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# GC-MS ANALYSIS, PHYTOCHEMICAL, AND ANTIMICROBIAL ACTIVITY OF SUDANESE *NIGELLA SATIVA* (L) OIL

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**ABSTRACT**
Preliminary phytochemical screening of ethanolic crude extract from *Nigella Sativa* seeds Indicated the presence of Alkaloids, Flavonoid, Carbohydrate, SaponinsTriterpen, Tannins, phenolic and Steroids. Maceration extraction of *Nigella Sativa* (L) seeds was extract with n-hexane gave 13 ml of crude oil. Eighteen components were detected by GC-MS analysis. Major constituents are Hexadecanoic acid, methyl ester (1.79%), n-Hexadecanoic acid (7.97% ,9, 12-Octadecadienoic acid (Z,Z) -, methyl ester (8.43 %),9-Octadecanoic acid (Z)-,methyl ester (3.48%), 9,12-Octadecadienoic acid (Z,Z)methyl ester (48.72%), Oleic acid (13.14 %), Heptadecanoic acid (1.59),9,12-Octadecadienoyl chloride (Z,Z) (8.10%), Methyl 2-hydroxy-octadeca-9,12,15-trienote (3.53%). The oil *Nigella Sativa* (L) seeds showed moderately activity against organisms.

**KEYWORDS:** GC-MS Analysis, Phytochemical, and Antibacterial activity.

## INTRODUCTION

Nigella Sativa (Family Ranunculaceae), commonly known as black seed or black cumin, is an annual plant that has been traditionally used in the Indian subcontinent. Arabian countries and Europe[1-3] for culinary and medicinal purposes. The black, angular seeds, in Arabia known as 'HabbaSauda', 'Habbet el Baraka', 'Kamun-aswad' and 'Shunez'. Previous studies showed that Nigella sativa seeds contains 36 - 38% fixed oils, proteins, alkaloids, saponins and 0.4 - 2.5% essential oils[4]. Found that Nigella sativa might be used in diabetic patients to prevent lipid per oxidation, increase anti-oxidant and defense system activity and prevent liver damage. Pharmacological and toxicological properties of Nigella sativa were investigated by[5]. They found that the pharmacological action of the crude extracts of the seeds caused protection against nephrotoxicity and hepatotoxicity induced by either disease or chemicals. They stated that the seeds were characterized by a very low degree of toxicity[6]. It has been reported that Nigella sativa oil possesses anticestode and antinematode actions. Besides, it produced a hepatoprotective effect by improving the immunological host system and to some extent by its antioxidant effect[7]. In a recent study have evaluated the possible protective effects of Nigella sativa against beta-cell damage from streptozotocininduced diabetes in rats and they found that Nigella sativa treatment exerts a therapeutic protective effect in diabetic animals by decreasing oxidative stress and preserving pancreatic beta-cell integrity[8].

## MATERIALS AND METHODS

### Plant material

Nigella Sativa seeds were bought from a local market at El Khartoum city, Sudan, and was identified at the herbarium of the Aromatic and Medicinal Plants Research Institute.

### Preparation of Crude Extracts

The powdered seeds (100g) were extracted successively with Ethanol in magnetic stirring apparatus for 4 hours at room temperature. The Ethanol extract was filtrated then was air dried after extraction, the residual of the powdered plants materials were desiccated.

### Phytochemical screening

Phytochemical screening for the active materials was carried out for extracts using the method described[9, 10] with verify some of modification.

### Extraction of oil

Powdered seeds were extracted with n-hexane using shaker extractor for four hour. The volume of n-hexane

was reduced under reduced pressure. The oil of Nigella Sativa Oil was obtained by evaporating the reduced n-hexane by air drying in a steady current. The oil was kept in a freezer for advance handling.

