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# GC-MS ANALYSIS, PHYTOCHEMICAL SCREENING, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF OIL FROM SUDANESE *PETROSELINUM CRISPUM (L) LEAVES.*

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## ABSTRACT
The present work aim to study phytochemical screening, examine the chemical constituents Oil of Sudanese *Petroselinum crispum* leaves and to assess its potential antimicrobial and antioxidant activity. Twenty-seven components were detected by GC-MS analysis. Major constituents are: 1, 3-Benzodioxole, 4-methoxy -6-(2-propenyl) (7.86%), Apiol (17.76%), Hexadecanoic acid, methyl ester (5.49%) and 9, 12-Octadecadienoic acid (Z, Z) -, methyl ester (12.78%), 9,-Octadecadienoic acid (Z) -, methyl ester (41.61%), Methyl stearate (2.45%), 13-Docosenoic, methyl ester (3.92 %). According to DPPH assay the sample showed the low antioxidant potential (10 ± 0.01). The oil showed activity against some organisms. Significant activity was observed against the Gram-positive bacteria: Bacillus subtilis Escherichia coli, Candida albicans organisms.

**KEYWORDS:** *Petroselinum crissum,* Phytochemical screening, GC-MS, Antimicrobial and antioxidant activity.

## INTRODUCTION
Reactive oxygen species are generated in the body as a result of the cellular metabolism and eliminated by defensive enzymes like superoxide dismutase (SOD). In the recent years, the medicinal plants have drawn interest against oxidative stress. The presence of various, natural antioxidants in herbs are well known. Phenolic compounds, especially flavonoids, can donate hydrogen to the harmful free radicals to prevent the oxidative damage at the first initiation step. They are not only scavenging radicals, but inhibiting their genesis.[1] Parsley (*Petroselinum crispum* L.) is a widely known culinary plant and herbal medicine in Europe since ancient times. It is easy to grow and many biological activities are attributed to its seed and leaf. The consumption of the leaf is beneficial to cardiovascular and diabetic diseases via anti-inflammatory, anti-hyperlipidimic and anti-hyperglycemic properties.[2] It is rich source of antioxidants such phenolic and flavonoids. *Petroselinum crispum* belongs to the Apiaceae family. It is a well-known spice and vegetable. Its herb and root are widely known for their effects on digestion, stomach, kidney, blood, and liver.[3] Parsley has been claimed in Arab Traditional Medicine to possess variety of properties including laxative, diuretic and antiurolithiatic. The leaves are used as hot application against inflammatory condition, mastitis and haematomata.[4] Parsley, widely used as a salad ingredient or as a healthy garnish, capable of masking foul odors, as it has a spicy scent. Parsley tea was given to the troops in the trenches suffering from dysentery. Various parts of the plant have been used for tumors of different organs including the stomach.[5] The two major phenolic compounds extracted from parsley flakes were identified as apiin and malonyl-apiin.[6]

## MATERIALS AND METHODS
### Plant material
(*Petroselinum crispum* L.) Leaves were purchased from a local market at Bahri city, Sudan, and was identified at the herbarium of the Aromatic and Medicinal Plants Research Institute.

### Extraction of oil
(40g) of the leaves were ground into fine powder. Powdered leaves were extracted with n-hexane using Soxhlet extractor for four hours. The volume of hexane was reduced under reduced pressure. The oil of (*Petroselinum crispum* L.) Was obtained by evaporating the reduced hexane by air drying in a steady current and was kept in a refrigerator for further manipulation.

**Phytochemical screening**

The fraction was screening for the presence of phenolic compounds, flavonoids, tannins, terpenoids, saponins, alkaloids and carbohydrates using standard methods.[7]

**Sample preparation (Methylation)**

2ml from sample was taken in test tube and 7 ml of NaOH was added to it the mixture was shacked for three minutes by vortex. Left the content to overnight and then 2 ml from supersaturated NaCl was added, add 2ml of normal hexane and shake for three minutes and collected the hexane layer, 5 µL from hexane collected and dilute it with 5 ml diethyl ether and 1 gram from sodium sulfate was added as drying agent. The mixture was filtered using syringe filter 0.45 mm, the filtrate was transferred to the GC/MS and I µm from sample was injected directly to GC-MS.

**GC /MS method**

The qualitative and quantitative analysis of the sample was carried out by using GC MS technique model ( GC /MS-QP2010-Ultra) from japan "Simadzu Company ,with capillary column (Rtx-5ms -30 m × 0.25 mm ×0.25 µm ).The sample was injected by using split mode ,helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60 C with rate 10 C /min to 300 c as final temperature degree , the injection port temperature was 300c, the ion source temperature was 200 c and the interface temperature was 250 c .The sample was analyzed by using scan mode in the range of m/z 40 – 550 charge to ratio .Identification of component for the sample was achieved by comparing their retention times and mass fragmentation patent with those available in the library, the National Institute of Standards and Technology (NIST).,results were recorded.

