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Research Article

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Sudanese *Petroselinum crispum* Fixed Oil: GC-MS Analysis and Antimicrobial Activity

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Abstract Information on plants used traditionally in Sudanese system of medicine is very scarce. Hence this study was undertaken to investigate the chemical constituents of *Petroselinum crispum* fixed oil and to evaluate its potential antimicrobial activity. 62 components were detected by GC-MS. Major components are: apiol (36.44%), 9-octadecenoic acid methyl ester (27.17%), 4-Methoxy-6-(2-propenyl)-1,3-benzodioxole12.12%) and 9, 12-octadecadienoic acid methyl ester (8.32%)

The antibacterial activity of the oil was evaluated via the diffusion bioassay against five standard human pathogens (Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseud*om*onasa aeruginos*a and the fungal species *Candida albicans*). The oil showed good activity against *Staphylococcus aureus* and *Bacillus subtilis* and also showed weak activity against *Escherichia coli* and *Pseudomonas aeruginosa*.

Keywords *Petroselinum crispum,* Fixed Oil, GC-MS, Antimicrobial Activity

Introduction

Petroselinum crispum (Mill) Nyman Ex AW Hill (Apiaceae) is worldwide cultivated for its nutritive value [1]. *Petroselinum crispum* is a herb growing up to 30-100 cm in height [2]. Different parts of the herb find many applications in pharmaceutical, food industries and cosmetics [3]. The plant is reported to improve memory and brain functions [4]. In Sudanese traditional medicine *Petroselinum crispum* is used to treat a wide spectrum of diseases including: hemorrhoids, inflammation and kidney stones [5]. Local healers also use it as: emmenagogic, carminative and abortifacient [6]. Several reports testified the potential hypoglycemic, diuretic and hypolipidemic effects of this herb [7]. The plant has been shown to possess hepatoprotective, antimicrobial and anticoagulant activities [7]. Some phytochemicals have been reported from *Petroselinum crispum* including: luteolin and myrecitin beside caretenoids, terpenes, coumarins, tocoferol, apiin , apiol and ascorbic acid [8-9]. It has been demonstrated that supplementing experimental animals with *Petroselinum crispum* leaves enhanced plasma radical scavenging capacity [10]. Also a constituent of *Petroselinum crispum* oil-benzo[α]pyrene- inhibited tumorigensis in lungs of model animals [11]. The essential oil from *Petroselinum crispum* is involved in the production of some perfumes and some kinds of soaps and creams [12].

Materials and Methods

Plant material

Seeds of *Petroselinum crispum* were purchased from the local market- Khartoum and authenticated by Institute of Aromatic and Medicinal Plants.

Instruments

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

Test organisms

Petroselinum crispum fixed oil was screened for antimicrobial activity using the standard microorganisms shown below:

Table 1: Test microorganisms

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	*Aspergillus niger*	fungus
6	*Candida albicans*	fungus

Methods

Phytochemical screening

Petroselinum crispum seeds (250 g) were extracted with 95% ethanol (soxhlet) until exhaustion. This prepared extract (PE) was used for phytochemical screening according to the method described by Harborne [13].

Extraction of *Petroselinum crispum* fixed oil

Powdered seeds of *Petroselinum crispum* (300g) were exhaustively macerated with n-hexane. The solvent was removed under reduced pressure to afford the oil. For GC-MS analysis, the oil (2 ml) was esterified via a methanoilc solution of NaOH and a methanoilc solution of H_2SO_4.

GC-MS analysis

The oil from seeds of *Petroselinum crispum* was analyzed by GC-MS. A Shimadzo GC-MS instrument was used. Tables (2) and (3) display the oven temperature program and chromatographic conditions respectively.