**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 of Nigella Sativa seeds were screened for its antimicrobial activity against six standard human pathogens (Bacilussubtilis (B.S), Staphylococcus aureus (Sa), Escherichia colli (Ec), Pseudomonas aeruginosa (Pa), Aspergillusniger (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 $37^{o}$ 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. (2ml) of the standard bacteria stock suspension were mixed with (200ml) of sterile molten nutrient agar which was maintained at 45o C. (20 ml) 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 $37^{o}$ C for 18 hours. After incubation, the diameter of the resultant growth inhibition zones were measured.

**RESULTS AND DISCUSSION**

**Phytochemical screening activity of Nigella Sativa:** The results of qualitative phytochemical analysis of Nigella Sativa Oil are given in Table (1).

**Table (1): Phytochemical screening of Nigella Sativa seeds extract.**

| No | Constituents | Test | Results |
|---|---|---|---|
| **1.** | Alkaloids | Mayer's,Wanger's reagent | ++ |
| **2.** | Flavonoid | Alkaline reagent | + |
| **3.** | Carbohydrate | Molish's | +++ |
| **4.** | Saponins | Forth | + |
| **5.** | Triterpen,Streol | Liberman | +++ |
| **6.** | Tannins,phenolic | Ferric chloride,Alminum chloride | +++ |

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

**Plant extracts**

Shaker extraction of *Nigella Sativa* seeds gave 13 ml of crude was extract with n-hexane.

**GC-MS analysis of Nigella Sativa fixed oil**

Lipid Constituents of Nigella Sativa 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.

**Constituents of oil**

The GC-MS analysis oil of Nigella Sativa revealed the presence of 18 components Table3.2.

The typical total ion chromatograms (TIC) are depicted in Fig3.9.


**Table 3.2.The GC-MS analysis oil of *Nigella Sativa.***

Peak Report TIC

| Peak# | R.Time | Area | Area% | Name |
|---|---|---|---|---|
| 1 | 4.743 | 90495 | 0.07 | o-Cymene |
| 2 | 7.910 | 155483 | 0.13 | Thymoquinone |
| 3 | 9.231 | 26267 | 0.02 | 3-Cyclohexene-1-methanol, .alpha.,.alpha., |
| 4 | 10.120 | 43238 | 0.03 | Longifolene |
| 5 | 10.953 | 73469 | 0.06 | Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-met |
| 6 | 15.782 | 2177498 | 1.76 | Hexadecanoic acid, methyl ester |
| 7 | 16.185 | 9873000 | 7.97 | n-Hexadecanoic acid |
| 8 | 17.437 | 10446185 | 8.43 | 9,12-Octadecadienoic acid (Z,Z)-, methyl e |
| 9 | 17.479 | 4306126 | 3.48 | 9-Octadecenoic acid (Z)-, methyl ester |
| 10 | 17.696 | 791739 | 0.64 | Methyl stearate |
| 11 | 17.910 | 60348701 | 48.72 | 9,12-Octadecadienoic acid (Z,Z)- |
| 12 | 17.936 | 16278422 | 13.14 | Oleic Acid |
| 13 | 18.942 | 503110 | 0.41 | Tributyl acetylcitrate |
| 14 | 19.227 | 1965587 | 1.59 | Heptadecanoic acid, heptadecyl ester |
| 15 | 20.692 | 10029657 | 8.10 | 9,12-Octadecadienoyl chloride, (Z,Z)- |
| 16 | 20.819 | 949648 | 0.77 | 2-Ethylbutyric acid, eicosyl ester |
| 17 | 22.059 | 1439096 | 1.16 | Cyclopropane, 1,1-dichloro-2,2,3,3-tetrame |
| 18 | 22.167 | 4373435 | 3.53 | Methyl 2-hydroxy-octadeca-9,12,15-trienoa |
| | | 123871156 | 100.00 | |

Table 3.2.The GC-MS analysis oil of *Nigella Sativa* revealed the presence of 18 components.

**Hexadecanoic acid, methyl ester (1.79%):** Fig. 2 shows the EI mass spectrum of Hexadecanoic acid, methyl ester. The peak at m/z 270, which appeared at R.T. 15.782 in total ion chromatogram, corresponds to M+[$C_{17}H_{34}O_2$]+.

