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Research Article

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Chemical Composition and antimicrobial activity of the Hexane Fraction of Sudanese Fennel (Apiaceae) Seeds

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Abstract Fennel is widely cultivated in many countries where it is used as flavouring agent. Fennel is rich in some minerals like calcium, sodium, iron, potassium and phosphorus. The plant also contains fibre (18.5%), protein (9.5 %); fats (10%) beside niacin, riboflavin and thiamine. Fennel hexane fraction was analyzed by GC-MS. Gas chromatogram revealed presence of: fatty acids (87.83%); aldehydes (5.80%); ketones (2.90%); alcohols (1.44%); mono- and sesquiterpenes (1.23%); hydrocarbons (0.80%).
Foeniculum vulgare hexane fraction was evaluated for antimicrobial activity against five standard human pathogens. The extract exhibited significant activity against *Staphylococcus aureus* in the concentration range: 100-50mg/ml. It also exhibited significant anticandidal activity at 100mg/ml.

Keywords *Foeniculum vulgare*, Hexane extract, GC-MS analysis, Antimicrobial activity

Introduction

Foeniculum vulgare Mill. (fennel) is a perennial herb in the family Apiaceae. Fennel is widely cultivated in many countries where it is used as flavouring agent in baked foods [1,2]. Fennel is rich in some minerals like calcium, sodium, iron, potassium and phosphorus. The plant also contains fibre (18.5%), protein (9.5%); fats (10%) beside niacin, riboflavin and thiamine [3]. Seeds of fennel which are hypotensive and diuretic are claimed to improve eyesight, while seed extract has been tested against glaucoma in experimental models [4]. Fennel essential oil contains some bioactive molecules like anethole, fenchone, estragol, p- anisaldehyde and α-phellandrene [2,5].
Sterols, acetylated kaempferol and some benzoisofuranone derivatives have been reported from fennel [6,7]. Also some flavonoids have been isolated from fennel [8-10]. These phenolics seem to be responsible for the antioxidant properties of fennel. The antispasmodic , diuretic, antinflammatory, analgesic, hepatoprotective properties of fennel essential oil have been documented [11-14]. It has been reported that fennel essential oil exhibited antimicrobial activity [15,16].
However, beside its health promoting properties, a constituent of fennel- leugenol- has become a cause of concern since the structurally related, methylleugenol has been listed as a potential carcinogenic agent [17].

Materials and Methods

Plant Material

Foeniculum vulgare seeds were purchased from the local market-Khartoum, Sudan. The plant was identified and authenticated by direct comparison with a reference herbarium sample.

Test organisms

- Gram +ve: *Bacillus subtilis and Staphylococcus aureus*

- Gram –ve: *Escherichia coli* and *Pseudomonas aeruginosa*

- Fungal strain: *Candida albicans.*

- Positive controls:

i. Ampicillin: for G+ve bacteria.

ii. Gentamicin: for G-ve bacteria.

iii. Clotrimazole: antifungal standard.

-Media for bacteria: Mueller –Hinton agar.

- Media for fungus: Sabouraud dextrose agar.

Methods

Hexane extract

Powdered seeds of *Foeniculum vulgare* (300g) were macerated with n-hexane for 72hr. The solvent was removed under reduced pressure to give the hexane extract.

GC-MS analysis

Foeniculum vulgare hexane fraction was analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument. chromatographic conditions are as follows:

-Column oven temperature	:	150.0 °C
-Injection temperature	:	300.0 °C
-Injection mode	:	Split
-Flow control mode	:	Linear velocity
-Pressure	:	139.3KPa
-Total flow	:	50.0ml/ min
-Column flow	:	1.54ml/sec.
-Linear velocity	:	47.2cm/sec.
-Purge flow	:	3.0ml/min.
-Spilt ratio	:	- 1.0

Antimicrobial assay

For bacteria an inoculum suspension (20 ml Mueller-Hinton Agar) was swabbed uniformly to solidify, and then allowed to dry. Holes of 6 mm in diameter were made in the seeded agar using glass Pasteur pipettes. Aliquots of the hexane extract (100 and 200 mg/ml) were added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at 37 ^{o}C for 24 h. The resulting inhibition zones were measured in millimeters (mm).The assays were repeated in duplicate and the concurrent values were taken.