Table 2: Oven temperature program

Rate	Temperature (°C)	Hold Time ($min.^{-1}$)
-	150.0	1.00
4.00	300.0	0.00

Table 3: Chromatographic conditions

Column oven temperature	150.0 ^oC
Injection temperature	300.0 ^oC
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3 KPa
Total flow	50.0 ml/ min
Column flow	1.54 ml/sec
Linear velocity	47.2 cm/sec
Purge flow	3.0 ml/min
Spilt ratio	- 1.0

Antimicrobial assay

Preparation of bacterial suspensions

Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungus respectively. The media was prepared according to the manufacturer instructions: one ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37 °C for

24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^8-10^9 colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37 °C for 24 hours.

Preparation of fungal suspensions

Fungal cultures were maintained on potato dextrose agar incubated at 25 °C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing for antimicrobial activity

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antimicrobial activity of the oil. (2 ml) 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. Each of these plates was divided into two halves. Two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for a sample. Separate Petri dishes were designed for standard antimicrobial chemotherapeutics.

The agar discs were removed, alternate cup were filled with 0.1 ml 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. After incubation, the diameters of the resultant growth inhibition zones were measured in duplicates and averaged.

Results and Discussion

Petroselinum crispum seeds were screened for major secondary metabolite. Qualitative tests were positive for flavonoids, alkaloids, tannins, saponins and carbohydrates.

GC-MS analysis

Fixed oil of *Petroselinum crispum* was extracted by maceration from seeds and analyzed by GC-MS where 62 components (Table 4) were detected in total ions chromatograms (Figure 1).

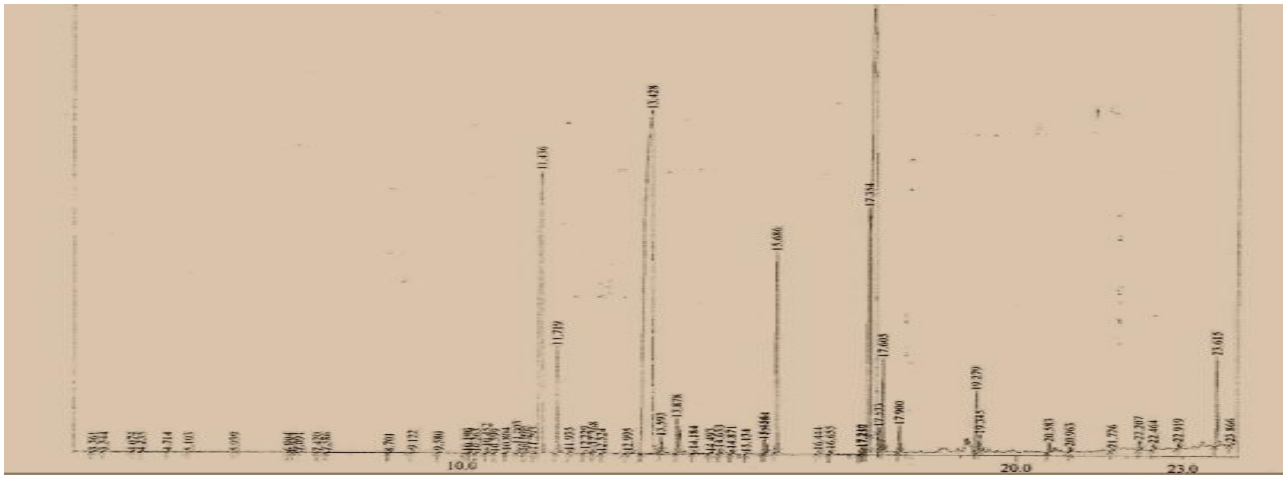

Figure 1: Total ions chromatograms of Petroselinum crispum oil

Different constituents of *Petroselinum crispum* oil were identified and quantified by retention times and their characteristic fragmentation pattern. A Tabulation of oil constituents is given in Table (4)