**Antimicrobial assay**

The oil *Petroselinum crispum* L.) Was screened for its antimicrobial activity against six standard human pathogens (Bacilus subtilis (B.S), Staphylococcus aureus (SA), Escherichia colli (Ec), Pseudomonas aeruginosa (Pa), Aspergillus niger (An) and Candida albacans (Ca).

**Preparation of bacterial suspensions**

One ml aliquot 24 hours' broth culture of the test organisms were aseptically distributed onto hutment agar slopes and incubated at 37o 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 producing a suspension containing about 108 - 104 colony forming units per ml. The suspension was stored in the refrigerator at 4o C until used. The average of viable organism per ml of the saline suspension was determined by means of the surface viable counting technique. Serial dilution of the stock suspension were made in sterile saline in tubes and one drop volumes (0-20ml) of the appropriate dilution were transferred by adjustable volume micropipette onto the surface of dried agar plates. The plates were allowed to stand for two hours at room temperature for drop to dry, and then incubated at 37o C for 24 hours.

**Testing for antibacterial activity**

To determine the antimicrobial activity of the oil, the cup- plate agar diffusion method was adopted with some minor modification.[8] (2ml) of the standard bacteria stock suspension were mixed with (200ml) of sterile molten nutrient agar which was maintained at 45o C. (20ml) aliquot of incubated agar were distributed into sterile Petri dishes. The agar was left to settle and each plate was cut using sterile cork-borer (No.4) and agar discs were removed. Alternates cups were filled with (0.1ml) of test sample using adjustable pipette and allowed to diffuse at room temperature. The Petri dishes were then incubated in the upright position at 37o C for 18 hours. After incubation, the diameter of the resultant growth inhibition zones was measured.

**DPPH radical scavenging assay**

The DPPH radical scavenging was determined according to the method of 1[9] with some modification. In 96 – well plate, were allowed to react with 2, 2 Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37 C. The concentration of DPPH was kept as (300µL M). The test sample was dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using mutilate reader spectrophotometer. Percentage radical scavenging activity by sample was determine in comparison with DMSO treated control group all testes and analysis were run in triplicate.

**RESULTS AND DISCUSSION**

**Plant extracts:** Soxhlet extraction of *Petroselinum crispum* L leaves gave 9 ml of crude was extract with n-hexane.

**Qualitative phytochemical analysis:** Results indicated the presence of many phyto-components in *Petroselinum crispum* L) Oil are given in Table 1

**Table 1: Phytochemical screening of *Petroselinum crispum* L.) Extract.**

| No | Constituents | Test | Results |
|---|---|---|---|
| 1 | Alkaloids | Mayer's,Wanger's reagent | ++++ |
| 2 | Flavonoid | Alkaline reagent | ++ |
| 3 | Saponins | Forth | - |
| 4 | Triterpen,Streol | Liberman Test | - |
| 5 | Tannins,phenolic | Ferric chloride,Alminum chloride | +++ |

(+++)-Heavy ;( ++)-Medium; (+)-Low ;(-)-indicates absent.

**GC-MS analysis of *Petroselinum crispum* L fixed oil**

Lipid Consituents of *Petroselinum crispum* L (L) oil were identified and quantified by GC-MS. Identification of the components was accomplished by comparison with the MS library (NIST). Furthermore, the observed fragmentation pattern was interpreted. Comparison of the mass spectra with the database on MS library revealed about 90-95% match. The GC-MS analysis oil of *Petroselinum crispum* L) revealed the presence of 27 components (Table 2). The typical total ion chromatograms (TIC) is depicted in Fig.1.