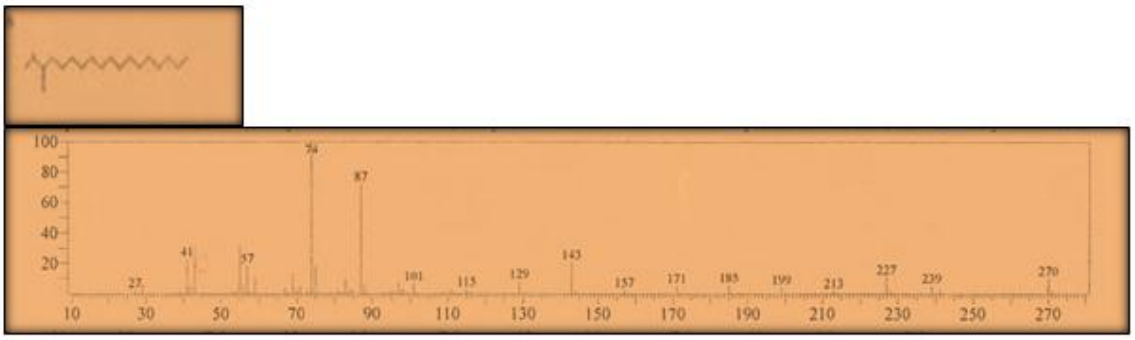



**Fig. 2: Mass spectrum of Hexadecanoic acid, methyl ester.**

**n-Hexadecanoic acid (7.97%):** Fig. 3 shows the EI mass spectrum of n-Hexadecanoic acid .The peak at m/w256, which appeared at R.T. 16.185 in total ion chromatogram, corresponds to M+[$C_{16}H_{32}O_2$]+. n-Hexadecanoic acid (plamitic acid) use to produce soaps and cosmetics.[11] It is used in small amounts as excipient in pharmaceutical industries.[12]

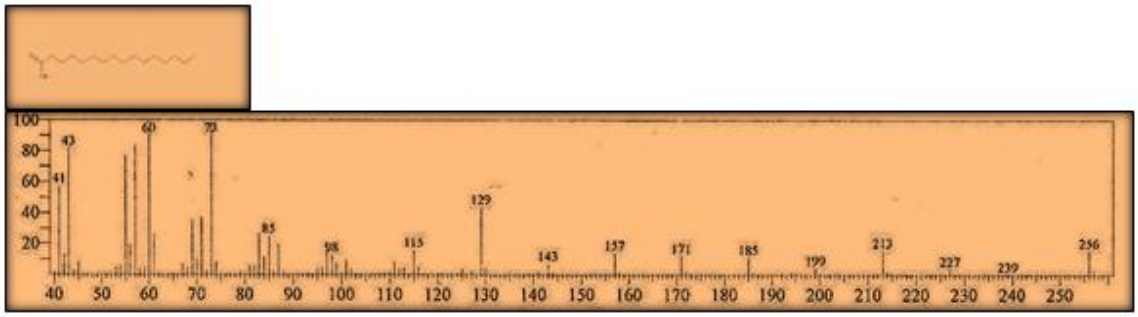


**Fig. 3: Mass spectrum of n-Hexadecanoic acid.**

**9, 12-Octadecadienoic acid (Z,Z) -, methyl ester (8.43 %)**

Fig. 4 shows the EI mass spectrum of 9 -octadecadienoic acid (Z) -, methyl ester .The peak at m/z 256, which appeared at R.T. 17.437 in total ion chromatogram, corresponds to M+[$C_{19}H_{34}O_2$]+ .

