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INVESTIGATION OF CONSTITUENTS AND BIOLOGICAL ACTIVITY AND ANTIOXADANT AND GC-MS ANALYSIS OF OIL FROM SAUDI ARABIA SPECIES: *LAVANDULA MULTIFIDA*

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ABSTRACT

The present study was designed to investigate the chemical constituents of saudi Lavandula Multifida seed oil and to evaluate its potential antimicrobial activity. 50 components were detected by GC-MS analysis. major constituents are: Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)- (27.11%), Eucalyptol (18.68%), 1,6-Octadien-3-ol, 3,7-dimethyl- (9.78%), Hentriacontane (8.87%) and Pentacosane (4.40%). The antimicrobial activity of the oil was evaluated via cup plate agar diffusion assay against five standard human pathogens (gram negative :staphylococcus aureus, bacillus subtilis and the fungi Candida albicans, gram positive: Escherichia coli and Pseudomonas aeruginosa. Lavandula Multifida oil showed partially active against Escherichia coli and good activity Pseudomonas aeruginosa. however, it was partially inactive against other test organisms. Percentage radical scavenging activity by samples was active.

KEYWORDS: Lavandula Multifida oil showed partially active against Escherichia coli and good activity Pseudomonas aeruginosa

INTRODUCTION

Lavandula multifida, commonly called fern leaf lavender, is a highly aromatic, fast- growing, everblooming plant that typically grows to 24"[1] tall on straight stems clad with dissected fern-like gray-green leaves. It is native to the warm climates of the northwestern Mediterranean plus the Canary Islands.[2] It looks like a perennial but is technically a sub-shrub because it will develop woody stems over time where it is winter hardy. Deeply-lobed, lacy, silver-green leaves (to 1.5" long) are usually twice pinnately divided into narrow segments. Blue-violet flowers (to 1/2" long) bloom in dense showy terminal spikes (to 2 ½" long) atop stems rising above the foliage to 6- 18" tall. Flowers bloom throughout the year in warm winter locations.[3] Flowers primarily in summer when plants are grown as annuals.[4]

Genus name comes from the Latin word lavo meaning I wash in reference to a former use of the plant as an aromatic wash.[5,6]

Specific epithet means much divided in reference to the plant's winged spikes.[7]

Herb garden plants can be used in a variety of ways including (1) add leaves as. flavoring to food dishes, (2) oil from stems and leaves has anti-septic properties (external applications to wounds, bruises, and insect bites), (3) dried flowers may be added to drawers of clothing as insect repellants, and (4) potpourris.[8,9]

Lavender oil, known as lavender oil, is extracted from the lavender herb, which grows in mountainous places in the Mediterranean and North Africa,[10] and lavender essential oil is one of the most widely used essential oils; it contains many antidepressants and microbes, in addition to being a sedative and natural sedative, which has been used throughout the ages in medicine and cosmetology[11] and warns against consuming this oil;

Because it may be toxic.[12]

Lavender oil is useful in treating many problems that affect the scalp[13], such as: infections, dandruff, and dryness of the scalp, as studies have indicated the ability of lavender oil to treat skin infections.[14] Lavender oil has a beautiful scent and therapeutic properties that heal the scalp There are many benefits of lavender oil for the skin As lavender oil perfumes the skin, It treats acne; Because of its anti- inflammatory and antibacterial properties, Lavender oil treats eczema, reduces skin irritation, and redness.[15]

MATERIALS AND METHODS

Plant material

Lavandula Multifida Seeds were collected from Saudi Arabia in august 2018. The plant was authenticated by direct comparison with a herbarium sample.

