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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SAUDI *RAPHANUS SATIVUS* L. (CRUCIFEREAE) FIXED OIL

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ABSTRACT

Raphanus sativus L. is an economically important vegetable native to Asia and Europe. This plant is known for its health promoting properties and is widely used in Sudanese system of medicine. In this work the seed oil was studied by GC-MS. The oil was also evaluated for antimicrobial activity. Fifteen components were detected by GC-MS analysis. Major constituents are: 13-docosenoic acid methyl ester(35.48%); 9-octadecenoic acid methyl ester(17.12%); cis-11-eicosenoic acid methyl ester(11.64%); 9,12-octadecadienoic acid methyl ester(10.77%); 9,12,15-octadecatrienoic acid methyl ester (7.14%) and hexadecanoic acid methyl ester(5.48%). he antimicrobial activity of the oil was evaluated via the diffusion bioassay against eight standard pathogenic bacteria At a concentration of 100mg/ml the oil showed partial activity against *Acinetobacter baumannii*, *Staphylococcus aureus* and *Aspergillus flavus.*

KEYWORDS: Raphanus sativus, Fixed Oil, GC-MS analysis, Antimicrobial Activity.

INTRODUCTION

Raphanus sativus L. is an economically important vegetable native to Asia and Europe. It grows in temperate climates.[1] The plant is used in ethnomedicine against liver diseases and respiratory disorders.[1] The antimicrobial activity of different extracts of *Raphanus sativus* has been documented.[2-4] Some alkaloids, amino acids, arabinogalactan proteins and enzymes have been isolated from *Raphanus sativus*.[5-17]

Raphanus sativus roots contain organic acids.[18,19] beside some flavonoids and other phenolics.[20-25] Some pectic substances have been reported from the leaves.[26,27]An allosteric enzyme effecter- adenosylmethionine – of remarkable biochemical function has been isolated from *Raphanus sativus* leaves.[28] The plant also contains β-carotene, β-sitosterol beside vitamin C.[19,29] The free radical scavenging capacity of *Raphanus sativus* has been documented.[30] Also the cytotoxicity of this plant has been studied.[6,31] It has been shown that *Raphanus sativus* exhibited a virucidal activity against several enveloped viruses.[32]

MATERIALS AND METHODS

Materials

Plant material

Raphanus sativus seeds were purchased from the local market Rhiad (Saudi Arabia) and authenticated by Dr. A. Abhari, Department of Biotechnology, Taibah University.

Methods

Extraction of oil

Dry powdered seeds (300g) of *Raphanus sativus* were macerated with n-hexane for 72h.The solvent was removed *in vacuo* and the oil was kept in the fridge for further manipulation.

GC-MS analysis

The target oil was analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness). Helium was used as carrier gas. Chromatographic conditions are displayed below.

Table 1: Oven Temperature Program.

Rate	Temperature (°C)	Hold Time (min.-1)
-	150.0	1.00
4.00	300.0	0.00

Column oven temperature: 150.0°C; *Injection temperature*: 300.0°C; *Injection mode*: *Split; 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

Diffusion method was the method used for screening the antimicrobial activity. Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungus respectively. The media were prepared according to the manufacturer's instructions, sterilized at 121°C for 15 minutes, poured into sterile Petri dishes and were allowed to cool and solidify. The sterilized media were sealed with 0.1ml of the standard inoculums of the test microbe (Mueller Hinton agar was sealed with the bacteria and Sabouraud dextrose agar sealed with the fungus). The inoculums were spread over the surface of the medium by the use of a sterile swab. By the use of a standard cork borer of 6mm in diameters, a well was cut at the centre of each inoculated medium. (0.1ml) of the oil (concentration of 100mg/ml) was then introduced into the well on the inoculated medium. Incubation of the inoculated medium was made at 37°C for 24 hours for the bacteria and at 30°C and for 4 days for the fungus. After incubation each plate of the medium was observed for the growth inhibition zone. The zone was measured with a transparent ruler and the results were recorded in millimeters.

RESULTS AND DISCUSSION

The oil from *Raphanus sativus* seeds was analyzed by GC-MS and the constituents of the oil were identified by their observed retention time and mass fragmentation pattern.

GC-MS analysis of *Raphanus sativus* oil

The GC-MS analysis of the target oil showed 15 components dominated by:-13-docosenoic acid methyl ester(35.48%); 9-octadecenoic acid methyl ester (17.12%); cis-11-eicosenoic a cid methyl ester(11.64%); 9,12-octadecadienoic acid methyl ester (10.77%); 9,12,15-octadecatrienoic acid methyl ester (7.14%) and hexadecanoic acid methyl ester(5.48%). The total ion chromatograms of *Raphanus sativus* oil is shown in Fig. 1, while the different constituents of the oil are given in Table 1.

