World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *FOENICULUM VULGARE* MILL. (APIACEAE.) OIL

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Article Received on 03/04/2020 **Article Revised on 24/04/2020** **Article Accepted on 14/05/2020**

ABSTRACT

In developing countries, ethnomedicine is still playing a vital role in primary health care and intensive pharmacological reports on the impact of bioactive phytochemicals on human physiology potentiated the applications of medicinal plants**.** Fennel (*Foeniculum vulgare* **Mill**.) is a perennial herb in the family Apiaceae. The plant is widely used as a flavouring agent in foods**.** Fennel seeds are hypotensive and diuretic. In this study, the oil from *Foeniculum vulgare* seeds has been analyzed by GC-MS. The GC-MS analysis showed 58 components. Major constituents are : 9- octadecenoic acid (Z)-, methyl ester(35.59%):9, 12-octadecadienoic acid (Z, Z)-, methyl ester (29.36%),and hexadecanoic acid, methyl ester (8.02%). The antmicrobial activity of the essential oil has been assessed. At a concentration of 100mg/ml, the oil showed significant anticandidal activity. It also exhibited good activity against Gram positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative *Escherichia coli.* However it failed to give inhibitory effect against Gram negative *Pseudomonas aeruginosa.*

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

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.) is a perennial herb in the family Apiaceae. The plant is widely used as a flavouring agent in foods.[1,2] Fennel contains some minerals(Ca, K, Na, Fe and P) and vitamins including niacin, riboflavin and thiamine. It also contains protein(9.5%) and fats (10%).[3] Fennel seeds are hypotensive and diuretic. Seed extract has been tested[4] against glaucoma *in vivo*. Fennel essential oil contains,among others, anethole, fenchone, estragol, p-anisaldehyde and α-phellandrene.[2] Anisole is claimed to possess estrogenic properties.[5] The potential pharmacological effects of *Foeniculum vulgare* is mainly associated with the biological activity of the constituents of its volatile oil. Some flavonoids-mainly quercetin and kaempferol conjugates- have been reported from fennel. The antioxidant activity of *Foeniculum vulgare* is associated with the presence of these flavonoids.[6-8] However, a major constituent of fennel-leugeno- has become a cause of concern since the structurally related,methylleugenol is a potential carcinogenic agent.[9]

MATERIALS AND METHODS

Plant material

The seeds of *Foeniculum vulgae* were purchased from the local market,Khartoum,Sudan.The plant material 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* 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* 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 Table 1.

Rate: - ; Tempt.,60.0°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 °C
Injection temperature	280.0 °C
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.(2ml) of the standardized bacterial stock suspension were mixed with (200 ml) of sterile molten agar which was maintained at 45°C. (20 ml) Aliquots of the incubated 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°C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured as average of two replicates.

GC-MS analysis of *Foeniculum vulgare* oil

The essential oil of the medicinally important *Foeniculum vulgare* was investigated. GC-MS analysis showed 58 constituents. Major components are: 9-octadecenoic acid (Z)-, methyl ester(35.59%), 9,12-octadecadienoic acid (Z,Z)-, methyl ester (29.36%),hexadecanoic acid methyl ester (8.02%). The total ion chromatogram is presented in Fig.1, while the oil costituents are displayed in Table 2.

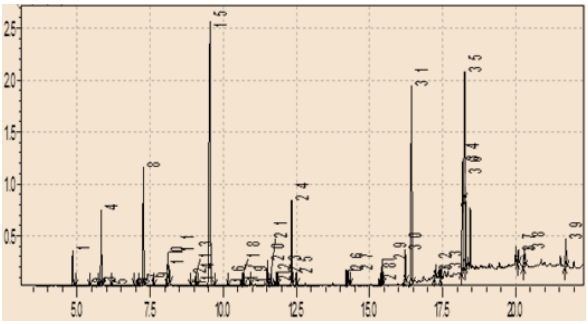
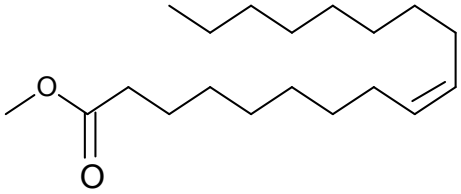


Fig.1: Total ions chromatograms.

Major components are discussed below:

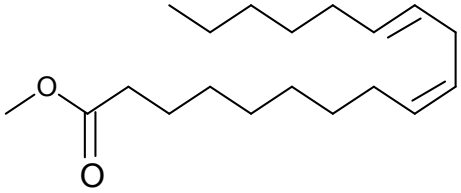
i)- 9-octadecenoic acid (Z)-, methyl ester(35.59%)



9-Octadecenoic acid methyl ester.