Methods

Phytochemical screening

Lavandula Multifida was screened for major secondary metabolites according to the method described by harborne. Lavandula Multifida was extracted with 80% aqueous methanol (soxhlet) until exhaustion. This prepared extract (PE) was used for phytochemical screening.[16]

Test for unsaturated sterols and triterpenes

10 ml of the (PE) were evaporated to dryness on a water bath and the cooled residue was stirred with petroleum ether to remove most of the coloring materials. The residue was then extracted with 10 ml chloroform. The chloroform solution was dehydrated over sodium sulphite anhydrous. 5ml portion of the solution was mixed with 0.5 ml of acetic anhydride, followed by two drops of concentrated sulphuric acid. Two separate layers (green, red) were observed.

Test for flavonoids

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

-To 3 ml of filtrate a fragment of magnesium ribbon was added, shaken and then few drops of concentrated hydrochloric acid were added. Red color was observed.

-To 3 ml of the filtrate few drops of aluminium chloride solution were added. A dark yellow color was formed.

-To 3 ml of the filtrate few drops of potassium hydroxide solution were added. A dark yellow color was observed.

Test for alkaloids

(10 ml) of the (PE) were evaporated to drynees on water bath and 5 ml of 0.2 N hydrochloric acid were added and the solution was heated with stirring for 10 minutes, then cooled and divided into two portions.

To one portion a few drops of Mayer reagent were added. A white precipitated appeared, to the other portion few drops of Wagner reagent were added. A brown precipitate appeared.

Test for tannins

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

Test for saponins

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

Extraction of oil from *Lavandula Multifida*

Dry powdered seeds of *Lavandula Multifida* (300 g) were macerated with hexane at room temperature for 48 h. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4°C for further manipulation.

Esterification of oil

2ml of the sample was mixed thoroughly with 7ml of alcoholic sodium hydroxide (NoaH) that was prepared by dissolving 2 g in 100 ml methanol. 7 ml from alcoholic sulfuric acid (1ml H_2SO_4 to 100 ml methanol) was then added. The mixture was then shaked for 5 minutes. The content of the test tube was left to stand overnight.1 ml of Super saturated sodium chloride (NaCl) was then added and the contents being shaken. 2ml of normal hexane was added and the contents were shaked thoroughly for three minutes. Then the n-hexane layer (the upper layer of the test tube) was taken using disposable syringe. 5 µl from the n-hexane extract was diluted with 5 ml of diethyl ether. Then the mixture was filtered through syringe filter 0.45 µm and dried with 1g of anhydrous sodium sulphate as drying agent and 1µl of the diluted sample was injected in the GC.MS instrument.

GC-MS analysis

The qualitative and quantitative analysis of the sample was carried out by using GM/MS technique model (GC/MS-QP2010-Ultra) from japans 'Simadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 was injected by using split mode, helium as the carrier gas mm×0.25µm). The sample passed with flow rate 1.61 ml/min, the temperature program was started from 60c with rate 10c/min to 300c as final temperature degree with 5 minutes hold time, the injection port temperature was 300c, the ion source temperature was 200c and the interface temperature was 250c. The sample was analyzed by using scan mode in the range of m/z 40-500

charges to ratio and the total run time was 29 minutes .Identification of components for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST)., results were recorded.

Antimicrobial assay

Lavandula Multifida oil was screened for antimicrobial activity against five standard human pathogens (Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger) using the cup plate agar method with some minor modifications.

Preparation of bacterial suspensions

One ml, aliquots of 24 hours broth culture of the test organisms were distributed onto agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce suspension containing about 10^8 -10^4 colony forming units per ml. The suspension was stored in refrigerator at 4°C until used. The average number of viable organism per ml of the saline suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volume (0.2 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature to dry and then incubated at 37°C for 24 hours.

Preparation of fungal suspensions

Fungal cultures were maintained on potato dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline and the suspension was stored in the refrigerator until used.

Antimicrobial assay

The cup plate agar diffusion method was adopted with some minor modification, to assess the antimicrobial activity of the *Lavandula Multifida* oil. Two ml of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in water bath.

(20ml) Aliquots of the incubated nutrient agar were distributed into sterile petri dishes and the agar was left to settle. In each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No.4). Each of the halves was designed for one of the test samples.