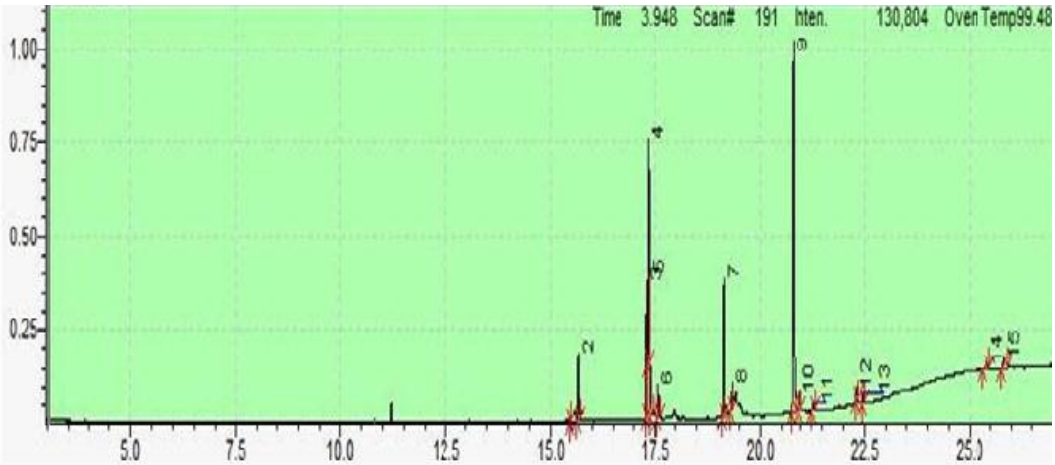

Fig. 1: Total ion chromatogram of *Raphanus sativus* oil.

Table 1: Constituents of *Raphanus sativus* oil.

Peak#	R.Time	Area	Area%	Name
1	15.472	61565	0.11	9-Hexadecenoic acid, methyl ester, (Z)-
2	15.664	3157853	5.48	Hexadecanoic acid, methyl ester
3	17.316	6204550	10.77	9,12-Octadecadienoic acid (Z,Z)-, methyl e
4	17.361	9856155	17.12	9-Octadecenoic acid (Z)-, methyl ester
5	17.385	4110173	7.14	9,12,15-Octadecatrienoic acid, methyl este
6	17.579	1415747	2.46	Methyl stearate
7	19.140	6705320	11.64	cis-11-Eicosenoic acid, methyl ester
8	19.338	1336160	2.32	Eicosanoic acid, methyl ester
9	20.786	20431171	35.48	13-Docosenoic acid, methyl ester, (Z)-
10	20.961	798998	1.39	Docosanoic acid, methyl ester
11	21.288	468825	0.81	Ethyl 9-hexadecenoate
12	22.312	695356	1.21	15-Tetracosenoic acid, methyl ester, (Z)-
13	22.468	544165	0.94	Tetracosanoic acid, methyl ester
14	25.432	767230	1.33	Olean-12-en-28-oic acid, 2.beta.,3.beta.,23-
15	25.844	1032770	1.79	Urs-12-en-28-al
		57586038	100.00	

The major components of *Raphanus sativus* oil are listed below

i)-13-Docosenoic acid methyl ester (35.48%)

In Fig. 2, the peak at m/z 352(RT,20.786) corresponds the molecular ion: $M^{+[}C_{23}H_{44}O_2]^+$. The signal at m/z 321 is due to loss of a methoxyl.

ii) 9-Octadecenoic acid(z)methyl ester (17.12%)

In figure 3, the peak at m/z 296(RT,17.361) corresponds to the molecular ion: $M^+[C_{19}H_{36}O_2]^+$, while the signal at m/z 265 accounts for loss of a methoxyl function.

iii) Cis-11-Eicosenoic acid methyl ester(11.64%)

In Fig. 4, the peak at m/z 324 which appeared at RT,19.140 accounts for the molecular ion: $M^+[C_{21}H_{40}O_2]^+$ while the signal at m/z 293 corresponds to loss of a methoxyl.

iv) 9,12-Octadecadienoic acid methyl ester (10.77%)

The mass spectrum of 9, 12-octadecadienoic acid methyl ester is displayed in Fig.5. The peak at m/z 294(RT, 17.316) corresponds to $M^+[C_{19}H_{34}O_2]^+$, while the signal at m/z 263 corresponds to loss of a methoxyl

(v) 9,12,15-octadecatrienoic acid,methyl ester (7.14%)

In Figure 6 ,the peak at m/z 292 which appeared at RT 17.385 is attributed to the molecular ion: $M^+[C_{19}H_{32}O_2]^+$.The signal at m/z 261 is due to loss of a methoxyl function.

v)-Hexadecanoic acid,methyl ester(5.48%)

The mass spectrum of hexadecanoic acid, methyl ester is displayed in Fig. 7. The peak at m/z 270(RT, 15.664) is due to the molecular ion: $M^+[C_{17}H_{34}O_2]^+$.The signal at m/z 239 corresponds to loss of a methoxyl group.