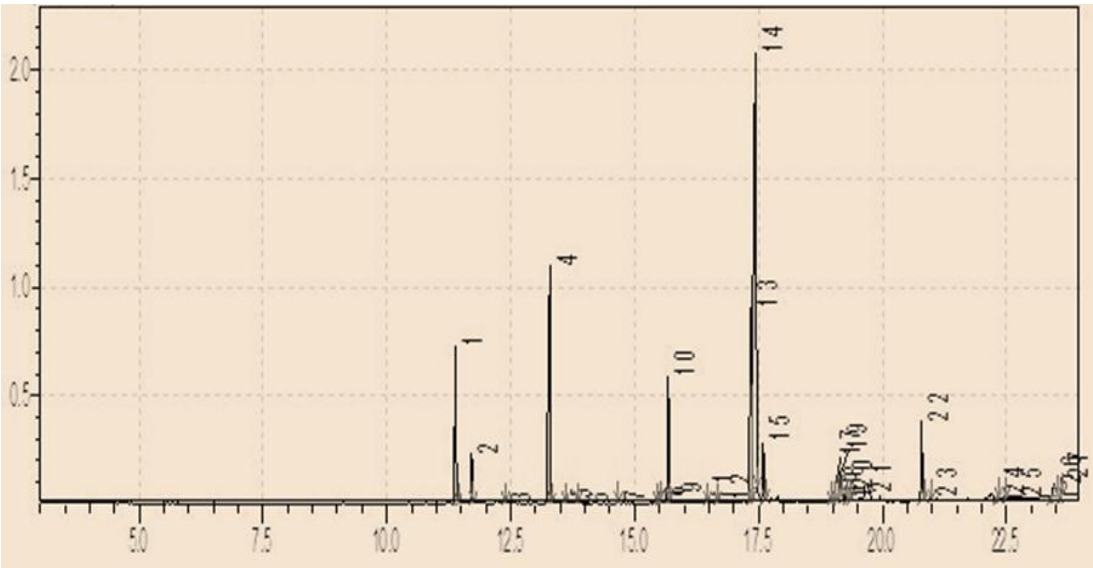


**Fig 1: Typical Total Ion Chromatograms of *Petroselinum crispum* L oil.**

**Table 2: Constituents of Sudanese *Petroselinum crispum L* Oil.**

Peak Report TIC

| Peak# | R.Time | Area | Area% | Name |
|---|---|---|---|---|
| 1 | 11.387 | 14570436 | 7.86 | 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl |
| 2 | 11.707 | 3628608 | 1.96 | Benzene, 1,2,3-trimethoxy-5-(2-propenyl)- |
| 3 | 12.361 | 98735 | 0.05 | Carotol |
| 4 | 13.293 | 32904956 | 17.76 | Apiol |
| 5 | 13.571 | 403322 | 0.22 | Methyl tetradecanoate |
| 6 | 13.842 | 82355 | 0.04 | Pyridinium, 1-ethyl-, hydroxide |
| 7 | 14.645 | 174167 | 0.09 | Pentadecanoic acid, methyl ester |
| 8 | 15.460 | 466613 | 0.25 | 7,10,13-Hexadecatrienoic acid, methyl este |
| 9 | 15.476 | 736242 | 0.40 | 6-Octadecenoic acid, methyl ester |
| 10 | 15.676 | 10179627 | 5.49 | Hexadecanoic acid, methyl ester |
| 11 | 16.438 | 137355 | 0.07 | cis-10-Heptadecenoic acid, methyl ester |
| 12 | 16.649 | 166023 | 0.09 | Heptadecanoic acid, methyl ester |
| 13 | 17.342 | 23677383 | 12.78 | 9,12-Octadecadienoic acid (Z,Z)-, methyl e |
| 14 | 17.442 | 77116409 | 41.61 | 9-Octadecenoic acid (Z)-, methyl ester |
| 15 | 17.593 | 4545031 | 2.45 | Methyl stearate |
| 16 | 18.949 | 183864 | 0.10 | Methyl 5,13-docosadienoate |
| 17 | 18.994 | 141744 | 0.08 | 9,12,15-Octadecatrienoic acid, methyl este |
| 18 | 19.083 | 671727 | 0.36 | 3-Hydroxy-2,6,6-trimethyl-hept-4-enoic aci |
| 19 | 19.147 | 2077501 | 1.12 | 11-Eicosenoic acid, methyl ester |
| 20 | 19.269 | 985448 | 0.53 | 1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4 |
| 21 | 19.342 | 676639 | 0.37 | Eicosanoic acid, methyl ester |
| 22 | 20.790 | 7263872 | 3.92 | 13-Docosenoic acid, methyl ester |
| 23 | 20.964 | 387295 | 0.21 | Docosanoic acid, methyl ester |
| 24 | 22.314 | 324985 | 0.18 | 15-Tetracosenoic acid, methyl ester, (Z)- |
| 25 | 22.468 | 261160 | 0.14 | Tetracosanoic acid, methyl ester |
| 26 | 23.461 | 2117507 | 1.14 | Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)- |
| 27 | 23.613 | 1348221 | 0.73 | Dotriacontane |
| | | 185327225 | 100.00 | |

**1, 3-Benzodioxole, 4-methoxy -6-(2-propenyl) (7.86%)**

Fig. 2 shows the EI mass spectrum of 1, 3-Benzodioxole,4-methoxy -6-(2-propenyl**)**. The peak at m/z192, which appeared at R.T. 11.387 in total ion chromatogram, corresponds to $M^{+}[C_{11}H_{12}O_2]^{+}$ .

