World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE ERUCA SATIVA (BRASSICACEAE) FIXED OIL

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Article Received on 13/09/2017 **Article Revised on 04/10/2017** **Article Accepted on 25/10/2017**

ABSTRACT

Eruca sativa seed oil was studied by GC-MS. The oil was also evaluated for antimicrobial activity. Twenty five components were detected by GC-MS analysis. Main constituents are: 13-docosenoic acid methyl ester (34.32%); (z,z)-9,12-octadecadienoic acid methyl ester(11.80%); cis-13-eicosenoic acid methyl ester(11.05%) ; 9-octadecenoic acid methyl ester (10.76%) ; hexadecanoic acid, methyl ester(6.98%) ; 9,12,15-octadecatrienoic acid methyl ester (6.41%) The antibacterial activity of the oil was evaluated via the disc diffusion bioassay against five standard pathogenic bacteria (Gram positive: *Staphylococcus aureus* and *Bacillus subtitlis; Gram* negative : *Esherichia coli* and *Pseudomonasa aeruginosa* and the fungus *Candida albicans*). The oil showed excellent 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 (also known as rocket) is a yearly-grown vegetable in the order Brassilicales of the family Brassicaceae.[1] The plant is native to the Mediterranean region[2]. The leaves and seeds contain-among others-alkaloids, saponins, tannins, flavonoids, cardiac glycosides, ascorbic acid and essential oil.[3,4,5] The plant is rich in vitamins C,K and A.[2]

The antimicrobial activity of *Eruca sativa* essential oil has been studied. This activity is probably due to the high content of erucic acid.[4] Seed extracts exhibited different antimicrobial responses and the methanol extract showed moderate activity.[6] The plant also showed free radical scavenging capacity[7,8,9] attributed to the presence of flavonoids, β-carotene and zeaxanthin. *Eruca sativa* essential oil reduced the effects of diabetes mellitus in model animals.[10] The essential oil is used traditionally against dandruff.[11] The insecticidal properties of *Eruca sativa* has been documented.[12,13]

MATERIALS AND METHODS

Plant material

Eruca sativa seeds were purchased from the local market, Khartoum, Sudan. The Plant was authenticated by Institute of Aromatic and Medicinal Plants-Khartoum, Sudan.

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 shown in Table (1).

Table 1: Test organisms.

Ser. No	Micro organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
5	*Candida albicans*	fungi

Methods

Extraction of oil

Powdered seeds of *Eruca sativa* (500g) were exhaustively extracted with n-hexane (soxhlet).The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further manipulation.

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 (min^{-1})
-	60.0	0.00
10.00	300.0	0.00

Table 2: Chromatographic conditions.

Table 2 : Chromatographic conditions

Column oven temperature	150.0°C
Injection temperature	300.0°C1-
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.
Split ratio	-1

Testing of anticrobial susceptibility

Muller Hinton 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 10^8cfu/ 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 in the inverted position. 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 oil

Eruca sativa seed oil was analyzed by GC-MS and the MS library was checked for identification of constituents. The analysis showed the presence of 25 components (Table 3). The typical total ion chromatograms (TIC) is shown in Fig.1.

Table 3: Contituents of *Eruca sativa* seed oil.

k#	R.Time	Area	Area%	Name
1	11.247	66488	0.02	Dodecanoic acid, methyl ester
2	13.559	716465	0.19	Methyl tetradecanoate
3	14.634	173673	0.05	Pentadecanoic acid, methyl ester
4	15.362	316919	0.08	7,10-Hexadecadienoic acid, methyl ester
5	15.428	703422	0.18	7-Hexadecenoic acid, methyl ester, (Z)-
6	15.466	1788082	0.47	9-Hexadecenoic acid, methyl ester, (Z)-
7	15.663	26587415	6.98	Hexadecanoic acid, methyl ester
8	16.429	377975	0.10	cis-10-Heptadecenoic acid, methyl ester
9	16.636	336216	0.09	Heptadecanoic acid, methyl ester
10	17.325	44978732	11.80	9,12-Octadecadienoic acid (Z,Z)-, methyl e
11	17.391	41010732	10.76	9-Octadecenoic acid (Z)-, methyl ester
12	17.410	24410432	6.41	9,12,15-Octadecatrienoic acid, methyl este
13	17.575	10613663	2.79	Methyl stearate
14	18.984	1099322	0.29	.gamma.-Linolenic acid, methyl ester
15	19.146	42113812	11.05	cis-13-Eicosenoic acid, methyl ester
16	19.190	9579059	2.51	cis-11-Eicosenoic acid, methyl ester
17	19.330	7965350	2.09	Eicosanoic acid, methyl ester
18	20.824	130774474	34.32	13-Docosenoic acid, methyl ester, (Z)-
19	20.954	9151644	2.40	Docosanoic acid, methyl ester
20	21.548	828323	0.22	cis-10-Nonadecenoic acid, methyl ester
21	21.714	428259	0.11	Tricosanoic acid, methyl ester
22	22.299	14194658	3.73	15-Tetracosenoic acid, methyl ester, (Z)-
23	22.451	4466316	1.17	Tetracosanoic acid, methyl ester
24	23.033	2722630	0.71	Stigmasterol
25	23.863	5646325	1.48	.gamma.-Sitosterol
		381050386	100.00	

