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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *CARUM CARVI* (APIACEAE) FIXED OIL

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ABSTRACT

Carum Carvi seed oil was studied by GC-MS. The oil was also assessed for antimicrobial activity. Fourty five components were detected by GC-MS analysis. Main constituents are: 9-octadecenoic acid (z)-methyl ester (45.82%), 9,12-octadecenoic acid(z,z)-methy1 ester(15%), estragole (11.48%). The antibacterial activity of the oil was evaluated via the diffusion assay against five standard pathogenic bacteria (Gram positive: *Staphylococcus aureus* and *Bacillus subtitlis; Gram* negative: *Esherichia coli* and *Pseudomonasa aeruginosa* and the fungus *Candida albicans).* With the exception of *Bacillus subtilis, Carum carvi* oil showed activity against all test organisms at: 50-25mg/ml.The oil exhibited excellent activity against *Staphylococcus aureus* in the concentration range: 50-25mg/ml. At: 50mg/ml it showed significant activity against the yeast: *Candida albicans*

KEYWORDS: *Carum Carvi,* Fixed Oil, GC-MS analysis, Antimicrobial Activity.

INTRODUCTION

Caraway (*Carum carvi* Linn.) is an erect, branched, biennial plant in the family Umbellifereae. The plant is a globally distributed spice, where it is used in salads and sauces.[1] Fruit is used traditionally for digestive disorders.[2] Seeds are rich in many nutrients including vitamins C, A, E and B complex. Seeds are claimed to treat gastrointestinal disorders and lack of appetite.[2,3]

All parts of the plant are edible, but the fresh leaves and the dried seeds are most traditionally used.

Pharmacological effects of seeds include: antiulcerogenic,[4] antimicrobial,[5,6] antitumor[7] and antioxidant.[8] Aqueous extract of seeds showed a diuretic effect. It also acts as expectorant.[9] The seeds are used by local healers to treat eye ailments, metritis, architis and gastrointestinal disorders.[10] Seeds vapor is used as emmenagague, galactogogue, stimulant, antispasmodic and tonic.[11] Seed oil showed anti-hyperglycemic activity in model animals.[12] *In vivo* studies demonstrated that aqueous extract of seeds showed significant lipid lowering activity.[13] The essential oil from seeds inhibited the growth of pathogenic G^+ and G^- bacteria.[5,6]

MATERIALS AND METHODS

Plant material

Carum Carvi seeds were purchased from the local market - Omdurman, Sudan. The plant was authenticated by Institute of Aromatic and Medicinal Plants,Khartoum, Sudan.

Test microorganisms

Carum Carvi oil was screened for antimicrobial activity using the standard bacterial isolates: *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans.*The agar diffusion bioassay was used.

Methods

Extraction of *Carum Carvi* fixed oil

The seeds of *Carum carvi* (400g) were macerated with n-hexane at ambient temperature for 48h with occasional shaking. The solvent was removed *in vacuo* to afford the oil.

Esterification of the oil, for GC-MS analysis, was accomplished via a methonolic solution of sodium hydroxide and a methanolic suphuric acid.

GC-MS analysis

The oil was analyzed by A Shimadzo Ultra instrument equipped with RTX-5MS column(30,length; 0.25 mm, diameater; 0.25µm, thickness). Analytical grade helium (purity;99.99%) was the carrier gas. Chromatographic conditions are shown below:

Table 1: Oven temperature program.

Rate	Temperature(C)	Hold time (mim.$^{-1}$)
-	60.0	0.00
10.00	300.0	0.00

Table 2: 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

The cork and bore diffusion method was used for screening the antimicrobial activity of the oil. Meuller Hinton and Sabouraud dextrose agars were used as growth media for the bacteria and fungi respectively. The media were prepared according to the manufacturer's instructions.

Briefly, (2ml) of standardized bacterial stock suspension were mixed with (200ml) of sterile molten nutrient agar which was maintained at 45^{o} C. (20ml) 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 (6mm in diameater) were cut using sterile cork borer (No4). Each half was designed for a test solution.

