

GC - MS AND ANTIMICROBIAL ACTIVITY OF  
VOLATILE OIL OF APIUM LEPTOPHYLLUM (BENTH.)

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**ABSTRACT:**

Volatile oil of Apium leptophyllum (Benth.), family Umbelliferae, growing in Egypt (0.3%) was studied. GC/MS of the oil resulted in identification of 12 components which constitute 96.8% of the oil. Also two unidentified sesquiterpene alcohols were reported. The oil showed a strong antifungal activity especially against Candida albicans and moderate activity against G +ve rods and G -ve cocci and rods.

**INTRODUCTION**

Apium leptophyllum (Pers.) F. Muell. ex Benth. (A. tenuifolium Thell), family Umbelliferae, is small delicate procumbent or ascending herb, it has almost fili-for leaf segments and minute white flowers in sessile leaf-opposed umbels. Fruits, are glabrous, ovate, and somewhat laterally compressed. The plant especially grows in lawns <sup>(1)</sup>.

Family Umbelliferae includes about 275 genera and 2850 species, most of them contain volatile oils of valuable pharmaceutical uses <sup>(2)</sup>. Genus Apium is represented in Egypt by only four species, the most common of which is Apium graveolens (celery) which is used for its unique texture and appetizing flavour. Celery oil is used commercially in flavouring food products, liqueur, perfume and soap<sup>(3)</sup>. Also it was reported to have a sedative and tonic effects upon the central nervous system and used in nerve tonics<sup>(4)</sup>.

Literature survey revealed no reports about Apium leptophyllum Benth. So, it was necessary to study this Egyptian plant. The present study deals with the analysis of the volatile oil content of the plant as well as its antimicrobial activity.

**EXPERIMENTAL**

**Material and apparatus:**

**1- Plant material:**

The fresh fruiting plant collected in May 1992 from Zagazig. The plant was kindly identified by Prof. Dr. Nabil El Hadidi, Department of Botany, Faculty of Science, Cairo University. Authentic sample has been kept in Pharmacognosy Department, Faculty of Pharmacy, Zagazig University.

**2- Strains:**

The microorganisms used were; Staphylococcus aureus (St.), Sarcina lutea (Sa.), Bacillus subtilis (Ba.), Escherishia coli (E.coli), Neisseria sp. (Ni.) and Candida albicans (Ca.).

3- Media: nutrient agar.

4- GC/MS analysis :

Gas chromatography coupled with mass spectrometry of the oil was carried out using HP GC - MS 5988A

a) GLC conditions: HP-ultra-1 (cross-linked methyl silicone) high speed capillary column 12 m x 0.2 mm x 0.33  $\mu$ m (U.S.A). Carrier gas helium at a flow rate 0.97 ml / min, initial temp. 70°C, final temp. 180°C for 38 min , rate 7°C / m. injector temp. 250°C ion source temp. 200°C and detector temp. 280°C.

b) Ms-conditions: Ms Mode: Ei, ionization voltage 15 ev. analyzer pressure  $16^{-6}$  torr, scan range 50-550.

Preparation of the Volatile Oil:

The volatile oil of the fresh fruiting plant was prepared by steam distillation using the E.P method<sup>(5)</sup>. The percentage of the yield was 0.3 V/W on fresh weight basis. The oil is colourless to pale yellow, has sp.gr. 0.89-0.91, aromatic characteristic odour and taste. A sample was submitted to GC/MS analysis and the results are shown in table 1.

Table (1): GC/MS analysis of the volatile oil of *Apium leptophyllum*.

Pk No	Rt (min)	%	M <sup>+</sup>	B.P	Major Peaks*	Identification
1	7.900	0.5965	136	93	121,107,79,69.	$\beta$ -pinene <sup>(3)</sup> .
2	8.552	17.511	134	119	91,79,77,65,51.	p-cymene <sup>(3)</sup> .
3	9.196	10.318	136	93	121,105,91,77.	$\beta$ -phellandrene <sup>(3)</sup> .
4	10.936	0.332	154	71	136,111,93,86,69.	$\beta$ -terpineol <sup>(3)</sup> .
5	11.922	22.715	164	149	134,119,91,77.	Thymyl-methyl-ether <sup>(6)</sup> .
6	11.986	7.475	164	164	149,134,119,91	Carvacryl-methyl ether.
7	14.553	31.717	194	179	164,149,134,119,91	Thymohydroquinone dimethyl-ether <sup>(7)</sup> .
8	14.885	3.353	194	194	179,149,134,119	Isomer of 7.
9	15.225	1.343	204	93	161,136,121,105,67	Germacrene C <sup>(8)</sup> .
10	15.488	0.715	204	161	189,133,120,105,81	Germacrene D <sup>(8)</sup> .
11	15.902	0.125	204	204	189,161,134,119,105	Cadinene <sup>(9)</sup> .
12	16.349	0.593	204	121	189,161,136,107,93	Bicyclogermacrene <sup>(10)</sup>
13	16.837	0.228	220	138	177,150,109,96,82	Undidentified sesquiterpene alcohol.
14	18.032	0.161	220	220	202,187,162,149,121	Undidentified sesquiterpene alcohol.

\* Compared fragments with the other oil constituents were slightly different in peak intensities due to different analysis conditions.



