

Full Length Research Paper

Analysis of bioactive chemical components of two medicinal plants (*Coriandrum sativum* and *Melia azedarach*) leaves using gas chromatography-mass spectrometry (GC-MS)

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The main objective of this study was to determine the phytochemical composition in the leaves of *Coriandrum sativum*, using methanolic extraction and report the main functional components by using IR technique. The phytochemical compounds in the extract were then screened by GC-MS method. Seven bioactive phytochemical compounds were identified in the methanolic extract of *C. sativum*: 1,6-octadien-3-ol, 3,7-dimethyl, 1,6-octadien-3-ol,3,7-dimethyl, 2-aminobenzoate, bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl., geranyl vinyl ether, 9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol., ascorbic acid 2,6-dihexadecanoate and 7 α H-cyclopenta[a] cyclopropa[f]cycloundecene. Thirteen bioactive phytochemical compounds were identified in the methanolic extract of *Melia azedarach*. In the present investigation, a variety of compounds have been detected in *M. azedarach* including trichloromethane, propanedioic acid, diethyl ester, 2-pyrrolidinyl-methylamine, butanedioic acid, diethyl ester, 2-piperidimethanamine, butanedioic acid, hydroxyl-, diethyl ester, 2,5-dimethylhexane-2,5-dihydroperoxide, dithiocarbamate, s-methyl-,n-(2-methyl-3-oxobutyl), triethyl citrate, γ -sitosterol, ethyl 9,12,15-octadecatrienoate, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, and octadecane, 3-ethyl-5-(2-ethylbutyl). It contains chemical constitutions which may be useful for various herbal formulation as anti-inflammatory, analgesic, antipyretic, cardiac tonic and antiasthmatic. *C. sativum* is highly active against *Aspergillus terreus* 6.01 ± 0.200 . Bioactive compounds of *C. sativum* and *M. azedarach* were assayed for *in vitro* antibacterial activity against *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aerogenosa*, *Escherichia coli* and *Klebsiella pneumonia* using the diffusion method in agar. The zone of inhibition was compared with different standard antibiotics. The diameters of inhibition zones ranged from 5.60 ± 0.320 to 1.96 ± 0.200 mm for all treatments.

Key words: Anti-bacterial, antifungal activity, *Coriandrum sativum*, GC-MS analysis, *Melia azedarach*, phytochemicals.

INTRODUCTION

Coriander is an annual popular culinary medicinal plant with a distinctive pungent, fatty, and aldehydic aroma (Msaada et al., 2009). Coriander is recognized as one of the most important spices in the world and is of great

significance in international trade (Msaada et al., 2007). The bioactive non-nutrient plant compounds in fruit, vegetables, grains, and other plant foods have been linked to reductions in the risk of major chronic diseases

(Altameme et al., 2015). Coriander has been cultivated since ancient times and is originally from the Mediterranean and Middle Eastern region and grows extensively in India, Russia, Central Europe, Asia and Morocco. The Coriander essential oil is generally obtained by steam distillation of the dried fully ripe fruits (seeds) and oil has a characteristic odor of linalool and a mild, sweet, warm and aromatic flavor (Ramadan and Moersel, 2006). The seeds have medical uses and traditionally applied for curing digestive disorder, pain in joints and rheumatism. Stomachic, spasmolytic, carminative, diarrhoea and dyspepsia of various origin's coriander are also used in aromatherapy (Gil et al., 2002; Eikani et al., 2007; Grosso et al., 2008, Hussein et al., 2015; Imad et al., 2015). It is used to treat female diseases such as menoxenia, ovulation type dysfunctional uterine bleeding (Paarakh, 2009). It is used for treating leucorrhoea; spermatorrhea. Coriander fruit possess stimulant and carminative properties (Khare, 2007). The fruits are used as astringent, anthelmintic, emollient, stomachic, antibilious, digestive, appetizer, constipating, diuretic, antipyretic, refrigerant, tonic, expectorant, anodyne, antidiabetic and dyspepsia (Paarakh, 2009; Hameed et al., 2015a). *Melia azedarach*, family Meliaceae is from west Asia (Sumathi, 2013). It is a moderate-sized deciduous tree 9 to 12 m in height dark grey and a cylindrical bole. *M. azedarach* is traditionally been used as anthelmintic, astringent and stomachic agent. It is widely distributed in Himalayan region. The leaves are bi- or trip innate, pinnate opposite or alternate, ovate or lanceolate, serrate, acuminate, glabrous on both surfaces (Bergsson et al., 2002; Dawson et al., 2002; Lee et al., 2002; Hameed et al., 2015b). *M. azedarach* is used for the treatment of inflammations, leprosy and cardiac disorders. Its fruits and leaves extracts possess antiviral, antifertility activity, ovicidal and larvicidal activity (Wandscheer et al., 2004; Corpinella et al., 2007; Mandal and Dhaliwal, 2007). The plant possesses antioxidant, antimalarial, antihepatotoxic, antibacterial, antiparasitic, and antiulcer properties (Dai et al., 1999; Devi et al., 2001; Bahuguna et al., 2009; Samudram et al., 2009; Nahak et al., 2010; Hameed et al., 2015c). The aim of the study was to investigate the presence of phytochemical compounds from the leaves of *Coriandrum sativum* and *M. azedarach* by using gas chromatography-mass spectrometry and evaluation antibacterial activity.

MATERIALS AND METHODS

Collection and preparation of plant material

The leaves were purchased from a local market in Hilla city, middle of Iraq. After thorough cleaning and removal of foreign materials,

the leaves were stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use (Jasim et al., 2015).

Preparation of sample

About 20 g of the plant sample powdered were soaked in 120 ml methanol for 18 h in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis. It was again filtered through sodium sulphate in order to remove the traces of moisture.

Gas chromatography - Mass spectrum analysis

GC-MS (Agilent 7890 A) was used in this study to identify the components present in the extract (Mohammed and Imad, 2013; Kareem et al., 2015). About 1 μ L of the methanol extract was injected into the GC-MS using a micro syringe (Imad et al., 2015a). Each of the peaks in the chromatogram represented the signal created when a compound eluted from the Gas chromatography column into the detector. The x-axis showed the retention time and the y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass (Imad et al., 2015b). The mass/charge (M/Z) ratio obtained was calibrated from the graph obtained, which was called as the mass spectrum graph which is the fingerprint of a molecule. Before analyzing the extract using gas chromatography and mass spectroscopy, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier. The flow rate of helium was set to 1 ml per minute. The electron gun of mass detector liberated electrons having energy of about 70 eV. The column employed here for the separation of components was Elite 1 (100% dimethyl poly siloxane). The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library (Muhanned et al., 2015; Imad et al., 2015c).

Determination of antibacterial activity of crude bioactive compounds of *C. sativum* and *M. azedarach*

Proteus mirabilis, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were swabbed in Mueller Hinton agar plates. 60 μ L of plant extract was loaded on the bored wells. The wells were bored in 0.5 cm in diameter. The plates were incubated at 37°C for 24 h and examined. After the incubation the diameter of inhibition zones around the discs was measured.

Determination of antifungal activity

Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 μ l of the samples solutions (*C. sativum* and *M.*

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azedarach) was delivered into the wells. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as control. Amphotericin B and fluconazole were used as reference antifungal agents. The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation (Huda et al., 2015; Ameer et al., 2015).

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at $p < 0.05$ using Duncan's multiple range test using SPSS software) version 9.1.

RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic leaves extract of *C. sativum* and *M. azedarach* shown in Tables 1 to 2. The GC-MS chromatogram of the twenty peaks of the compounds detected is shown in Figures 1 and 2. Chromatogram GC-MS analysis of the methanol extract of *C. sativum* showed the presence of 20 major peaks and the components corresponding to the peaks were determined as follows. The first set up peak was determined to be 1,6-octadien-3-ol,3,7-dimethyl (Figure 2). The second peak was indicated to be 1,6-octadien-3-ol,3,7-dimethyl, 2-aminobenzoate (Figure 3). The next peaks was considered to be bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl, geranyl vinyl ether, 9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol, ascorbic acid 2,6-dihexadecanoate, 7ah-cyclopenta[a]cyclopropa[f]cycloundecene (Figures 4 to 9). Coriander oil may have future use as a free radical scavenger, preventing oxidative deterioration in foods. In a report by Ramadan and Moersel (2006) coriander oil was shown to have greater activity against the radical generating activity of 1,1-diphenyl-2-picrylhydrazyl in several oils. Recently, Coriander oil has been reported to possess many medicinal properties, including antimicrobial properties against selected pathogenic (Martins et al., 2003; Ishikawa et al., 2003) antioxidant (Quynh et al., 2009), antidiabetic (Pourmortazavi and Hajimirsadeghi, 2007), anticancer and antimutagenic activities (Mohammad et al., 2011). *Coriandrum sativum* can act as source for oleic acid as the percentage found was 38.55% and soxhlation method can be used to extract it from the fruit (Padmaa, 2014). Shahidi (2008) reported that their synergistic effects are rendered by a combination of phytochemicals present in source materials, and complementary nature of phytochemicals from different sources are important factors to consider in the formulation of functional foods and in the choice of a healthy diet.

Medicinal plants are used in traditional treatments to cure variety of diseases (Hariprasad and Ramakrishnan, 2011). Various scientific studies reported the analgesic,

anticancer, antiviral, antimalarial, antibacterial, antifeedent and antifertility activity of this plant (Vishnukanta, 2008; Sen and Batra, 2011). Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic leaves extract of *M. azedarach* as shown in Table 2. Chromatogram GC-MS analysis of the methanol extract of *M. azedarach* showed the presence of 13 major peaks and the components corresponding to the peaks were determined as follows. The first set up peak was determined to be Trichloromethane (Figure 10). The second peak indicated to be Propanedioic acid and diethyl ester (Figure 11). The next peaks considered to be 2-pyrrolidiny-methylamine, butanedioic acid, diethyl ester, 2-piperidimethanamine, butanedioic acid, hydroxyl, diethyl ester, 2,5-dimethylhexane-2,5-dihydroperoxide, dithiocarbamate, s-methyl-,n-(2methyl-3-oxobutyl), triethyl citrate, y-sitosterol, ethyl 9,12,15-octadecatrienoate, hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester, and octadecane, 3-ethyl-5-(2-ethylbutyl) (Figures 12 to 22). Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects. Continued further exploration of plant derived antimicrobials is needed today.

Antibacterial and antifungal activity

K. pneumoniae, *P. aeruginosa*, *E. coli*, *S. aureus* and *P. mirabilis* were five clinical pathogens selected for THE antibacterial activity maximum zone formation against *E. coli* (Table 3). Methanolic extraction of plant showed notable antifungal activities against *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus flavus* and *Aspergillus fumigates* (Table 4). *C. sativum* and *M. azedarach* was very highly active against *A. terreus* (6.01 ± 0.200). *Aspergillus* was found to be sensitive to all test medicinal plants and mostly comparable to the standard reference antifungal drug amphotericin B and fluconazole to some extent.

Conclusion

C. sativum and *M. azedarach* are native plant of Iraq. Thus, the GC-MS analysis of methanolic leaves extract of *C. sativum* and *M. azedarach* showed a highly complex profile containing approximately 20 components. This study may be useful to explore the pharmacological and biosynthetic activity of the plants further.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENT

The authors thank Dr. Abdul-Kareem Al-Bermani,

Table 1. Major phytochemical compounds identified in methanolic extract of *Coriandrum sativum* leaves.

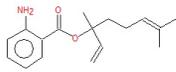
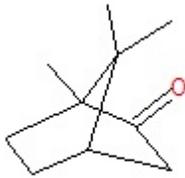
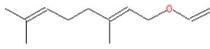
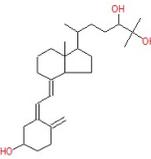
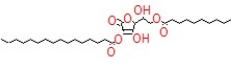
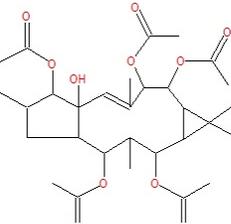
Phytochemical compound	RT (min)	Formula	Molecular weight	Exact mass	Chemical structure	MS Fragmentation	Pharmacological actions
1,6-Octadien-3-ol,3,7-dimethyl	4.3	C ₁₀ H ₁₈ O	154	154.1358		55,71,80,93,107,121,136,154	Anti-inflammatory and anti-cancer properties.
1,6-Octadien-3-ol,3,7-dimethyl, 2-aminobenzoate	4.643	C ₁₇ H ₂₃ N ₂ O ₂	273	273.1729		55,69,80,93,105,121,136,154	Anti-inflammatory, antiseptic, anti-depressant
Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl.	5.908	C ₁₀ H ₁₆ O	152	152.1201		55,81,95,137,152	Immune enhancement and anti-microbial activity
Geranyl vinyl ether	8.534	C ₁₂ H ₂₀ O	180	180.1514		53,69,93,136,152,178	anti-microbial, anti-cancer and anti-malaria
9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol.	10.248	C ₂₇ H ₄₄ O ₃	416	416.329		55,69,91,118,136,158,176,189,207,21,253,281,291	Anti-cancer, anti-inflammatory, and hepatoprotective
Ascorbic acid 2,6-dihexadecanoate	15.212	C ₃₈ H ₆₈ O ₃	652	652.4914		57,73,85,98,115,129,143,157,185,19,9,213,256,299,327,396,414	Antimetastatic and anti-invasive and antioxidant action
7aH-Cyclopenta[a]cyclopropano[f]cycloundecene	19.52	C ₃₀ H ₄₄ O ₁₁	580	580.2884		55,69,111,149,237,281,358,400,46	Immune enhancement and anti-micro-organism.

Table 2. Major phytochemical compounds identified in methanolic extract of *Melia azedarach* leaves.

