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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE FOENICULUM VULGARE (APIACEAE) ESSENTIAL OIL

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ABSTRACT

Foeniculum vulgare essential oil was studied by GC-MS. The oil was also assessed for antimicrobial activity. Thirty five components were detected by GC-MS analysis. Main constituents are: (1-phenyl)-1, 2-ethanediol (21.43%), 4-(1-methyethyl) benzaldehyde(18.03%), gamma,-terpinene(15.35%), 2-caren-10-al(14.15%), and β-pinene (10.45%). The antibacterial activity of the oil was evaluated via the diffusion assay against five standard human pathogens (Gram positive: *Staphylococcus aureus* and *Bacillus subtitlis; Gram* negative: *Esherichia coli* and *Pseudomonasa aeruginosa* and the fungus *Candida albicans*). *Foeniculum vulgare* oil showed excellent activity against all test organisms at 50mg/ml. At 25mg/ml the oil exhibited significant activity against *Staphylococcus aureus* and the yeast *Candida albicans*. The oil was active against all test organisms in the range: 50-12.5mg/ml.It seems that the oil is a lead for further optimization.

KEYWORDS: *Foeniculum vulgare*, Essential Oil, GC-MS, Antimicrobial Activity.

INTRODUCTION

*Foeniculum vulgare***,** which is marketed worldwide as spice , is a perennial plant in the family Apiaceae. The herb is widely used as flavoring agent.[1,2] *Foeniculum vulgare* contains, among others, minerals, fiber, protein, niacin, thiamine and riboflavin.[3]

Seeds are diuretic, hypotensive and are traditionally claimed to improve eye sight. They were tested *in vivo* against glaucoma.[4] Fennel essential oil contains many bioactive constituents including: p-anisole, fenchone, estragol, p- anisaldehyde and α-phellandrene.[2] p-Anisole is claimed to possess estrogenic properties.[5] Some phenolics including: kaempferol and quercetin conjugates were reported from fennel. Such phenolics are associated with the antioxidant capacity of this herb.[6,8] Some benzoisofuranone derivatives, sterols, sugars and acetylated kaempferol were reported from seeds.[9]

In continuation of our interest in the bioconstituents of plants used in Sudanese system of medicine, this study was designed to investigate the constituents of Sudanese fennel essential oil.

MATERIALS AND METHODS

Plant material

The seeds of *Foeniculum vulgare* were purchased from the local market – Omdurman, Sudan. The plant was authenticated by direct comparison with a herbarium sample.

Test microorganisms

Foeniculum vulgare oil was screened for antimicrobial activity using the standard bacterial strains: (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*). The cork and cup diffusion bioassay was used with some minor modifications.

Methods

Extraction of *Foeniculum vulgare* oil

Foeniculum vulgare seeds (400g) were steam –distilled to afford the essential oil.

GC-MS analysis

Fennel essential oil was analyzed for the presence of different volatiles via the GC-MS technique. Analysis was performed by using A Shimadzo GC-MS-$QP_2$2010 Ultra instrument equipped with RTX-5MS column, (30, length; 0.25mm diameter; 0.25µm, thickness) and gas chromatogram interfaced to a Mass Selective Detector .

Analytical grade Helium was used as carrier gas. Column flow rate is 50ml/sec.; injection temperature is 280°C. The oven was programed from 60°C to 300°C at a rate of 10°C /min. Relative abundance of different constituents were expressed as % age with peak area normalization.

Identification of oil constituents was accomplished by retention time and fragmentation pattern. Library source – NIST[10] was also used for identification purposes.

Antimicrobial assay

The cork and bore diffusion method[11]was used to assess the antimicrobial activity of the oil. Meuller Hinton and Sabouraud dextrose agars were used as growth media for the bacteria and fungi respectively.

Pure cultures of microorganisms were inoculated into Mueller Hinton agar and incubated for 24h at 37°C. Bacterial growth was harvested and washed off with sterile normal saline, then it was suspended in (100ml) of normal saline to afford about 108-109 colony forming unit per ml. Average number of viable organism per ml of the stock suspension was determined by the means of surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline. (0.02ml) 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.Sabouraud dextrose agar was used for fungal cultures and incubation continued for 4 days at 25°C.After incubation, the diameters of resultant growth inhibition zones were measured in duplicates and averaged.

RESULT AND DISCUSSION

GC-MS analysis of *Foeniculum vulgare* oil

GC-MS analysis of *Foeniculum vulgare* oil was conducted.The analysis revealed the presence of 35 components. GC-MS chromatogram is displayed in Fig.1.The spectra of different components were matched with NIST library[10]. A tabulation of major active principles and peak area is given in Table 1.

