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Research Article

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GC-MS analysis and Antimicrobial Activity of Saudi *Eruca sativa* (Brassicaceae) Fixed Oil

Sufian A[1], Abdel Karim M[2]*, Weam AK[3]

[1]Taiba University,Faculty of Science, Dept. of Chemistry
[2]Sudan University of Science and Technology, Faculty of Science, Sudan
[3]Omdurman Islamic University, Faculty of Pharmacy

Abstract *Eruca sativa* seed oil was studied by GC-MS. The oil was also evaluated for antimicrobial activity. Sixteen components were detected by GC-MS analysis being dominated by: 13-docosenoic acid methyl ester (33.72%). The antibacterial activity of the oil was evaluated via the disc diffusion bioassay against. The oil showed significant activity against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.* However, it showed partial activity against *Bacillus subtilis* and it was inactive against the yeast *Candida albicans*.

Keywords *Eruca sativa* , Fixed Oil, GC-MS analysis, Antimicrobial Activity

Introduction

Eruca sativa is a perennial plant in the family Brassicaceae native to the Mediterranean region and globally cultivated for its economic value and health promoting properties [1,2]. It has been reported that *Eruca sativa* has depurative, tonic, rubefacient, laxative, emollient and diuretic effects [3-6]. The anticancer properties of this herb has been documented [7,8]. Beside vitamin C, *Eruca sativa* contains flavonoids, carotenoids and glucosinolates which are known for their bioactivity [9-13]. It has been shown that *Eruca sativa* seeds have a free radical scavenging capacity and can protect against mercuric chloride – mediated renal toxicity2.Different extracts of *Eruca sativa* showed significant antimicrobial [14-16], antifngal [17] and fungicidal properties [18,19] beside insecticidal activity.

Materials and Methods

Plant Material

Eruca sativa seeds were purchased from the local market Riyadh, Saudi Arabia. The Plant was authenticated by Dr. Ahmed, A., Department of Biotechnology, Taiba University, Faculty of Science.

Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 025μm, thickness) was used for GC-MS analysis.

Test organisms

Eruca sativa oil was screened for antibacterial and antifungal activities using the standard microorganisms; G-ve: *Escherichia coli, Pseudomonas aureginosa,* ; G+ve : *Staphylococ*us *aureus* , *Bacillus subtilis* and the fungal species: *Candida albicans.*

Methods

Extraction of oil

Powdered seeds of *Eruca sativa* (250g) were exhaustively extracted with n-hexane at room temperature. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4 ^{o}C.

The oil (2ml) was placed in a test tube and 7ml of alcoholic sodium hydroxide were added followed by 7ml of alcoholic sulphuric acid. The tube was stoppered and shaked vigorously for five minutes and then left overnight. 2ml of supersaturated sodium chloride were added, then 2ml of normal hexane were added and the tube was vigorously shaked for five minutes .The hexane layer was then separated.(5µl) of the hexane extract were mixed with 5ml diethyl ether . The solution was filtered and the filtrate (1µl) was injected in the GC-MS vial.

GC-MS analysis

The oil of *Eruca sativa* was analyzed by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument. Chromatogrphic conditions are shown below:

Oven temperature program:

Rate	**Temperature**	**Hold time (per min.)**
1.00	-	150.0
0.00	40	300.0

Chromatographic conditions:

Column oven temperature	:	150 °C
Injection temperature	:	300 °C
Injection mode	:	Split
Flow control mode	:	Linear velocity
Pressure	:	139.3 KPa
Total flow	:	50.0 ml/min
Column flow	:	1.54 ml/sec
Linear velocity	:	47.2 cm/sec
Purge flow	:	3.0 ml/min
Split ratio	:	-1.0

Testing of antimicrobial susceptibility

Mueller Hinton (MH) agar and Sabouraud dextrose agars were used as media for growth of bacteria and fungi respectively. They were prepared according to the manufacturer instructions.

The disc diffusion bioassay was used to assess the antibacterial potency of the oil. Bacterial suspension was diluted with sterile physiological solution to 10^{8}cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of the bacterial suspension were swabbed uniformly on surface of MH-agar and allowed to dry for 5 minutes. Sterilized filter

paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MH-agar and soaked with (20 µl) of the test solution. The inoculated plates were incubated at 37 °C for 24h. The diameters (mm) of the inhibition zones were measured in duplicates and averaged.

The above procedure was also used for antifungal activity, but instead of Muller Hinton agar, Sabouraud dextrose agar was used. Samples were used here by the same concentrations used above.