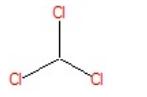
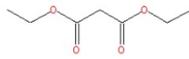
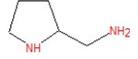
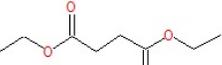
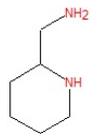
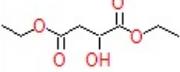
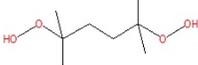
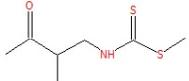
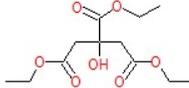
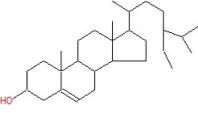
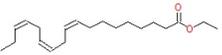
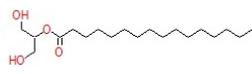
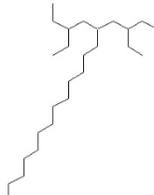
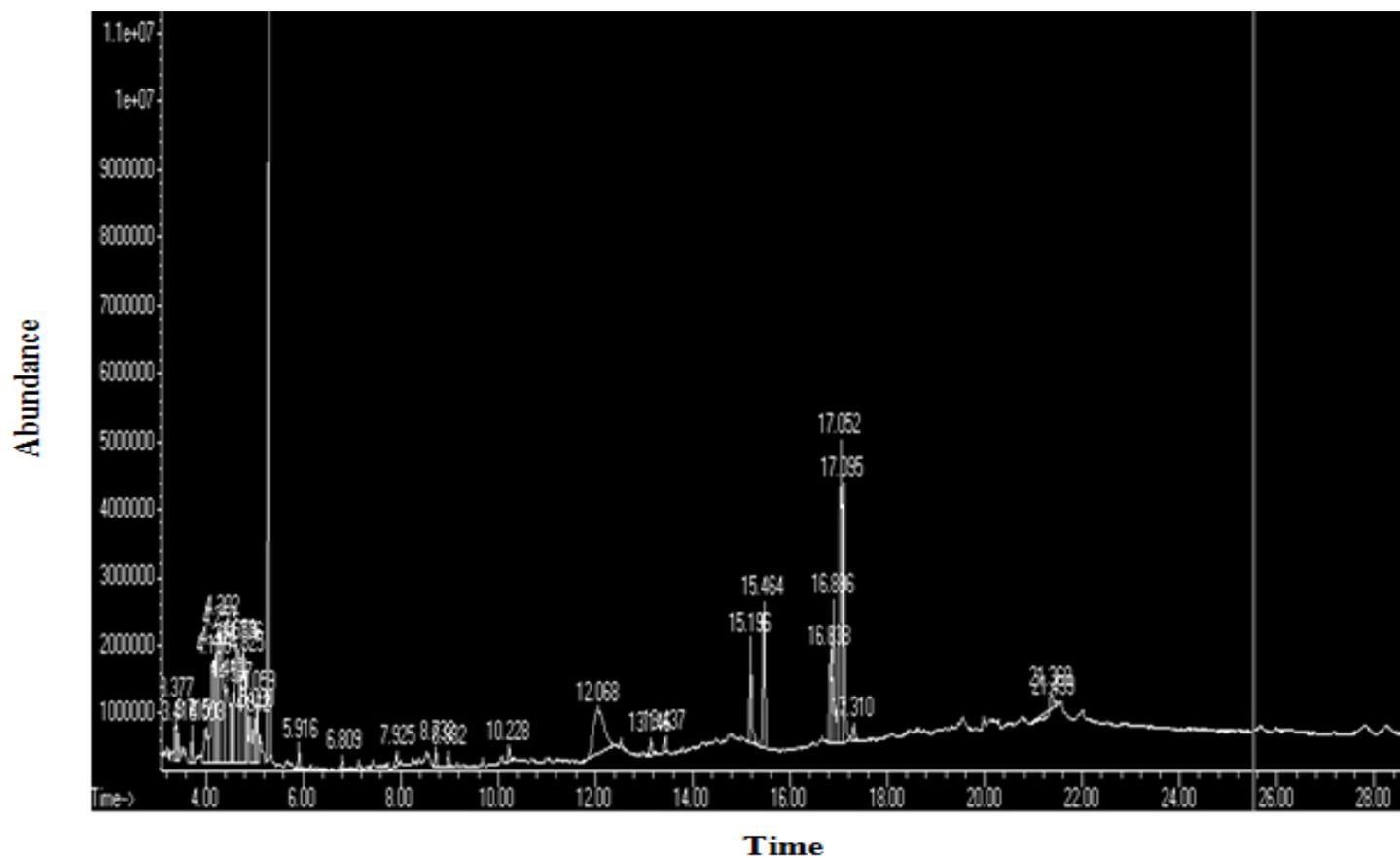
Phytochemical compound	RT(min)	Formula	Molecular weight	Exact mass	Chemical structure	MS Fragment -ions	Pharmacological actions
Trichloromethane	4.58	CHCL3	117	117.9438		50,59,70,83,118	anti-virus, anti-cancer, anti-mutagenic, anti-allergic and anti-ulcer
Propanedioic acid, diethyl ester	4.683	C7H12O4	160	160.0376		53,60,70,88,115,133,160	anti-inflammatory
2-pyrrolidinyl-methylamine	5.233	C5H12N2	100	100.1		55,77,84,99	anti-Allergic
Butanedioic acid, diethyl ester	6.39	C8H14O4	174	174.0892		55,73,84,101,129,147,174	antimicrobial, antispasmodic and anti-inflammatory effects
2-Piperidimethanamine	6.537	C6H14N2	114	114.1157		56,67,84,96,114	anti-depressant activity and anti-tumor
Butanedioic acid, hydroxyl-, diethyl ester	7.27	C8H14O5	190	190.8141		60,71,75,89,102,117,145	anti-ulcer
2,5-Dimethylhexane-2,5-dihydroperoxide	8.391	C8H18O4	178	178.1502		55,69,75,85,95,111,127,144,,178	anti-aging agents and anti-oxidant
Dithiocarbamate,S-methyl-,N-(2methyl-3-oxobutyl)	10.056	C7H13NOS2	191	191.0439		57,85,143,191	anti-cancer agents
Triethyl citrate	12.122	C12H20O7	276	276.1209		60,69,87,115,129,157,167,185,203,213,231	Anti-Ulcer and anti-inflammatory agent
Y-Sitosterol	13.044	C29H50O	414	414.3862		55,69,81,119,145,161,213,255,303,329,345,381,396,414	Anti-inflammatory activity
Ethyl 9,12,15-octadecatrienoate	17.106	C20H34O2	306	306.2559		55,67,79,95,121,135,191,221,261,306	antioxidant, anti-inflammatory, antimicrobial and pesticide
Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	20.001	C19H38O4	330	330.277		57,74,84,98,112,134,154,182,213,239,275,270,299,330	antioxidant, anti-inflammatory and anthelmintic activities

Table 2. Contd.

Octadecane, 3-ethyl-5-(2-ethylbutyl)	20.596	$C_{26}H_{54}$	366	366.422552		57,71,85,99,127,183,197,225,281,295,337,364	anti-oxidant and anti-inflammatory effect
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Figure 1. GC-MS profile of methanolic seeds extract of *Coriandrum sativum*.

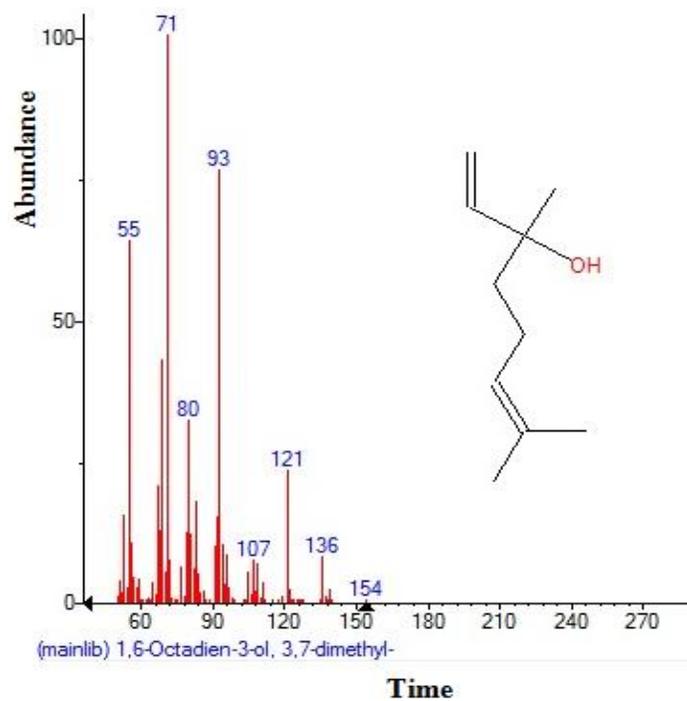


Figure 3. Structure of 1,6-Octadien-3-ol,3,7-dimethyl present in the methanolic leaves extract of *Coriandrum sativum* by using GC-MS analysis.

