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ANTIMICROBIAL AND PHYTOCHEMICAL STUDY OF *MATRICARIA CHAMOMOLLA* L., *MENTHALONGI FOLIA* L. AND *SALVIA OFFICINALIS* L.

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Abstract

The chemical composition and the antimicrobial activity of the aqueous and alcohol extracts of *Matricaria chamomilla* L., *Menthalongi folia* L. and *Salvia officinalis* L. were investigated in the present study. The results showed a prominent inhibitory effect of both aqueous and alcohol extracts on all the microbial isolates, alcoholic extract of *Salvia officinalis* appear the most inhibitory effect on microbial growth according to the diameter of inhibition zone (4.71 mm), while the aqueous extract of *Matricaria chamomilla* appear the lowest inhibitory effect on microbial growth (1.64 mm). All the concentrations of extracts that used in this study (5, 10, 20, 40%) showed inhibitory effect on microbial growth and this effect increase with the concentration increasing. *S.aureus* was more inhibition by extracts especially with alcohol extract (inhibition zone was 3.4 mm), while the lowest microorganism affected was *C. albicans* when using aqueous extracts (inhibition zone was 2.25 mm).24 bioactive phytochemical compounds which was a good environmental choice and these compounds were identified in the ethanolic extract of *S.officinalis* by GC-Mass technique. The identification of phytochemical compounds is based on the peak area, retention time molecular weight, molecular formula, chemicalstructure and MS Fragment ions. The FTIR analysis of *S.officinalis* proved the presence of Alkenes, alkyl halides and Aromatic Compounds.

Key words : Molecular formula, aromatic compounds, alcohol extract, volatile oil.

Introduction

Bioactive compounds in plants are compounds produced by plants having pharmacological or toxicological effects in man and animals. Medicinal plants may contain inherent active ingredients used to cure disease or relieve pain (Okigbo *et al*, 2008). The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemicals in them (Adesokan *et al*, 2008).

Mentha longifolia L. (Lamiaceae) comprises herbaceous, perennial plants, these plants are used in pharmaceutical, cosmetics and food industries. These properties are most often offered by volatile oils produced by the secretory hairs. The chemical composition of volatile oil derived from M.longifolia was investigated by Kaddem *et al* (2009). Dzamic *et al*. (2010) analyzed the volatile oil extracted from *M. longifolia* obtaining as main components and appear their containing trans- and cis-dihydrocarvone, piperitone, 1,8-cineole and neoisodihydrocarveol.

Matricaria chamomilla L. (Asteraceae) is one of the most common herbs used for medicinal purposes, by using tea and herbal extracts which prepared from dried flowers of Matricaria species. Chamomile is one of the oldest, most widely used and well documented medicinal plants in the world and has been recommended for a variety of healing applications. Raal *et al*. (2013) reported that in Estonia, *M. chamomilla* were frequently used as a self-medication to treat cold and flu. Malik *et al*. (2013) reported the increasing use of chamomile in homeopathy in Pakistan. Carvalho *et al*. (2014) showed that the crude ethanolic extract of *M. chamomilla* L. flowers, which contains apigenin, caused growth inhibition of *Pseudomonas aeruginosa* in broth dilution and was confirmed by agar diffusion (10 mm diameter inhibition zone).

Salvia officinalis L. (Lamiaceae) is a small evergreen subshrub (a woody perennial plant), with

woody stems, grayish leaves, and blue to purplish flowers native to southern Europe and the Mediterranean region ,its family reported to include more than 900 species (Pierozan, 2009; Ilkiu-Vidal *et al*, 2010). Oils of *S. officinalis* is important in pesticide, pharmaceutical, flavouring, perfumery, fragrance and cosmetic industries (Ozkam, 2008).

The aim of this study was evaluate the antimicrobial activity of some medicinal plants (*Mentha longifolia, Matricariacha momolla*, *Salvia officinalis*) by using two solvent : water and ethanol.

Materials and Methods

Preparation of extracts

Aqueous extract

10 g from each plant (air-dried powder) was taken and put in a conical flask (500 ml) and 250 ml of distilled water was added , and boiled on slow heat for 2h, then filtered through 8 layers of muslin cloth and centrifuged at 5000 RPM for 10 min. The supernatant was collected (Parekh and Chanda, 2008). The extract was concentrated in an oven at 45°C until dryness. Dried extracts were stored at 4°C for further use (Jain and Singh, 2013).

Alcoholic extract

10 gm of the powdered plant was soaked in the conical flask (250 ml) containing 200 ml of ethanol, plugged with cotton and then put in a horizontal shaker at 140-220 RPM for 24 h. Then it was filtered through 8 layers of muslin cloth and centrifuged at 5000 RPM for 10 min, the supernatant was collected (Parekh and Chanda, 2008). The extract was concentrated in an oven at 45°c until dryness. Dried extracts was stored at 4°C for further use (Jain and Singh, 2013).

Qualitative tests of phytochemicals

Test for alkaloids

A- Tannic acid reagent

2 ml from 10% Tannic acid solution was added to 5 ml from extract, Alkaloids give orange color precipitate (Neelima *et al*, 2011).

B- Hager's test

Hager's reagent is saturated solution of Picric acid ($C_6H_3N_3O_7$), after added a few drops from this this reagent appear yellow color precipitate, that's positive indicator to the presence of alkaloids (Neelima *et al*., 2001).

Test for phenols

Lead acetate test : The extract (50 mg) was dissolved in 5 mL of distilled water. To this, 3ml of 10% lead acetate were added. A bulky white precipitate indicated the presence of phenol compounds (Tamilselvi *et al*, 2012).

Test for saponins

Mixed one ml of extract with one ml of distilled water, then shaked vigorously, a stable foam indicate the presence of saponin (Anyasor *et al*, 2010).

Test for tannins

Ferric chloride : A portion of extract was dissolved in water, then filtrate it, Ferric chloride ($FeCl_3$) (1%) was added. The appearance of bluish green color indicates the presence of tannins (Anyasor *et al*, 2010).

Test for flavonoids

Melted 10 gm of plant powder in 50 ml of ethanol (95%) and then nominated by A. 10 ml of ethanol (50%), added to 10 ml of potassium hydroxide (50%) and nominated by B. Mix equal amount of solution A and B, the appearance color yellow as evidence of a flavonoids (Jaffer *et al.*, 1983).

Test for glycosides

Using Fehling's test to determination the presence of glycosides by mixed equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and sodium hydroxide in distilled water), few drops of this reagent was added to 5 ml of the extract and boiled, red precipitate was formed, if sugars are present (Neelima *et al*., 2011).

Identification of component by gas chromatography – mass spectrum analysis

Interpretation of GC-MS was conducted using data base of national Institute standard and technology (NIST) having more than 62000 patterns. The spectrum of the unknown components compared with the spectrum of known components stored in the NIST library.