Results and Discussion

GC-MS analysis of the oil

The GC-MS analysis of *Eruca sativa* oil showed 16 components dominated by 13-docosenoic acid methyl ester (33.72%), cis- 13-eicosenoic acid methyl ester (14.67%)**,** 9- octadecenoic acid (Z)- methyl ester (13.21%), 9,12-octadecenoic acid (Z,Z)-, methyl ester (11.08%)**,** 9,12,15- octadecatrienoic acid methyl ester (8.57%) and

hexadecanoic acid, methyl ester (6.48%) .The total ions chromatograms is shown in Figure 1 and the constituents of the oil are illustrated in Table 1.

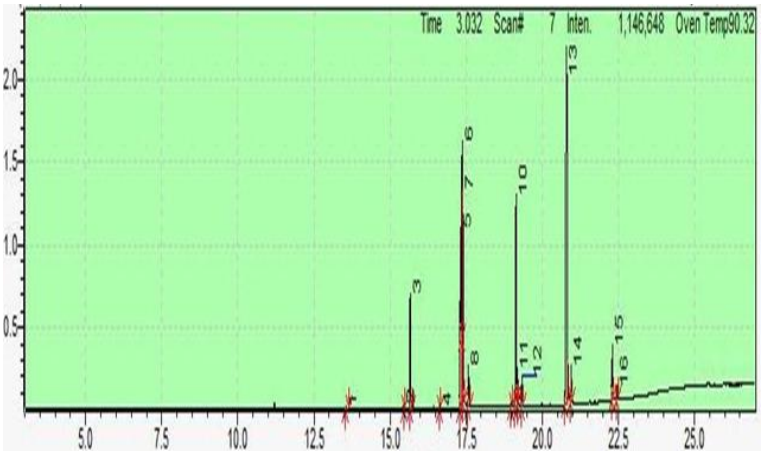

Figure 1: Total ions chromatograms of Eruca sativa oil

Table 1: Constituents of *Eruca sativa* oil

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	13.569	150412	0.08	Methyl tetradecanoate
2	15.471	434850	0.24	9-Hexadecenoic acid, methyl ester, (Z)-
3	15.664	11899523	6.48	Hexadecanoic acid, methyl ester
4	16.642	46679	0.03	Heptadecanoic acid, methyl ester
5	17.321	20347539	11.08	9,12-Octadecadienoic acid (Z,Z)-, methyl e
6	17.374	24248131	13.21	9-Octadecenoic acid (Z)-, methyl ester
7	17.395	15728679	8.57	9,12,15-Octadecatrienoic acid, methyl este
8	17.580	4080332	2.22	Methyl stearate
9	18.994	622158	0.34	.gamma.-Linolenic acid, methyl ester
10	19.143	26932687	14.67	cis-13-Eicosenoic acid, methyl ester
11	19.192	3594527	1.96	cis-11-Eicosenoic acid, methyl ester
12	19.337	3072317	1.67	Eicosanoic acid, methyl ester
13	20.806	61902166	33.72	13-Docosenoic acid, methyl ester, (Z)-
14	20.961	3523364	1.92	Docosanoic acid, methyl ester
15	22.311	5806666	3.16	15-Tetracosenoic acid, methyl ester, (Z)-
16	22.466	1208209	0.66	Tetracosanoic acid, methyl ester
		183598239	100.00	

The major components of *Eruca sativa* oil are briefly discussed below:

a) 13-Docosenoic acid, methyl ester (33.72%)

In Fig. 2, the peak at m/z 352(RT, 20.806) corresponds to the molecular ion: $M^+ [C_{23} H_{44} O_2]^+$. The signal at m/z 322 accounts for loss of a methoxyl.

b) cis- 13- Eicosenoic acid, methyl ester (14.67%)

In Fig. 3, the peak at m/z 324(RT, 19.143) is due to $M^+ [C_{21} H_{40} O_2]^+$. The signal at m/z 294 corresponds loss of a methoxyl function.

c) 9- octadecenoic acid (Z)-, methyl ester (13.21%)

The peak at m/z 296 (Fig.4) with retention time 17.374 accounts for the molecular ion: $M^+ [C_{19} H_{36} O_2]^+$, while the signal at m/z 266 is attributed to loss of a methoxyl.

d)-9, 12- octadecadienoic acid (Z, Z)-, methyl ester (11.08%)