Table 4: Total ions chromatograms of *Petroselinum crispum* oil

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	3.361	53417	0.00	Hexanoic acid, methyl ester
2	3.544	27962	0.00	.alpha.-Pinene
3	4.074	17027	0.00	.beta.-Pinene
4	4.235	33062	0.00	Pentanoic acid, 4-methyl-, methyl ester
5	4.714	32398	0.00	D-Limonene
6	5.103	32502	0.00	.gamma.-Terpinene
7	5.939	31056	0.00	Octanoic acid, methyl ester
8	6.904	58507	0.00	Thymol
9	6.984	85559	0.01	L-.alpha.-Terpineol
10	7.091	164344	0.01	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-di
11	7.420	38982	0.00	Citronellol
12	7.586	191489	0.01	3-(2-Hydroxy-cyclopentylidene)-2-methyl-p
13	8.701	54674	0.00	Decanoic acid, methyl ester
14	9.122	687866	0.05	3-Cyclohexene-1-methanol, .alpha.,.alpha.,
15	9.580	475090	0.04	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7
16	10.100	246718	0.02	1H-3a,7-Methanoazulene, octahydro-3,8,8-
17	10.150	257761	0.02	Caryophyllene
18	10.263	183234	0.01	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4
19	10.452	1348679	0.10	(E)-.beta.-Famesene
20	10.599	169551	0.01	1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-oc
21	10.804	364047	0.03	.beta.-copaene
22	11.003	2135670	0.16	Naphthalene, decahydro-4a-methyl-1-meth
23	11.104	332939	0.02	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4
24	11.162	857025	0.06	.beta.-Bisabolene
25	11.293	701743	0.05	Dodecanoic acid, methyl ester
26	11.436	164112211	12.12	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl
27	11.719	28343982	2.09	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-
28	11.935	551533	0.04	Asarone
29	12.229	1422825	0.11	1,3,5-Trimethoxy-2-propenylbenzene
30	12.368	1846488	0.14	Carotol
31	12.524	342394	0.03	1H-1,3-Benzimidazole-2-methanol, 5-meth
32	12.995	1088069	0.08	cis-3-Butyl-4-vinyl-cyclopentene
33	13.428	493477272	36.44	Apiol
34	13.593	3409538	0.25	Methyl tetradecanoate
35	13.878	8483012	0.63	1,3-Benzenediamine
36	14.184	1038200	0.08	2',4'-Dimethoxy-3'-methylpropiophenone
37	14.493	431625	0.03	6-Octadecenoic acid, methyl ester, (Z)-
38	14.653	1317809	0.10	Pentadecanoic acid, methyl ester
39	14.871	1037869	0.08	2-Pentadecanone, 6,10,14-trimethyl-
40	15.134	274448	0.02	.alpha.-Santalol
41	15.456	3622787	0.27	7,10,13-Hexadecatrienoic acid, methyl este
42	15.484	3712592	0.27	9-Hexadecenoic acid, methyl ester, (Z)-
43	15.686	55253847	4.08	Hexadecanoic acid, methyl ester
44	16.444	837198	0.06	7-Hexadecenoic acid, methyl ester, (Z)-
45	16.655	1030433	0.08	Heptadecanoic acid, methyl ester
46	17.210	2423051	0.18	6,9-Octadecadienoic acid, methyl ester
47	17.252	1556285	0.11	Cyclohexadecane
48	17.354	112655745	8.32	9,12-Octadecadienoic acid (Z,Z)-, methyl es
49	17.475	367962054	27.17	9-Octadecenoic acid (Z)-, methyl ester
50	17.533	4879794	0.36	Phytol
51	17.605	21959540	1.62	Methyl stearate
52	17.900	6525907	0.48	Tricyclo[5.1.0.0(3,5)]octane-2,6-dione, 1,3,4
53	19.279	15321503	1.13	2-Butenoic acid, 2-methyl-, 2-(acetyloxy)-1.
54	19.345	3892483	0.29	Eicosanoic acid, methyl ester
55	20.583	2845037	0.21	9-Octadecenoic acid, 1,2,3-propanetriyl est
56	20.963	1354208	0.10	Docosanoic acid, methyl ester
57	21.726	690902	0.05	Tricosanoic acid, methyl ester
58	22.207	2722116	0.20	Tetracontane
59	22.464	1603442	0.12	Tetracosanoic acid, methyl ester
60	22.919	1454924	0.11	Tetratriacontane
61	23.615	25362901	1.87	Hexatriacontane
62	23.866	634637	0.05	Hexacosanoic acid, methyl ester
		1354059963	100.00	

