Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/4866
DOI URL: http://dx.doi.org/10.21474/IJAR01/4866
ISSN NO. 2320-5407

RESEARCH ARTICLE

GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SAUDI *FOENICULUM VULGARE* MILL.(APIACEAE) FIXED OIL.

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Manuscript Info

Manuscript History

Received: 16 May 2017
Final Accepted: 18 June 2017
Published: July 2017

Key words:-
Foeniculum vulgare, Fixed oil, GC-MS, Antimicrobial activity

Abstract

Foeniculum vulgare seed oil was studied by GC-MS. The oil was also assessed for antimicrobial activity. Thirty two components were detected by GC-MS analysis. Main constituents are: 9-octadecenoic acid methyl ester(48.54%), 9,12-octadecadienoic acid methyl ester(28.50%), γ-terpinene(4.98%), and β-pinene(3.51%).The antibacterial activity of the oil was evaluated via the diffusion assay against five standard human pathogens(Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative : *Escherichia coli* and *Pseudomonasa aeruginosa* and the fungus *Candida albicans*) . *Foeniculum vulgae* oil showed excellent activity against *Staphylococcus aureus* in the concentration range : 100-25mg/ml. It also exhibited significant activity against the yeast *Candida albicans* at 100mg/ml..It seems that the oil is a lead for further optimization.

Introduction:-

Fennel (*Foeniculum vulgare* Mill.) is a perennial herb, with feathering leaves, in the family Apiaceae. The plant is cultivated worldwide for its economic value as a flavouring agent in baked foods(Diaaz, 2005 , 2006). The plant contains : protein(9.5%) ; fats (10%); minerals(Ca , K , Na, Fe and P),13.4%) ; fibre(18.5%) beside niacin, riboflavin and thiamine(Manzoor,2016).

Fennel is added to purgatives to allay their side effects. Seeds are claimed to improve eyesight if taken raw while seed extract has been tested against glaucoma in model animals. Seeds are also diuretic and hypotensive.(Agrawal,2008).

The potential pharmacological effect of fennel seems to be associated with its volatile oil which contains, among others, anethole, fenchone, estragol, p- anisaldehyde and α-phellandrene(Diiaz,2006). Anisole is claimed to possess estrogenic properties(Tognolini, 2007).

Some quercetin and kaempferol conjugates have been isolated from from fennel(Faudale,2008; park,1996 , Parejo,2004). Such phenolics are responsible for the free radical scavengaging capacity of fennel. Sterols , sugars , acetylated kaempferol and some benzoisofuranone derivatives were identified in fruits(Marino,2007, Soliman,2002). Beside its health promoting properties , a main constituent of fennel- leugenol- has become a cause of concern since the structurally related ,methylleugenol was reported as a potential carcinogenic agent(Zeller,2006).

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Materials and Methods:-

Plant material:-

The seeds of *Foeniculum vulgare* were collected from around Najran, Saudi Arabia.The plant was authenticated by direct comparison with a herbarium sample.

Test organisms:-

Foeniculum vulgae oil was screened for antimicrobial activity using the standard bacterial strains: *Bacillus subtilis* (Gram +ve) , *Staphylococcus aureus* (Gram +ve), *Pseudomonas aeroginosa* (Gram –ve) , *Escherichia coli* (Gram – ve) and the fungal species *Candida albicans*.

Methods:-

Extraction of *Foeniculum vulgare* seed oil:-

Powdered seeds of *Foeniculum vulgare* (300g) were macerated with n-hexane at ambient temperature for 48h..The solvent was removed *in vacuo* to afford the oil. Esterification of the oil, for GC-MS analysis, was accomplished via a methanolic solution of sodium hydroxide and a methanolic sulphuric acid.

GC-MS Analysis:-

A Shimadzo Ultra instrument was used for GC-MS analysis of *Foeniculum vulgare* fixed oil . RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 μm, thickness) was used. Analytical grade helium (purity; 99.99 %) was the carrier gas. Oven temperature program is displayed below , while other chromatographic conditions are depicted in Tables 1 .

Rate: - ; Tempt.,60.0^{o}C ; Hold time(min^{-1}) ,0.00
Rate, 10.0 ; Tempt.,300.0 ; Hold time(min^{-1}) ,0.00

Table 1:- Chromatographic conditions

Column oven temperature	1300.0 ℃
Injection temperature	280.0 ℃
Injection mode	Split
Flow control mode	Linear velocity
Pressure	93.1KPa
Total flow	50.0ml/ min
Column flow	1.50ml/sec
Linear velocity.	44.7cm/sec
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial assay:-

Preparation of bacterial suspensions:-

Diffusion method was the method used for screening the oil. Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungi respectively. The media were prepared according to the manufacturer's instructions.