Microorganisms tested

Microbial cultures of three different species of microorganisms were used for determination of the antimicrobial activity, they were *Staphylococcus aureus*, *Eschrichia coli* and *Candida albicans*. All the test cultures were maintained on nutrient agar media with regular sub-culturing.

Fourier transform infrared spectrophotometer (FTIR)

The powdered sample of *P. nigrum* specimen was treated for FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan). The sample was run at infrared region between

Table 1 : Qualitative tests of phytochemicals.

Phytochemicals	*M. chamomilla*		*M. longifolia*		*S. officinalis*	
	Aqueous	Alcoholic	Aqueous	Alcoholic	Aqueous	Alcoholic
Alkaloids	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Tannins	_	_	_	_	+	+
Flavonoids	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+

Table 2 : Effect of aqueous extract of different plants on microorganisms growth.

Plant extract / Microoganisms	*M.chamomolla*				*M.longifolia*				*S. officinalis*			
	Concentration (%)											
	5	10	20	40	5	10	20	40	5	10	20	40
S. aureous	0.60	1.00	2.63	3.55	0.56	1.20	3.01	4.66	1.83	3.43	5.40	7.10
E.coli	0.60	0.90	2.00	3.60	0.40	1.00	2.23	3.23	1.46	2.50	4.50	7.43
C. albicans	0.26	0.53	1.53	2.56	0.93	1.73	2.70	4.10	1.50	2.56	3.50	5.10
Average	480.	0.81	2.05	3.23	0.65	1.13	2.67	4.08	1.60	2.83	4.46	6.54

LSD(0.05)=0.405

400 nm and 4000 nm.

Test antimicrobial activity of the plant extracts

Preparation of inoculum

Suspended isolated colonies from nutrient agar was added to 5 ml from 0.85% sterile normal saline to achieve 0.5 McFarland turbidity to form a stock suspension of 1 × 106 to 5 × 106 cells/mL, which should produce semi confluent growth with most micoorganisms isolates (Lee *et al*., 2009).

Agar well diffusion method

Determine the antimicrobial activity of plant extracts was followed by agar well diffusion method. Nutrient agar plates were swabbed (sterile cotton swabs), wells in about 10 mm in diameter were made in each plate using sterile cork borer. Stock solution of each extract was prepared at a concentration 40% in different plant extract (aqueous and alcoholic), about 100 µl of different concentration of extract were added using micropipette into the well and allowed to diffuse at room temperature for 2 hrs. The plates incubated at 28°C for 24-48 hrs. After that measured the diameter of inhibition zone (mm), The experiment were maintained triplicates, for each replicate the reading were taken in three different fixed directions and recorded the average values (Sen and Batra, 2012).

Results and Discusion

Table 1 represent the secondary metabolites constituents that present in the plants under the study by using aqueous and ethanol solvents. The qualitative analysis of all extracts appear the presence of phenols, alkaloids, terpenes and glycosides in all plants by using all solvents that used in this study, tannin was appeared only in S. *officinal*.

Aqueous extracts appeared antimicrobial potential indifferent concentrations (5, 10, 20, 40%) of all medicinal plants (*M. chamomilla*, *M. longifolia*, *S. officinalis*) as shown in fig. 1, which appear the diameter of inhibition zone as following 0.91, 1.59, 3.06 and 4.61mm respectively. *S. officinalis* had more inhibitory effect (3.85mm) then *M. longifolia* (2.13mm) and *M. chamomilla* (1.64mm), on growth of *S. aureous*, *E. coli* and *C. albicans* (fig. 2). *S. aureous* appear more effective than *E. coli* and *C. albicans* (2.91, 2.48, 2.25 mm) as shown in fig. 3. Table 2 appears the interaction among type of plant, type of microorganism and the concentration.

Ethanol extracts also appear antimicrobial potential of different concentrations (5, 10, 20, 40%) of all medicinal plants (*M. chamomilla*, *M. longifolia*, *S. officinalis*) as shown in fig. 4, which appear the diameter of inhibition zone as 1.13, 2.17, 4.19 and 5.23 mm respectively for all ethanol plant extracts as *M. chamomilla* which appear inhibition zone 2.46 mm and *M. longifolia* (2. 27mm), *S. officinalis* appear heights inhibitory effect on bacteria growth (inhibition zone was 4.71mm) (fig. 5). *S. aureous* appear more effective than *E. coli* and *C. albicans* (3.4, 3.36, 3.23mm) as shown in fig. 6. Table 3 appears the interaction among type of

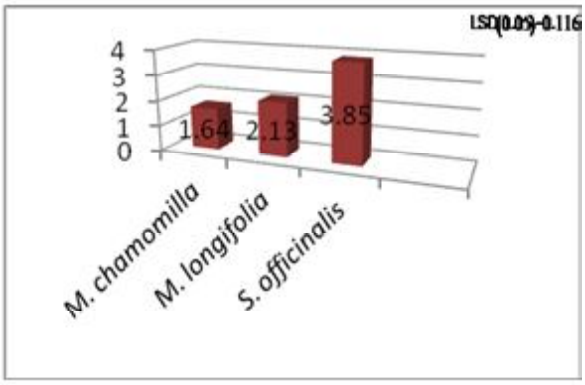

Fig. 1 : Effect of concentration on microorganisms growth (aqueous extract).

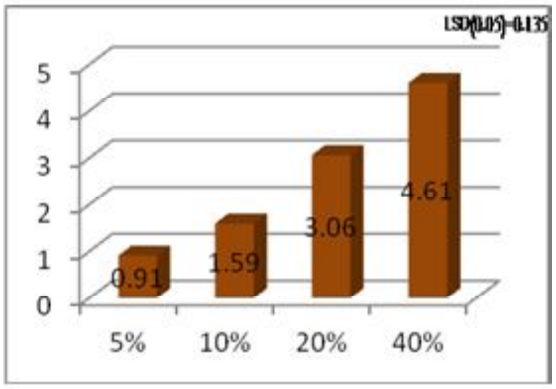

Fig. 2 : Inhibitory effect of plant type on microorganisms growth.

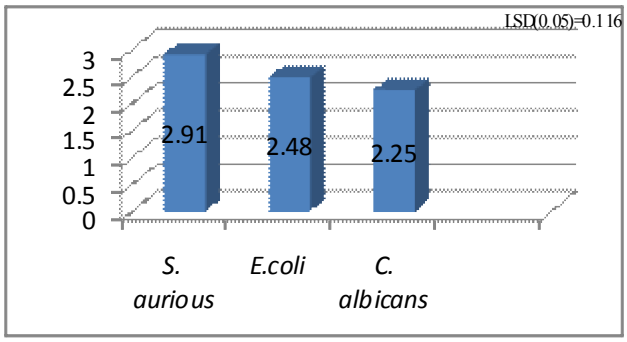

Fig. 3 : Inhibitory effect of aqueous extracts according to type of micro oragnisms.