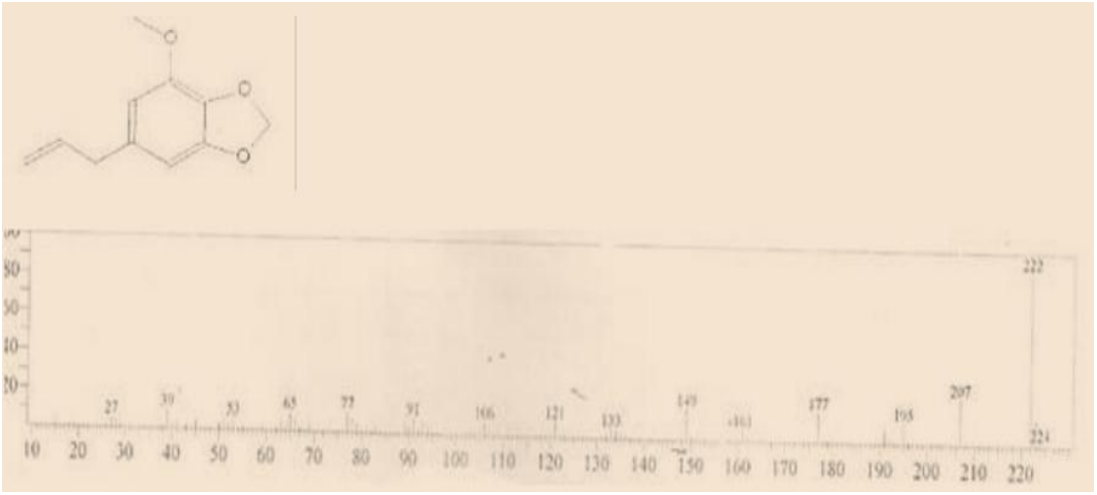

**Fig (2): Mass spectrum of 1, 3-Benzodioxole,4-methoxy -6-(2-propenyl).**

**Benzene, 1, 2, 3-trimethoxy -5-(2-propenyl) (1.96%)**

Fig. 3 shows the EI mass spectrum of Benzene,1,2,3-trimethoxy -5-(2-propenyl).The peak at m/z 208, which appeared at R.T. 13.707 in total ion chromatogram, corresponds to $M^+[C_{12}H_{16}O_3]^+$.

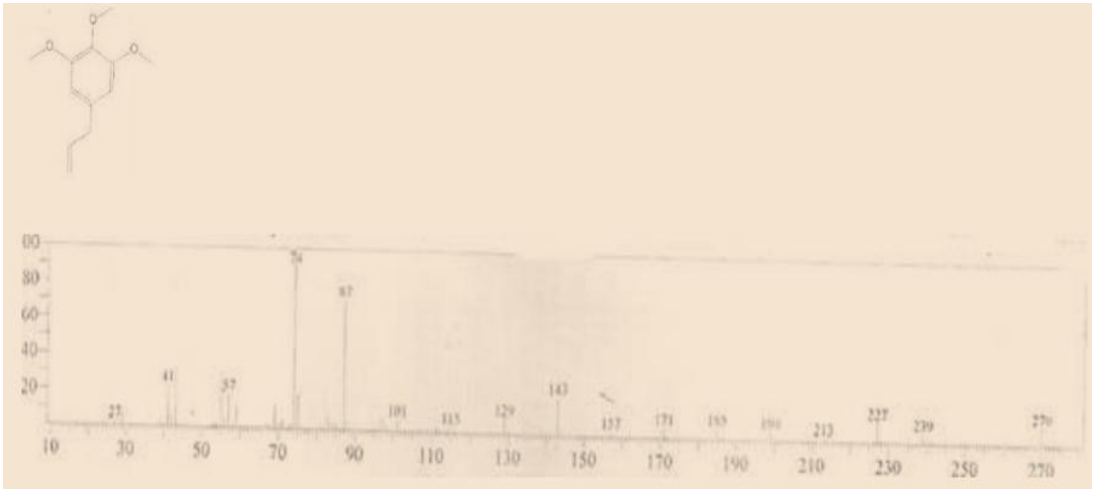

**Fig (3): Mass spectrum of Benzene, 1,2,3-trimethoxy -5-(2-propenyl),3-Benzodioxole,4-methoxy -6-(2-propenyl). Apiol (17.76%).**

Fig. 4 shows the EI mass spectrum of Apiol**.** The peak at m/z 208, which appeared at R.T. 13.293 in total ion chromatogram, corresponds to $M^+[C_{15}H_{26}O]^+$.