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

RESULTS AND DISCUSSION

GC-MS analysis of *Carum carvi* fixed oil

GC-MS analysis of *Carum carvi* oil was performed. MS library (NIST)[14] was checked for identification of constituents (a 90-95% match was observed). Furthermore, retention time and observed fragmentation pattern were also used for identification purposes. The GS-MS spectrum of the studied oil revealed the presence of 45 components (Table 3 and Fig.1).

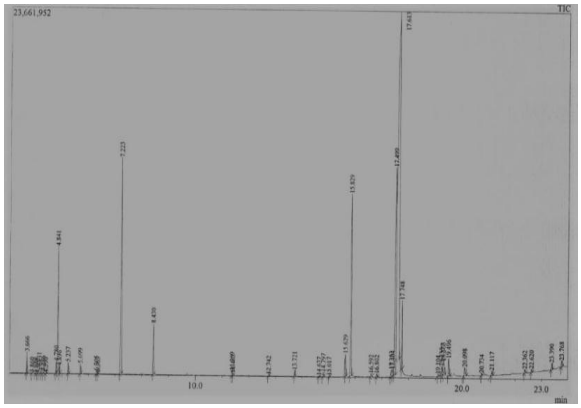


Fig. 1: Total ion chromatograms.

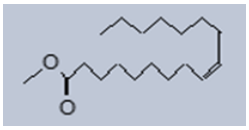
Table 3: Constituent of *Carum carvi* oil.

Peak#	R.Time	Area%	Name
1	3.666	0.65	.alpha.-Pinene
2	3.860	0.01	Camphene
3	3.980	0.01	Benzene, 1-ethyl-3-methyl-
4	4.061	0.00	Benzene, 1,2,3-trimethyl-
5	4.131	0.18	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-me
6	4.202	0.07	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-meth
7	4.286	0.07	.beta.-Myrcene
8	4.390	0.02	Mesitylene
9	4.780	0.40	o-Cymene
10	4.841	4.54	D-Limonene
11	4.896	0.25	Eucalyptol
12	5.237	0.38	.gamma.-Terpinene
13	5.699	0.37	L-Fenchone
14	6.305	0.10	3-Oxatricyclo[4.1.1.0(2,4)]octane, 2,7,7-tri
15	6.363	0.06	4-Cyclohexylidenebutyraldehyde
16	7.223	11.48	Estragole
17	8.430	1.94	Anethole
18	11.369	0.18	Butylated Hydroxytoluene
19	11.403	0.08	Dodecanoic acid, methyl ester
20	12.742	0.05	Apiol
21	13.721	0.23	Methyl tetradecanoate
22	14.637	0.05	cis-5-Dodecenoic acid, methyl ester
23	14.797	0.17	Pentadecanoic acid, methyl ester
24	15.017	0.03	2-Pentadecanone, 6,10,14-trimethyl-
25	15.629	0.83	Methyl 5-eicosenoate
26	15.829	8.91	Hexadecanoic acid, methyl ester
27	16.592	0.13	6-Octadecenoic acid, methyl ester, (Z)-
28	16.802	0.12	Heptadecanoic acid, methyl ester
29	17.353	0.45	Methyl 5,11,14-eicosatrienoate
30	17.404	0.29	6,9-Octadecadienoic acid, methyl ester
31	17.499	15.00	9,12-Octadecadienoic acid (Z,Z)-, methyl e
32	17.613	45.82	9-Octadecenoic acid (Z)-, methyl ester
33	17.748	2.74	Methyl stearate
34	19.104	0.11	Methyl 6,11-octadecadienoate
35	19.235	0.40	2-Hexadecenoic acid, methyl ester, (E)-
36	19.277	0.32	11-Eicosenoic acid, methyl ester
37	19.305	0.94	13-Docosenoic acid, methyl ester
38	19.496	0.76	Methyl 18-methylnonadecanoate
39	20.098	0.55	7-Octadecanone
40	20.734	0.11	9-Octadecenoic acid, 1,2,3-propanetriyl es
41	21.117	0.23	Methyl 20-methyl-heneicosanoate
42	22.362	0.17	Dotriacontane
43	22.620	0.16	Tetracosanoic acid, methyl ester
44	23.390	0.35	Oxirane, heptadecyl-
45	23.768	0.30	Tetracosane
		100.00	

Main constituents of the oil are briefly discussed below:

9-z-Octadecenoic acid methyl ester (45.82%)

Fig.2 shows the mass spectrum of 9-octadecenoic acid methyl ester. The peak at m/z 296 with retention time 17.613, in total ion chromatogram matches: M^{+} $[C_{19}H_{36}O_2]^{+}$, while the peak at m/z266 accounts for loss of methoxyl.