The same procedure was adopted for antifungal activity, but Sabouraud dextrose agar was used instead of Mueller Hinton agar.

Results and Discussion

The hexane fraction of *Foeniculum vulgare* was investigated by GC-MS. The analysis revealed detection of 53 components. The retentions times and percentages of these constituents are illustrated in Table 1. Fig. 1 shows the total ion chromatograms. The hexane fraction was dominated by fatty acids (87.83%) followed by aldehydes (5.80%), ketones (2.90%), alcohols (1.44%), mono-and sesquiterpenes (1.23%) and hydrocarbons (0.80%)-see Fig. 2.

The essential oil of Sudanese material of *Foeniculum vulgare* has been investigated by Omnia *et.al* [18]. These authors reported that monoterpenoids were the major constituent (98.06%), while sesquiterpenes were present as a minor constituent (0.66%).

Table 1: Constituents of the hexane fraction

Peak#	R.Time	Area	Area%	Name
1	4.203	84570	0.06	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-meth
2	4.525	30413	0.02	.alpha.-Phellandrene
3	4.680	11635	0.01	1,3-Cyclohexadiene, 1-methyl-4-(1-methyle
4	4.781	117328	0.08	o-Cymene
5	5.239	790094	0.53	.gamma.-Terpinene
6	7.169	1770355	1.20	1-Cyclohexene-1-carboxaldehyde, 4-(1-met
7	7.254	32642	0.02	Ethanol, 2-(3,3-dimethylcyclohexylidene)-,
8	7.838	7377623	4.98	Benzaldehyde, 4-(1-methylethyl)-
9	8.340	105596	0.07	1-Cyclohexene-1-carboxaldehyde, 4-(1-met
10	8.461	978631	0.66	2-Caren-10-al
11	8.500	84529	0.06	p-Cymen-7-ol
12	8.736	65607	0.04	3-Cyclopenten-1-one, 2-hydroxy-3-(3-meth
13	8.786	151033	0.10	Bicyclo[2.2.1]heptan-2-ol, 7,7-dimethyl-, ac
14	9.014	107999	0.07	1,4-Cyclohexadiene-1-methanol, 4-(1-meth
15	9.496	139965	0.09	Silane, (4-ethylphenyl)trimethyl-
16	9.625	73030	0.05	Benzoic acid, 4-(1-methylethyl)-, methyl es
17	9.685	88198	0.06	2,4-Pentadienoic acid, 3,4-dimethyl-, isopr
18	9.738	172439	0.12	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7
19	9.998	98576	0.07	Benzaldehyde dimethyl acetal
20	10.188	29159	0.02	2,5-Dimethylbenzenethiol, S-pentafluoropr
21	10.310	177806	0.12	Caryophyllene
22	10.421	65563	0.04	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4
23	10.605	255455	0.17	(E)-.beta.-Famesene
24	10.750	30200	0.02	1,4,7,-Cycloundecatriene, 1,5,9,9-tetrameth
25	10.804	24453	0.02	.beta.-copaene
26	10.964	129754	0.09	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene
27	10.999	453512	0.31	Di-epi-.alpha.-cedrene
28	11.320	94523	0.06	.beta.-Bisabolene
29	11.374	209527	0.14	Butylated Hydroxytoluene
30	11.408	113605	0.08	Dodecanoic acid, methyl ester
31	12.520	377438	0.25	Carotol
32	13.726	605076	0.41	Methyl tetradecanoate
33	14.642	54816	0.04	5-Octadecenoic acid, methyl ester
34	14.802	246515	0.17	Pentadecanoic acid, methyl ester
35	14.922	100059	0.07	5H-3,5a-Epoxynaphth[2,1-c]oxepin, dodeca
36	15.023	44048	0.03	2-Pentadecanone, 6,10,14-trimethyl-
37	15.534	42935	0.03	7,10-Hexadecadienoic acid, methyl ester
38	15.607	1058379	0.71	7,10,13-Hexadecatrienoic acid, methyl este
39	15.637	1395519	0.94	Methyl hexadec-9-enoate
40	15.834	9906577	6.69	Hexadecanoic acid, methyl ester
41	16.598	463306	0.31	Methyl 18-fluoro-octadec-9-enoate
42	16.809	180927	0.12	Heptadecanoic acid, methyl ester
43	17.361	817919	0.55	Methyl 5,11,14-eicosatrienoate
44	17.411	860594	0.58	Methyl 6,11-octadecadienoate
45	17.519	40550698	27.38	9,12-Octadecadienoic acid (Z,Z)-, methyl e
46	17.600	59585782	40.24	9-Octadecenoic acid (Z)-, methyl ester
47	17.671	817221	0.55	Phytol
48	17.750	2720097	1.84	Methyl stearate
49	19.141	399938	0.27	Methyl 5,13-docosadienoate
50	19.243	2970620	2.01	3-Hydroxy-2,6,6-trimethyl-hept-4-enoic aci
51	19.430	4046234	2.73	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4
52	19.501	533479	0.36	Methyl 18-methylnonadecanoate
53	19.659	417377	0.28	6,9,12,15-Docosatetraenoic acid, methyl est