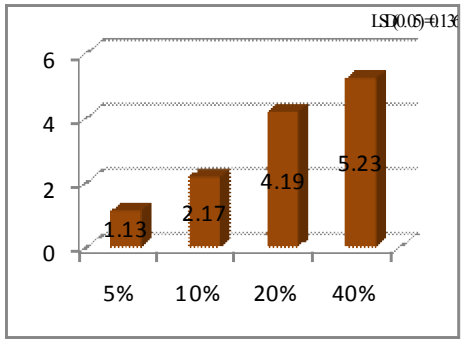

Fig. 4 : Effect of concentration on microorganisms growth (alcoholic extract).

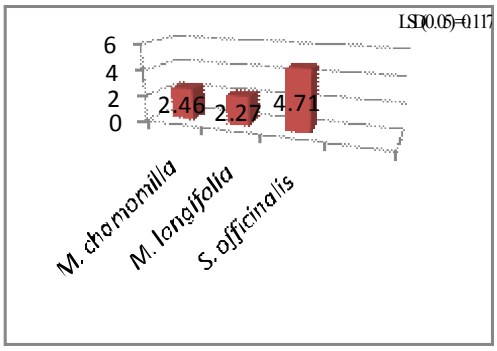

Fig. 5 : Inhibitory effect of plant type on microorganisms growth.

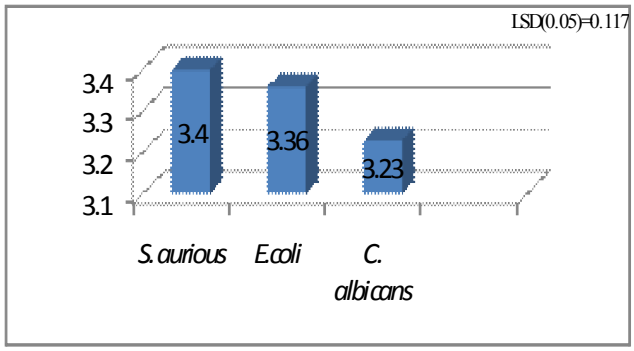

Fig. 6 : Inhibitory effect of aqueous extracts according to type of micro oragnisms.

plant, type of microorganism and the concentration of extracts.

Analysis of *S. officinalis* ethanol extract under FTIR technique showed that the presence of 24 functional groups including Alkenes, alkyl halides and Aromatic Compounds (fig. 32, table 5).

The results of this study agreed with results of other research which indicated that ethanol extract of *S. officinalis* with concentration of 50, 400,100 mg/mL has prevented the growth of both Gram negative and Gram positive bacteria. Thus, the research represents the antibacterial effects on this medicinal herb on Gram negative and Gram positive multidrug resistant bacteria. By gradual increase of concentration, inhibition zone increases as well. The most sensitivity was observed in *S. aureus* (Mosafa *et al*., 2004).

According to the above results, Gas chromatography and mass spectroscopy analysis was done to *S. officinalis* to determine the phytochemical compounds in this plant.

Gas chromatography and mass spectroscopy analysis of compounds was carried out in ethanolic extract, shown in table 4. The GC-MS chromatogram of the 24 peaks of

Table 4 : Major phytochemical compounds identified in ethanolic extract of *S. officinalis*.

MS Fragment- ions	Exact Mass	Molecular weight	RT (min)	Phytochemical compound	Serial No.
53, 69, 93, 121	102.1156983	102	3.247	B-Pinene	1.
51, 77, 91, 119, 134	134.10955	134	3.562	2, 6-Dimethyl-1,3,5,7-Octatetraene	2.
55,69,18,93,121,139,154	154.135765	154	3.711	2-Cyclohexen-1, 1-methyl-4-(1-methy-lethyle)	3.
55, 81, 121, 136, 154	154.135765	154	4.002	exo-2,7,7-trimethylbicyclo[1.2.2]	4.
55, 69, 83, 109, 126, 139, 154	154.09938	154	4.506	Cyclohexanecarboxaldehyde, 3,3,-dimethyl	5.
67, 91, 165, 205, 303	394.28718	394	4.758	5,8,11,14-Ecosatetraenoic acid, phenylmethyl ester	6.
55, 69, 85, 95, 121, 139	185.105193	185	4.843	1-Cyclopropyl-nitro-methyl-Cyclopentanol	7.
55, 69, 77, 93, 111, 120, 138, 152	153.115364	153	4.489	1- Hydroxy-4,4-dimethylcyclohexanecarbon	8.
55, 79, 91, 105, 119, 147, 232, 287, 357	388.334131	388	5.072	Methyl 10, 12-pentacosadiynoate	9.
55, 69, 95, 110, 139, 154	154.13576	154	5.433	1,7,7-Trimethylbicyclo[2.2.1.]	10.
55, 67, 79, 91, 105, 120, 137, 150	167.094628	167	6.291	Cyclohexene	11.
55, 67, 79, 91, 109, 134, 152	152.120115	152	6.486	cis-p-metha	12.
53, 67, 79, 91, 105, 119, 134, 151, 164	196.14633	196	7.201	Cyclohexene, 4-isopropenyl	13.
51, 107, 135	150.06808	150	7.493	2-Methoxy, 4-vinylphenol	14.
55, 69, 81, 93, 121, 139, 154	154.135765	154	7.744	2-Cyclohexen-1, methyl-4-(methylethyl)	15.
55, 67, 77, 93, 105, 119, 133, 161, 189, 204	204.1878	204	7.870	q-ylangene	16.
51, 55, 67, 79, 91, 105, 117, 131, 145, 145, 159	290.22458	290	8.036	2,5-Octadecadiynoic acid, methyl ester	17.
55, 69, 77, 91, 105, 119, 133, 147, 161, 175, 189, 204	204.1878	204	8.225	alfa,-Copaene	18.
51, 65, 77, 91, 105, 133, 149, 169, 197, 232	232.005785	232	8.500	1,1-Dichloro-2-methyl-3-(4,4-diformyl)	19.
55, 69, 79, 93, 105, 119, 133, 147, 161, 175, 189, 204	204.1878	204	8.814	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl	20.
55, 67, 79, 91, 108, 133, 147, 161, 175, 189, 204	204.1878	204	9.112	1, 4-Methano-1Hindene, Octahydro-4-methyl	21.
55, 67, 79, 93, 105, 121, 133, 161, 189, 204	204.1787	204	9.301	cis-q-Bisabolene	22.
55, 67, 79, 91, 105, 119, 133, 147, 189, 204	204.1878	204	9.570	ß-Copaene	23.
59, 93, 119, 204	222.198365	222	9.804	q-acorenol	24.