The agar discs were removed and cups were filled with (0.1 ml) of test sample using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. After incubation the diameters of the resultant growth inhibition zones were measured in duplicates and averaged.

Testing for antifungal activity

The above mentioned method was adopted for antifungal activity, but instead of nutrient agar potato dextrose agar was used. Samples were used here by the same concentrations mentioned above.

Antioxidant Activities

DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the method of Shimada et.al. (1992). With some modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octy1phenyl)-1-picryl-hydrazyl stable free radical(DPPH) for half an hour at $37C^0$. The concentration of DPPH was kept as (300µm).

The test samples were dissolved in DMSO while DPPH was prepared in ethanol.

After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.[17]

RESULTS AND DISCUSSION

Phytochemical screening

In the present study, Saudi Arabia *Lavandula Multifida* extracts were screened for the occurrence of bioactive compounds. The results (Table:1) positively showed the presence of flavonoids, saponins, tannins and alkaloids in both aqueous and methanolic extract of *Lavandula Multifida* . These findings suggested that phytochemicals present in *Lavandula Multifida* are potentially beneficial as therapeutic and antioxidative agents in pharmaceuticals, food and other related industries.

Table 1: Phytochemical screening of *Lavandula Multifida.*

Species	Flavonoids	Saponins	Alkaloids	Tannins
Lavandula Multifida	+ve	+ve	+ve	+ve

GC-MS analysis of *Lavandula Multifida*

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

Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 50 components (Table 2). The typical total ion chromatogram (TLC) in shown in fig.1.

Table 2: Constituents of Lavandula Multifida oil.

ID#	Name	Ret.Time	Area	Area%
1.	1-Pentylcyclopentanol	3.187	273417	0.31
2.	erythro-(trans)(1,4),(trans)(1',4')-4,4'-Dihydroxybicyclooctyl	3.461	166488	0.19
3.	.alpha.-Pinene	3.684	123908	0.14
4.	Camphene	3.882	460292	0.51
5.	.beta.-Pinene	4.246	115961	0.13
6.	Eucalyptol	4.994	16736855	18.68
7.	p-Menth-8-en-1-ol, stereoisomer	5.525	68931	0.08
8.	trans-Linalool oxide (furanoid)	5.575	2525239	2.82
9.	trans-Linalool oxide (furanoid)	5.801	1715884	1.91
10	1,6-Octadien-3-ol, 3,7-dimethyl-	5.961	8763151	9.78
11	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	6.676	24289056	27.11
12	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-	6.943	579697	0.65
13	endo-Borneol	6.987	3260889	3.64
14	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-	7.073	142782	0.16
15	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	8.127	2516281	2.81
16	Tricyclo[2.2.1.0(2,6)]heptane, 1,7-dimethyl-7-(4- methyl-3-pentenyl)-, (-)-	10.393	234901	0.26
17	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	10.444	659023	0.74
18	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl- 3-pentenyl)-	10.581	59806	0.07
19	Cedrene	11.226	66012	0.07
20	Neryl (S)-2-methylbutanoate	11.454	69805	0.08
21	.gamma.-Muurolene	11.623	126716	0.14
22	Tricyclo[2.2.1.0(2,6)]heptane-3-methanol, 2,3- dimethyl-	11.684	101379	0.11
23	trans-Z-.alpha.-Bisabolene epoxide	12.137	115383	0.13
24	trans-Z-.alpha.-Bisabolene epoxide	12.524	2780906	3.10
25	Spiro[2.4]heptane-5-methanol, 5-hydroxy-	13.101	54227	0.06
26	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1.alpha.,4.beta.,4a.beta.,8a.beta.)]-	13.165	322460	0.36
27	Patchouli alcohol	13.463	136714	0.15
28	Caryophyllene oxide	13.542	144991	0.16
29	11-Hexadecyn-1-ol	13.702	231838	0.26
30	2-Pentadecanone, 6,10,14-trimethyl-	15.228	237072	0.26
31	Hexadecanoic acid, methyl ester	16.034	1954817	2.18
32	Heptacosanoic acid, methyl ester	16.747	53709	0.06
33	9,12-Octadecadienoic acid, methyl ester, (E,E)-	17.677	440331	0.49
34	9-Octadecenoic acid (Z)-, methyl ester	17.721	439845	0.49
35	1,3-Cyclooctadiene	17.745	345882	0.39
36	Phytol	17.848	905510	1.01
37	Methyl stearate	17.945	228762	0.26
38	Eicosanoic acid, methyl ester	19.696	160472	0.18
39	Triacontanoic acid, methyl ester	20.299	48213	0.05
40	Nonadecane, 2-methyl-	20.736	191796	0.21
41	Isosteviol methyl ester	20.803	118062	0.13
42	Octadecane, 2-methyl-	21.029	226088	0.25
43	Eicosane	22.535	346281	0.39
44	Eicosane, 3-methyl-	23.064	325184	0.36
45	Tetracosane	23.938	786770	0.88
46	Tridecane, 3-methyl-	24.434	671473	0.75