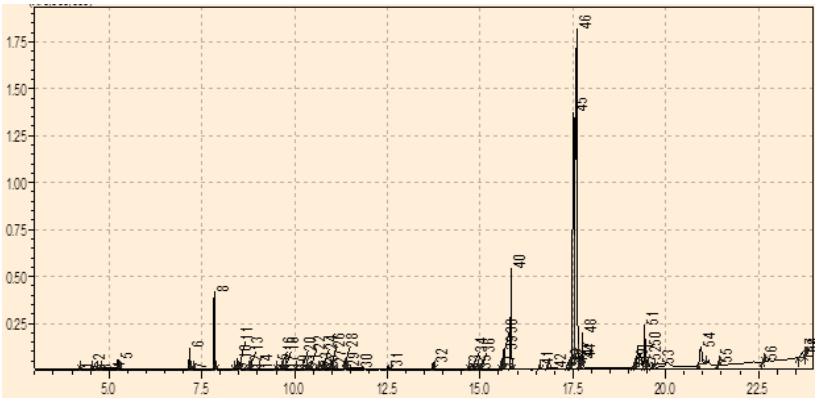

Figure 1: Total ions chromatograms

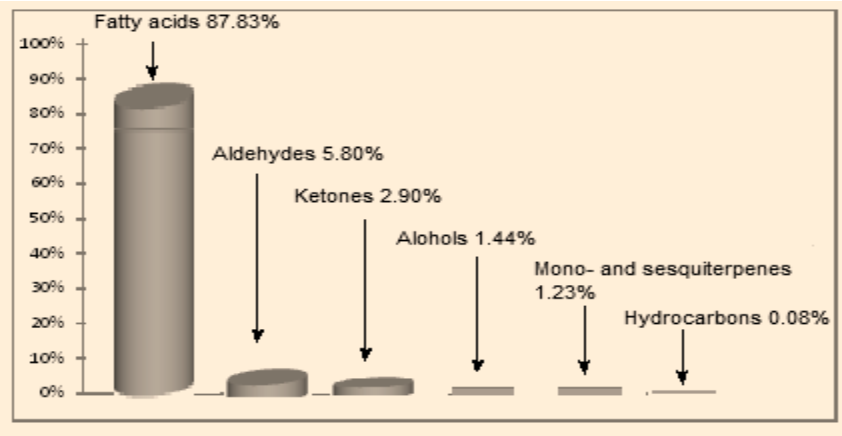

Figure 2: Abundance of oil constituents

Major constituents of the hexane fraction are discussed below:

a-9-Octadecenoic acid methyl ester(40.24%)

Fig. 3 shows the mass spectrum of 9-octadecanoic acid methyl ester .The peak at m/z 296(R.T. 17.600) accounts for : $M^+[C_{19}H_{36}O_2]^+$, while the signal at m/z265 corresponds to loss of a methoxyl .

b-9,12-Octadecadienoic acid methyl ester(27.38%)

The EI mass spectrum of 9,12-octadecanoic acid methyl ester is shown in Fig. 4.The peak at m/z 294, which appeared at R.T. 17.519 in total ion chromatogram,is due to the molecular ion : $M^+[C_{19}H_{34}O_2]^+$ .The peak at m/z263 corresponds to loss of a methoxyl group.

c-Hexadecanoic acid methyl ester(6.69%)

The EI mass spectrum of hexadecanoic acid methyl ester is shown in Fig. 5.The peak at m/z 270, which appeared at R.T. 15.834 in total ion chromatogram, corresponds to $M^+[C_{17}H_{34}O_2]^+$ .The peak at m/z239 corresponds to loss of a methoxyl function.