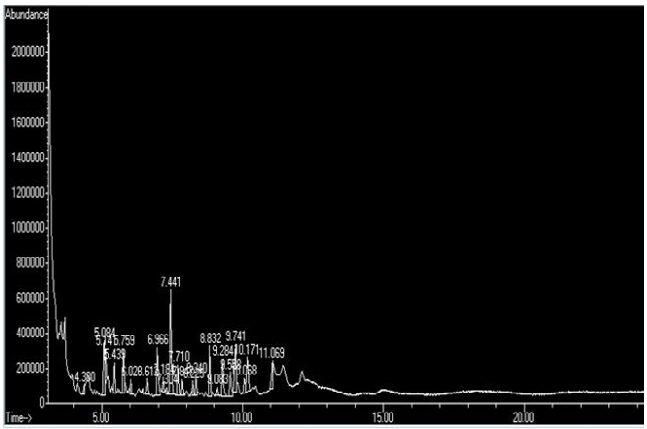

Fig. 7 : GC-MS chromatogram of methanolic extract of *S. officinalis*.

the compounds detected was shown in fig. 7. Chromatogram GC-MS analysis of the ethanol extract of *S.officinalis* showed the presence of twenty four major peaks and the components corresponding to the peaks were determined as follows. The first set up peak were determined to be B-Pinene (fig. 8) and the last set up peak were determined to beq-acorenol (fig. 31). Said-Al Ahl *et al*. (2015) revealed the presence of 31 phytochemical compounds in the volatile oils in *S. officinalis*by GC- mass mostly aromatic compounds.

FTIR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition (Marimuthu and Gurumoorthi, 2013; Geethu *et al*., 2014; Mishra *et al*., 2015). The antimicrobial and cytotoxic

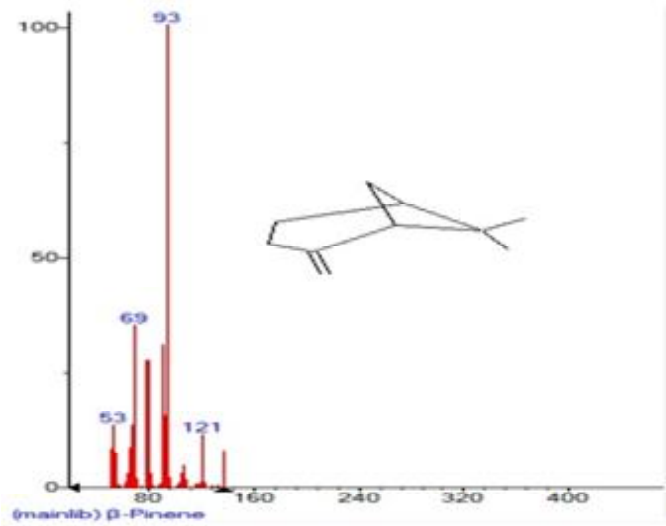

Fig. 8 : Mass spectrum of B-Pinene.

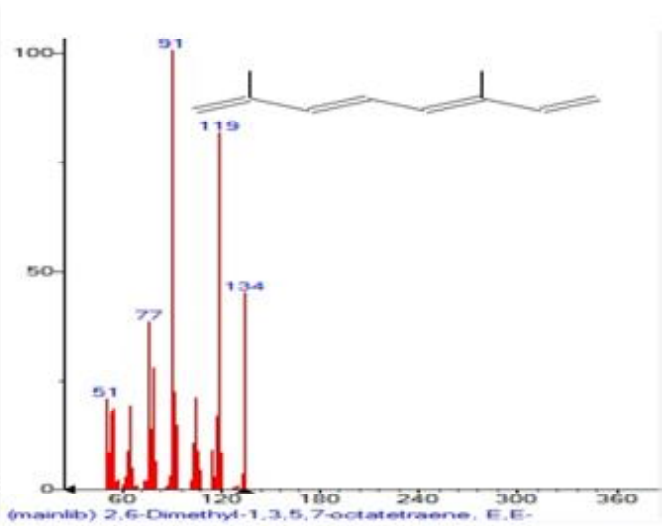

Fig. 9 : Mass spectrum of 2,6-Dimethyl-1,3,5,7-Octatetraene.

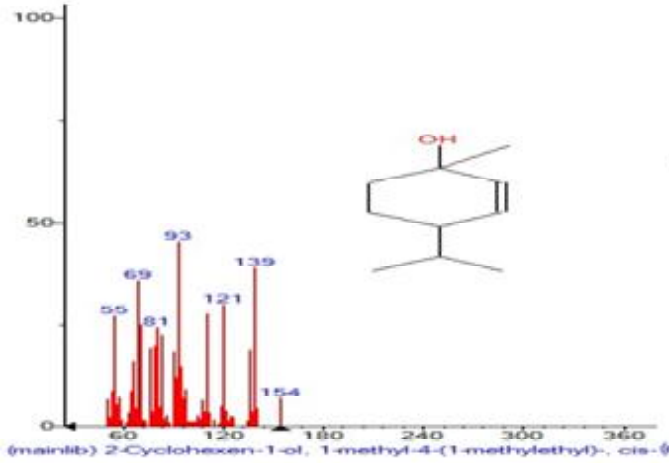

Fig. 10 : Mass spectrum of 2-Cyclohexen-1, 1-methyl-4-(1-methylethyle).

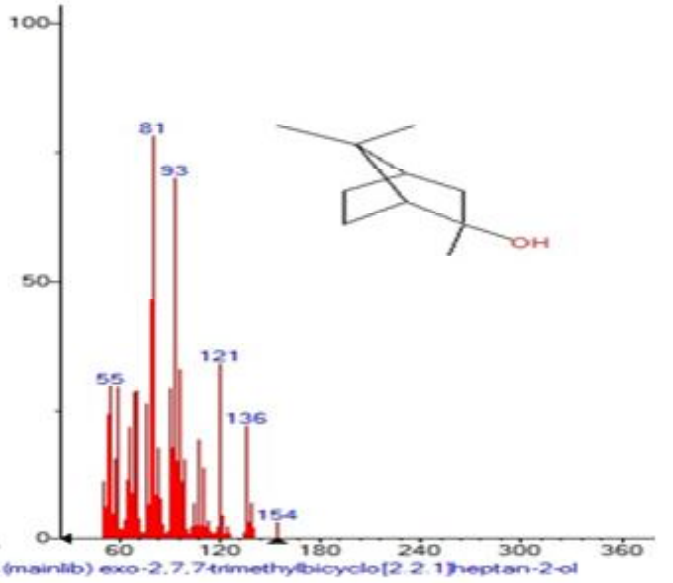

Fig. 11 : Mass spectrum of exo-2,7,7-trimethylbicyclo[1.2.2].