Aliquots (1ml) of 24 hours broth culture of the test microorganisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. Bacterial growth was harvested and washed off with sterile normal saline , then it was suspended in (100 ml) of normal saline to afford about 108-109 colony forming units per ml. Average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline.(0.02 ml) of the appropriate dilutions were transferred onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature and then incubated at 37°C for 24 hours.

Fungal cultures were maintained on Sabouraud 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.

Testing for Antibacterial Activity:-

(2ml) of the standardized bacterial stock suspension were mixed with (200 ml) of sterile molten nutrient agar which was maintained at 45°C. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle . Each plate was divided into two halves. In each half two cups (10mm in diameter) were cut using sterile cork borer (No 4). Each half was designed for a test solution.

Agar discs were removed, alternate cups were filled with(0.1 ml) samples of each test solution and allowed to diffuse at room temperature for two hours. The plates were then incubated at 37^{o}C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured in duplicates and averaged.

Results and Discussion:-

GC-MS analysis of *Foeniculum vulgae* fixed oil:-

GC-MS analysis of *Foeniculum vulgae* fixed oil was carried out. The MS library (NIST) was checked for identification of the constituents (a 90-95% match was observed) . Furthermore, the observed fragmentation pattern was interpreted.

The GC-MS analysis of the studied oil revealed the presence of 32 components(Table 2).The typical total ion chromatograms (TIC) is depicted in Fig.1.

