



ANTIMICROBIAL ACTIVITY OF *PIMPINELLA ANISUM* (UMBELLIFERAE) GROWN IN JORDAN

Abdel Karim M.^{1*}, Magid E.¹ and M. Alla²

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

²Delta College of Science and Technology (Sudan).

Corresponding Author: Abdel Karim M.

Sudan University of Science and Technology, Faculty of Science.

Article Received on 03/08/2020

Article Revised on 24/08/2020

Article Accepted on 14/09/2020

ABSTRACT

Pimpinella anisum L. (Anise) is a herb in the family Umbelliferae. The plant is a key species in Sudanese system of medicine. Anise seeds are used as analgesic, carminative, disinfectant, and diuretic in traditional medicine. In this study *Pimpinella anisum* seed oil was analyzed by GC-MS. The analysis revealed 34 components dominated by: 9-octadecenoic acid methyl ester(44.82%); apiol(15.33%);9,12-octadecadienc acid methyl ester (11.22%); hexadecanoic acid methyl ester(6.57%) and cis-10-nonadecenoic acid methyl ester (5.22%). The oil was evaluated for antimicrobial activity against five standard pathogenic microbes. It exhibited significant anticandidal potency and moderate activity against *Staphylococcus aureus* *Bacillus subtilis* and *Escherichia coli*:

KEYWORDS: *Pimpinella anisum*, Oil Constituents, Antimicrobial Activity.

INTRODUCTION

Pimpinella anisum L. (Anise) is a plant in the family Umbelliferae. It is one of the oldest medicinal plants. It is an annual grassy herb reaching 30–50 cm in height with white flowers and small green to yellow seeds.^[1] *P. anisum* is primarily grown for its fruits (seeds) which contain 1.5–5% essential oil. Seeds are used as flavouring, digestive, carminative, and against gastrointestinal spasms. Consumption of seeds by lactating women increases milk and also reliefs their infants from gastrointestinal problems.^[2] In the food industry, anise is used as flavoring and aromatic agent for fish products, ice cream, sweets, and gums.^[1,3]

Anise seeds are used as analgesic in migraine and also as carminative, aromatic, disinfectant, and diuretic in traditional medicine.^[4] Seeds are also effective in polishing of teeth. In some traditional pharmacopoeia, anise is mentioned for melancholy and also in treatment of epilepsy.^[5,6]

MATERIALS AND METHODS

Seeds of *Pimpinella anisum* were purchased from the local market, Amman (Jordan). The plant was identified and authenticated by direct comparison with reference herbarium sample.

Instruments

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

Test organisms

Pimpinella anisum seed oil was screened for antimicrobial activity using the standard microorganisms: *Bacillus subtilis* (G+ve), *Staphylococcus aureus* (G+ve), *Pseudomonas aeruginosa* (G-ve), *Escherichia coli* (G-ve) and the fungal species *Candida albicans*.

Methods

Extraction of oil

Powdered seeds of *Pimpinella anisum* (350 g) were macerated with n-hexane for 72hr. The solvent was removed under reduced pressure to give the oil.

GC-MS analysis

The oil from *Pimpinella anisum* seeds was analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument. Chromatographic conditions are displayed below.

Table 1: Oven temperature program.

Rate	Temperature(°C)	Hold Time (min. ⁻¹)
-	150.0	1.00
4.00	300.0	0.00

Table 2: Chromatographic conditions.