Table 1: Constituent of *Foeniculum vulgare* volatile oil.

Peak#	R.Time	Area%	Name
1	4.765	0.32	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-met
2	4.906	0.66	.alpha.-Pinene
3	5.639	0.94	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-mc
4	5.722	10.45	.beta.-Pinene
5	5.929	0.70	.beta.-Myrcene
6	6.237	2.07	.alpha.-Phellandrene
7	6.364	0.04	3-Carene
8	6.481	0.29	(+)-4-Carene
9	6.587	0.09	Cyclohexene, 1-methyl-4-(1-methylethyl)-,
10	6.643	3.24	o-Cymene
11	6.748	0.79	Cyclohexene, 1-methyl-5-(1-methylethenyl
12	6.810	0.23	Eucalyptol
13	7.355	15.35	.gamma.-Terpinene
14	7.984	0.08	(+)-4-Carene
15	8.182	3.27	1,6-Octadien-3-ol, 3,7-dimethyl-
16	8.709	0.04	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylet
17	9.117	0.11	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-r
18	9.253	0.10	(+)-2-Bornanone
19	9.329	0.05	3,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-
20	9.697	0.05	3-Undecen-5-yne, (Z)-
21	9.917	0.20	Terpinen-4-ol
22	10.194	0.09	.alpha.-Terpineol
23	10.263	4.10	1-Cyclohexene-1-carboxaldehyde, 4-(1-mc
24	10.423	0.25	Decanal
25	11.260	18.03	Benzaldehyde, 4-(1-methylethyl)-
26	12.001	0.31	1-Cyclohexene-1-carboxaldehyde, 4-(1-mc
27	12.193	14.15	2-Caren-10-al
28	12.320	21.43	1,2-Ethanediol, 1-phenyl-
29	13.053	0.81	1,4-Cyclohexadiene-1-methanol, 4-(1-metl
30	14.038	0.13	Geranyl acetate
31	15.968	0.59	Spiro[4.5]dec-7-ene, 1,8-dimethyl-4-(1-me
32	18.230	0.22	Carotol
33	22.718	0.28	Hexadecanoic acid, methyl ester
34	24.636	0.28	Methyl 10-trans,12-cis-octadecadienoate
35	24.686	0.26	9-Octadecenoic acid, methyl ester, (E)-
		100.00	

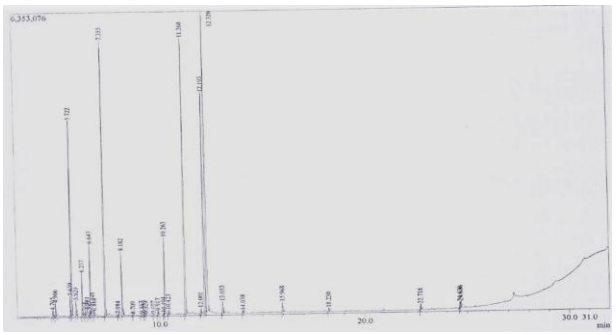
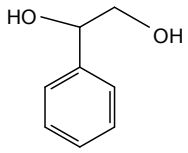


Fig. 1: Total ion chromatograms.

Main constituents of the oil are discussed below:

(1-phenyl)-1, 2-ethanediol (21.43%).

Fig. 2 shows the EI mass spectrum of (1-phenyl)-1, 2-ethanediol. The peak at m/z138, which appeared at R.T. 12.320 in total ion chromatogram, corresponds M^+ $[C_8H_{10}O_2]^+$.



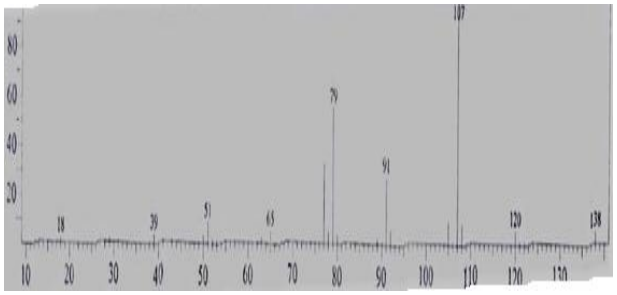
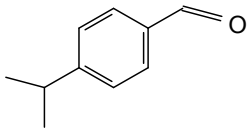


Fig. 2: Mass spectrum of (1-phenyl)-1, 2-ethanediol.

4-(1-methylethyl)-benzaldehyde(18.03%)

The mass spectrum of 4-(1-methylethyl)-benzaldehyde is displayed in the Fig.3. The peak at m/ z148 (R.T. 11.260- in total ion chromatogram) corresponds M^+ $[C_{10}H_{12}O]^+$. The signal at m/z133 corresponds to loss of a methyl function.