In Fig. 5, the signal which appeared at m/z 294(RT,17.321) corresponds $M^+ [C_{19} H_{34} O_2]^+$, while the signal at m/z 263 corresponds loss of a methoxyl group.

e)-9, 12, 15- Octadecatrienoic acid, methyl ester (8.57%)

The molecular ion: $M^+ [C_{19} H_{32} O_2]^+$ appeared at m/z 292 (Fig. 6) with retention time 17.395.The signal at m/z 261 is due to loss of a methoxyl function.

f) Hexadecanoic acid, methyl ester (6.48%)

In Fig. 7, the peak at m/z 270(RT, 15.664) corresponds to the molecular ion $M^+ [C_{19} H_{38} O_2]^+$, whilst the signal at m/z 239 accounts for a methoxyl.

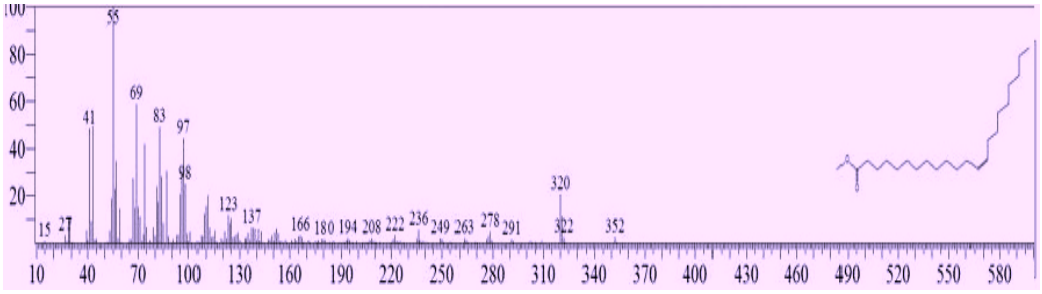

Figure 2: The mass spectrum of 13- docosenoic acid, methyl ester

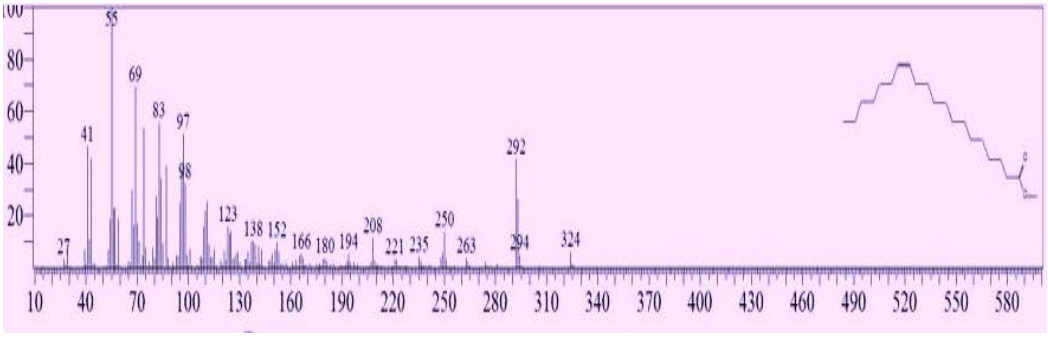

Figure 3: The mass spectrum of cis- 13- eicosenoic acid, methyl ester

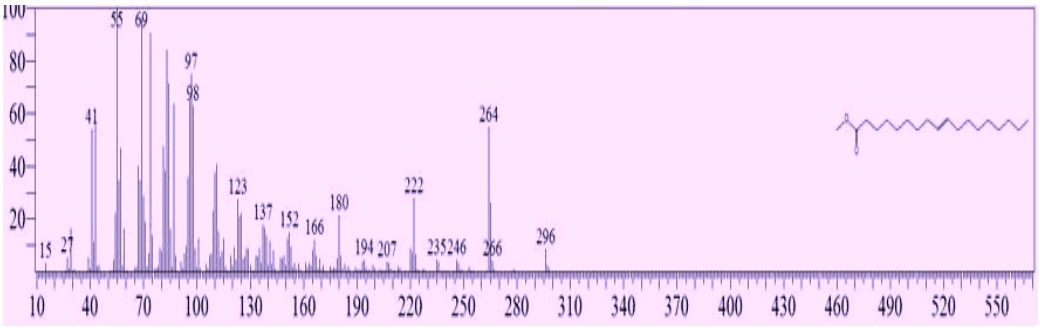

Figure 4: Mass spectrum of 9- octadecenoic acid (Z)-, methyl ester

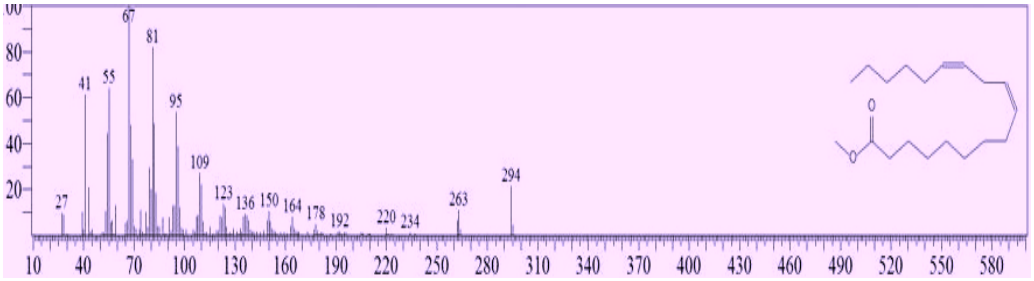

Figure 5: Mass spectrum of 9, 12- octadecadienoic acid (Z, Z)-, methyl ester

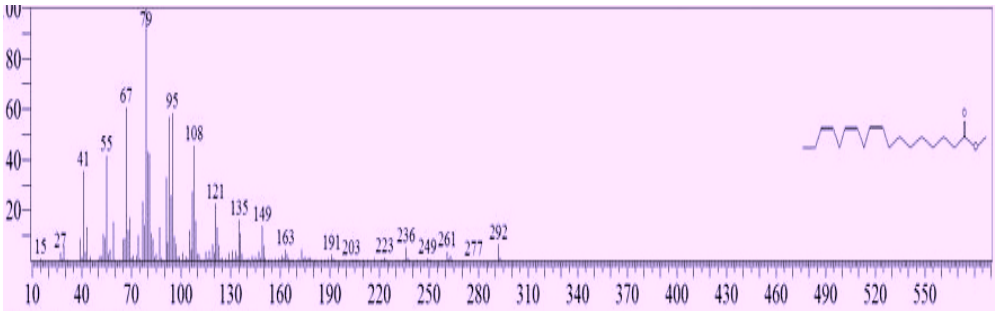

Figure 6: Mass spectrum of 9, 12, 15- octadecatrienoic acid, methyl ester