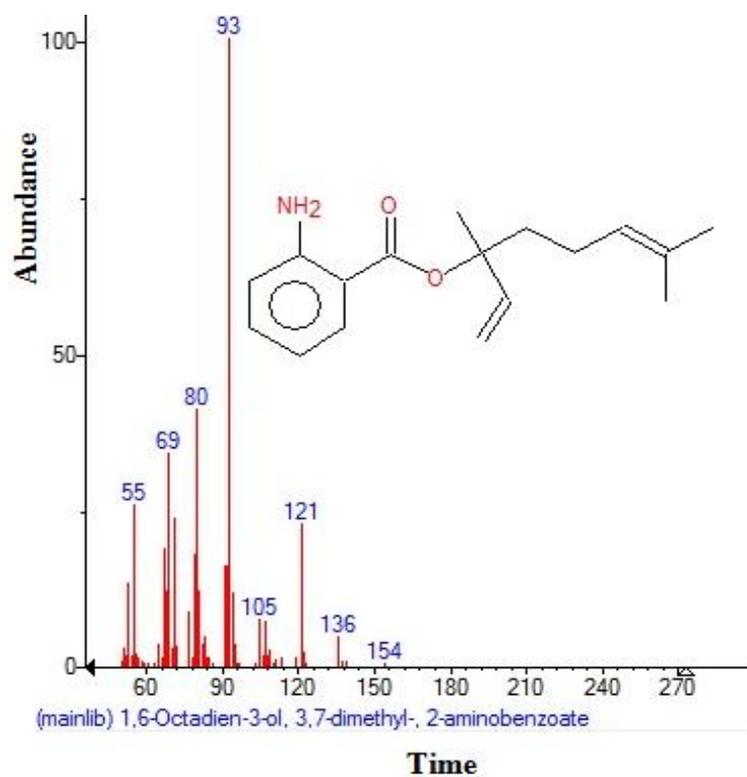


Figure 4. Structure of 1,6-Octadien-3-ol,3,7-dimethyl, 2-aminobenzoate present in the methanolic leaves extract of *Coriandrum sativum* by using GC-MS analysis.

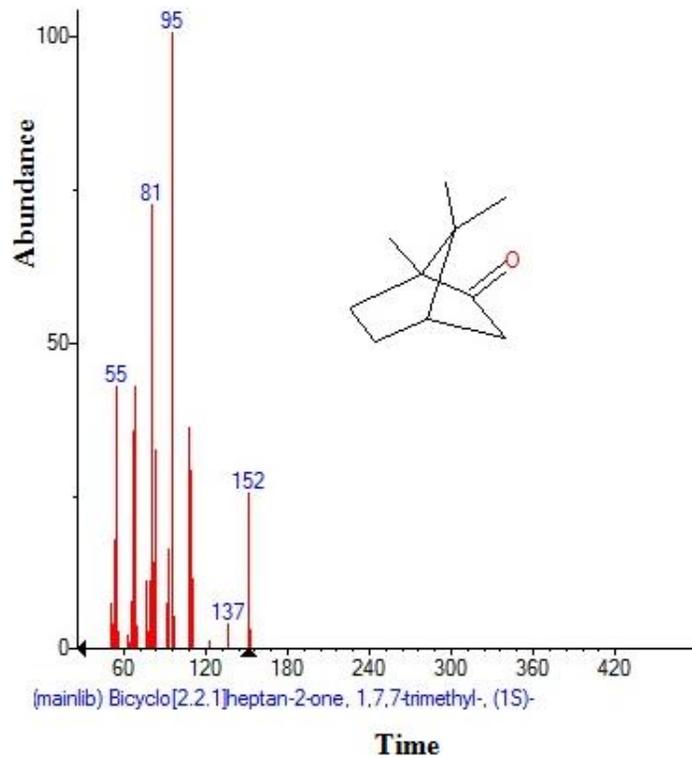


Figure 5. Structure of Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl present in the methanolic leaves extract of *Coriandrum sativum* by using GC-MS analysis.

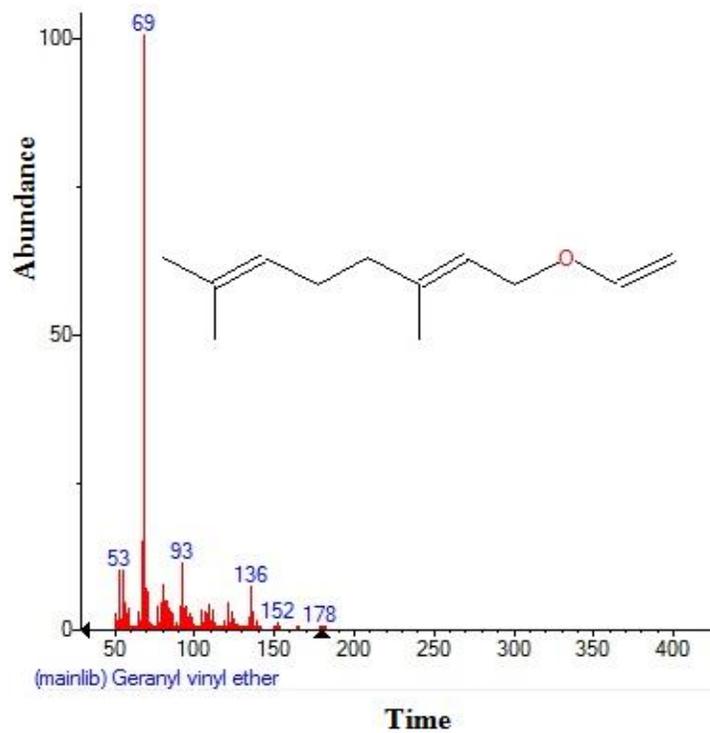


Figure 6. Structure of Geranyl vinyl ether present in the methanolic leaves extract of *Coriandrum sativum* by using GC-MS analysis.

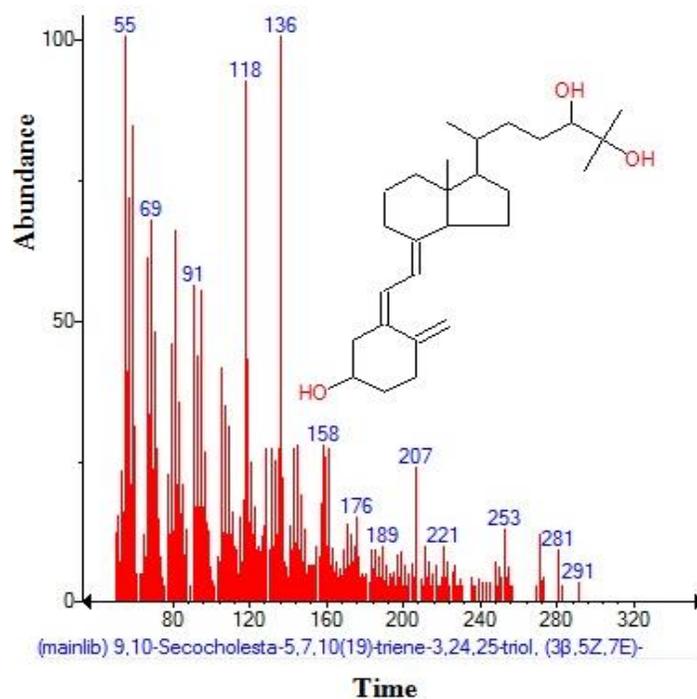


Figure 7. Structure of 9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol present in the methanolic leaves extract of *Coriandrum sativum* by using GC-MS analysis.

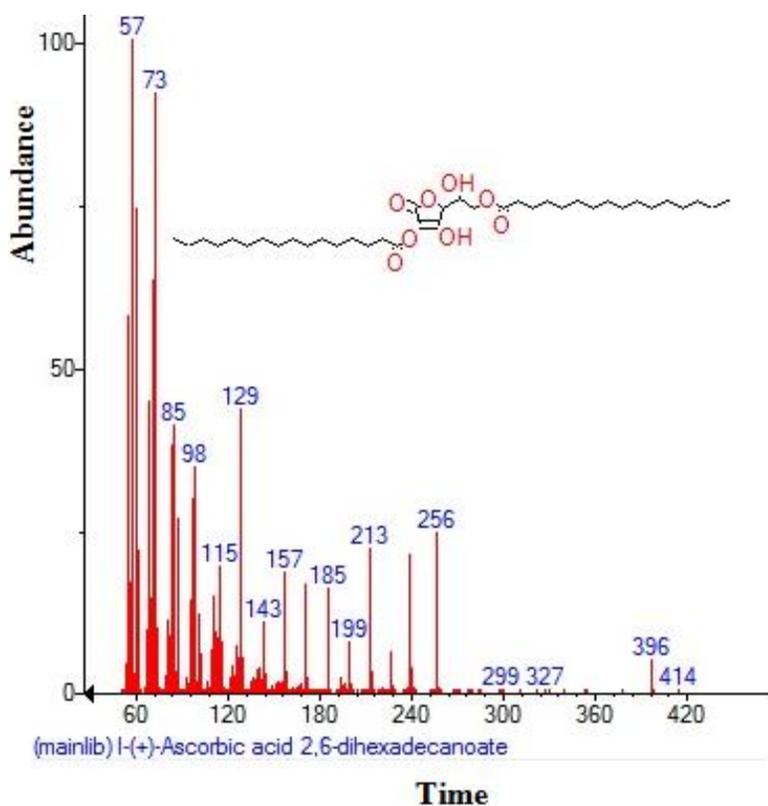


Figure 8. Structure of Ascorbic acid 2,6-dihexadecanoate present in the methanolic leaves extract of *Coriandrum sativum* by using GC-MS analysis.