47	Pentacosane	25.265	3945697	4.40
48	Hexadecane, 3-methyl-	25.795	1870439	2.09
49	Hentriacontane	26.822	7945289	8.87
50	Nonadecane, 1-chloro-	27.500	1517940	1.69

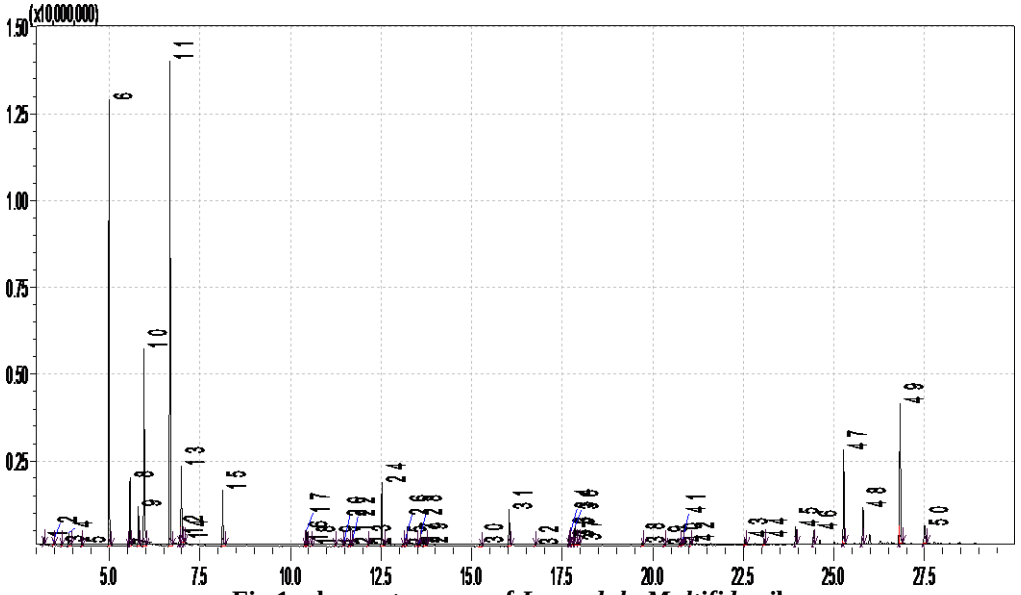
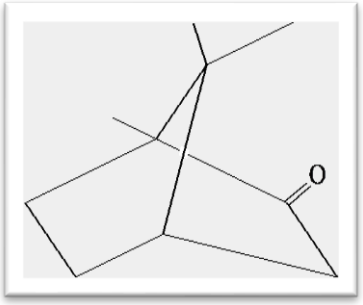
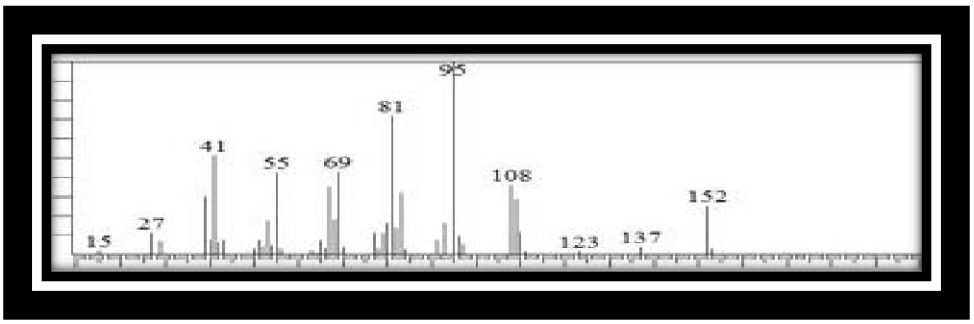