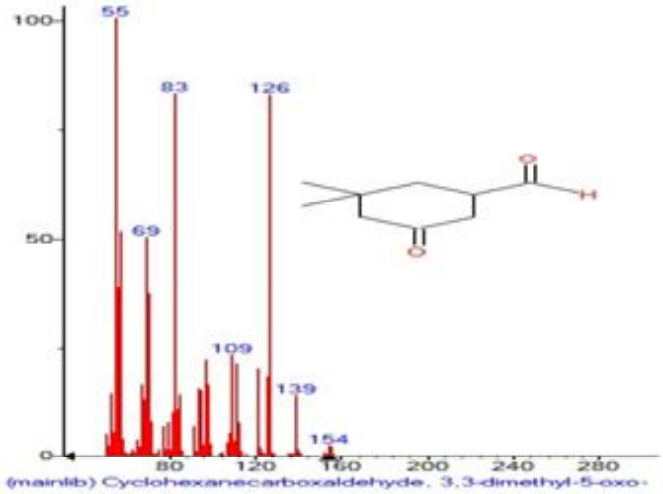

Fig. 12 : Mass spectrum of Cyclohexanecarboxaldehyde, 3,3,-dimethyl.

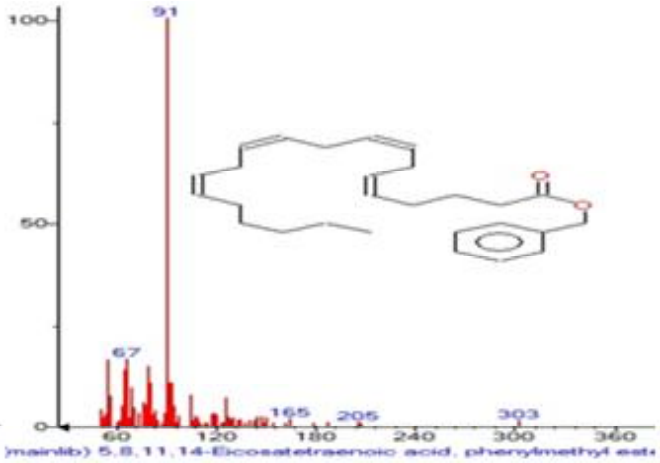

Fig. 13 : Mass spectrum of 5,8,11,14- Ecosatetraenoic acid, phenylmethyl ester .

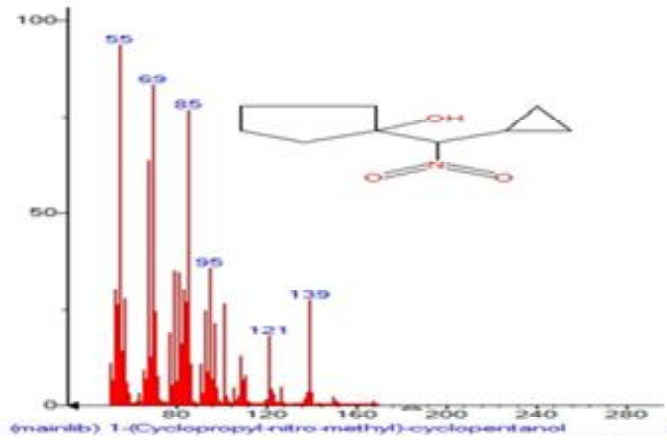

Fig. 14 : Mass spectrum of 1-Cyclopropyl-nitro-methyl-Cyclopentanoldimethyl.

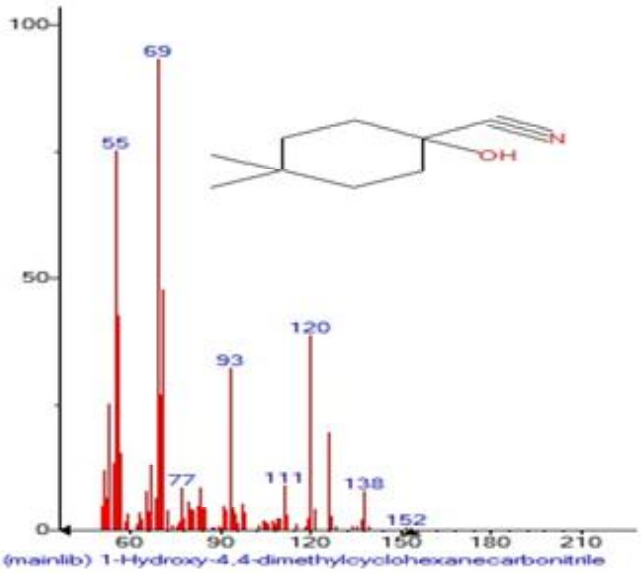

Fig. 15 : Mass spectrum of 1- Hydroxy-4,4- cyclohexanecarbon.

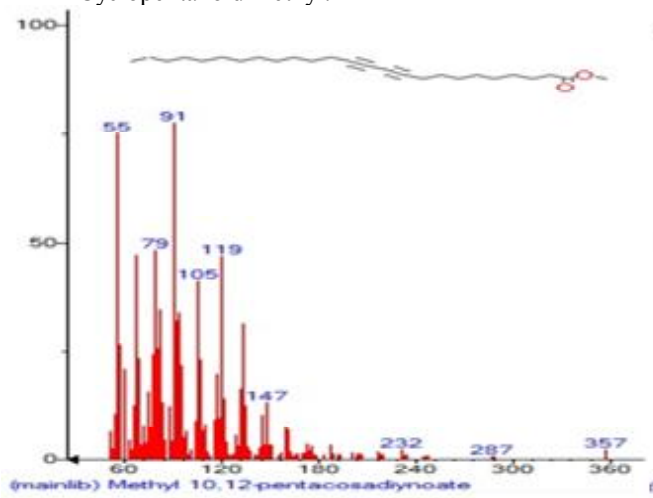

Fig. 16 : Mass spectrum of Methyl 10, 12-pentacosadiynoate.