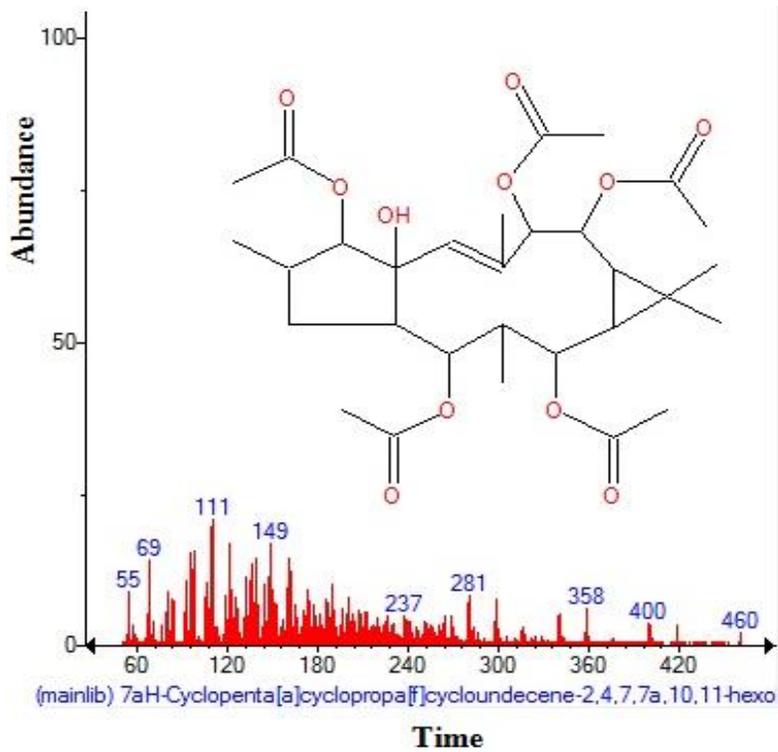


Figure 9. Structure of 7aH-Cyclopenta[a]cyclopropa[f]cycloundecene present in the methanolic leaves extract of *Coriandrum sativum* by using GC-MS analysis.

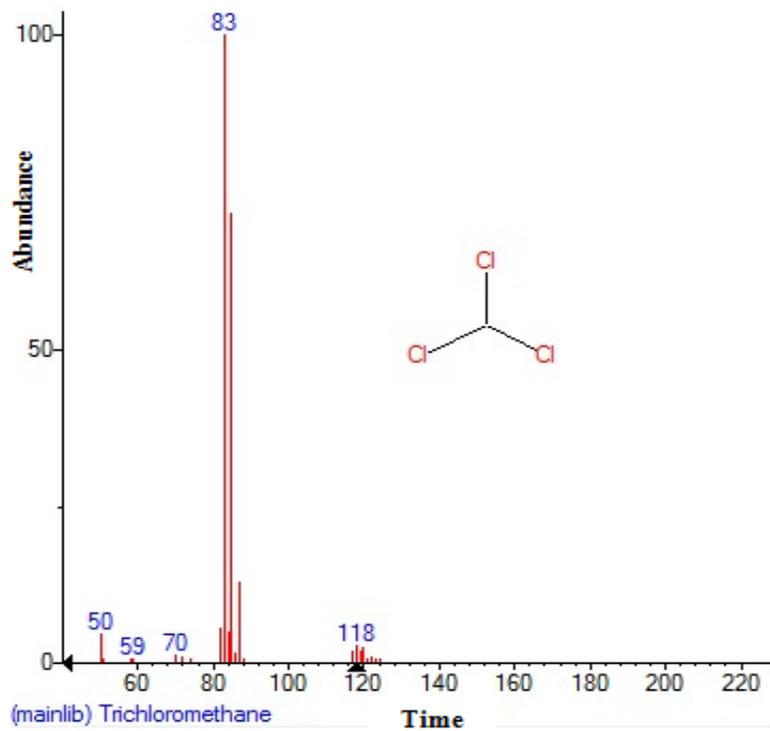


Figure 10. Mass spectrum of trichloromethane with retention time (RT) = 4.580.

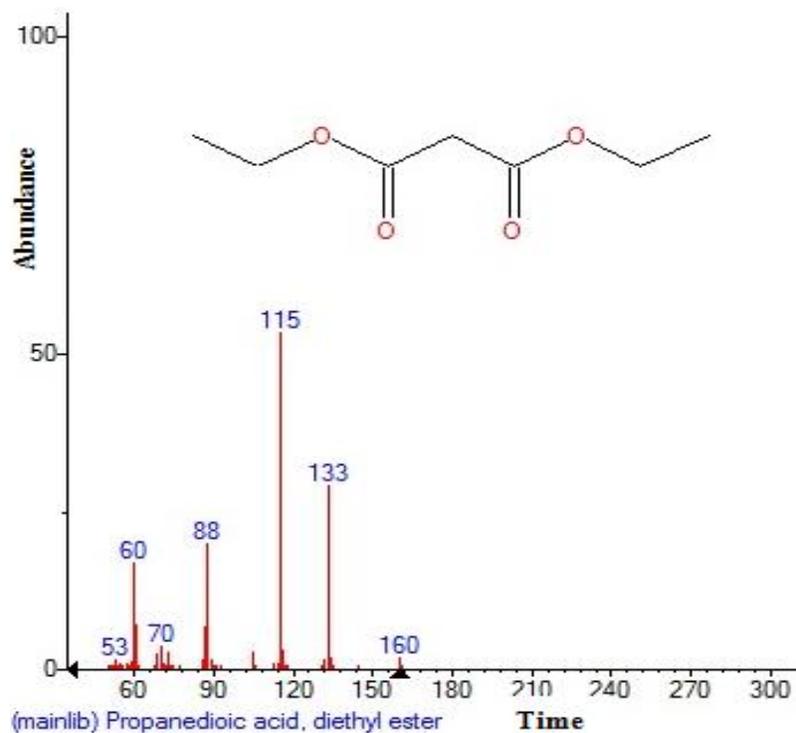


Figure 11. Mass spectrum of propanedioic acid, diethyl ester with retention time (RT) = 4.683.