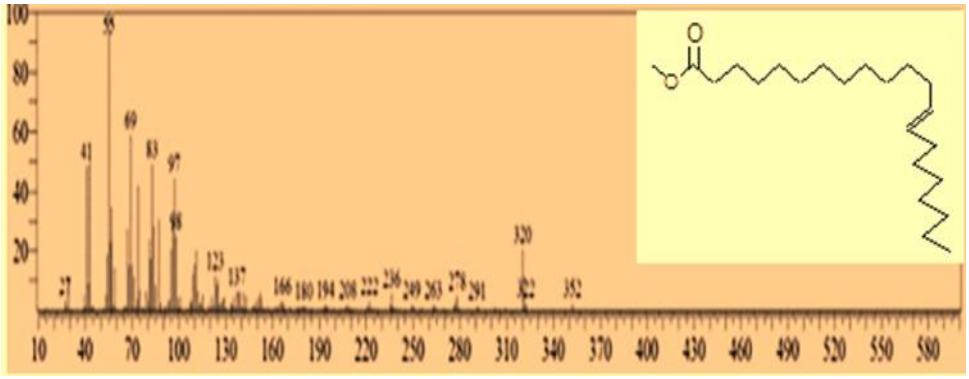

Fig. 2: The mass spectrum of 13- docosenoic acid methyl ester.

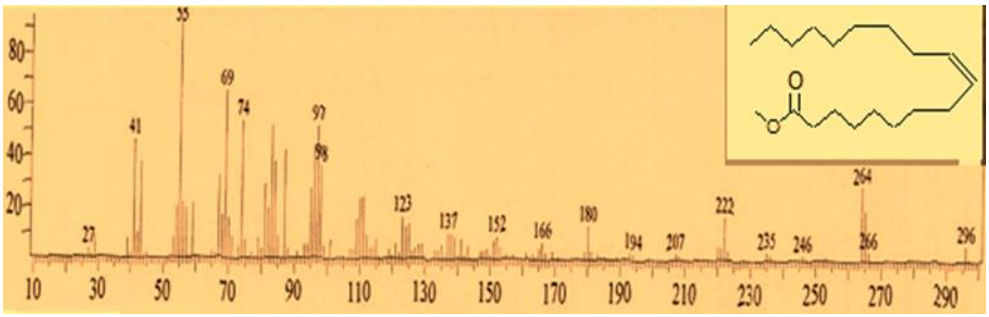

Fig. 3: The mass spectrum of 9-octadecenoic acid (z)methyl ester.

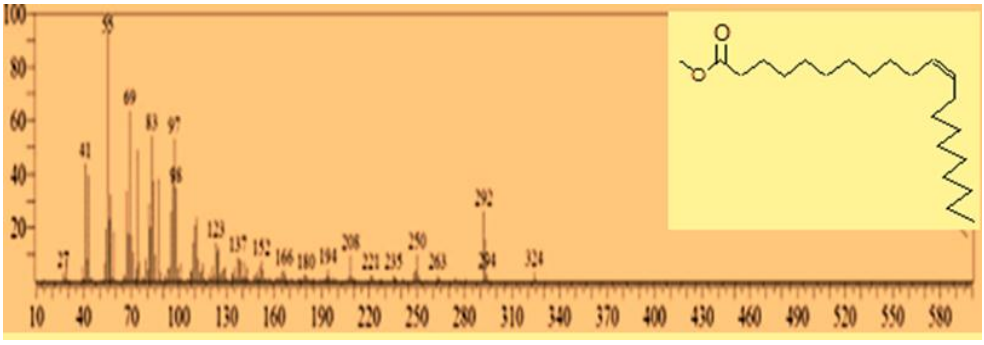

Fig. 4: The mass spectrum of cis-11-eicosenoic acid methyl ester.