Major co

Apiol (36.44%)

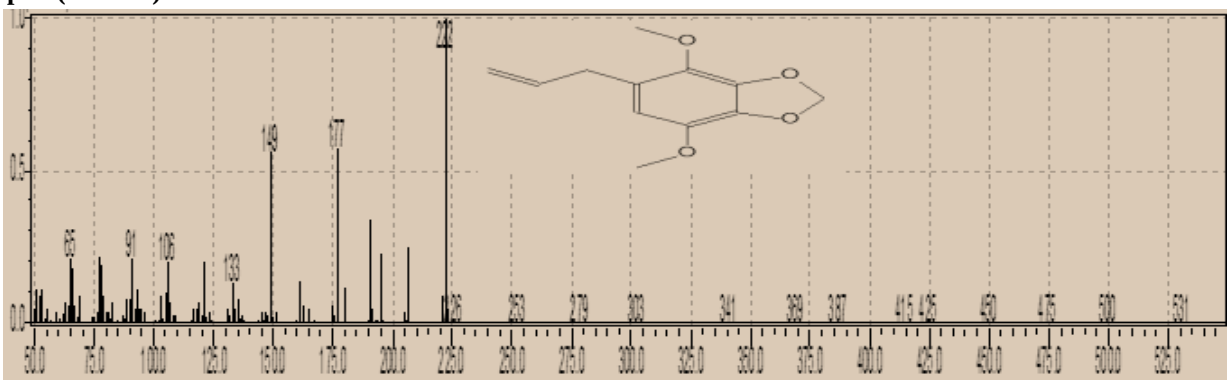

Figure 2: Mass spectrum of apiol

The EI mass spectrum of apiol is shown in Figure 2.The peak at m/z 222, which appeared at R.T. 13.428 in total ion chromatogram, corresponds $M^+[C_{12}H_{14}O_4]^+$. The peak at m/z 207 corresponds to loss of a methyl function and the peak at m/z 191 corresponds to loss of a methoxyl function.

9-Octadecenoic acid methyl ester (27.17%)

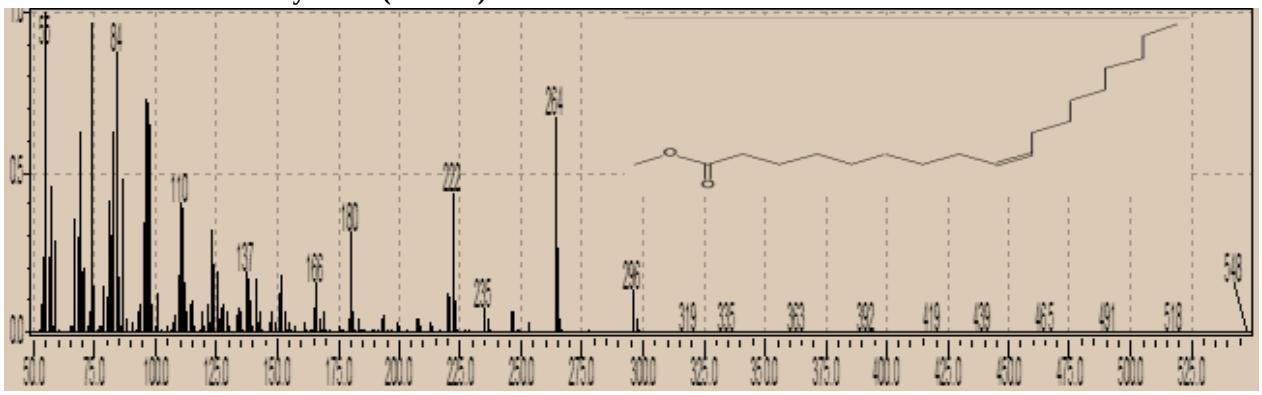

Figure 3: Mass spectrum of 9-octadecenoic acid methyl ester

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig.3.The signal which appeared at m/z 296(R.T. 17.475 in total ion chromatogram) corresponds $M^+[C_{19}H_{36}O_2]^+$. The peak at m/z 265 accounts for loss of a methoxyl.