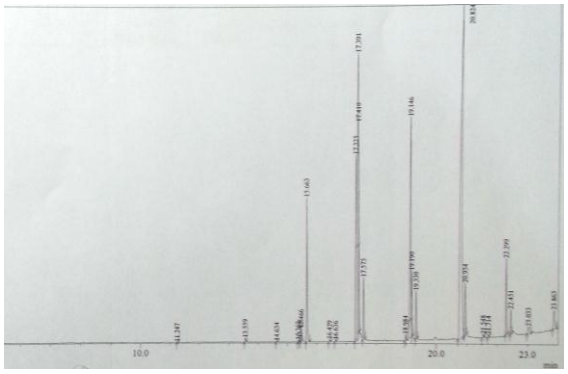

Fig. 1: Typical total ion chromatograms.

Some important constituents are discussed below

13-Docosenoic acid methyl ester (34.32%)

The EI mass spectrum of 13-docosenoic acid methyl ester is displayed in Fig.2. The peak at m/z352 which appeared at R.T.20.824 in total ion chromatogram, corresponds $M^+[C_{23}H_{44}O_2]^+$. The peak at m/z322 corresponds to loss of a methoxyl function.

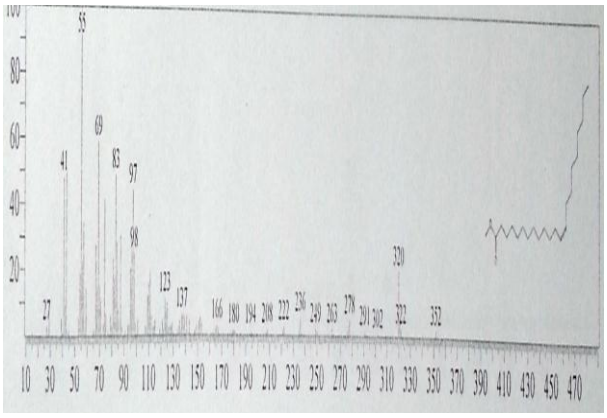

Fig. 2: Mass spectrum of 13-docosenoic acid methyl ester.

(z,z)-9, 12-octadecadienoic acid methyl ester (11.80%)

Fig. 3 shows the mass spectrum of 9, 12-octadecadienoic acid (z,z)-,methyl ester. The signal at m/z294 (R.T.17.325) corresponds $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z263 is due to loss of a methoxyl function.

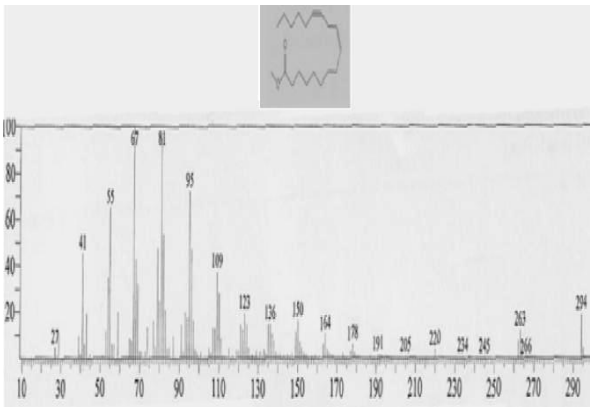

Fig. 3: Mass spectrum of 9, 12-octadecadienoic acid methyl ester.

Cis-13-Eicosenoic acid methyl ester (11.05%)

The mass spectrum of cis 13-eicosenoic acid methyl ester is depicted in Fig.4. The peak at m/z324 which appeared at R.T.19.146 corresponds $M^+[C_{21}H_{40}O_2]^+$. The signal at m/z292 accounts for loss of a methoxyl.

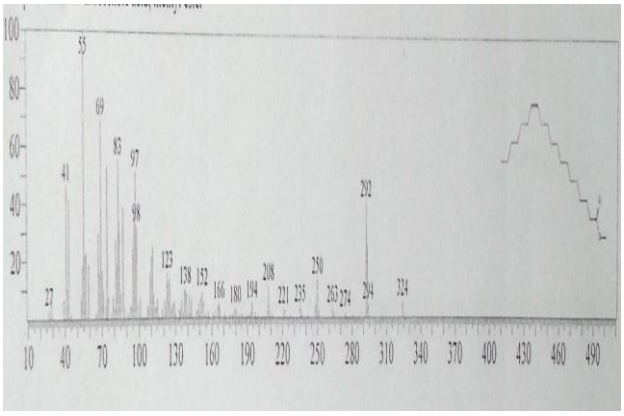

Fig. 4: Mass spectrum of cis -13-docosenoic acid methyl ester.