Fig. 2 presents the mass spectrum of 9-octadecenoic acid (Z)-, methyl ester. The peak at m/z 296 which appeared at retention time (17.452) is due to M^+ $[C_{19}H_{36}O_2]^+$.The signal at m/z264 is due to loss of methoxyl

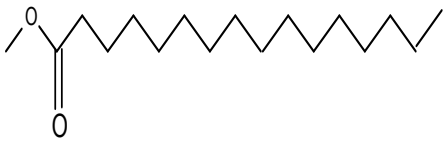
.ii)-9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (29.36%)



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

The mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester is depicted in Fig.3.The signal at m/z 294 (R.T. 17.364) corresponds $M^+[C_{19}H_{34}O_2]^+$ while the signal at m/z263 is due to loss of methoxyl.

iii)-Hexadecanoic acid, methyl ester (8.02%)



Hexadecanoic acid, methyl ester

The mass spectrum of hexadecanoic acid, methyl ester is displayed in Fig.4.The peak at m/z 270 -which appeared at R.T. 15.668 - accounts for the molecular ion: $M^+[C_{17}H_{34}O_2]^{+\cdot}$The peak at m/z239 accounts for loss of methoxyl.

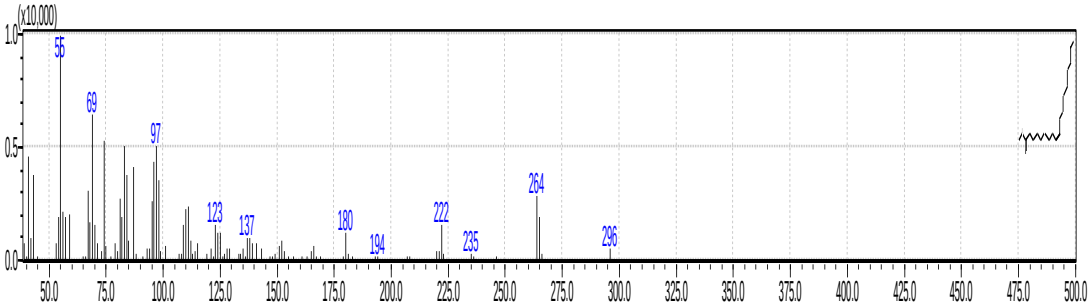


Fig. 2: Mass spectrum of 9-octadecenoic acid methyl ester.

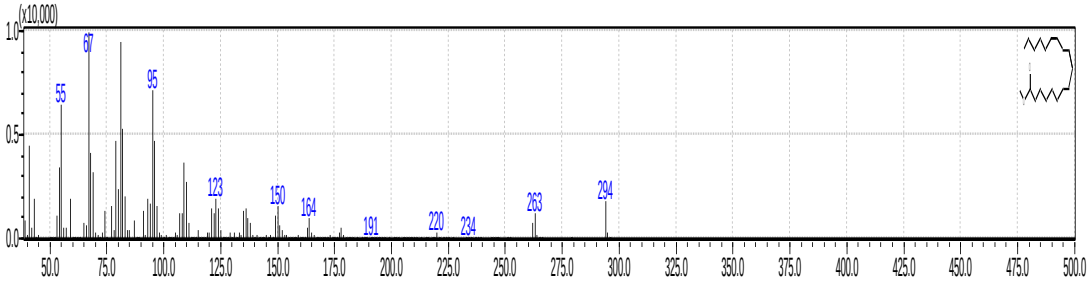


Fig. 3: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester.

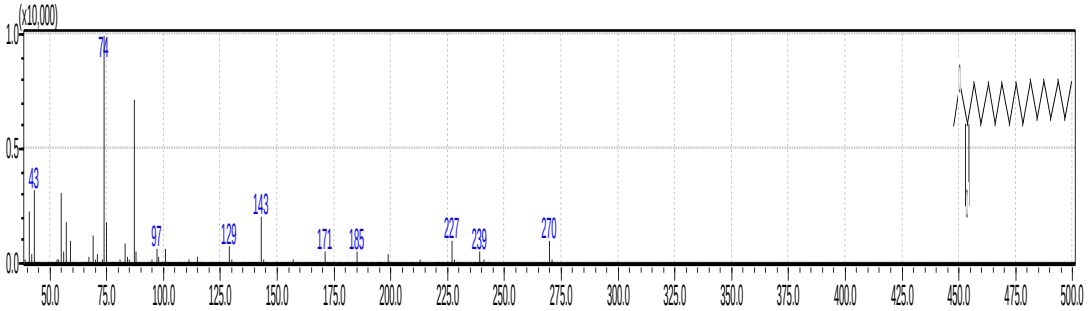


Fig. 4: Mass spectrum of hexadecanoic acid, methyl ester.

Table 2: Constituents of the oil.