4-Methoxy-6-(2-propenyl) -1, 3-benzodioxole(12.12%)

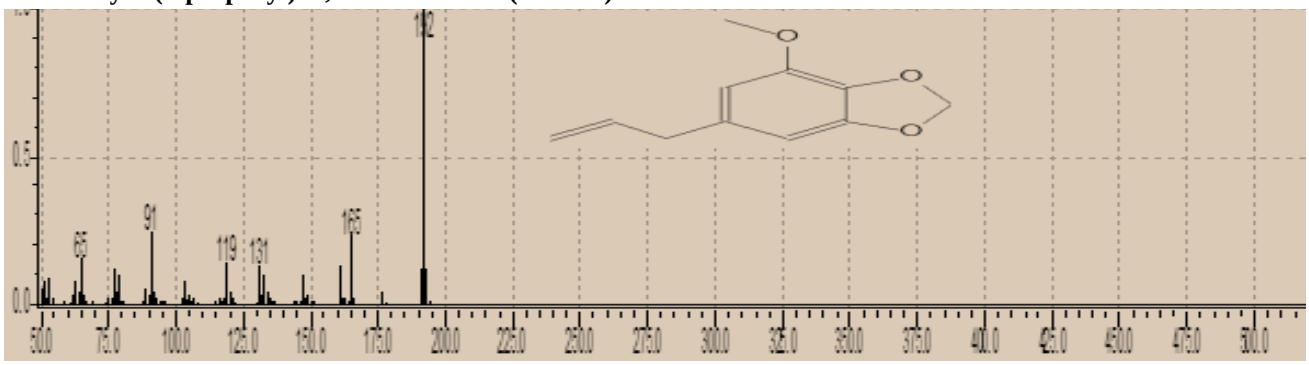

Figure 4: Mass spectrum of 4-methoxy-6-(2-propenyl)-1,3-benzodioxole

Figure 4 shows the mass spectrum of 1, 3-benzodioxole, 4-methoxy-6-(2-propenyl) .The peak at m/z 192(with R.T. 11.436 in total ion chromatogram) corresponds $M^+[C_{11}H_{12}O_3]^+$ .The signal at m/z 177 is due to loss of a methyl function.

9, 12-Octadecadienoic acid methyl ester (8.32%)

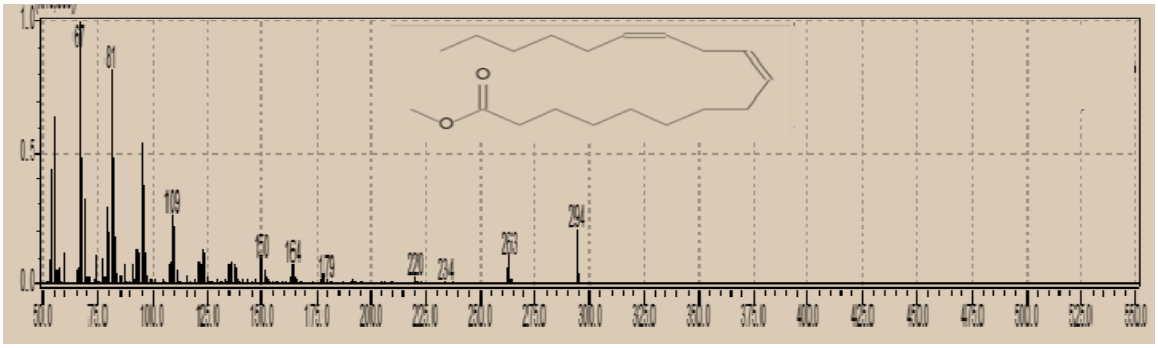

Figure 5: *Mass spectrum of 9, 12-octadecadienoic acid methyl ester*

The mass spectrum of 9, 12-octadecadienoic acid methyl ester is shown in Fig. 5.The peak at m/z 294 (R.T. 17.354 in total ion chromatogram) corresponds $M^+[C_{19}H_{34}O_2]^+$ .Loss of a methoxyl gave m/z 263 .

