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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *PETROSELINUM CRISPUM*(MILL) NYMAN EX AW HILL (APIACEAE) ESSENTIAL OIL

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ABSTRACT

Information on the phytoconstituents of medicinal plants used traditionally in Sudan is very scarce. Hence this study was set to investigate the chemical constituents of the medicinally important species *Petroselinum crispum* oil and to evaluate its potential antimicrobial activity. 47 components were detected by GC-MS analysis being dominated by: apiol (38.28%); 4-methoxy-6-(2-propenyl)-1,3-enzodioxole(28.82%); 1,2,3-trimethoxy-5-(2-propenyl)benzene(6.63%); 2,6,6-trimethyl-bicycl[3.1.1]heptane(6.31%). The antibacterial activity of the oil was evaluated via cup plate agar diffusion bioassay against six standard human pathogens(Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseudomonasa aeruginos*a and the fungi *Candida albicans* and *Aspergillus niger*). The oil showed activity only against *Staphylococcus aureus*. It seems that the oil is a lead for further optimization.

KEYWORDS: *Petroselinum crispum*, Essential Oil, GC-MS, Antimicrobial Activity.

INTRODUCTION

Petroselinum crispum (Mill)Nyman Ex AW Hill (Apiaceae) is a herb that grows up to 30-100 cm high.[1] Nowadays, the plant is worldwide cultivated[2] for its economic value. Different parts of the herb find many applications in pharmaceutical,cosmetic and food industries.[3] *Petroselinum crispum* is used traditionally to treat an array of human disorders including hemorrhoids, inflammation and kidney stones.[4] The plant is claimed to improve memory

and brain function.[5] The herb is also used as emmenagogic, carminative and abortifacient.[6] Several investigations demonstrated the potential hypoglycemic ,diuretic and hypolipidemic properties of this species.[7] Also the plant is claimed to possess antimicrobial, anticoagulant and hepatoprotective activities.[7]

The plant is reported to contain: flavonols, luteolin and myrecitin beside caretenoids, pthalides, terpenes, coumarins, tocoferol, apiin, apiol and ascorbic acid.[8,9] In model animal studies, supplementation of diets with *Petroselinum crispum* leaves enhanced plasma radical scavenging capacity.[10] A major constituent of *Petroselinum crispum* essential oil-benzo[α]pyrene- inhibited tumorigensis in lungs of model animals.[11] The essential oil is manipulated in the production of soaps, perfumes and creams.[12] *Petroselinum crispum* volatiles include; α-pinene, α- and β-phellandrenes, β-myrcene,cis-ocimene, isopropenyl-4-methylbenzene, α-terpinolene, p-mentha-1,3,8-triene, α-copaene, caryophylene, β-famesen, β-selinene, γ-cadinene, β-bisabolene, β –sesquiphellandrene.[12]

With view of the titivation of phenyl propanoid metabolism[13], *Petroselinum crispum* is used as system for investigating non-host plant/pathogen interactions. Antioxidant and antibacterial properties of *Petroselinum crispum* make it propitious in food systems.[14]

Culinary herbs[15], like *Petroselinum crispum*, have been used to reduce food spoilage and controlling the growth of food-borne pathogens. Lipid peroxidation seems to deteriorate food quality. *In vitro* studies demonstrated that constituents of *Petroselinum crispum* leaves scavenge superoxide anion[16] while the methanolic extract scavenge hydroxyl radical.[17] Nielsen *et. al*.[18] claimed that *Petroselinum crispum* leaves decreased oxidative stress in human subjects.[15]

Freez-dried and irradiated *Petroselinum crispum* leaves and stems were evaluated for antimicrobial and antioxidant activities.[15] Several mechanisms of antioxidant activity including ion chelating and free radical scavenging capacity as well as reducing power were examined.[15]

Different fractions of *Petroselinum crispum* were evaluated for their DPPH radical scavenging capacity.[19] The dichloromethane fraction showed significant protection against H_2O_2-induced DNA damage in model animals suggesting a potential in cancer prevention. The extract also inhibited H_2O_2-induced MCF-7 cell migration which is necessary for

metastasis of cancer cells[1]. This suggests that: *Petroselinum crispum* has health-promoting properties with the potential to prevent oxidative stress-related diseases and can be developed into functional food. In ADP-induced platelet aggregation, two constituents of *Petroselinum crispum* leaves –apigenin and cosmosiin – exhibited strong antiplatelet aggregation activity.[20]

MATERIALS AND METHODS

Plant material

Seeds of *Petroselinum crispum* were purchased from the local market-Khartoum and authenticated by direct comparison with a 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**.**

Test organisms

Petroselinum crispum oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in table(1).