9-octadecenoic acid methyl ester (10.76%)

In Fig. 5, the peak at m/z 296 (R.T.17.391) corresponds $M+[C_{19}H_{36}O_2]^+$, while the peak at m/z266 accounts for loss of a methoxyl group.

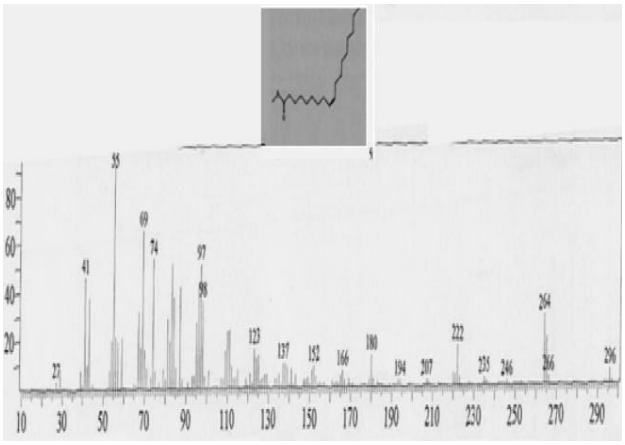

Fig. 5: Mass spectrum of 9-octadecenoic acid methyl ester.

Hexadecanoic acid, methyl ester (6.98%)

The mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.6. Peak at m/z270 (R.T.15.663) corresponds $M^+[C_{17}H_{34}O_2]^+$ .The signal at m/z239 is attributed to loss of a methoxyl function.

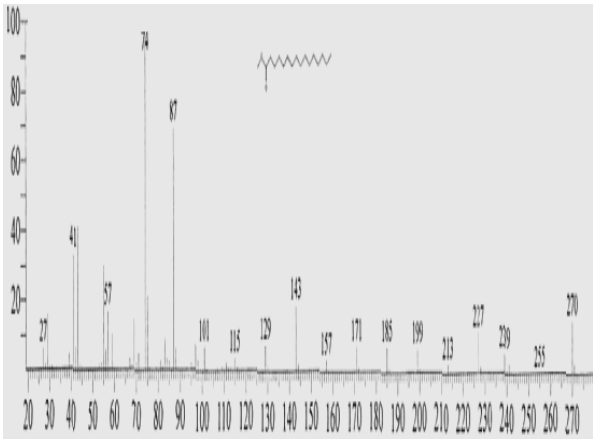

Fig. 6: Mass spectrum of hexadecanoic acid methyl ester.

9, 12, 15-Octadecatrienoic acid methyl ester (6.41%)

Fig.7 shows the mass spectrum of 9, 12, 15-Octadecatrienoic acid methyl ester. The signal at m/z292(R.T.17.410) corresponds $M^+[C_{19}H_{32}O_2]^+$,

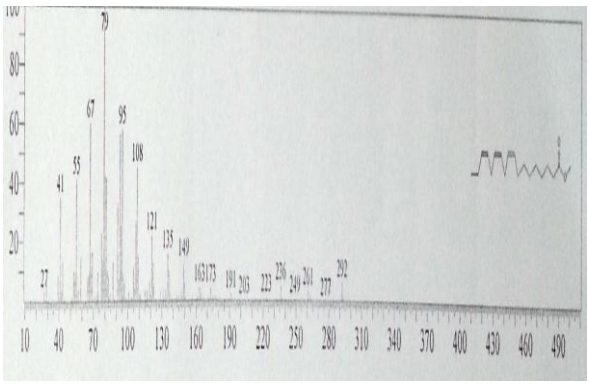

Fig. 7: Mass spectrum of 9, 12, 15-octadecatrienoic acid.

Antimicrobial activity

The oil was screened for antimicrobial activity against standard microorganisms using the disc diffusion bioassay. The average of the diameters of the growth inhibition zones are shown in Table (4) .The results were interpreted as follows: (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (5) and (6) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 4: Antibacterial activity of *Eruca sativa*: M. D. I. Z (mm).

Type	Conc.mg/ml	Sa	Bs	Ec	Ps	Ca
Oil	100	20	12	18	23	-

Table 5: Antibacterial activity of standard drugs.

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

Table 6: Antifungal activity of standard drug.

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
M.D.I.Z: Mean diameter or growth inhibition zone (mm).

The oil showed excellent 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*.

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