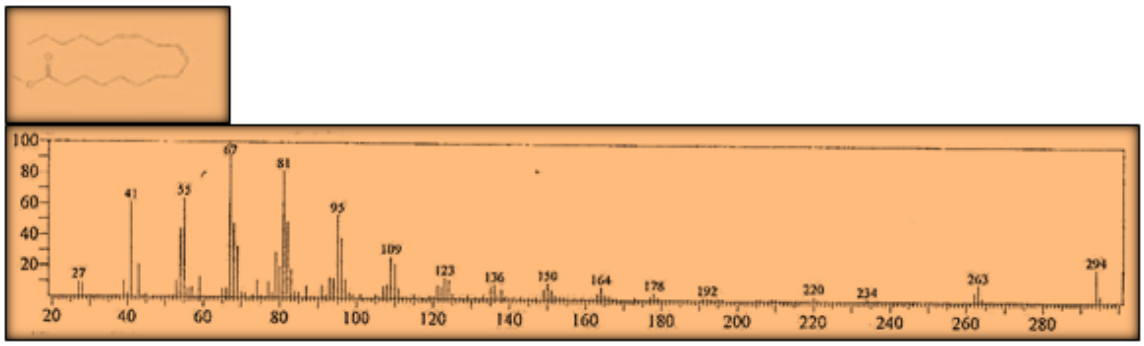


**Fig3.4. Mass spectrum of 9, 12-Octadecadienoic acid (Z,Z) -, methyl ester.**

**9-Octadecanoic acid (Z)-,methyl ester (3.48%) :** Fig. 5 shows the EI mass spectrum of 9 -octadecadienoic acid (Z),methyl ester. The peak at m/z 294, which appeared at R.T. 17.479 in total ion chromatogram, corresponds to M+[$C_{19}H_{36}O_2$]+. 9-octadecenoic acid is a common monounsaturated fat in human diet. It may be responsible for the hypotensive potential of olive oil[13], It was use anti-oxidant activity, anti-carcinogenic Human Blood and serve as Endogenous peroxisome proliferator activated receptor ligand dermatitigenic flavor.[14]

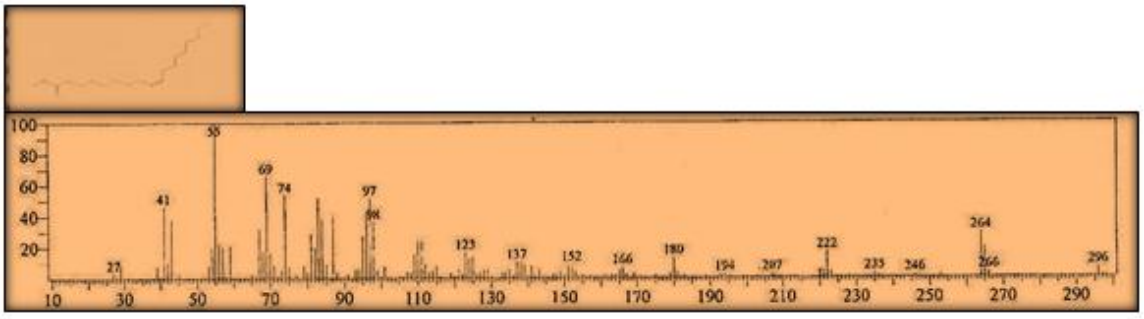


**Fig3.5. Mass spectrum of 9-Octadecadienoic acid (Z,Z) -, methyl ester.**

**9,12-Octadecadienoic acid (Z,Z)methyl ester (48.72%)**

Fig. 6 shows the EI mass spectrum of 9,12-Octadecdienoic acid(Z,Z) methyl ester. The peak at m/z 280, which appeared at R.T. 17.910 in total ion chromatogram, corresponds to M+[$C_{18}H_{32}O_2$]+. 9,12-Octadecadienoic acid (Z,Z) -, methyl ester use fuel and fuel additives[9]It was found to have potential cancer preventive, anti-inflammatory and anti-arthritic activities[10].