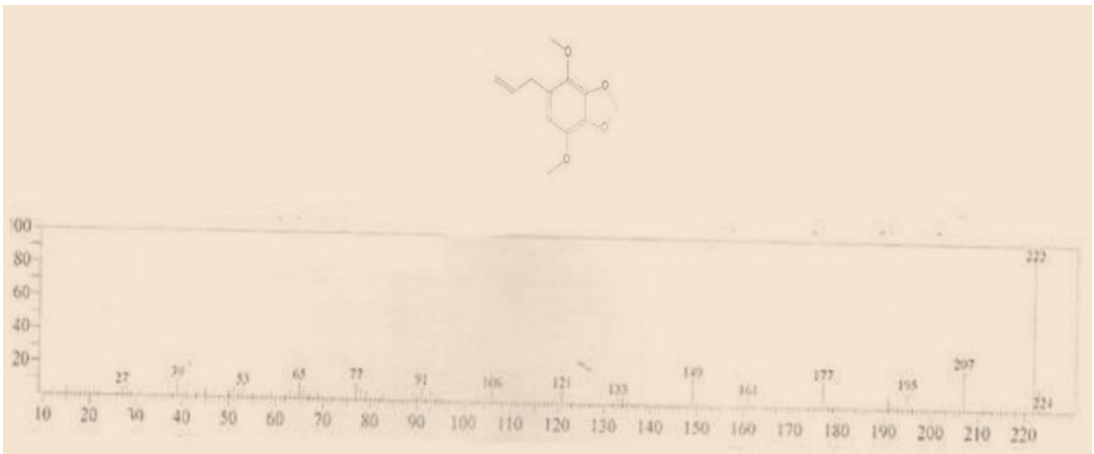

**Fig (4): Mass spectrum of Apiol (17.76%**

**Hexadecanoic acid, methyl ester (5.49%)**

Fig. 5 shows the EI mass spectrum of Hexadecanoic acid, methyl ester.The peak at m/z 226, which appeared at R.T. 15.676 in total ion chromatogram, corresponds to $M^+[C_{17}H_{28}O_2]^+$. Palmitic acid (hexadecanoic acid) is a saturated fatty acid. It is wide-spread in plants and humans. This acid is produced first during the synthesis of fatty acids[10] and is considered as precursor of long-chain fatty acids. Palmitic acid is a major lipid component of human breast milk.[11] The acid finds applications in soap and cosmetics industries. It is also used in food industry.

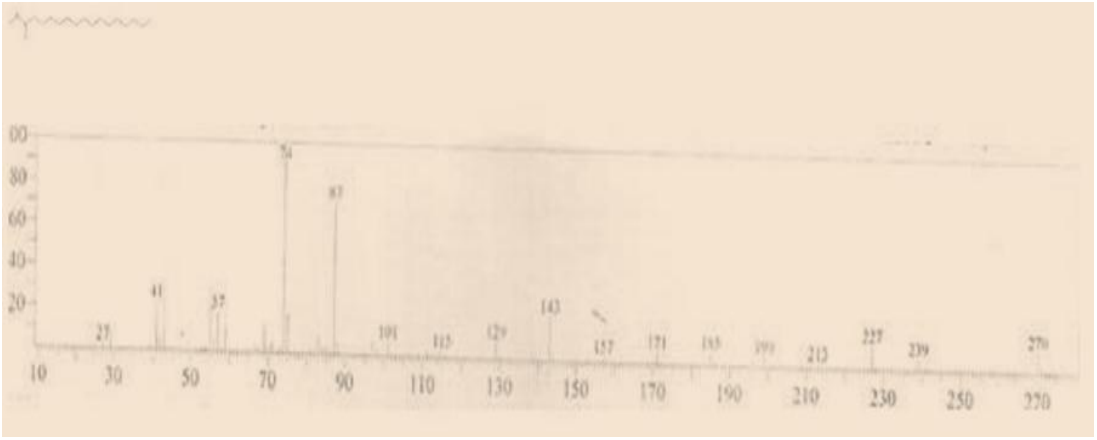

**Fig (5): Mass spectrum of Hexadecanoic acid, methyl ester.**

**9, 12-Octadecadienoic acid (Z, Z) -, methyl ester (12.78%)**

Fig. 6 shows the EI mass spectrum of 9, 12-octadecadienoic acid (Z, Z) -, methyl ester. The peak at m/z 294, which appeared at R.T. 17.342 in total ion chromatogram, corresponds to $M^+[C19H34O2]^+$.9,12-Octadecadienoic **(**linoleic ) acid cannot be synthesized by human bodies and is available through diet(Burr *et.al*.,1930).It belongs to one of the two families of essential fatty acids. It exists in lipids of cell membrane and is used in the biosynthesis of arachidonic acid. Oleic acid is converted enzymatically into mono-hydroxy products which are subsequently oxidized by some enzymes to keto metabolites.These metabolites are implicated in human physiology and pathology. Deficiency of linolate caused hair loss and poor wound healing in model animals.[12-14]