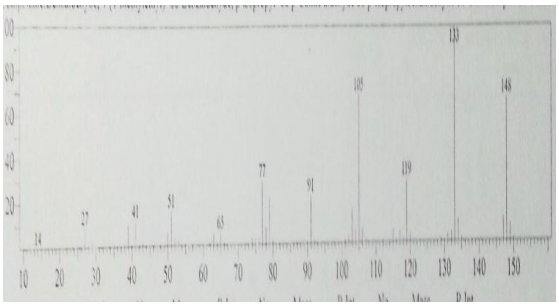
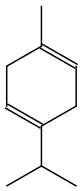


Fig. 3: Mass spectrum of 4-(1-methylethyl)-benzaldehyde.

gama-Terpinene(15.35)

Fig.4. shows the mass spectrum of gamma,-terpinene .The peak at m/z136, which appeared at R.T.7.355 in total ion chromatogram, corresponds M^+ $[C_{10}H_{16}]^+$. . The signal at m/z121 is due to loss of a methyl function.



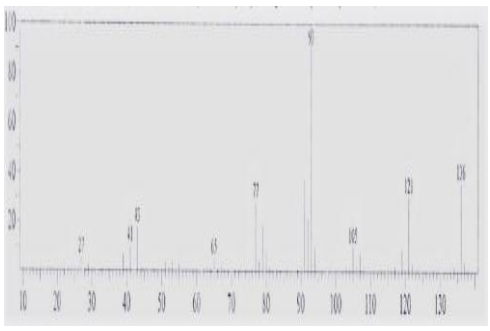
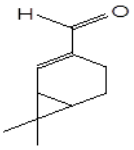


Fig. 4: Mass spectrum of gama,-terpinene.

2-Caren-10-al (14.5%)

The mass spectrum of 2-caren-10-al is shown in Fig.5. The molecular ion $M^+[C_{10}H_{14}O]^+$ appeared at m/z150 with R.T.12.193 in total ion chromatogram.



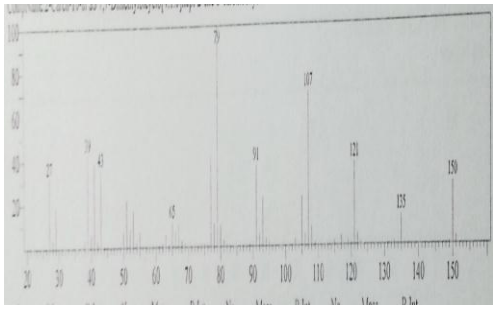
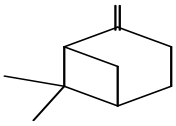


Fig. 5: Mass spectrum of 2-caren-10-al.

β- Pinene(10.43%)

The masss spectrum of β-pinene is shown in Fig.6. The molecular ion $M^+[C_{10}H_{16}]^+$ appeared at m/z136 with R.T.5.722 in total ion chromatogram.



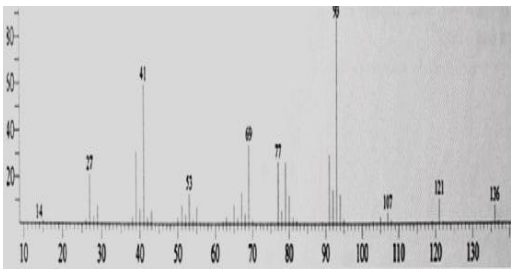


Fig. 6: Mass spectrum of β- pinene.

Antibacterial activity

Foeniculum vulgare oil was screened for antimicrobial activity against five standard bacterial strains. The diameters of the growth of inhibition zones are shown in Table (2). Conventional terms were used for interpretation of the results : (<9mm: inative;9-12mm:partially active;13-18mm: active; 13-18mm: active 18> very active). Tables (3) and (4) represents the antimicrobial activity of standard drugs.

Table 2: Antibacterial activity of *Foeniculum vulgare* oil.

Type	Conc.(mg/ml)	Sa.	Bs.	Ec.	Pa.	Ca.
oil	50	20	17	20	18	20
	25	18	16	15	16	18
	12.5	16	16	12	14	16
	6.25	14	15	-	12	14

Table 3: Antibacterial activity of standard chemotherapeutic agent.

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

Table 4: Antifungal activity of standard chemotherapeutic agent.

Drug	Conc.(mg/ml)	An	Ca
Clotramizole	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 vulgare oil showed excellent activity against all test organisms at 50mg/ml. At 25mg/ml the oil exhibited significant activity against *Staphylococcus aureus* and the yeast *Candida albicans*. The oil was active against all test organisms in the range: 50-12.5mg/ml.

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