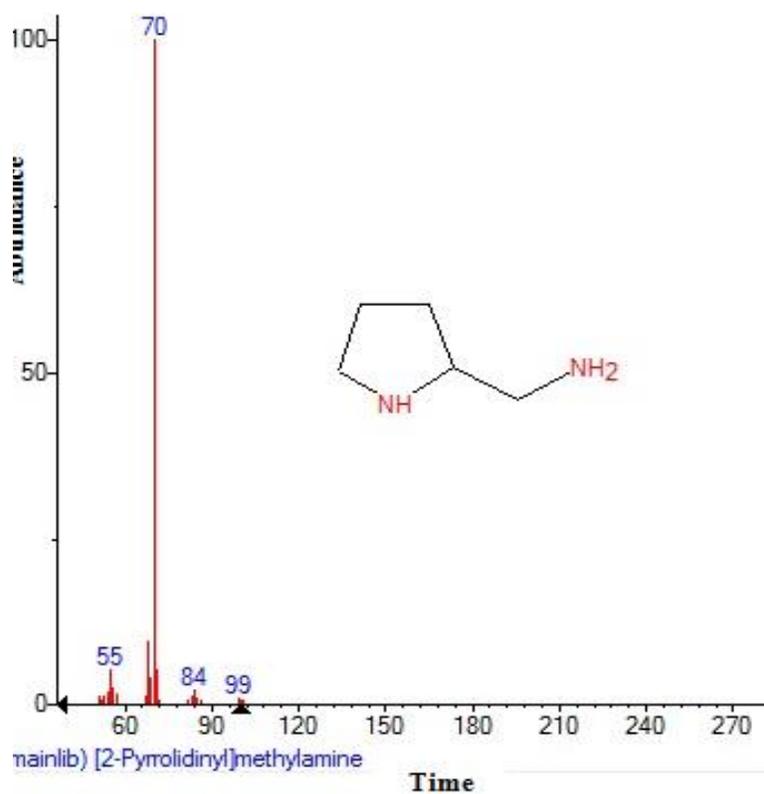


Figure 12. Mass spectrum of 2-pyrrolidinyl-methylamine with retention time (RT) = 5.233.

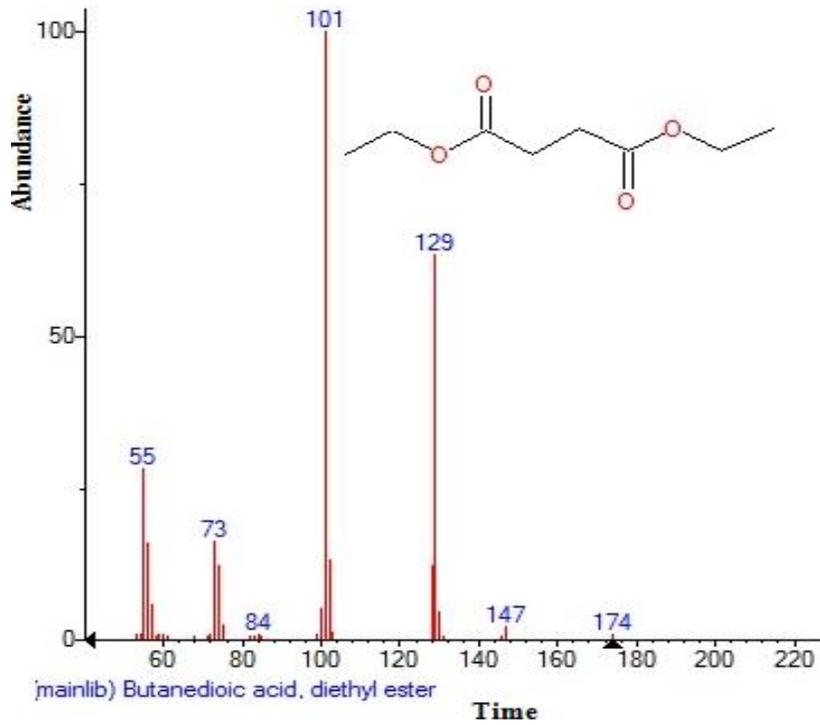


Figure 13. Mass spectrum of butanedioic acid, diethyl ester with retention time (RT) = 6.39.

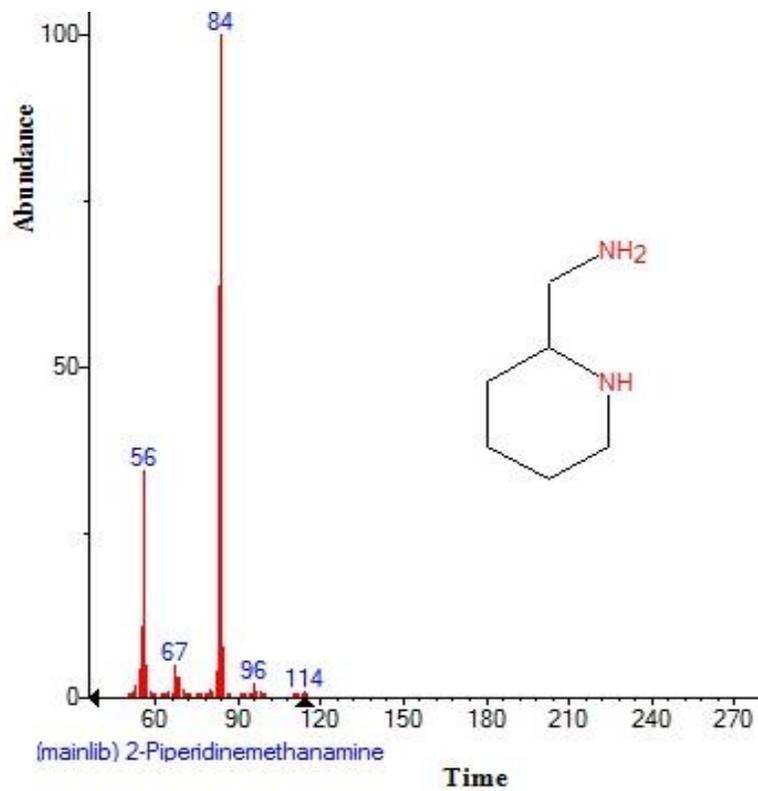


Figure 14. Mass spectrum of 2-piperidinemethanamine with retention time (RT) = 6.537.

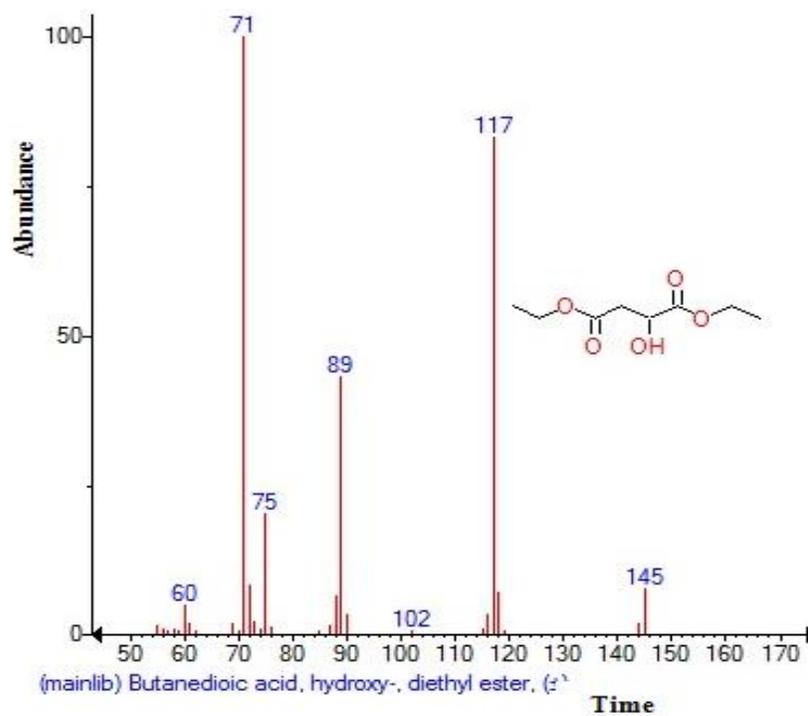


Figure 15. Mass spectrum of butanedioic acid, hydroxyl-, diethyl ester with retention time (RT) = 7.270.