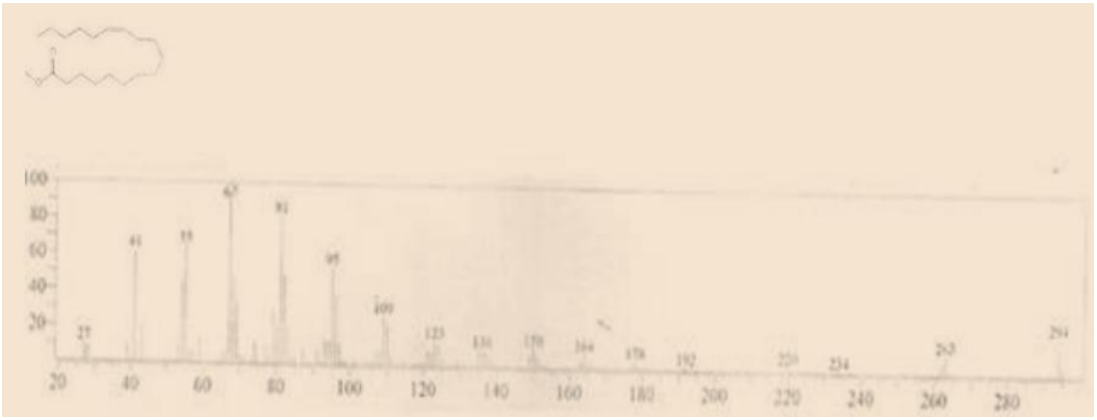

**Fig (6): Mass spectrum of 9, 12-Octadecadienoic acid (Z, Z) -, methyl ester.**

**9, -Octadecadienoic acid (Z) -, methyl ester (41.61%)**

Fig. 7 shows the EI mass spectrum of 9 -octadecadienoic acid (Z) **-,** methyl ester.The peak at m/z296, which appeared at R.T. 17.442 in total ion chromatogram, corresponds to M+$[C19H36O2]^+$ .

9-octadecenoic acid (oleic acid) is a common monounsaturated fat in human diet. It may be responsible for the hypotensive potential of olive oil.[15] Oleic acid finds some applications in soap industry and it is used in small amounts as excipient in pharmaceutical industries. It is also used as soldering flux in stained glass work. Oleic acid is employed as emollient.[16] The consumption of oleate in olive oil has been associated with decreased risk of breast cancer.[17]

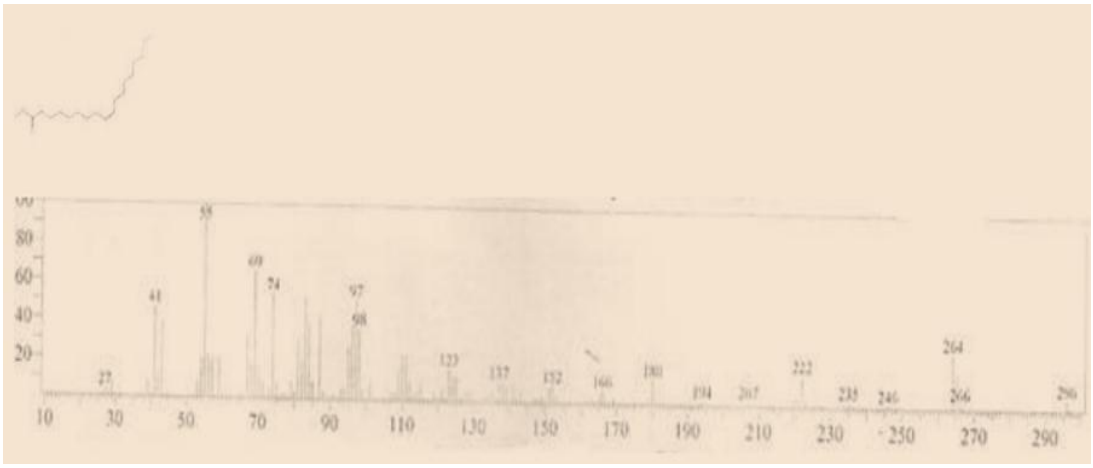

**Fig (7): Mass spectrum of 9, - Octadecadienoic acid (Z) -, methyl ester.**

**Methyl stearate (2.45%)**

Mass spectrum of methyl stearate is depicted in Fig. 8. The peak at m/z 296, which appeared at R.T. 17.593 corresponds to M+[$C_{19}H_{36}O_2$]+.

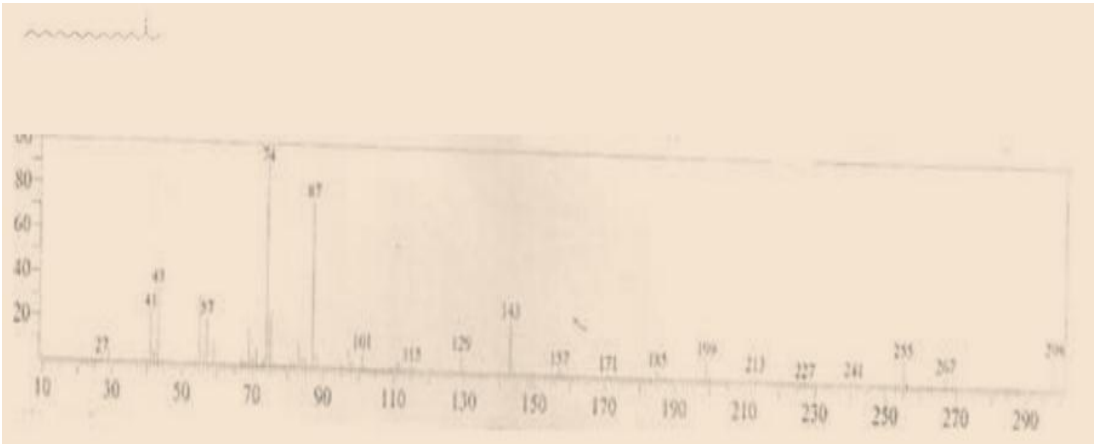

**Fig (8): Mass spectrum of Methyl stearate.**

**11-Eicosanic acid methyl ester (1.12%)**

Fig. 9 shows the EI mass spectrum of 11. Eicosanic acid methyl ester**. The** peak at m/z 324, which appeared at R.T. 19.147 in total ion chromatogram, corresponds to M+[$C_{21}H_{40}O_2$]+.