Hexadecanoic acid, methyl ester (4.08%)

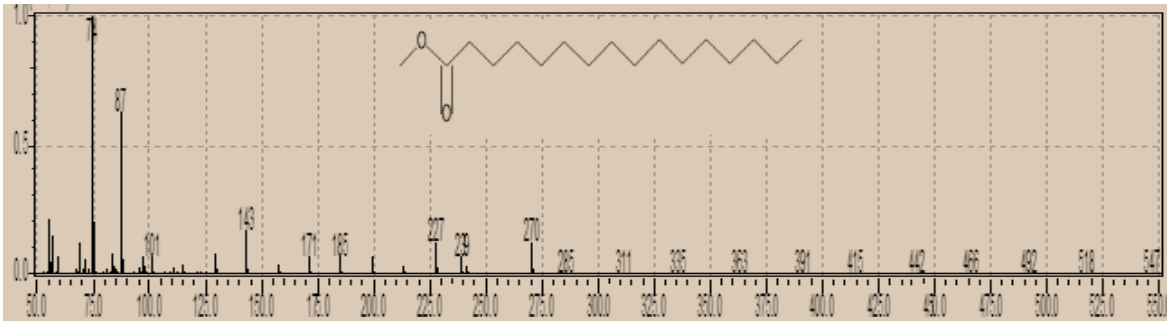

Figure 6: Mass spectrum of hexadecanoic acid, methyl ester

The EI mass spectrum of hexadecanoic acid, methyl ester is depicted in Fig. 6.The peak at m/z 270, which appeared at R.T. 15.686 in total ion chromatogram, corresponds $M^+[C_{17}H_{34}O_2]^+$ while the signal at m/z 239 is attributed to loss of a methoxyl function.

5-(2-propenyl) -1, 2, 3-trimethoxybeneze (2.09%)

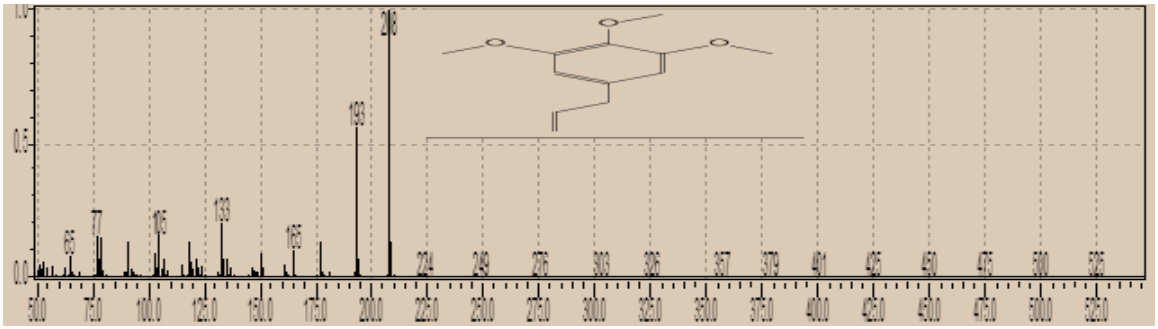

Figure 7: Mass spectrum of 5-(2-propenyl) -1, 2, 3-trimethoxybeneze

Mass spectrum of 5-(2-propenyl) -1, 2, 3-trimethoxybeneze is shown in Fig. 7.The peak at m/z 208, which appeared at R.T. 11.719 in total ion chromatogram, corresponds to $M^+[C_{12}H_{16}O_3]^+$ .The peak at m/z 193 corresponds to loss of a methyl function.