Fig.1: chromatograms of *Lavandula Multifida* oil.

1-Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)- (27.11%)
2- Eucalyptol (18.68%)
3-1,6-Octadien-3-ol, 3,7-dimethyl- (9.78%)
4- Hentriacontane (8.87%)

1- The major constituent are discussion (Fig 2) shows the mass spectrum of Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-**, The peak at m/z 152 which appeared at R.T. (6.67) in total ion chromatogram, corresponds to the moleculer ion M^+ [**$C_{12}H_{16}O$**].**

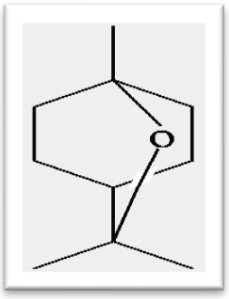


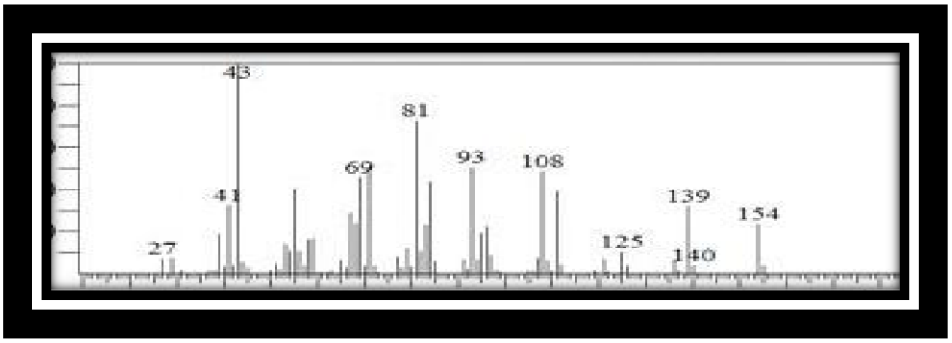


Fig(2): Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S).

2- The major constituent are discussion (Fig 3) shows the mass spectrum of Eucalyptol (18.68**%), The peak at**

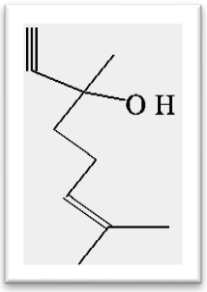
m/z 154 which appeared at R.T. (4.99) in total ion chromategram, corresponds to the moleculer ion M^+ [$C_{12}H_{16}O$].