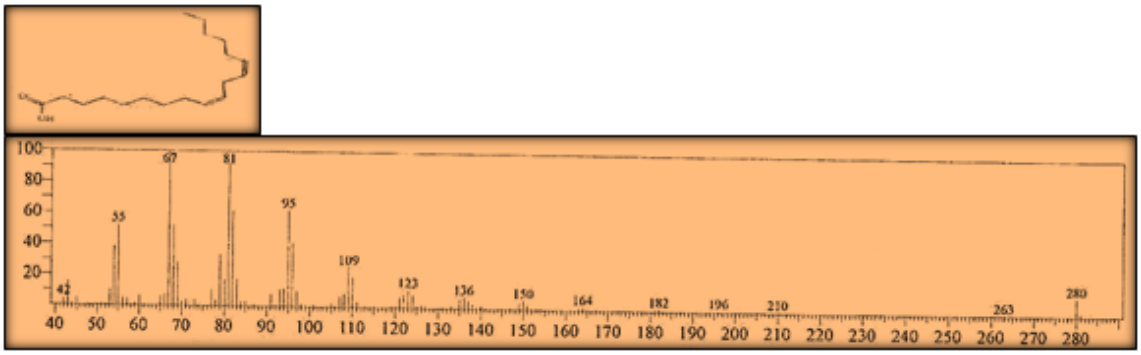
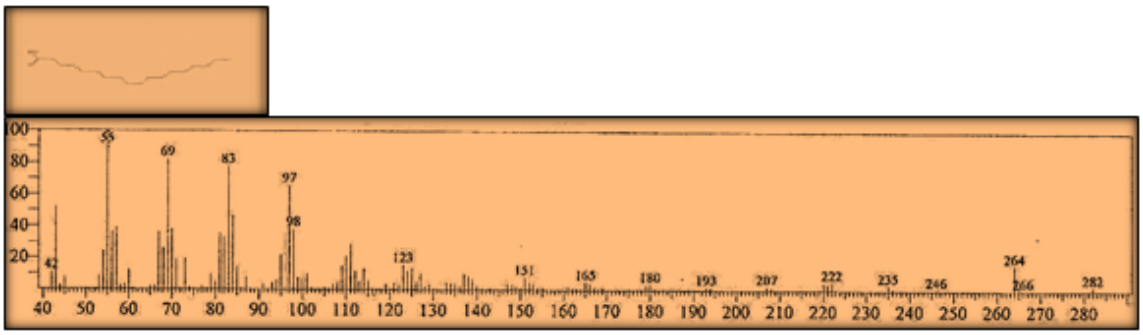


**Fig (3-6) Mass spectrum of 9,12-Octadecadienoic acid (Z,Z)methyl ester.**

**Oleic acid (13.14 %)**

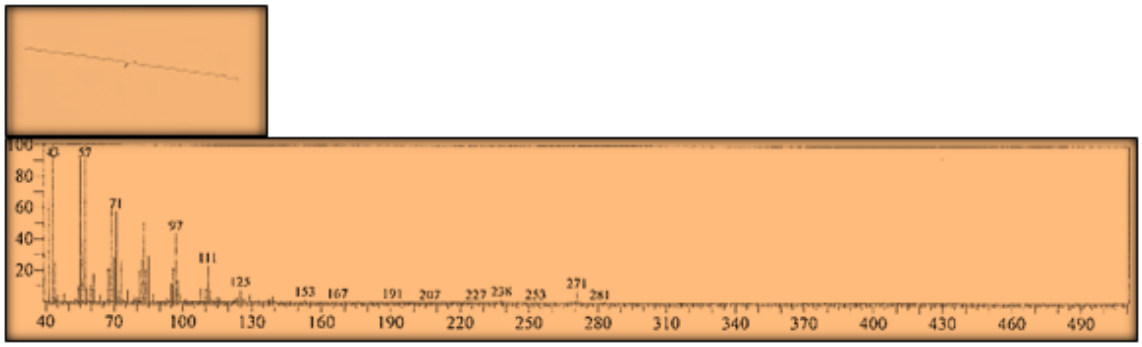
Fig. 7 .shows the EI mass spectrum of Oleic acid .The peak at m/z 282, which appeared at R.T. 17.936 in total ion chromatogram, corresponds to M+[$C_{18}H_{34}O_2$]+. Oleic acid finds some applications in soap industry and it is used in small amounts as excipient in pharmaceutical industries. It is also used as soldening flux in stained glass work. Oleic acid is employed as emollient.[13] The consumption of oleate in olive oil has been associated with decreased risk of breast cancer.[15]