Column oven temperature	150 ^o C
Injection temperature	300 ^o C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0ml/min.
Column flow	1.54ml/sec.
Linear velocity	47.2cm/ sec.
Purge flow	3.0 ml/ min.
Split ratio	-1.0

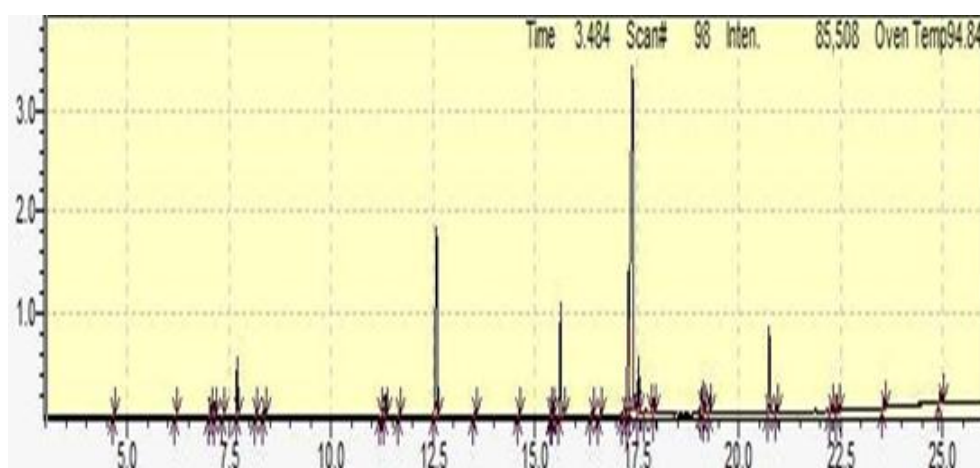
Antimicrobial assay

The cup-plate agar diffusion bioassay was adopted, with some minor modifications, to assess the antimicrobial activity of the studied oil. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at

45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and in each of these plates, which were divided into two halves, two cups in each half (6 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for a test solution. The agar discs were removed, alternate cups were filled with 0.1 ml of test samples and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours (for bacteria) and for 3 days for the fungus. After incubation, the diameters of the resultant growth inhibition zones were measured and recorded as average of two replicates.

RESULTS AND DISCUSSION

GC-MS analysis of *Pimpinella anisum* oil was conducted and the identification of the constituents was initially accomplished by comparison of the retention times and consulting the MS library (NIST). The GC-MS analysis revealed the presence of 34 components (Table 3). The typical total ion chromatograms (TIC) is depicted in Fig.1.

**Fig. 1: Total ions chromatograms.****Table 3: Constituents of the oil.**

ID#	Name	Ret.Time	Area%
1.	D-Limonene	4.670	0.06
2.	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-	6.186	0.01
3.	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-	7.040	0.77
4.	3-Nonyne	7.156	0.13
5.	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-	7.341	0.02
6.	(-)-Carvone	7.700	3.04
7.	1-methyl-4-(prop-1-en-2-yl)-7-oxabicyclo[4.1.0]heptan-2-one	8.164	0.38
8.	Thymol	8.351	0.39
9.	Dodecanoic acid, methyl ester	11.216	0.06
10.	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-	11.336	1.15
11.	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	11.671	0.11
12.	Apiol	12.589	15.33
13.	Methyl tetradecanoate	13.530	0.26
14.	Pentadecanoic acid, methyl ester	14.605	0.11

15.	7-Hexadecenoic acid, methyl ester, (Z)-	15.411	0.44
16.	9-Hexadecenoic acid, methyl ester, (Z)-	15.436	0.60
17.	Hexadecanoic acid, methyl ester	15.635	6.57
18.	cis-10-Heptadecenoic acid, methyl ester	16.399	0.11
19.	Heptadecanoic acid, methyl ester	16.610	0.09
20.	n-Nonadecanol-1	17.191	0.79
21.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.299	11.22
22.	9-Octadecenoic acid (Z)-, methyl ester	17.392	44.82
23.	Methyl stearate	17.552	2.91
24.	Oleic Acid	17.731	0.39
25.	Ethyl Oleate	17.927	0.17
26.	cis-11-Eicosenoic acid, methyl ester	19.104	1.41
27.	cis-13-Eicosenoic acid, methyl ester	19.153	0.29
28.	Eicosanoic acid, methyl ester	19.301	0.79
29.	cis-10-Nonadecenoic acid, methyl ester	20.748	5.22
30.	Docosanoic acid, methyl ester	20.921	0.35
31.	15-Tetracosenoic acid, methyl ester, (Z)-	22.270	0.30
32.	Tetracosanoic acid, methyl ester	22.424	0.23
33.	Hexatriacontane	23.567	0.45
34.	10-Nonadecanone	24.936	1.03