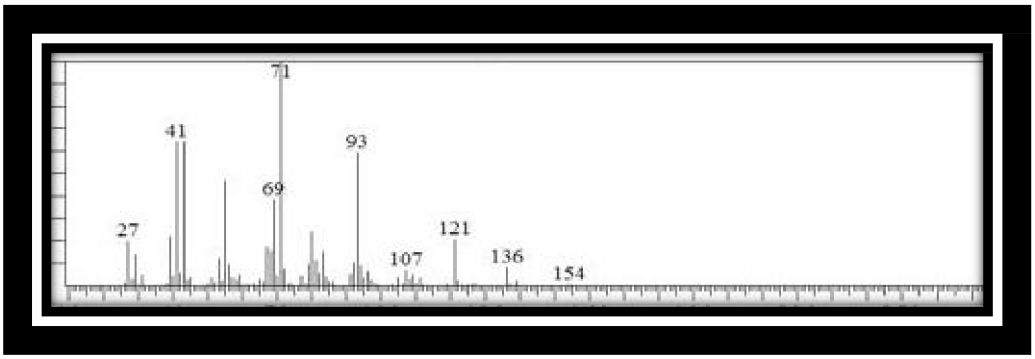




Fig(3): Eucalyptol.

3- The major constituent are discussion (Fig 4) shows the mass spectrum of 1,6- Octadien-3-ol, 3,7-dimethyl- (9.78%)**, The peak at m/z 154 which appeared at R.T. (5.96) in total ion chromategram, corresponds to the moleculer ion M^+ [$C_{12}H_{16}O$].**

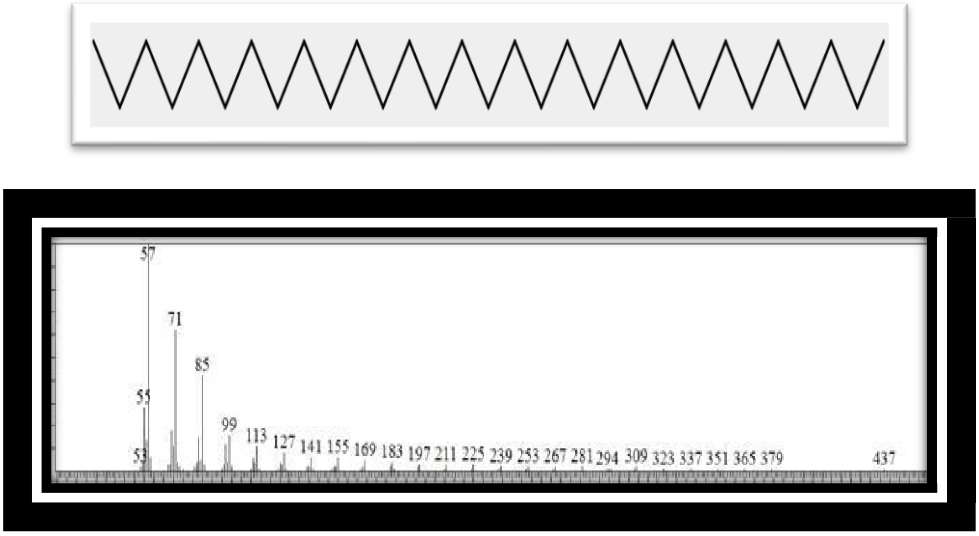




Fig(4): Octadien-3-ol, 3,7-dimethyl-.

4- The major constituent are discussion (Fig 5) shows the mass spectrum of Hentriacontane (8.87%)**, The peak at**

m/z 436 which appeared at R.T. (26.82) in total ion chromategram, corresponds to the moleculer ion M^+ [$C_{31}H_{64}$].



Fig(5): Hentriacontane.

Antimicrobial assay

In cup plant agar diffusion bioassay, the oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table3. The results were expressed in terms of the diameter of the inhibition zone:< 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active ;> 18 mm, very active.

	Conc.(mg/ml)	E.C	Ps.a	S.a	B.s	C.a
Oil	100	10-14	12-14	10-11	8-8	8-10

E.c: Escherichia coli
Ps.a: Pseudomonas aeruginosa
S.a: Staphylococcus aureus
B.s : Bacillus subtilis
C.a: Candida albicans

In cup plate agar diffusion method *Lavandula Multifida* oil showed partially active against Escherichia coli and Pseudomonas aeruginosa, but inactive against Bacillus subtilis and Candida albicans.

Antioxidant

percentage radical scavenging activity by samples in comparison with a DMSO (standard 90±0.01) treated control group is 25± 0.03.

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