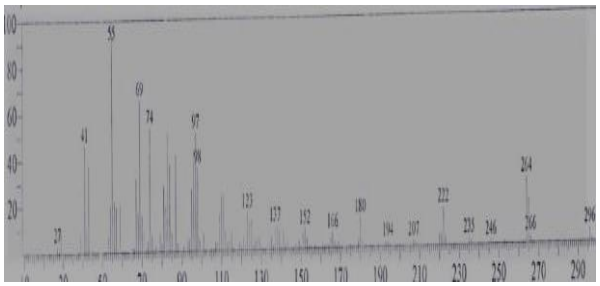
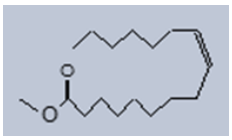


Fig. 2: Mass spectrum of 9-octadecenoic acid methyl ester.

9,12-z,z -Octadecadienoic acid methyl ester (15%)

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



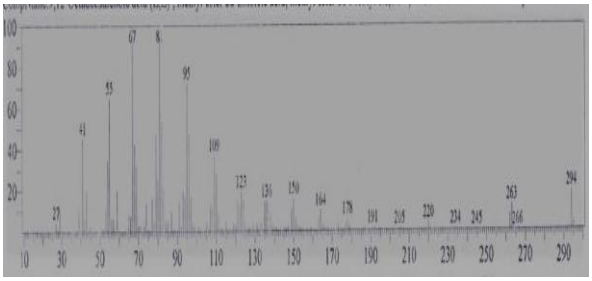
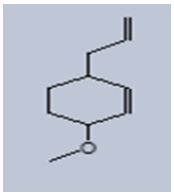


Fig. 3: Mass spectrum of 9,12-z,z-octadecenoic acid methyl ester.

Estragole (11.48%)

Fig.4. shows the mass spectrum of estragole.The peak at m/z148 with R.T.7.223 in total ion chromatogram corresponds $M^{+\cdot}$.



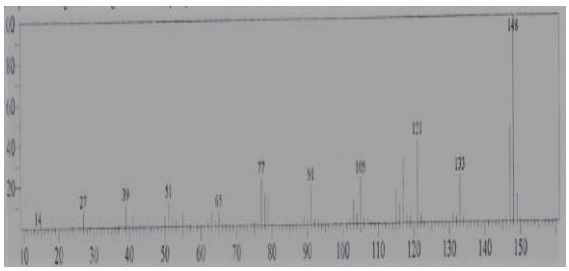


Fig. 4: Mass spectrum of estragole.

Antibacterial activity

Carum carvi oil was screened for antimicrobial activity against five standard pathogenic bacteria. The diameters of the growth of inhibition zones are shown in Table (4).Results of Table 4 were interpreted in conventional terms: (<9mm: inative;9-12mm:partially active;13-18mm: active; 13-18mm: active;>18 very active). Tables (5) and (6) represents the antimicrobial activity of standard drugs.

Table 4: Antibacterial activity of *Carum carvi* oil.

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
oil	50	18	-	16	16	17
	25	17	-	14	15	15
	12.5	16	-	-	14	13
	6.25	12	-	-	-	12

Table 5: Antibacterial activity of standard drug.

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 6: 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*

With the exception of *Bacillus subtilis*, *Carum carvi* oil showed activity against all test organisms at: 50-25mg/ml.The oil exhibited excellent activity against *Staphylococcus aureus* in the concentration range: 50-25mg/ml. At: 50mg/ml it showed significant activity against the yeast: *Candida albicans*.

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