**Antimicrobial Activity OF The Volatile Oil :**

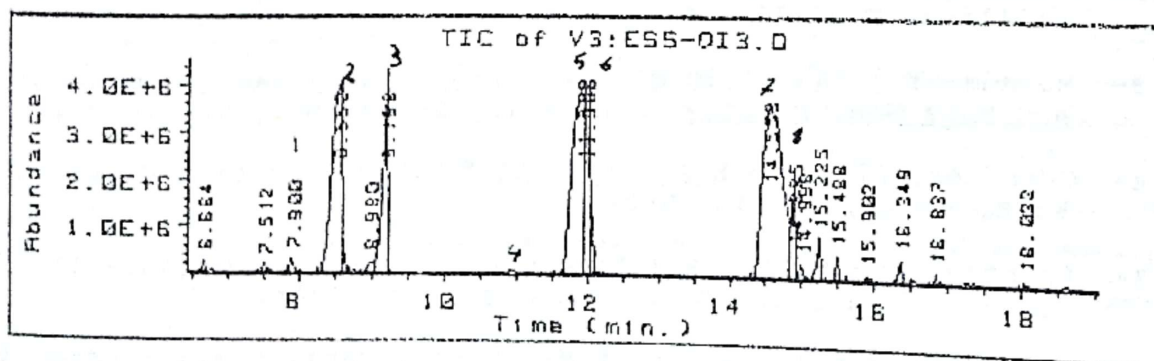
The disc-agar diffusion method (11) was adopted using volatile concentration 100 mg/ml DMF and the results are shown in table (2).

Table (2): The results of the antimicrobial test.

Inhibition-Zone Diameter (I Z D) in mm					
Bacteria					Fungi
G +ve			G -ve		Candida albicans
cocci		rods	cocci	rods	
St.	Sa.	Ba.	Ni.	E.coli	
--	--	10	12	8	

**RESULTS AND DISCUSSION**

The volatile oil of *Apium leptophyllum* (Benth.) was prepared (0.3% v/w). A sample was submitted to Gc/Ms analysis. Identification of the various constituents present in the oil were carried out using combined Gc/Ms and comparing the resulted data with several reported mass fragmentation pattern of previously investigated oils of different plants(3,6,7,8,9,10). The Gc /Ms analysis revealed the presence of 32 components. 12 components constitute 96.8% of the oil were identified. The identified oxygenated compounds form about 66% while the identified hydrocarbons form about 31% . The presence of the aromatic hydrocarbon P-cymene in high percentage (17.511) in the oil give stability to the oil.



(Fig. 1)-GLC chromatogram of volatile oil of *Apium leptophyllum*.

Peaks No 5 & 6 (Fig. 1) showed two components with parent ion  $M^+$  m/z 164, 149 ( $M^+-15$ ), 134 ( $M^+-30$ ), 119 ( $M^+-45$ ), 91 ( $M^+-73$ ), then fragmentation were identical to p-cymene. This suggested the possible conclusion of having two isomeric mono methoxylated p-cymene derivatives. By comparing with mass fragmentation of previously investigated oils of different plants (6), it was found that compounds 5 & 6 are thymyl-methyl-ether (22.715%) and its isomer carvacryl-methyl-ether (7.475%).

Peaks No 7 & 8 (Fig.1) also showed two isomeric components with parent ion  $M^+$  m/z 194, 179 ( $M^+-15$ ), 164 ( $M^+-30$ ), 149 ( $M^+-45$ ), then fragmentation were identical to those compounds 5&6 with the exception of  $M^+$  is more only by 30 which suggest another methoxyl group, this led to the possible conclusion of having two isomeric dimethoxylated p-cymene. Compound 7 was previously isolated from Sphaeranthus suaveolens (7) and named thymo hydroquinone dimethyl ether, but it seems to be identified for the first time as volatile oil content.

The antimicrobial study for the oil revealed a strong activity against Candida albicans, moderate activity against G - ve cocci and rods and G + ve cocci.

From the above mentioned results one rise to conclusion that the volatile oil of Apium leptophyllum can be used as antifungal and in soap and cosmetic industries for its high content of the oxygenated compounds.

#### ACKNOWLEDGEMENTS

The author is grateful to Dr. Hassan Abd El salam, Microbiology Department, Faculty of Pharmacy, Zagazig University, who provided the authentic microorganisms and carried out the antimicrobial test for the oil.

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دراسة الزيت الطيار لنبات أبيم لبثيلم ( بنث )  
من الفصيلة الخيمية بواسطة كروماتوجرافيا الغاز وجهاز طيف الكتلة

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الملخص العربي :-

في هذا البحث تم تحضير الزيت الطيار لنبات أبيم لبثيلم بواسطة التقطير  
بالبخار ووجدت نسبته ٣ و٪ . كما تم تحليل الزيت باستخدام كروماتوجرافيا الغاز المتصله  
بجهاز طيف الكتلة وقد تم التعرف علي ١٢ مركب تكون ٨ و ٩٦٪ من مكونات الزيت ووجد ان  
المركبات الاكسجينية التي تم التعرف عليها تمثل حوالي ٦٦٪ ، اما المواد الهيدروكربونية التي تم  
التعرف عليها فتمثل ٣١٪ .  
وكذلك تم اختبار فاعلية الزيت ضد الميكروبات والفطريات فوجد ان له تأثير قوي ضد فطر  
كانديدا ألبيكانس وبعض الانواع من البكتريا .  
ونتيجة لهذه الدراسة يتضح امكانيه استخدام هذا الزيت في صناعة الصابون ومستحضرات  
التجميل الاخري لما يحتويه من نسبة عالية من المركبات الاكسجينية .