Mass spectra of the major constituents are briefly discussed below:

Fig. 2 presents the mass spectrum of 9-octadecenoic acid (Z)-, methyl ester. The peak at m/z 296 which appeared at retention time (17.392) is due to $M^+ [C_{19}H_{36}O_2]^+$. The signal at m/z 264 is due to loss of a methoxyl.

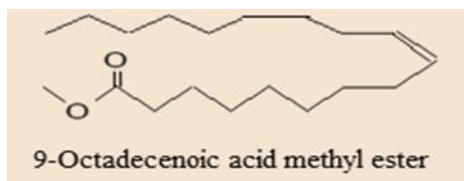


Fig. 3: shows the mass spectrum of apiol. The signal at m/z 222 (RT.12.589) is due to $M^+ [C_{12}H_{14}O_4]^+$.

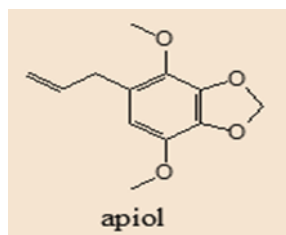
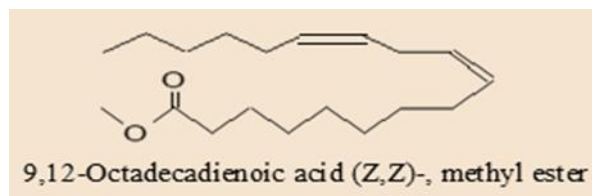


Fig. 4 illustrates the mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester. The peak at m/z 294 (RT.17.299) accounts for the molecular ion: $M^+ [C_{19}H_{34}O_2]^+$. The signal at m/z 263 accounts for loss of a methoxyl.



The mass spectrum of hexadecanoic acid, methyl ester is shown in Fig.5. The peak at m/z 270, which appeared at retention time (15.635) accounts for the molecular ion: $M^+ [C_{17}H_{34}O_2]^+$. The signal at m/z 239 accounts for loss of a methoxyl.

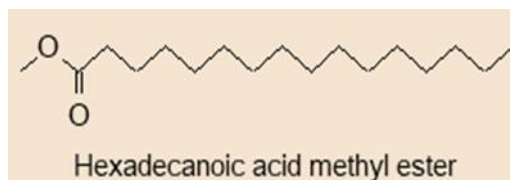
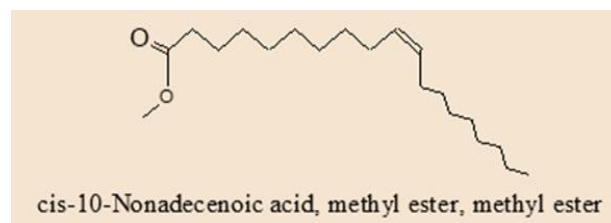


Fig. 6 displays the mass spectrum of cis-10-nonadecenoic acid, methyl ester. The signal which appeared at m/z 310 (RT.20.748) coincides with the molecular ion: $M^+ [C_{20}H_{38}O_2]^+$. The signal at m/z 278 accounts for loss of a methoxyl.