Table 1: Test organisms.

Ser. 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	*Aspergillusniger*	fungus
6	*Candida albicans*	fungus

METHODS

Phytochemical screening

(100 g) Of powdered shade- dried leaves of *Petroselinum crispum* were extracted with 95% ethanol (soxhlet) until exhaustion. This prepared extract(PE) was used for phytochemical screening according to the method describe by Harborne.[21]

Test for unsaturated sterols and for triterpenes

(10 ml)of the (PE) was evaporated to dryness on a water bath, and the cooled residue was stirred with petroleum ether to remove most of the coloring materials. The residue was then extracted with 10 ml chloroform. The chlorform solution was dehydrated over anhydrous

sodium sulphite.(5 ml) portion of the solution was mixed with(0.5 ml) of acetic anhydride, followed by two drops of concentrated sulphuric acid.

Test for flavonoids

(20 ml) of the (PE) were evaporated to dryness on water bath. The cooled residue was defatted with petroleum ether and then dissolved in 30 ml of 30% aqueous methanol and filtered. The filtrate was used for the following tests.

- To 3 ml. of filtrate a fragment of magnesium ribbon was added, shaken and then few drops of concentrated hydrochloric acid were added.
- To 3 ml. of the filtrate few drops of aluminium chloride solution were added.
- To 3 ml. of the filtrate few drops of potassium hydroxide solution were added.

Test for alkaloids

(10 ml) of the (PE) were evaporated to dryness on a water bath and 5 ml of 0.2N hydrochloric acid were added and the solution was heated with stirring for 10 minutes, then cooled and filtrated.

Filtrate was divided into two portions.

To one portion a few drops of Maeyer reagent were added., to the other portion few drops of Wagner reagent were added.

Test for tannins

(10 ml) of (PE) were evaporated to dryness and the residue was extracted with n-hexane and then filtrated. The insoluble residue was stirred with n-hexane and (10 ml) of hot saline (0.9% w/v of sodium chloride and freshly prepared distilled water) were added. The mixture was cooled, filtrated and the volume adjusted to 10 ml. with more saline solution. (5 ml) of this solution were treated with few drops of ferric chloride solution.

Test for saponins

(1g) of dried powdered plant material was placed in a test tube. (10 ml) of distilled water were added and the tube was stoppered and vigorously shaken for about 30 seconds, and allowed to stand.

Extraction of oil from seeds of *Petroselinum crispum*

Powdered seeds of *Petroselinum crispum* (300g) were exhaustively extracted with n-hexane (soxhlet). The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further manipulation.

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 alcoholic sodium hydroxide were added followed by 7ml of alcoholic sulphuric acid. The tube was stoppered and shaken vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added, then (2ml) of normal hexane were added and the tube was vigorously shaken for five minutes. The hexane layer was then separated.(5µl) of the hexane extract were mixed with 5ml diethyl ether. The solution was filtered and the filtrate(1µl) was injected in the GC-MS vial.

GC-MS analysis

The oil from seeds of *Petroselinum crispum* was analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness)was used. Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program is given in Table 2, while other chromatographic conditions are depicted in Table 3.

Table 2: Oven temperature program.

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

Table 3: Chromatographic conditions.

Column oven temperature	150.0^{o}C
Injection temperature	300.0^{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	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial assay

Preparation of bacterial suspensions

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 10^8-10^9colony 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 antibacterial activity

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antibacterial activity of the 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. 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 using adjustable volume microtiter pipette 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 triplicates and averaged.

RESULTS AND DISCUSSION

Phytochemical screening

Preliminary phytochemical screening of *Petroselinum crispum* leaves gave positive reactions for: flavonoids, tannins, alkaloids, saponins and carbohydrates(Table 4).