**Fig(3-7) Mass spectrum of Oleic acid.**

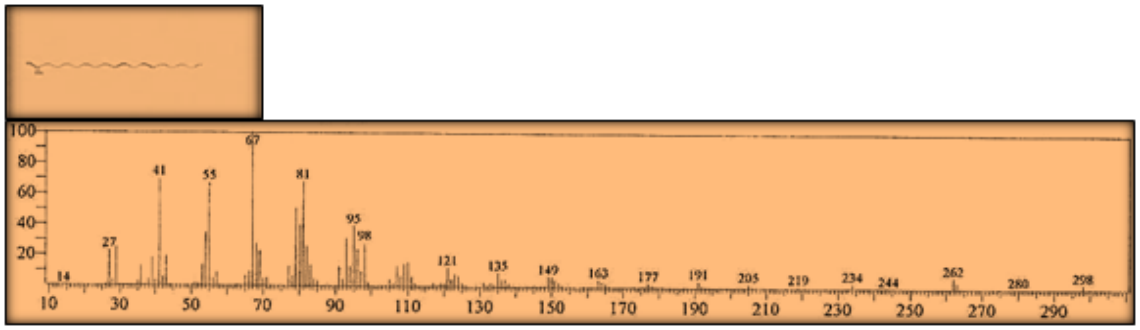
**Heptadecanoic acid (1.59)**

Fig. 8 shows the EI mass spectrum of Heptadecanoic acid .The peak at m/z 508, which appeared at R.T. 19.227 in total ion chromatogram, corresponds to M+[$C_{34}H_{68}O_2$]+. Heptadecanoic acid (Margaric acid) may e use a reference during the analysis of medium chain fatty acid from biomaterials such as seed oil and plant and animals oil.[16]



**Fig(3-8) Mass spectrum of Heptadecanoic acid9,12-Octadecadienoyl chloride (Z,Z) (8.10%).**

Fig. 9 shows the EI mass spectrum of 9,12-Octadecadienoyl chloride (Z,Z).The peak at m/z 298, which appeared at R.T. 20.692 in total ion chromatogram, corresponds to M+[$C_{18}H_{31}C_{lO}$]+. 9,12-Octadecadienoyl chloride (Z,Z) (Linoleic acid) use rom industrial beauty product, antiflammatory, acne reductive, and moisture retentive properties when applied topically on the skin.[17]



**Fig(3-9) Mass spectrum of 9,12-Octadecadienoyl chloride (Z,Z).**

**Cyclopropane, 1,1-dichloro-2,2,3,3-tetramethyl (1.16%)**

Fig. 10 shows the EI mass spectrum of Cyclopropane, 1,1-dichloro-2,2,3,3-tetramethyl .The peak at m/z 166, which appeared at R.T. 22.059 in total ion chromatogram, corresponds to M+[$C_7H_{12}Cl_{12}$]+.