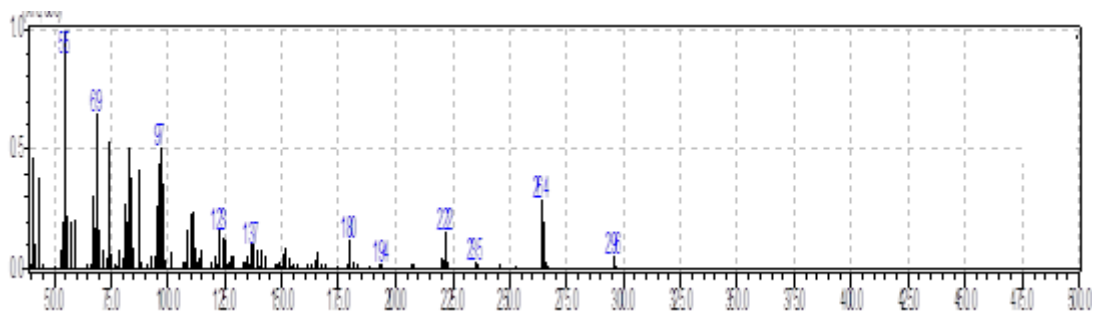


Fig. 2: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester.

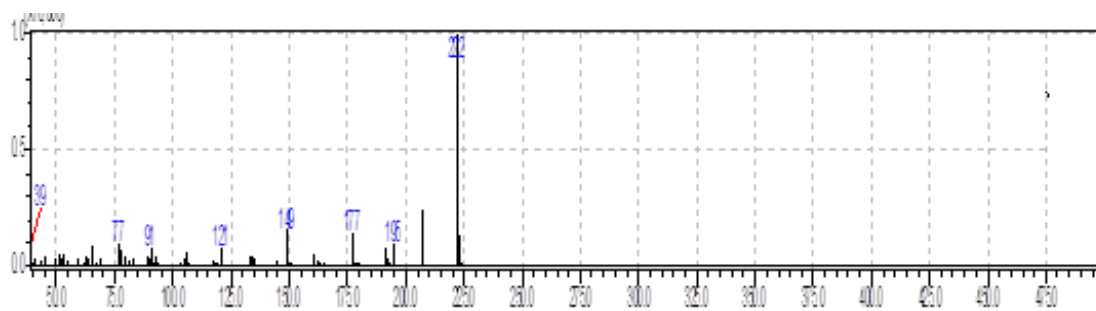


Fig. 3: Mass spectrum of apiol.

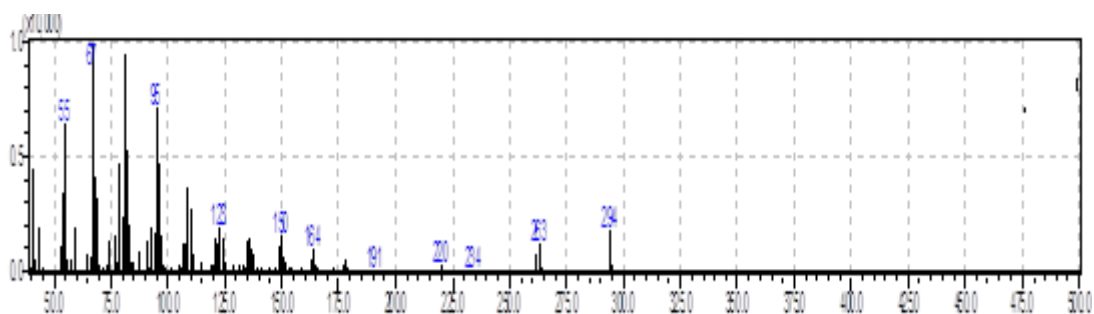


Fig. 4: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester.

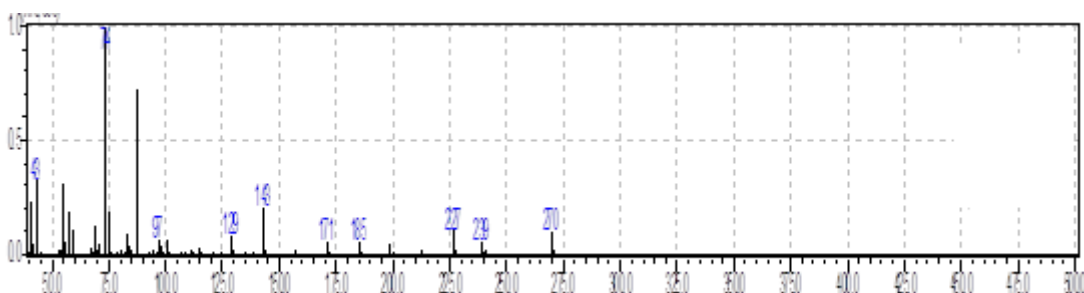


Fig. 5: Mass spectrum of hexadecanoic acid, methyl ester.