Table 4: Phytochemical screening of *Petroselinum crispum*.

Species	Flavonoids	Tannins	Alkaloids	Saponins	Carbohydrates
Petroselinum crispum	+ve	+ve	+ve	+ve	+ve

GC-MS analysis of *Petroselinum crispum* essential oil

GC-MS analysis of *Petroselinum crispum* oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern. Comparison of the mass spectra with the database on MS library revealed about 90-95% match.

Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 47 components(Table 5). The typical total ion chromatograms(TIC) of hexane extract is shown in Fig.1.

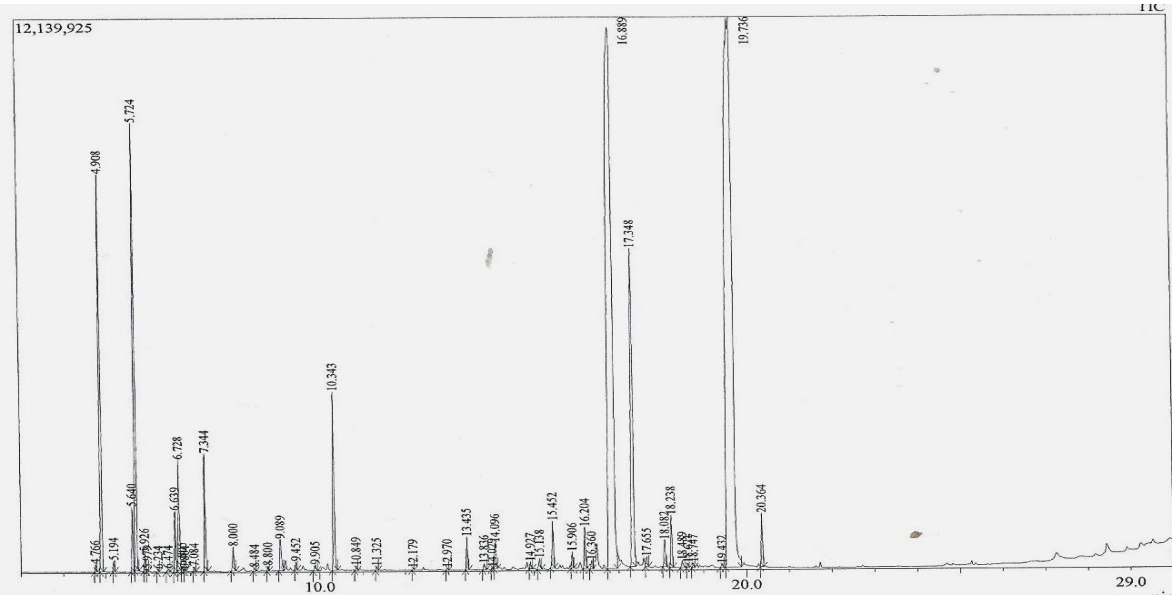

Fig 1: Cromatograms of *Petroselinum crispum* oil.

The following major constituents were detected in the chromatograms.

Apiol (38.28%)

The EI mass spectrum of apiol is shown in Fig. 2.The peak at m/z 222, which appeared at R.T. 19.736 in total ion chromatogram, corresponds to $M^+[C_{12}H_{14}O_4]^+$ .The peak at m/z207 corresponds to loss of a methyl function.

4-Methoxy-6-(2-propenyl)-1,3-enzodioxole(28.82%)

The EI mass spectrum of 4-methoxy-6-(2-propenyl)-1,3-benzodioxole is shown in Fig. 3.The peak at m/z 192, which appeared at R.T.16.889 in total ion chromatogram, corresponds to $M^+[C_{11}H_{12}O_3]^+$ .The peak at m/z177 corresponds to loss of a methyl function.

1,2,3-Trimethoxy-5-(2-propenyl)benzene(6.63%)

The EI mass spectrum of 1,2,3-trimethoxy-5-(2-propenyl)benzene is shown in Fig. 4.The peak at m/z 270, which appeared at R.T. 17.348 in total ion chromatogram, corresponds to $M^+[C_{17}H_{34}O_2]^+$ .The peak at m/z239 corresponds to loss of a methoxyl function.