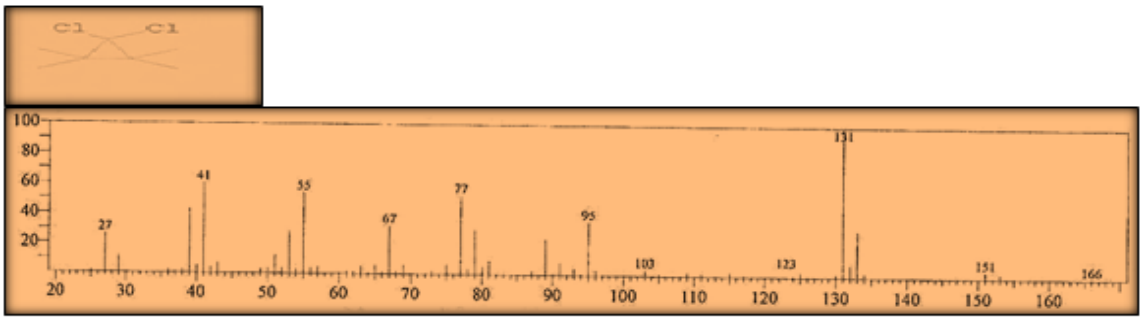


**Fig (3-10) Mass spectrum of Cyclopropane, 1,1-dichloro-2,2,3,3-tetramethyl.**

**Methyl 2-hydroxy-octadeca-9,12, 15-trienote (3.53%)**

Fig. 11 shows the EI mass spectrum of Methyl 2-hydroxy-octadeca-9,12,15-trienote. The peak at m/z 308, which appeared at R.T. 22.167 in total ion chromatogram, corresponds to M+[$C_{19}H_{32}O_3$]+. Methyl 2-hydroxy-octadeca-9,12,15-trienotic was may be teratment cancer, anti-inflammatory, anti-andorgenic and 5-alpha reductase inhibitor.[18]

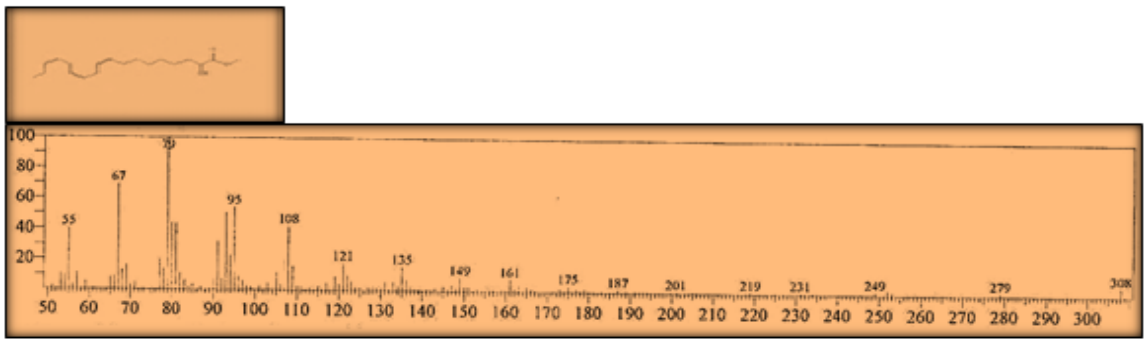


**Fig (3-11) Mass spectrum of Methyl 2-hydroxy-octadeca-9,12,15-trienote.**

### 3.4. Antibacterial Activity

In The disc diffusion method, *Nigella Sativa* **(**L) seeds oil was evaluated for bacterial activity. The averages of the diameters of the growth inhibition zones are shown in Table (2). The results were interpreted in commonly used terms ; > 9 mm: 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 3:- Antibacterial activity of *Nigella Sativa L* oil: M.D.I.Z (, mm).**

| Drug | Conc.mg/ml | Bs. | Ps. | Ba. | Ec. | Ca |
|---|---|---|---|---|---|---|
| Oil | 100 | 16 | - | 11 | - | - |
| Ampicillin | 100 | 14 | 14 | - | 13 | - |

## CONCLUSION

- Phytochemical screening was carried out the plant part was showed to contain alkaloids, flavonoids, tannins, tri terpenes and carbohydrate.
- The extracts of seed was gave normal results ranged between strong to moderate activities against all bacterial organisms were used.
- In this study, nearly eighteen chemical constituents have been identified from n-hexane extract of the seeds swellings of Nigella Sativa plant by GC-MS analysis.

## Recommendation

I. The medicinal plants are continued source of new active compounds that are used for medicinal care or in drug synthesis, which must be to give more attention and support of scientific research in this area.
II. More research needed on this plant Nigella Sativa to specify the active component that makes inhibition for growth of microorganisms
III. Future work to purification of the plant extracts to isolate the bioactive metabolites and their structure must be elucidated.
IV. The toxicity of other extracts of this plant must be done.
V. Cytotoxicity Bioassay is very important test for medicinal plants research.

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