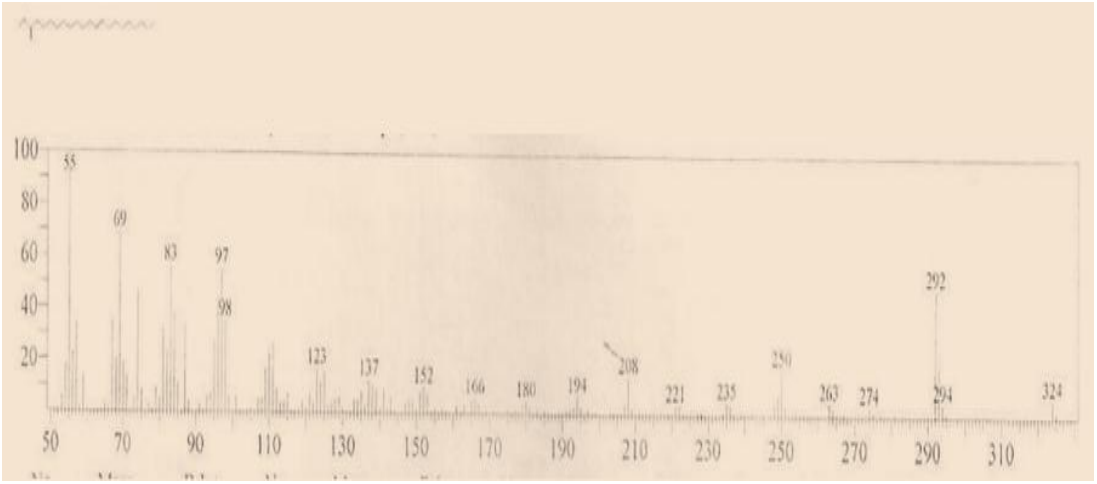

**Fig (9): Mass spectrum of 11-Eicosanic acid methyl ester.**

**13-Docosenoic, methyl ester (3.92 %)**

Mass spectrum of 13-Docosenoic , methyl ester is depicted in Fig. 10.The peak at m/z 352, which appeared at R.T. 22.034 corresponds to M+[$C_{23}H_{44}O_2$]+.

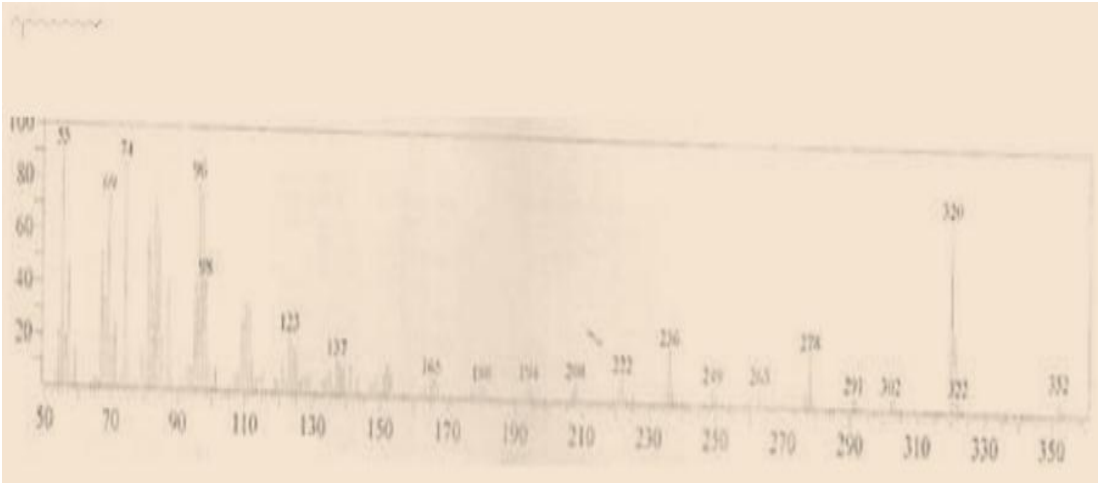

**Fig (10): Mass spectrum of 13-Docosenoic, methyl ester.**

**Stigmast-7-en-3-ol, (3. beta. 5. alpha., 24S) - (1.14 %)**

Mass spectrum of Stigmast-7-en-3-ol, (3. beta, 5. alpha. 24S)- (1.14 %) is depicted in Fig. 11. The peak at m/z414, which appeared at R.T. 23.461 corresponds to M+[$C_{29}H_{50}O$]+.