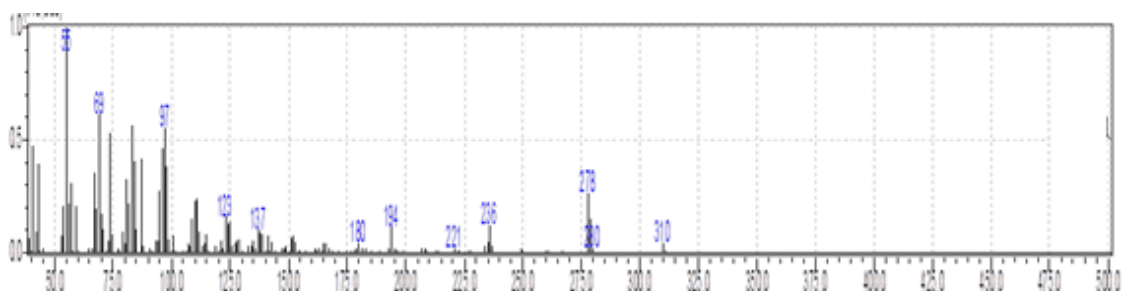


Fig. 6: Mass spectrum of cis-10-nonadecenoic acid, methyl ester.

Antimicrobial activity

Pimpinella anisum oil was investigated for antimicrobial activity via the cup plate agar diffusion bioassay using five standard pathogenic microbes. The average of the diameters of the growth inhibition zones are displayed in

Table (4). Results were interpreted as follows: less than 9 mm: considered inactive; 9-12mm: weak activity; 13-18mm: active and more than 18: very active. Ampicilin, gentamycin and clotrimazole have been used as positive controls.

Table 4: Antimicrobial activity of the oil.

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	15	16	15	--	17
Ampicilin	40	30	15	--	--	--
Gentacycin	40	19	25	22	21	--
Clotrimazole	30	--	--	--	--	38

Sa: *Staphylococcus aureus*

Ec.: *Escherichia coli*

Pa.: *Pseudomonas aeruginosa*

Bs.: *Bacillus subtilis*

Ca.: *Candida albicans*

At a concentration of 100mg/ml, the oil showed significant anticandidal activity. It also exhibited moderate activity against Gram positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative *Escherichia coli*. However it failed to give inhibitory effect against Gram negative *Pseudomonas aeruginosa*.

REFERENCES

1. Salehi-Surmaghi, M.H., Medicinal Plants and Phytotherapy, vol. 1, Donyay Taghziah Press, Tehran, Iran, 2010.
2. Zargari, A. *Medicinal Plants*, Tehran University Press, Tehran, Iran, 1996.
3. Zcan, M.O. and Chalchat, J.C., Chemical composition and antifungal effect of anise (*Pimpinella anisum*L.) fruit oil at ripening stage, *Annals of Microbiology*, 2006; 56(4): 353–358.
4. Amin, G.R. , Popular Medicinal Plants of Iran, Vice-Chancellorship of Research, Tehran University of Medical Science Press, Tehran, Iran, 2005.
5. Mirheydar, H. Herbal Information: Usage of Plants in Prevention and Treatment of Diseases, Islamic Culture Press Center, Tehran, Iran, 2001.
6. Aghili-Khorasani, M.H., Makhzan al Advieh, Bavardaran Press, Institute for Islamic and Complementary Medicine, Iran University of Medical Sciences, Tehran, Iran, 2001.