Antimicrobial activity

The oil was screened for antimicrobial activity against five standard clinical isolates. The results are shown in Table (5). Results were interpreted in conventional terms: (<9mm: inactive; 9-12mm: partially active; 13-18mm: active; >18mm:very active) .Tables (6) and (7) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 5: Antibacterial activity of *Petroselinum crispum* oil: M.D.I.Z (mm)

Drug	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca
Petroselinum crispum oil	100	9	10	15	14	8

Table 6: Antibacterial activity of standard chemotherapeutic agents

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

Table 7: Antifungal activity of standard chemotherapeutic agent

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

Sa.*: Staphylococcus aureus*
Ec.*: Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: Bacillus subtilis

The oil showed good activity against *Staphylococcus aureus* and *Bacillus subtilis*. It also showed weak activity against *Escherichia coli* and *Pseudomonas aeruginosa*.

References

1. Bailey, L. H., Hortorium, L. H. B., & Bailey, E. Z. (1976). Hortus third; a concise dictionary of plants cultivated in the United States and Canada.
2. Menglan, S., Fading, P., Zehui, P., Watson, M. F., Cannon, J. F., Holmes-Smith, I., Kljuykov, E.V. Phillippe, L.R., & Pimenov, M. G. (2005). Apiaceae (Umbelliferae). *Flora of China*, *14*, 1-205.
3. Lopez, M. G., Sanchez-Mendoza, I. R., & Ochoa-Alejo, N. (1999). Compartive study of volatile components and fatty acids of plants and in vitro cultures of parsley (Petroselinum crispum (Mill) nym ex hill). *Journal of agricultural and food chemistry*, *47*(8), 3292-3296.
4. Adams, M., Gmünder, F., & Hamburger, M. (2007). Plants traditionally used in age related brain disorders—A survey of ethnobotanical literature. *Journal of Ethnopharmacology*, *113*(3), 363-381.
5. Ezer, N., & Arisan, Ö. M. (2006). Folk medicines in Merzifon (Amasya, Turkey). *Turkish Journal of Botany*, *30*(3), 223-230.
6. Soysal, Y. (2004). Microwave drying characteristics of parsley. *Biosystems engineering*, *89*(2), 167-173.
7. Sener, G., Saçan, Ö., Yanardag, R., & Ayanoglu-Dülger, G. (2003). Effects of parsley (Petroselinum crispum) on the aorta and heart of STZ induced diabetic rats. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum)*, *58*(3), 1-7.
8. Fejes, S., Kery, A., Blazovics, A., Lugasi, A., Lemberkovics, E., Petri, G., & Szöke, E. (1998). Investigation of the in vitro antioxidant effect of Petroselinum crispum (Mill.) Nym. ex AW Hill. *Acta Pharmaceutica Hungarica*, *68*(3), 150-156.

9. Vora, S. R., Patil, R. B., & Pillai, M. M. (2009). Protective effects of Petroselinum crispum (Mill) Nyman ex AW Hill leaf extract on D-galactose-induced oxidative stress in mouse brain. Indian *Journal of Experimental Biology*, *47*, 338-342.
10. Hempel, J., Pforte, H., Raab, B., Engst, W., Böhm, H., & Jacobasch, G. (1999). Flavonols and flavones of parsley cell suspension culture change the antioxidative capacity of plasma in rats. *Molecular Nutrition & Food Research*, *43*(3), 201-204.
11. Nielsen, S. E., Young, J. F., Daneshvar, B., Lauridsen, S. T., Knuthsen, P., Sandström, B., & Dragsted, L. O. (1999). Effect of parsley (Petroselinum crispum) intake on urinary apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subjects. *British Journal of Nutrition*, *81*(6), 447-455.
12. Tahan, M., & Bayram, I. (2011). Effect of using black cumin (Nigella sativa) and parsley (Petroselinum crispum) in laying quail diets on egg yield, egg quality and hatchability. *Archiva Zootechnica*, *14*(4), 39-44.
13. Harborne,J.B., (1976). "Phytochemical Methods", Chapman and Hall, London.