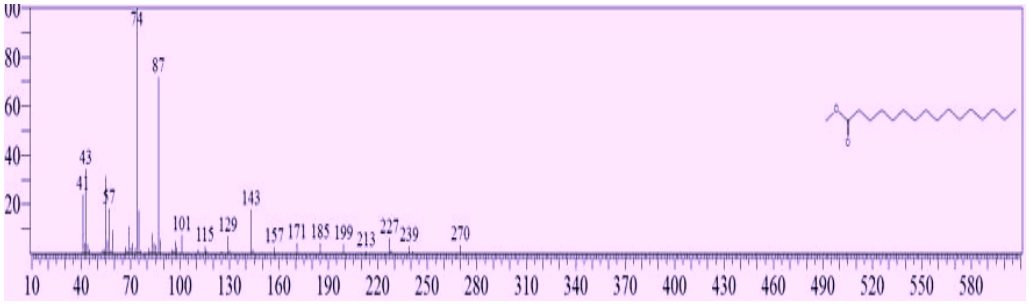

Figure 7: Mass spectrum of hexadecanoic acid, methyl ester

Antimicrobial activity

Eruca sativa oil was assessed for antimicrobial activity via the disc diffusion assay using five standard human pathogens ; G-ve: *Escherichia coli, , Pseudomonas aureginosa* ; G+ve : *Staphylococus aureus* , *Bacillus subtilis* and the fungal species: *Candida albicans*. The inhibition zones are illustrated in Table 2. Ampiclin and nystatin were used in this study as positive controls. At 100mg/ml, the oil showed significant activity against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.* However, it showed partial activity against *Bacillus subtilis* and it was inactive against the yeast *Candida albicans*.

Table 2: Diameter of inhibition zone (mm)

		Oil		Amp
	Concentration(µg/ml)	**100**	**10**	**10**
Gram -ve	*Escherichia coli*	18	25	-
Gram -ve	*Pseudomonas aureginosa*	23	29	-
Gram +ve	*Staphylococus aureus*	20	32	-
Gram +ve	*Bacillus subtilis*	12	19	-
Fungal	*Candida albicans*	-	N	12

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