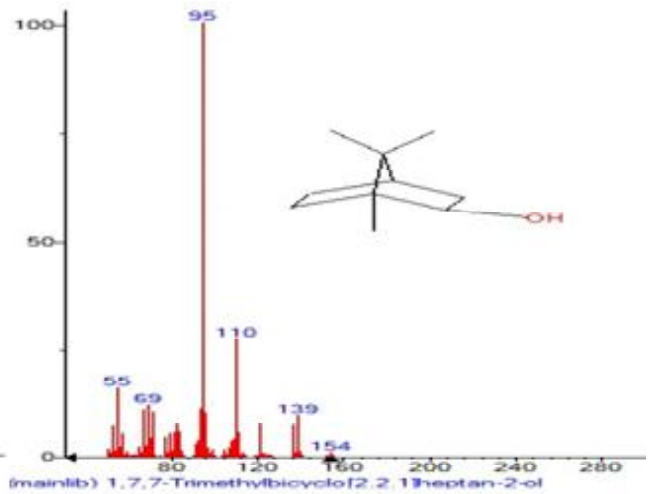

Fig. 17 : Mass spectrum of 1,7,7-Trimethylbicyclo [2.2.1.]

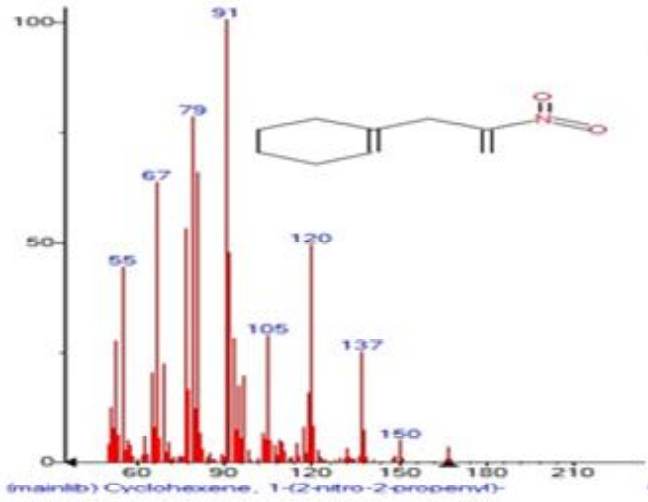

Fig. 18 : Mass spectrum of Cyclohexene.

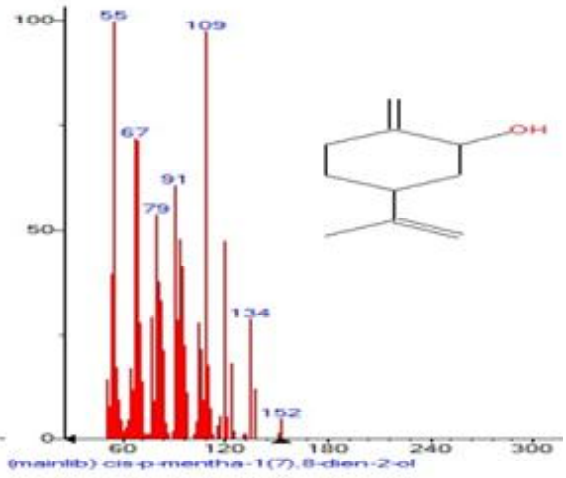

Fig. 19 : Mass spectrum of cis-p-metha.

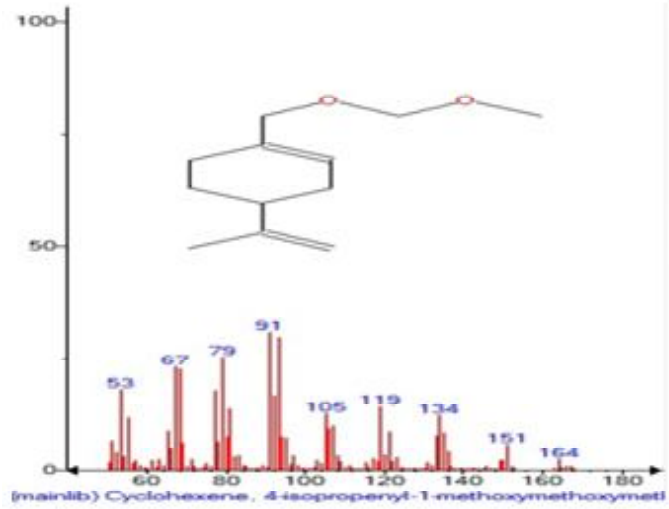

Fig. 20 : Mass spectrum of Cyclohexene, 4-isopropenyl.

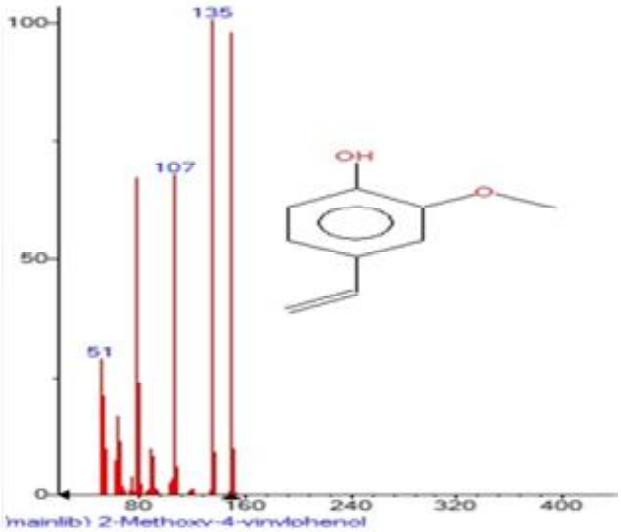

Fig. 21 : Mass spectrum of 2-Methoxy, 4-vinylphenol.

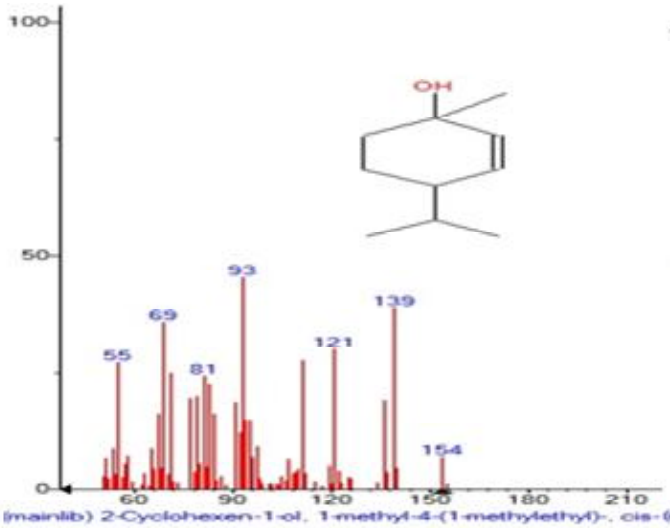

Fig. 22 : Mass spectrum of 2-Cyclohexen-1, methyl-4-(methylethyl).