2,6,6-trimethyl-bicycl[3.1.1]heptane(6.31%)

The EI mass spectrum of 2,6,6-trimethyl-bicycl[3.1.1]heptane is shown in Fig. 5.The peak at m/z 136, which appeared at R.T.5.724 in total ion chromatogram, corresponds to $M^+[C_{10}H_{16}]^+$ .The peak at m/z121 corresponds to loss of a methyl function.

Table 5: Consitituents of *Petroselinum crispum* oil.

No.	RT	Area%	Name
1	4.766	0.08	Alpha-Phellandrene
2	4.908	5.68	Alpha-Pinene
3	5.194	0.12	Camphene
4	5.640	0.74	Bicyclo[3.1.0]hexane , 4-methylene-1-(methyl)
5	5.724	6.31	Bicyclo[3.1.1]heptanes, 6,6-dimethyl-2-methyl
6	5.926	0.21	Beta myrcene
7	5.979	0.03	2,3-Dehydro-1,8-cineole
8	6.234	0.03	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methyl)
9	6.474	0.04	Bicyclo[310]hexan-2-ol, 2-methyl-5-(1-methyl)
10	6.639	0.73	p- Cumene
11	6.728	1.73	D-Limonene
12	6.805	0.05	Eucalyptol
13	6.864	0.02	Trans-beta-Ocimene
14	7.085	0.05	1,3,6-Octatriene, 3,7-dimethyl- , Z-
15	7.344	1.39	Gamma-Terpinene
16	8.000	0.36	Benzene, 1-methyl-4-(1-methylethenyl)-
17	8.484	0.04	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-
18	8.800	0.05	Camphenol, 6-
19	9.089	0.60	Benzyl nitrile

20	9.452	0.10	6-Butyl-1,4-cycloheptadiene
21	9.905	0.10	Terpinen-4-ol
22	10.343	2.50	(IR)-(-)-Myrtenal
23	10.849	0.06	Trans-3-caren-2-ol
24	11.325	0.05	(-)-Carvone
25	12.179	0.02	Acetic acid, 1,7,7-triethyl-bicyclo[2.2.1.]hexane
26	12.970	0.03	Myrtenyl acetate
27	13.435	0.42	3-Cyclohexene-1-methanol, alpha., alpha.
28	13.836	0.11	Benzene, (isothiocyanatomethyl)-
29	14.029	0,02	Alpha, Cubebene
30	14.096	0.39	Gamma, Muurolene
31	14.927	0.09	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-triene
32	15.138	0.18	lBicyclo[3.1.1]hept-ene, 2,6-dimety l-6
33	15.452	0.64	(E)-beta-Famesene
34	15.906	0.21	Beta, Copaene
35	16.204	0.57	Alpha, Guaiene
36	16.360	0.12	Guaia-1(10), 11-diene
37	16.889	28.82	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-
38	17.348	6.63	Benzen, 1,2,3-trimethoxy-5-(2-propenyl)-
39	17.655	0.13	Asarone
40	18.082	0.44	1,2-Dimethoxy-4-(2,3-dimethoxy-1-propenyl)
41	18.238	0.75	Carotol
42	18.489	0.14	2-Allyl-1,4-Dimethoxy-4-(2,3-dimethoxy-1-propenyl)-
43	18.621	0.07	2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexyl)-
44	18.747	0.06	6-Isopropenyl-4,8a-dimethy L,1,2,3,4,5,6,7,8,8
45	19.736	0.06	Cyclohexanemethanol, 4-ethenyl-alpha
46	19.736	38.28	Apiol
47	20.364	0.75	Pyridinium, 1-ethyl, hydroxide

α-Pinene(5.68%)

The EI mass spectrum of α-pinene is shown in Fig.6.The peak at m/z136, which appeared at R.T.4.908 in total ion chromatogram, corresponds to $M^{+}[C_{10}H_{16}]^{+}$ .The peak at m/z121 corresponds to loss of a methyl function.