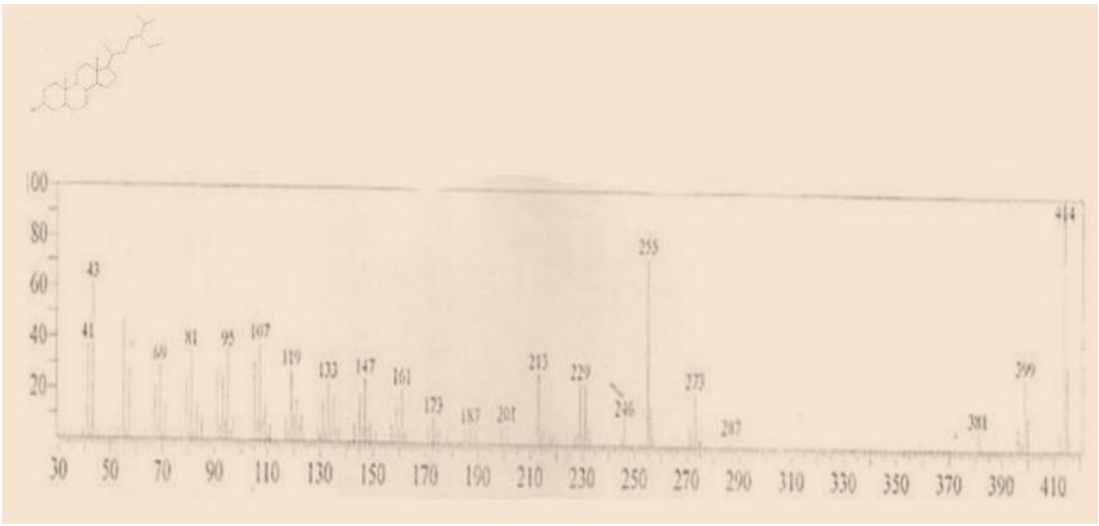

**Fig (11): Mass spectrum of Stigmast-7-en-3-ol, (3. beta.,5. alpha.,24S).**

**Antioxidant activity**

The antioxidant potential of sample was assayed by standard methods (DPPH). According to DPPH assay the sample showed the low antioxidant potential are given in Table (3).

**Table 3: Antioxidant activity results.**

| No | Sample | RSA ± % SD (DPPH) |
|---|---|---|
| **1** | Oil | 10 ± 0.09 |
| **Standard** | Propyl Gallate | 89 ± 0.01 |

**Antibacterial activity.**

In The paper disc diffusion method, *Petroselinum crispum* (L) leaves oil was evaluated for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table (2). The results were interpreted in commonly used terms; >9mm: inactive; 9-12mm: partially active; 13-18mm: active; < 18mm: very active). Tables (3) and (4) represent the antimicrobial activity of standard antibacterial against standard bacteria.

**Table 4: Antibacterial activity *Petroselinum crispum L* Oil and standard: M.D.I.Z (, mm).**

| Drug | Conc.mg/ml | Bs. | Ps. | Ba. | Ec. | Ca |
|---|---|---|---|---|---|---|
| **Oil** | 100 | 14 | - | - | 15 | 12 |
| **Ampicillin** | 100 | 12 | 12 | 15 | 13 | - |

**(Ba = Bacillus subtilis, Ec = Escherichia coli, Pa = Pseudomonas aeruginosa, Ca = Candida albicans, Bs = Bacillus subtilis)**

## DISCUSSION

Preliminary phytochemical screening of ethanolic crude extract from *Petroselinum crispum* L leaves indicated the presence of Alkaloids, Flavonoid, Carbohydrate, Saponins Triterpen, and Steroids. Soxhlet extraction of *the* sample was extract with n-hexane gave 9 ml of oil. Twenty-seven components were detected by GC-MS analysis. Major constituents are: [(1,3-Benzodioxole,4-methoxy -6-(2-propenyl) (7.86%), Apiol (17.76%), Hexadecanoic acid, methyl ester (5.49%) and 9, 12-Octadecadienoic acid (Z, Z) -, methyl ester (12.78%),9, -Octadecadienoic acid (Z) -, methyl ester (41.61%), Methyl stearate (2.45%),13-Docosenoic, methyl ester (3.92 %)]. According to DPPH assay the sample showed the low antioxidant potential (10 ± 0.01). The oil showed activity against some organisms. Significant activity was observed against the Gram-positive bacteria: Bacillus subtilis, Candida albicans, and Escherichia coli.

## CONCLUSIONS

Future studies will identify and quantify individual flavonoids, Alkaloids, and phenolic acids of Petroselinum *crispum* Leaves.

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