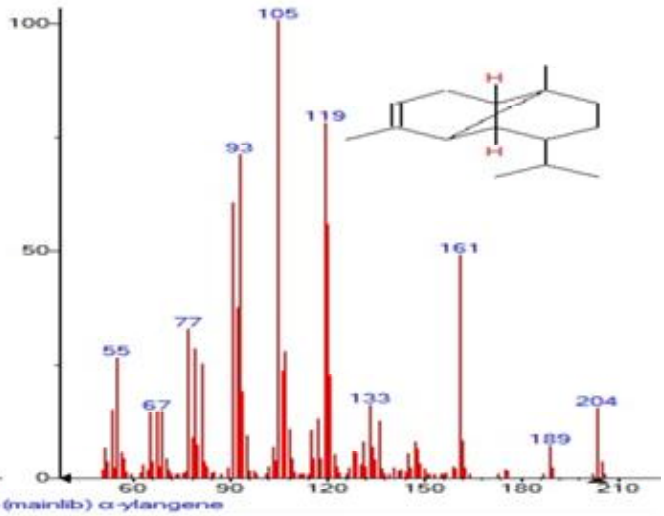

Fig. 23 : Mass spectrum of q-ylangene.

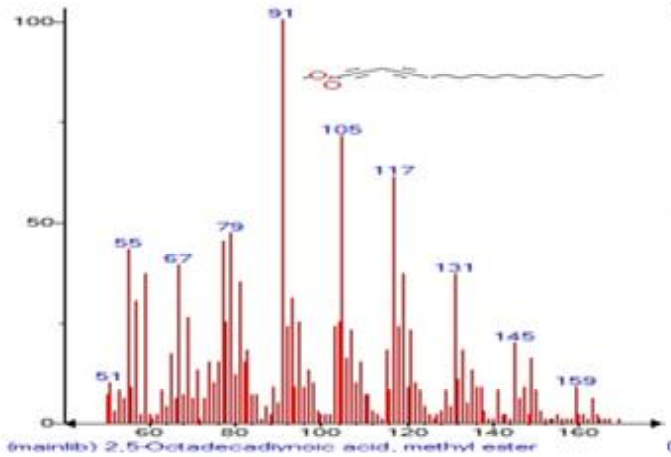

Fig. 24 : Mass spectrum of 2,5-Octadecadiynoic acid, methyl ester.

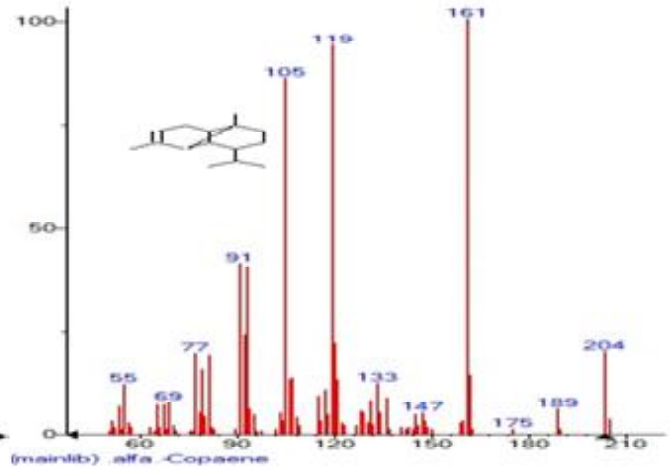

Fig. 25 : Mass spectrum of alfa,-Copaene.

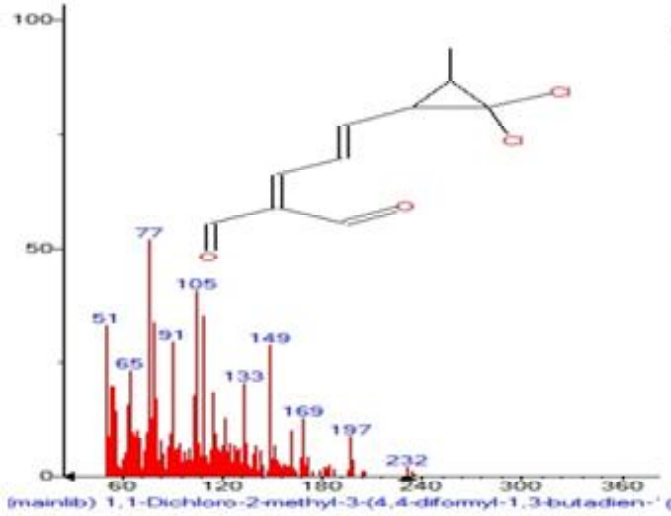

Fig. 26 : Mass spectrum of 1,1-Dichloro-2-methyl-3-(4,4-diformyl)

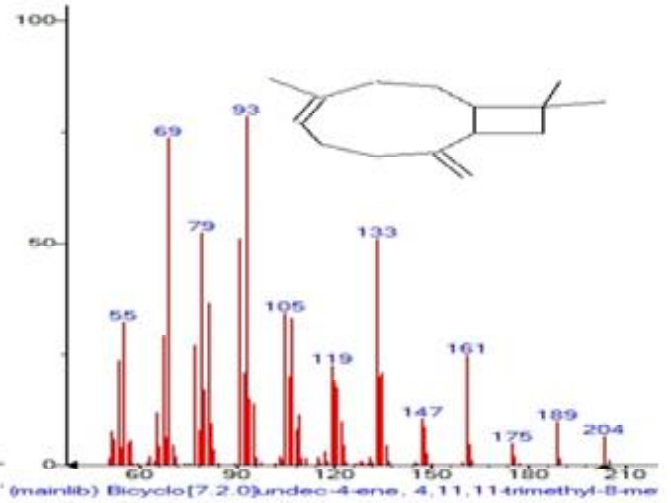

Fig. 27 : Mass spectrum of Bicyclo[7.2.0]undec-4- ene, 4,11,11-trimethyl.

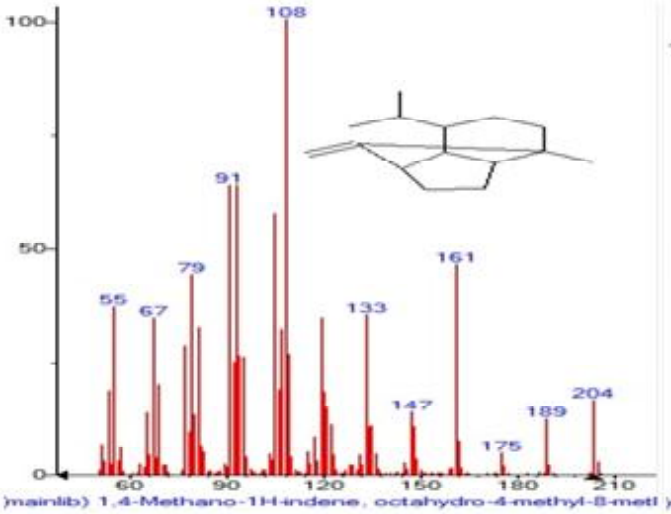

Fig. 28 : Mass spectrum of 1, 4-Methano-1Hindene, Octahydro-4-methyl.