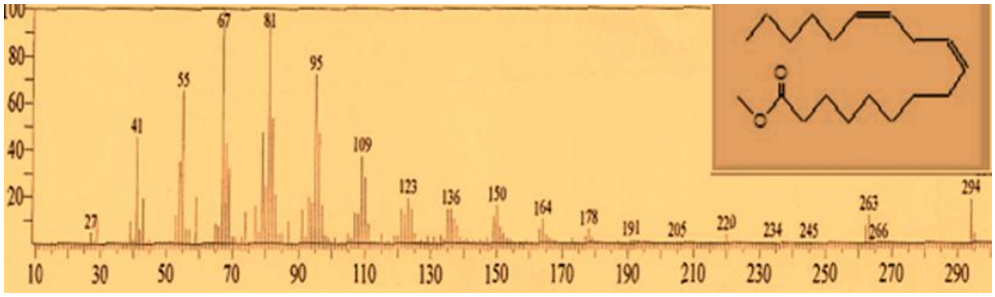

Fig. 5: The mass spectrum of 9,12-octadecadienoic acid methyl ester.

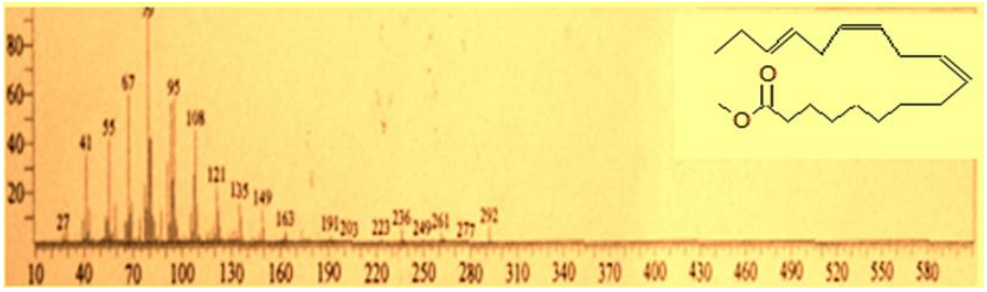

Fig. 6: The mass spectrum of 9,12,15-octadecatrienoic acid methyl ester.

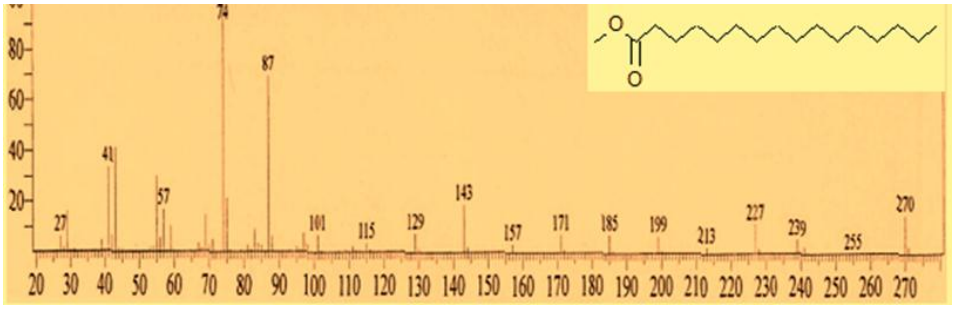

Fig. 7: The mass spectrum of hexadecanoic acid methyl ester.

Table 2: Minimum inhibition zone (mm).

	Concn. mg/ml					
	oil			Amp*	Kan*	Nys*
Strain	0%	50%	100%	10	10	10
Escherichia coli	-	6±0.1	6±0.7	25±0.4	21±0.3	N
Klebsiella pneumoniae	-	6±0.2	7±0.6	22±0.4	24±0.0	N
Acinetobacter baumannii	-	7±0.4	10±0.2	22±0.3	16±0.3	N
Pseudomonas aureginosa	-	6±0.1	7±0.6	29±0.3	11±0.3	N
Staphylococcus aureus	-	6±0.6	9±0.6	32±0.5	19±0.5	N
Bacillus subtilis	-	6±0.1	8±0.6	19±0.4	21±0.4	N
Candida albicans	-	6±0.1	7±0.6	N	N	12±0.5
Aspergillus flavus	-	6±0.7	9±0.6	N	N	15±0.5

-: no activity, N: Not Valid

* = +ve control

Antimicrobial activity

Raphanus sativus oil was assessed for antimicrobial activity via the cup plate agar diffusion method using eight standard human pathogens. Table (2) displays the average of the diameters of the growth inhibition zones. Results were interpreted as follows: *<9mm: inative; 9-12mm: partially active;13-18mm: active; >18mm: very active.*

At a concentration of 100mg/ml, the oil showed partial activity against *Acinetobacter baumannii, Staphylococcus aureus* and *Aspergillus flavus.*

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