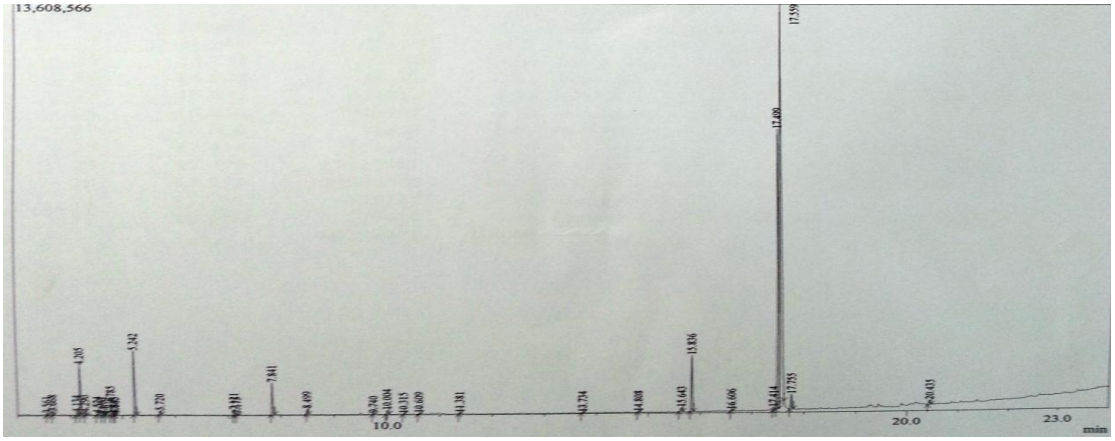

Fig.1:- Total ion chromatograms

Table 2: Contituents of *Foeniculum vulgae* oil

Peak#	R.Time	Area	Area%	Name
1	3.561	78697	0.13	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-met
2	3.668	149117	0.25	.alpha.-Pinene
3	4.134	163802	0.27	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-me
4	4.205	2134541	3.51	.beta.-Pinene
5	4.290	123090	0.20	.beta.-Myrcene
6	4.524	62880	0.10	.alpha.-Phellandrene
7	4.603	16082	0.03	3-Carene
8	4.679	18436	0.03	(+)-4-Carene
9	4.785	512747	0.84	o-Cymene
10	4.843	48379	0.08	D-Limonene
11	4.865	42651	0.07	.beta.-Phellandrene
12	5.242	3027403	4.98	.gamma.-Terpinene
13	5.720	172319	0.28	Undecane
14	7.121	253023	0.42	Dodecane
15	7.175	86329	0.14	1-Cyclohexene-1-carboxaldehyde, 4-(1-me
16	7.841	1719357	2.83	Benzaldehyde, 4-(1-methylethyl)-
17	8.499	281517	0.46	Tridecane
18	9.740	54609	0.09	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-
19	10.004	210440	0.35	Benzenepropanol, 4-methoxy-
20	10.315	30972	0.05	Caryophyllene
21	10.609	70725	0.12	(E)-.beta.-Famesene
22	11.381	49224	0.08	Butylated Hydroxytoluene
23	13.734	37259	0.06	Methyl tetradecanoate
24	14.808	33780	0.06	Pentadecanoic acid, methyl ester
25	15.643	336214	0.55	9-Hexadecenoic acid, methyl ester, (Z)-
26	15.836	2902997	4.78	Hexadecanoic acid, methyl ester
27	16.606	76436	0.13	Methyl 8-heptadecenoate
28	17.414	159302	0.26	6,9-Octadecadienoic acid, methyl ester
29	17.499	17325881	28.50	9,12-Octadecadienoic acid (Z,Z)-, methyl e
30	17.559	29508900	48.54	9-Octadecenoic acid (Z)-, methyl ester
31	17.755	825729	1.36	Methyl stearate
32	20.435	279634	0.46	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl

Main constituents of the oil are discussed below:

9-Z-Octadecenoic acid methyl ester(48.54%%)

Fig. 2 shows the EI mass spectrum of 9-octadecenoic acid methyl ester.The peak at m/z 296, which appeared at R.T. 17.559 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{36}O_2]^{+}$, while the peak at m/z266 accounts for loss of a methoxyl function

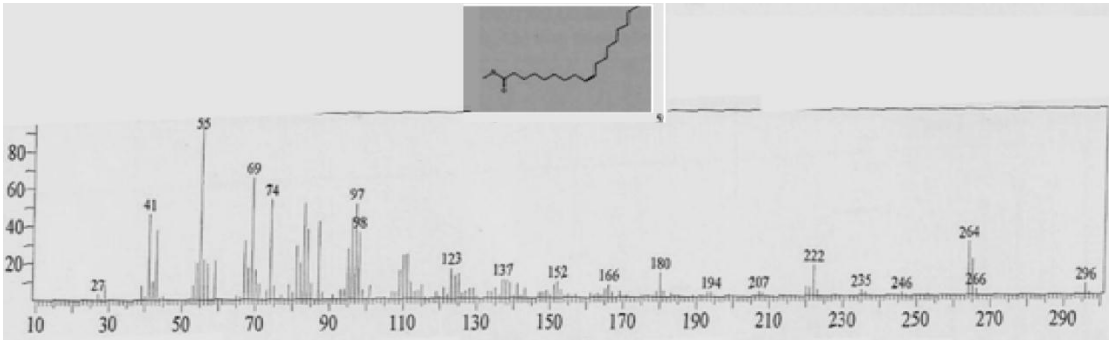

Fig. 2:- Mass spectrum of 9-octadecenoic acid methyl ester.

9,12-Z,Z-Octadecadienoic acid methyl ester (28.50%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is displayed in Fig.3.The peak at m/z294(R.T. 17.499-in total ion chromatogram)) corresponds $M^{+}[C_{19}H_{34}O_2]^{+}$ .The signal at m/z263 corresponds to loss of a methoxyl function.

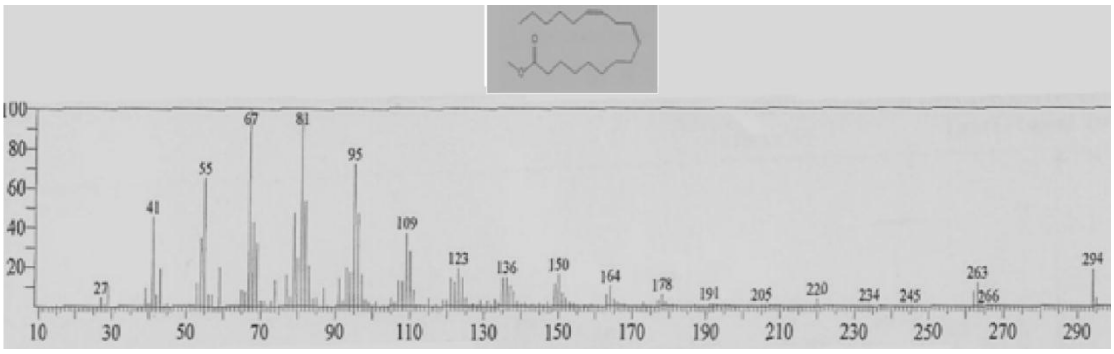

Fig. 3:- Mass spectrum of 9,12-octadecadienoic acid methyl ester

γ – Terpinene(4.98%)