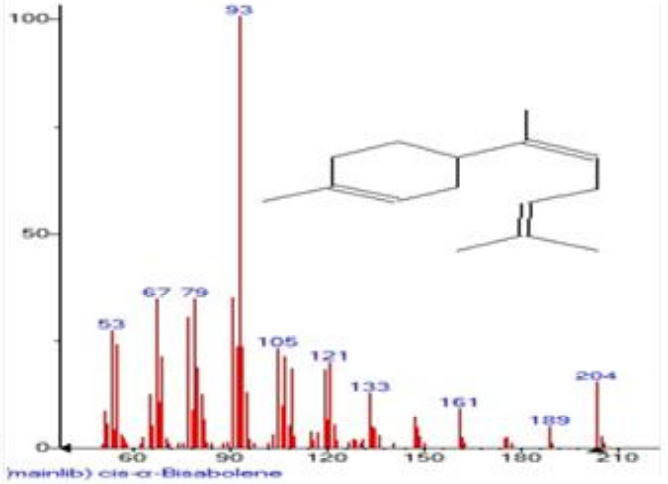

Fig. 29 : Mass spectrum of cis-q-Bisabolene.

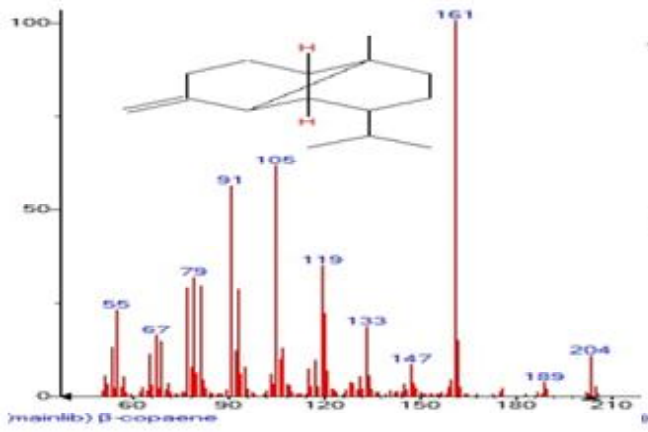

Fig. 30 : Mass spectrum of ß-Copaene.

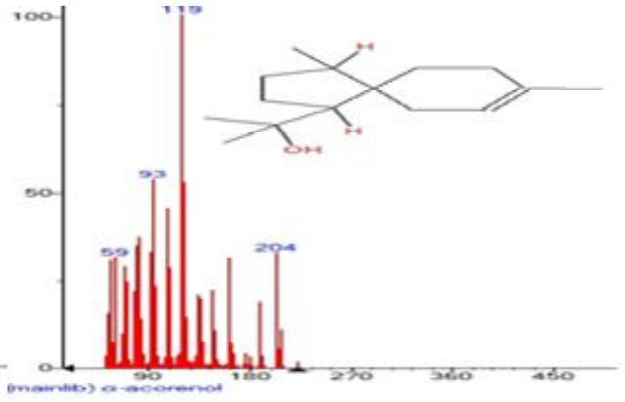

Fig. 31 : Mass spectrum of q-acorenol.

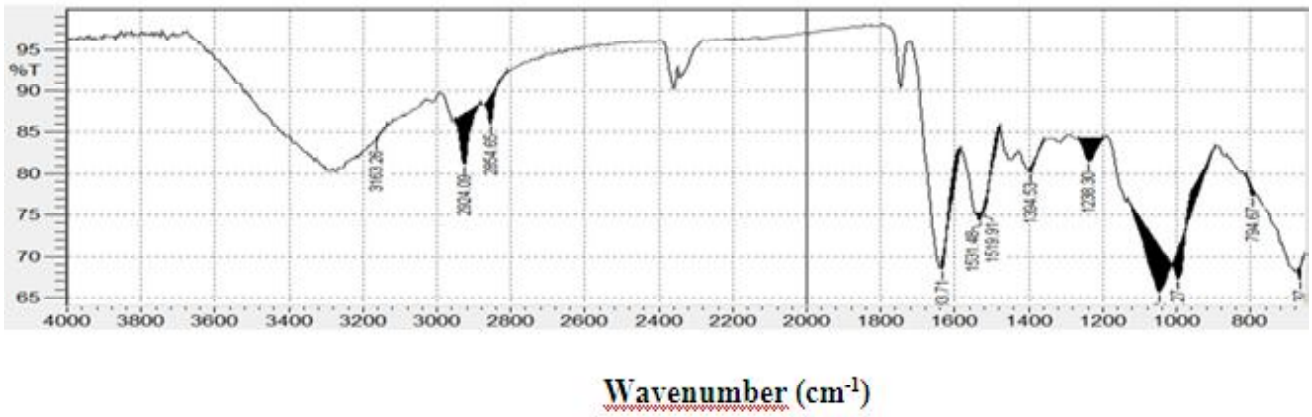

Fig. 32 : Fourier-transform infrared spectroscopic profile analysis of *S. officinalis*.

Table 5 : FT-IR peak values of analysis of *S. officinalis*.

Group frequency	Functional group assignment	Bond	Intensity	Peak (Wave number cm-α)	No.
600–800	alkyl halides	C-Cl	67.245	667.37	1.
600–800	alkyl halides	C-Cl	77.258	794.67	2.
675-1000	Alkene	=C-H	67.253	995.27	3.
1000-1400	alkyl halides	C-F	65.642	1045.42	4.
1000-1400	alkyl halides	C-F	81.459	1238.30	5.
1000-1400	alkyl halides	C-F	80.196	1394.53	6.
1400-1600	Aromatic	C=C	75.457	1519.91	7.
1400-1600	Aromatic	C=C	74.327	1531.48	8.
1620-1680	Alkene	C=C	68.420	1633.71	9.
2850-3000	Alkane	C-H	86.016	2854.37	10.
2850-3000	Alkane	C-H	81.209	2924.09	11.
3010-3100	Alkene	C-H	84.032	3163.63	12.

effects related to these substances are due to the fact that aliphatic chains (long chain alkanes and alkenes) and fatty acids, fatty acid ester that normally found in the lipid layer of the cell membrane and mitochondria. Consequently, they disturb the integrity of cell structure which becomes permeable (Santos and Novales, 2011).

Conclusion

Results revealed a strong activity of plant extracts on the growth of the microorganisms, Ethanol extract of *S. officinalis* was most inhibitory effect on microorganisms growth than other extracts and this effect increase with concentration increasing. *S. aureus* was most affected than *E. coli* and *C. albicans*.

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