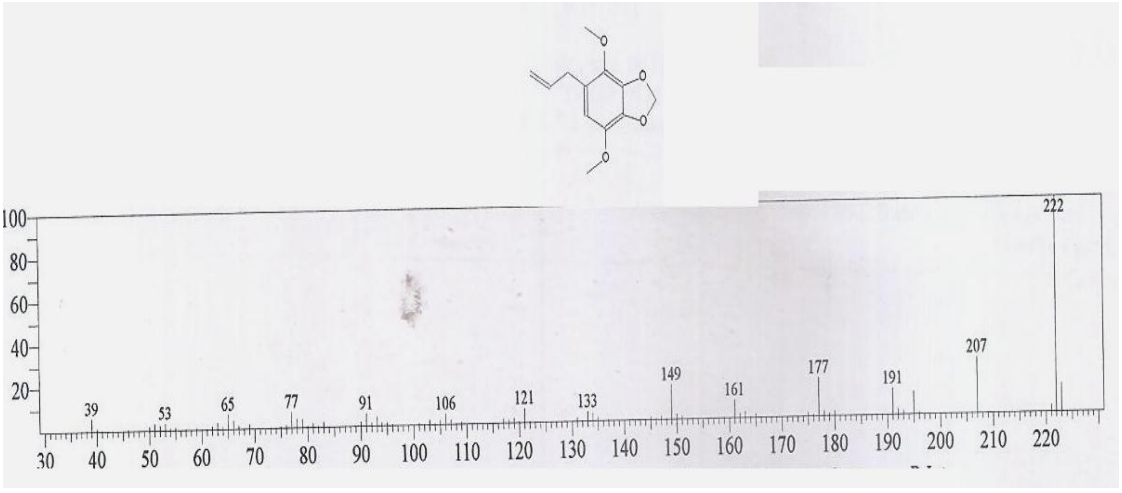

Fig 2: Mass spectrum of apiol.

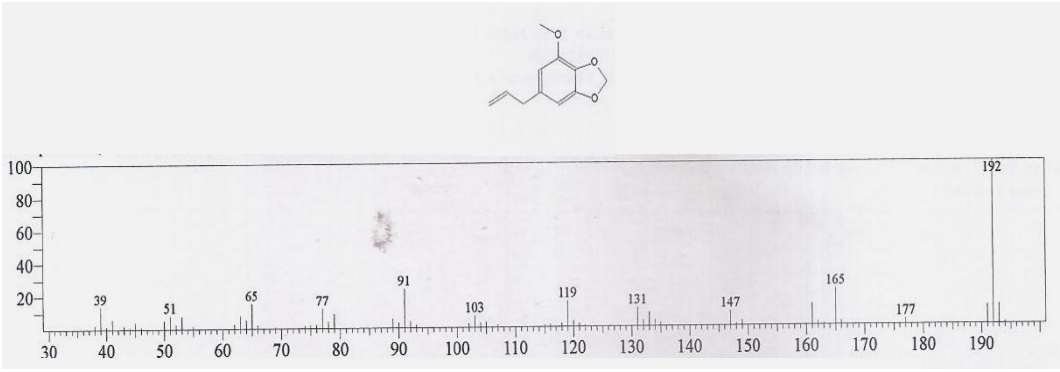

Fig. 3: Mass spectrum of 4-methoxy-6-(2-propenyl)-1,3-benzodioxole.

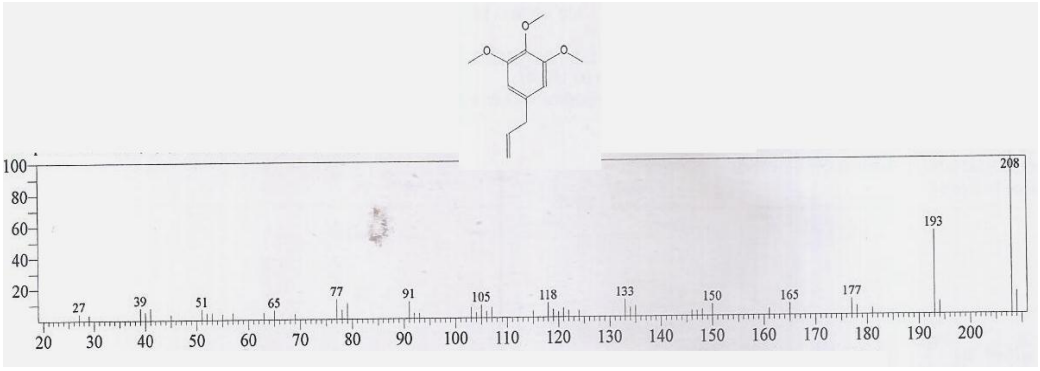

Fig. 4: Mass spectrum of 1,2,3-trimethoxy-5-(2-propenyl)benzene.