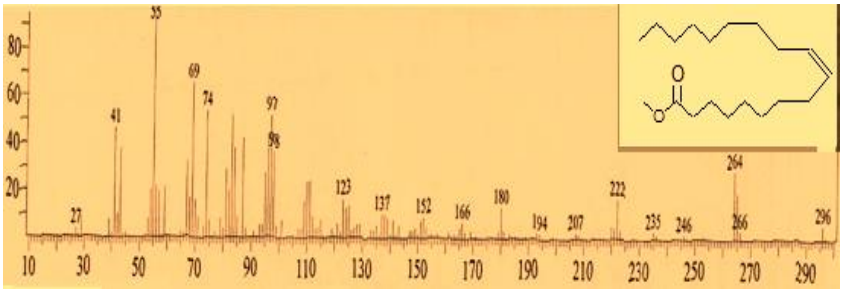

Figure 3: Mass spectrum of 9-octadecanoic acid methyl ester

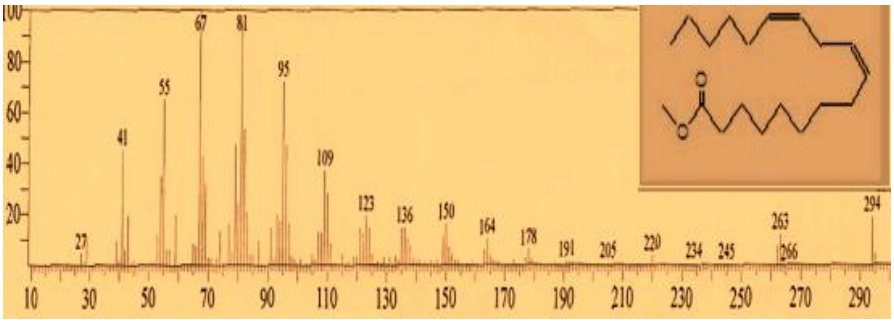

Figure 4: Mass spectrum of 9,12-octadecanoic acid methyl ester

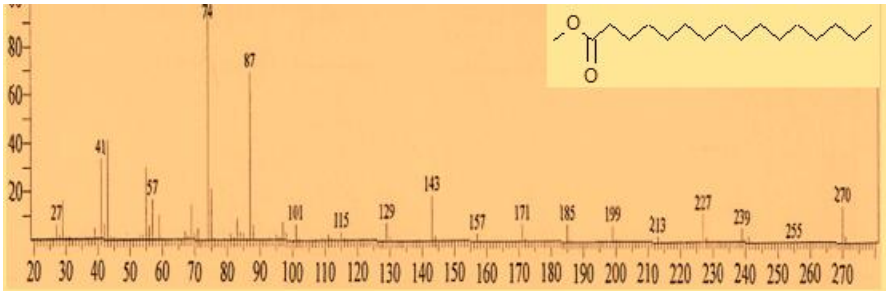

Figure 5: Mass spectrum of hexadecanoic methyl ester

Antimirobial activity

Foeniculum vulgae hexane fraction was evaluated for antimicrobial activity against five standard human pathogens. The diameters of the growth of inhibition zones are shown in Table (2). Ampicillin, gentamicin and clotrimazole were used as positive contol(Tables 3 and 4). *Foeniculum vulgare* hexane fraction exhibited significant activity against *Staphylococcus aureus* in the concentration range: 100-50mg/ml. It also exhibited excellent anticandidal activity at 100mg/ml.

Table 2: Antimicrobial activity of hexane fraction

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 3: Antibacterial activity of standard chemotherapeutic agents

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

Table 4: 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*

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