyl ester	15.119	12.05
29.	cis-10-Heptadecenoic acid, methyl ester	15.869	0.17
30.	Heptadecanoic acid, methyl ester	16.081	0.14
31.	6,9-Octadecadienoic acid, methyl ester	16.618	0.26
32.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.774	13.47
33.	9-Octadecenoic acid (Z)-, methyl ester	16.933	56.04
34.	Methyl stearate	17.042	4.69
35.	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	18.515	1.39
36.	Eicosanoic acid, methyl ester	18.765	0.56
37.	13-Docosenoic acid, methyl ester, (Z)-	20.204	0.05
38.	Docosanoic acid, methyl ester	20.384	0.21
39.	Tricosanoic acid, methyl ester	21.149	0.05
40.	Tetracosanoic acid, methyl ester	21.887	0.22
41.	Squalene	22.605	0.11
42.	Hexacosanoic acid, methyl ester	23.284	0.07
43.	Octacosanoic acid, methyl ester	24.641	0.07
44.	Stigmasterol	26.067	0.25
45.	.gamma.-Sitosterol	26.639	0.10

Antimicrobial activity

Apium graveolens seed 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 3. The results were interpreted in the

following manner: (<9mm: inactive; 9-12mm: partially active; 13-18mm: active; >18mm:very active). Tables 4 and 5 display the antimicrobial activity of standard antibacterial and antifungal drugs respectively.

At a concentration of 100mg/ml the oil showed partial activity against *Escherichia coli*, *Staphylococcus aureus* and the fungal species *Candida albicans.*

Table 3: Antimicrobial Activity of the *Azadirachta Indica* seed oil

Oil	**Antibacterial activity**				
	Gram positive		**Gram negative**		
mg/ml	*Bs.*	*Sa.*	*Ec.*	*Pa.*	*Ca.*
100	--	10	10	--	12

Table 4: Antibacterial activity of standard chemotherapeutic agents

Drug	**Conc. mg/ml**	**Bs.**	**Sa.**	**Ec.**	**Ps.**
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamicin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 5: Antifungal activity of standard chemotherapeutic agent

Drug	**Conc. mg/ml**	**An.**	**Ca.**
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

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