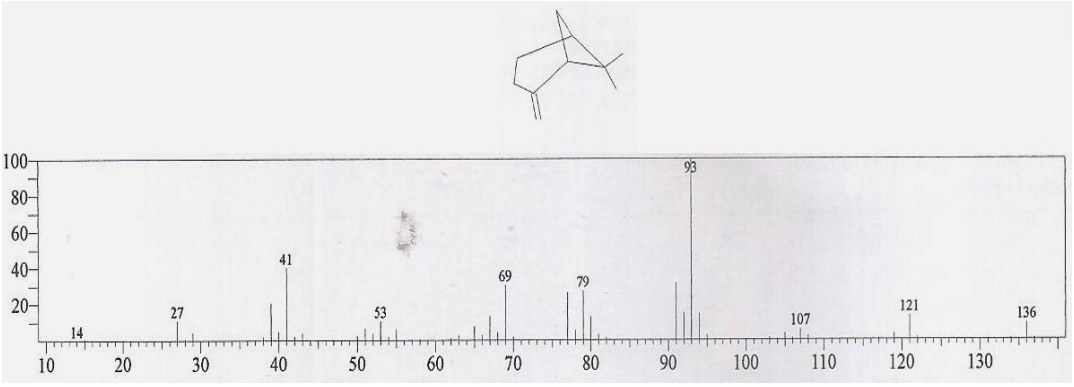

Fig. 5: Mass spectrum of 2,6,6-trimethyl-bicycl[3.1.1]heptanes.

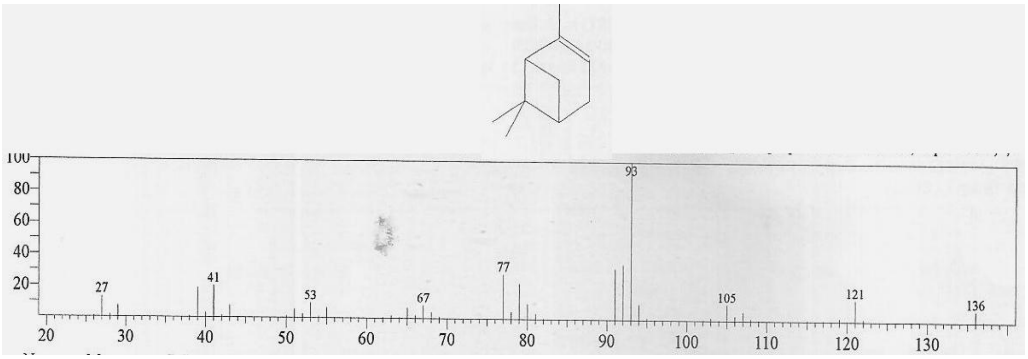

Fig. 6: Mass spectrum of α-pinene.

Antimicrobial activity

The oil was screened for antimicrobial activity against standard organisms. The average of the diameters of the growth inhibition zones are shown in Table (6) .The results were interpreted in terms of the commonly used terms (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (7) and (8) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 6: Antibacterial activity of *Petroselinum crispum* oil: M.D.I.Z (mm)

Drug	Conc. (mg/ml)	Ec	Ps	Sa	Bs	Ca	An
Petroselinum crispum	100	-	-	13	-	-	-

Table 7: Antibacterial activity of standard chemotherapeutic agents: M.D.I.Z (mm)

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 8: 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*
- M.D.I.Z: Mean diameter or growth inhibition zone (mm). Average or two replicates, inhibition zone >=15: sensitive, <15: resistant.

The oil showed activity only against *Staphylococcus aureus* (Table 6).

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