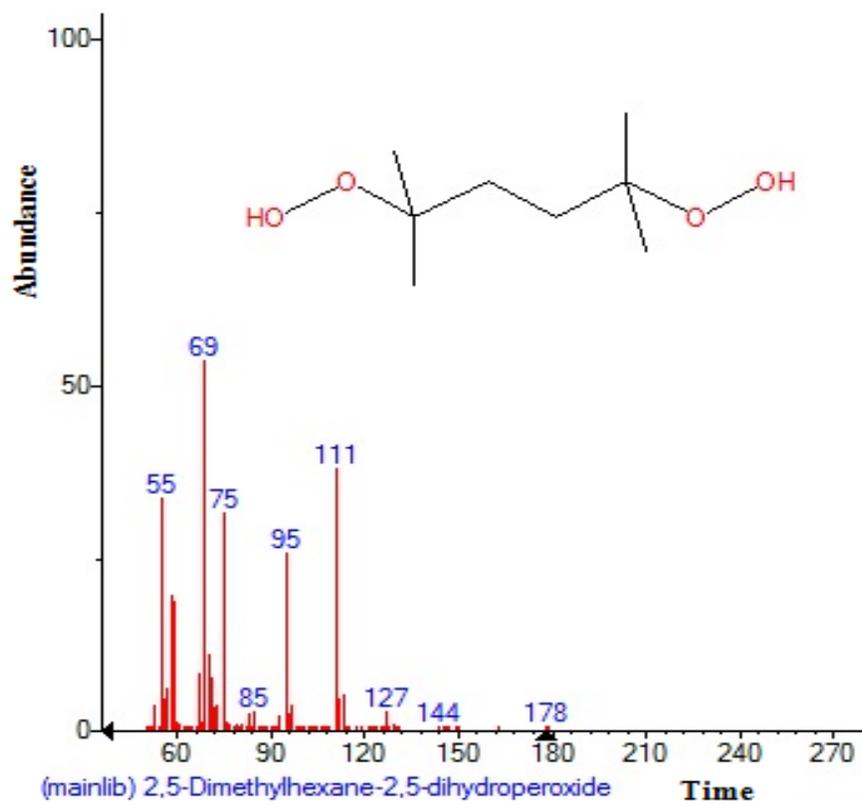


Figure 16. Mass spectrum of 2,5-dimethylhexane-2,5-dihydroperoxide with retention time (RT)= 8.391.

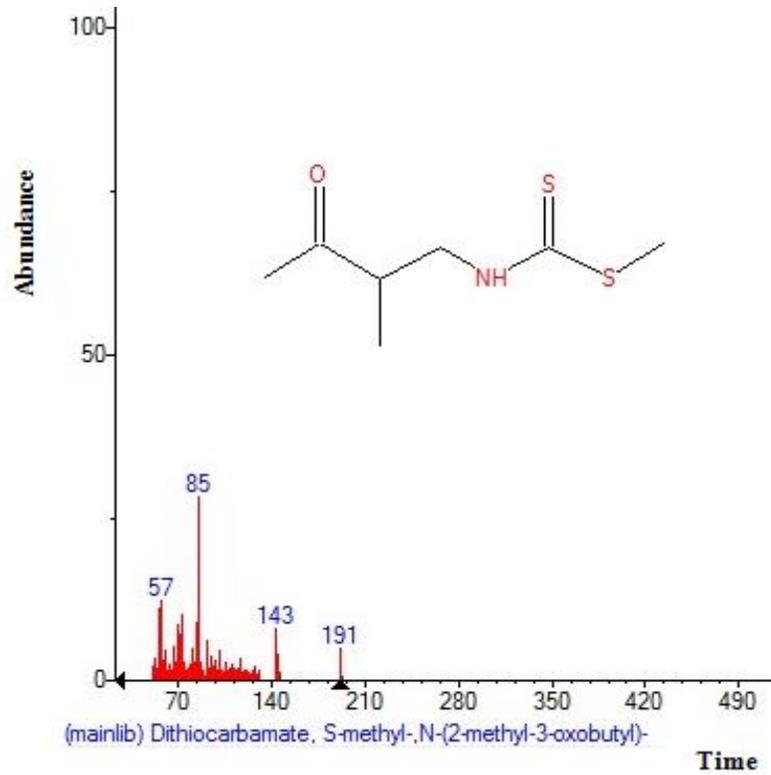


Figure 17. Mass spectrum of dithiocarbamate, S-methyl-,N-(2-methyl-3-oxobutyl) with retention time (RT) = 10.056.

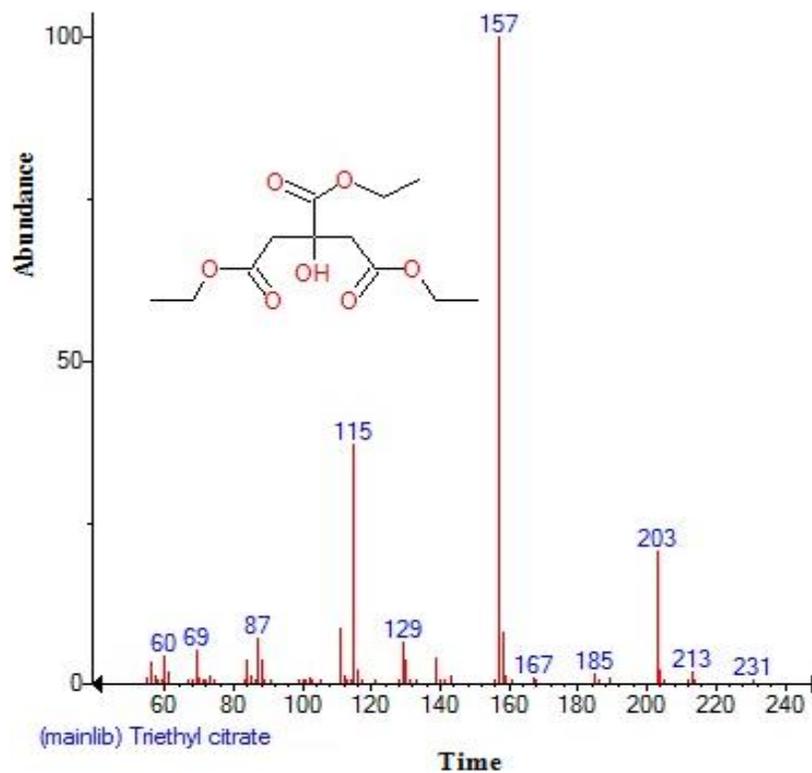


Figure 18. Mass spectrum of Triethyl citrate with Retention Time (RT) = 12.122.

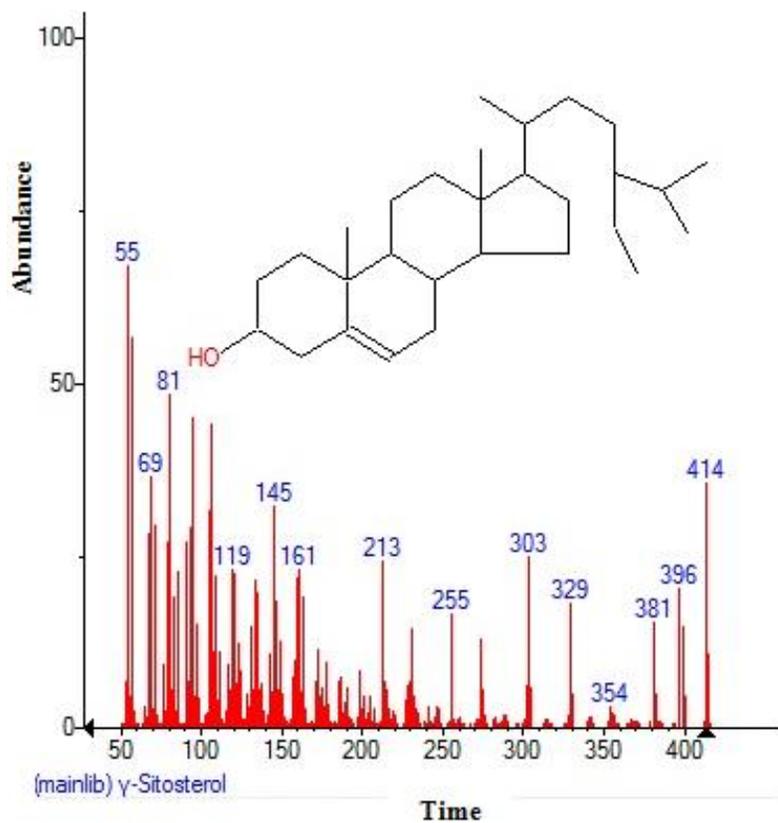


Figure 19. Mass spectrum of γ -sitosterol with retention time (RT) = 13.044.