Fig. 4 shows the mass spectrum of **γ –** terpinene .The peak at m/z136 , which appeared at R.T. 5.242 in total ion chromatogram, corresponds $M^+[C_{10}H_{16}]^+$ **.**

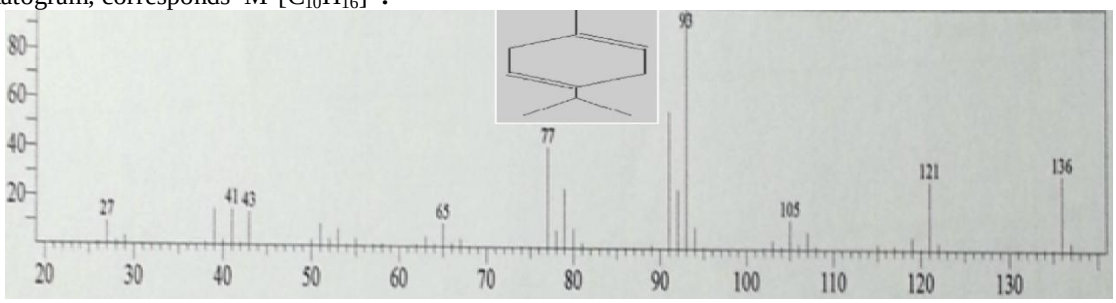

Fig. 4:- Mass spectrum of **γ –** terpinene

β-Pinene(3.51%)

The masss spectrum of β-Pinene is shown in Fig.5. The molecular ion $M^+(C_{10}H_{16})$ appeared at m/z136 with RT,4.205 in total ion chromatogram.

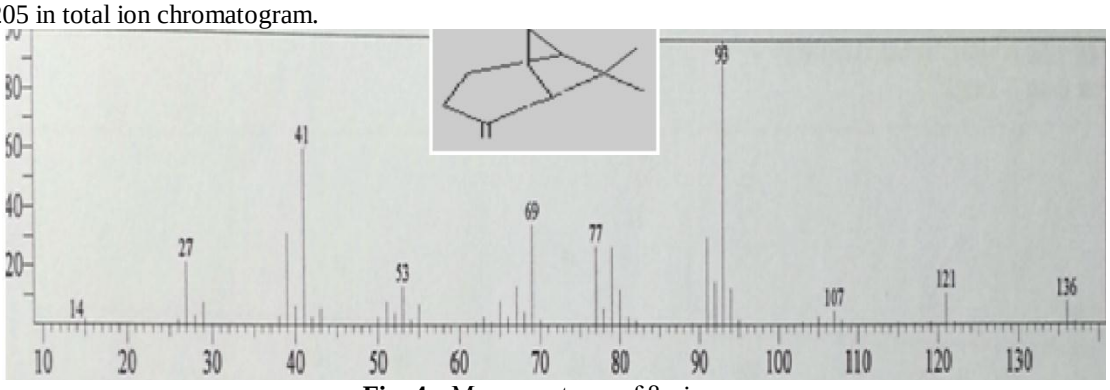

Fig. 4:- Mass spectrum of β-pinene

Antibacterial activity

Foeniculum vulgae oil was screened for antimicrobial activity against five standard bacterial strains . The diameters of the growth of inhibition zones are shown in Table (3) . Conventional terms were used for interpretation of the results : (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (4) and (5) represent the antimicrobial activity of standard drugs.

Table 3:- Antibacterial activity of *Foeniculum vulgae* oil

Type	Conc.(mg/ml)	**Sa**	Bs	Ec	Ps	Ca
Oil	100	20	14	15	15	17
	50	18	-	14	14	15
	25	17	-	13	13	10
	12.5	15	-	12	12	9
	6.25	11	-	10	7	-

Table 4:- Antibacterial activity of standard chemotherapeutic agents

Drug	Conc.(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 5:- Antifungal activity of standard chemotherapeutic agent

Drug	Conc.(mg/ml)	An	Ca
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

Foeniculum vulgae oil showed excellent activity against *Staphylococcus aureus* in the concentration range : 100-25mg/ml. It also exhibited significant activity against the yeast *Candida albicans* at 100mg/ml.

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