No.	Name	Ret.Time	Area%
1.	.alpha.-Pinene	3.541	0.06
2.	Bicyclo[3.1.0]hexane,4-methylene-1-(1-methylethyl)-	4.001	0.09
3.	Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene-, (1S)-	4.070	1.63
4.	.beta.-Myrcene	4.158	0.15
5.	.alpha.-Phellandrene	4.388	0.03
6.	o-Cymene	4.647	1.13
7.	D-Limonene	4.704	0.17
8.	.beta.-Phellandrene	4.725	0.04
9.	Eucalyptol	4.760	0.03
10.	.gamma.-Terpinene	5.100	4.32
11.	1,6-Octadien-3-ol, 3,7-dimethyl-	5.630	0.03
12.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-	5.678	0.02
13.	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	6.801	0.01
14.	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethyl)-	7.019	0.83
15.	Estragole	7.066	0.17
16.	Propanal, 2-methyl-3-phenyl-	7.691	4.97
17.	2-Caren-10-al	8.311	0.64
18.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-	9.569	0.18

19.	Benzaldehyde dimethyl acetal	9.840	0.03
20.	Caryophyllene	10.138	0.10
21.	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	10.253	0.05
22.	(E)-.beta.-Famesene	10.442	0.24
23.	.beta.-copaene	10.792	0.08
24.	Spiro[4.5]dec-7-ene, 1,8-dimethyl-4-(1-methylethenyl)-, [1S-(1.alpha.,4.beta.,5.alpha.)]-	10.827	0.15
25.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-	11.154	0.09
26.	Dodecanoic acid, methyl ester	11.254	0.02
27.	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	11.352	0.02
28.	11,11-Dimethyl-spiro[2,9]dodeca-3,7-dien	12.202	0.02
29.	Carotol	12.354	0.10
30.	Methyl tetradecanoate	13.565	0.15
31.	6-Octadecenoic acid, methyl ester, (Z)-	14.373	0.01
32.	cis-5-Dodecenoic acid, methyl ester	14.479	0.04
33.	Pentadecanoic acid, methyl ester	14.637	0.16
34.	1,4-Eicosadiene	14.769	0.08
35.	7,10-Hexadecadienoic acid, methyl ester	15.365	0.03
36.	7-Hexadecenoic acid, methyl ester, (Z)-	15.450	0.47
37.	9-Hexadecenoic acid, methyl ester, (Z)-	15.469	0.92
38.	Hexadecanoic acid, methyl ester	15.668	8.02
39.	cis-10-Heptadecenoic acid, methyl ester	16.430	0.35
40.	Heptadecanoic acid, methyl ester	16.641	0.12
41.	6,9-Octadecadienoic acid, methyl ester	17.192	0.51
42.	8,11-Octadecadienoic acid, methyl ester	17.245	0.45
43.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.364	29.36
44.	9-Octadecenoic acid (Z)-, methyl ester	17.452	35.59
45.	Methyl stearate	17.587	3.05
46.	8,11-Eicosadienoic acid, methyl ester	18.939	0.15
47.	9,12-Octadecadienoyl chloride, (Z,Z)-	18.973	0.24
48.	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	19.076	2.72
49.	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl-	19.261	0.96
50.	Eicosanoic acid, methyl ester	19.332	0.30
51.	Heneicosanoic acid, methyl ester	20.157	0.03
52.	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	20.252	0.03
53.	Docosanoic acid, methyl ester	20.951	0.17
54.	1,5-Heptadien-4-one, 3,3,6-trimethyl-	21.249	0.05
55.	Tricosanoic acid, methyl ester	21.716	0.05
56.	Tetracontane	22.195	0.15
57.	Tetracosanoic acid, methyl ester	22.455	0.18
58.	Hexatriacontane	23.598	0.26

Antimicrobial assay

Foeniculum vulgar essential oil was investigated for antimicrobial activity via the cup plate agar diffusion bioassay using five standard human pathogens. The average of the diameters of the growth inhibition zones are displayed in Table (3). Results were interpreted as follows: < 9considered inactive; 9-12:weak activity ; 13-18: active and >18: very active. Ampicilin, gentamicin and clotrimazole have been used as positive controls.

Table 3: Inhibition zones(mm/mg sample)

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	15	16	15	--	17
Ampicilin	40	30	15	--	--	--
Gentacycin	40	19	25	22	21	--
Clotrimazole	30	--	--	--	--	38

Sa:*Staphylococcusaureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillusniger*
Ca.:*Candida albicans*
Bs.: Bacillus subtilis

At a concentration of 100mg/ml, the oil showed significant anticandidal activity. It also exhibited good activity against Gram positive *Staphylococcus aureus, Bacillus subtilis* and Gram negative *Escherichia coli.* However it failed to give inhibitory effect against Gram negative *Pseudomonas aeruginosa.*

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