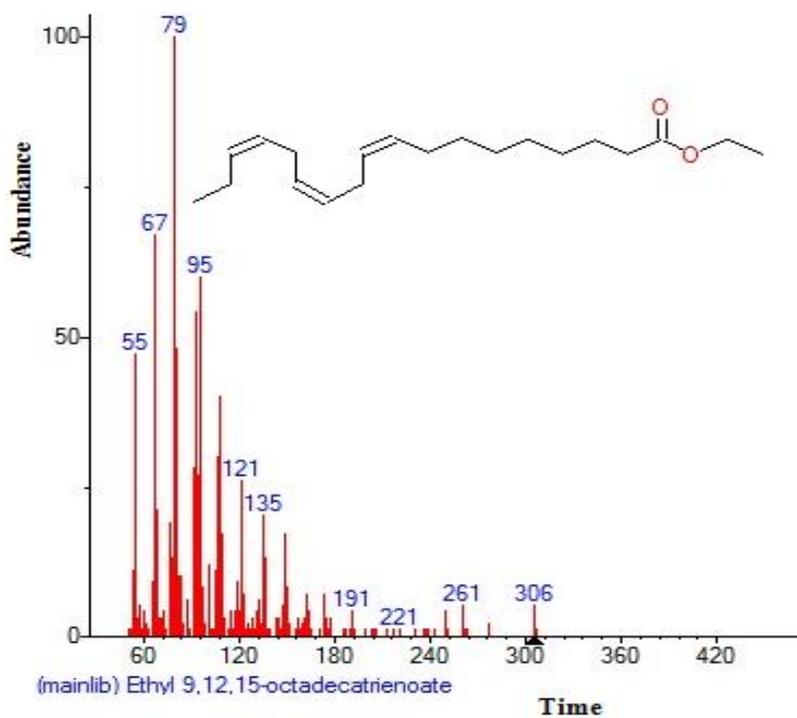


Figure 20. Mass spectrum of ethyl 9,12,15-octadecatrienoate with retention time (RT) = 17.106.

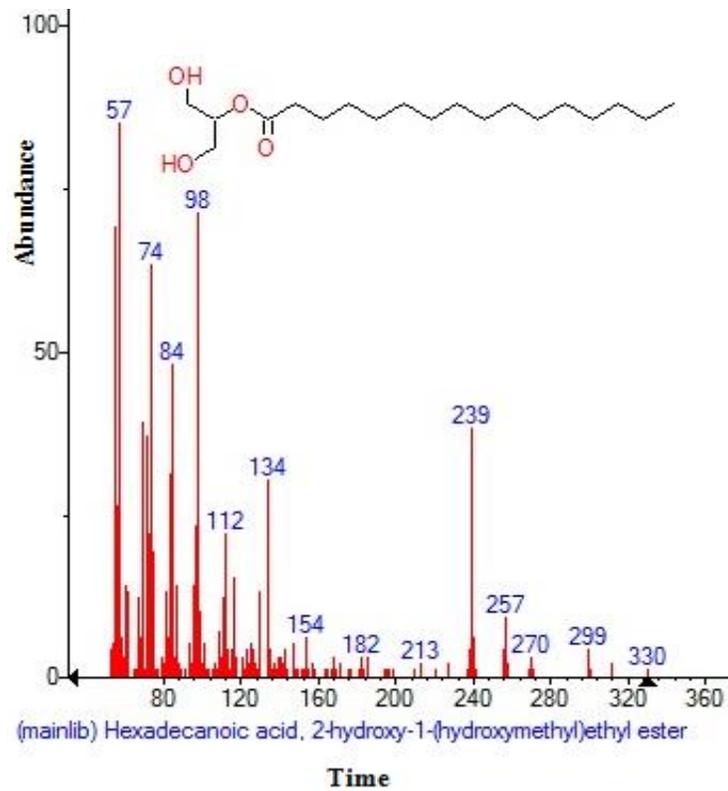


Figure 21. Mass spectrum of hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester with retention time (RT) = 20.001.

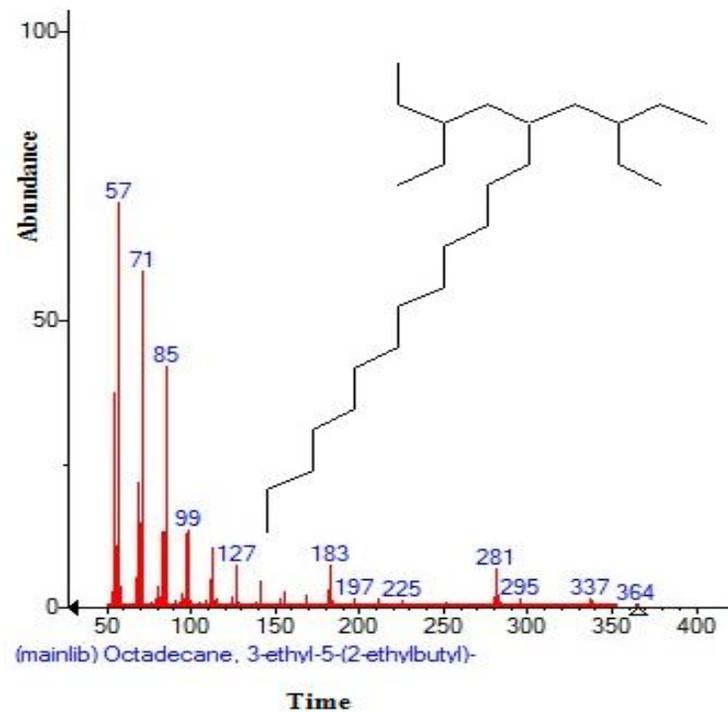


Figure 22. Mass spectrum of octadecane, 3-ethyl-5-(2-ethylbutyl) with retention time (RT) = 20.59.

Table 3. Zone of inhibition (mm) of test bacterial strains to *Coriandrum sativum* and *Melia azedarach* bioactive compounds and standard antibiotics.

Bacteria	Plants/antibiotics			
	<i>Coriandrum sativum</i>	<i>Melia azedarach</i>	Rifambin	Cefotaxime
<i>Pseudomonas aeruginosa</i>	4.08±0.120	3.99±0.110	1.96±0.200	2.99±0.160
<i>Escherichia coli</i>	5.60±0.320	4.11±0.200	2.91±0.310	2.99±0.620
<i>Klebsiella pneumonia</i>	3.00±0.510	3.99±0.170	3.23±0.300	2.00±0.310
<i>Staphylococcus aureus</i>	1.97±0.810	3.04±0.240	2.08±0.220	3.68±0.230
<i>Proteus mirabilis</i>	3.30±0.860	2.80±0.300	2.00±0.250	2.90±0.520

Table 4. Zone of inhibition (mm) of *Aspergillus* spp. test to *Coriandrum sativum* and *Melia azedarach* bioactive compounds and standard antibiotics.

Plant/Antibiotics	<i>Aspergillus niger</i>	<i>Aspergillus terreus</i>	<i>Aspergillus flavus</i>	<i>Aspergillus fumigatus</i>
<i>Coriandrum sativum</i>	3.00±0.180	6.01±0.200	5.94±0.300	5.08±0.140
<i>Melia azedarach</i>	2.07±0.100	5.01±0.310	4.66±0.130	6.00±0.130
Amphotericin B	2.05±0.120	4.00±0.340	4.06±0.200	4.36±0.180
Fluconazol	4.07±0.311	2.96±0.155	3.00±0.265	4.90±0.620
Control	0.00	0.00	0.00	0.00

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