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CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF *BRASSICA JUNCEA* L. CZERN. COSS. (BRASSICACEAE) MARKETED IN SUDAN

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ABSTRACT

Brassica juncea is a herb in the family (Brassicaceae). For centuries, *Brassica juncea* has been used as a natural remedy. Seeds are traditionally used against rheumatism, vomiting and jaundice. Seeds, mixed with *moringa olefera*, is a remedy for spleen and liver diseases. In this study *Brassica juncea* seed oil was analyzed by GC-MS which revealed 15 constituents. Major components of the oil are: 13-docosenic acid methyl ester(43.61%); 9 12-octadecadienoic acid methyl ester(17.50%); 9 12 15-octadectrienoic acid methyl ester(12.49%);9-hexadecenoic acid methyl ester(8.11%) and cis-13-eicosenoic acid methyl ester(7.83%). The oil was assessed for antimicrobial activity against five standard human pathogens.It exhibited partial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. However, it failed to exhibit activity against *Staphylococcus aureus* and the yeast *Candida albicans*.

KEYWORDS: Brassica juncea, Oil, Constituents, Antimicrobial Activity.

INTRODUCTION

Brassica is a genus comprising more than 150 species in the family Brassicaceae. Diffferent *Brassica* species are cultivated worldwide for their economic value. Leaves of these plant species are edible and diverse medicinal uses of seeds are known in many communities.

Brassica juncea **L. Czern. Coss.** is a palnt in the mustard family.The plant is widely cultivated in many countries for its nutritive and medicinal values.[1] For centuries *Brassica juncea* hs been used as a natural remedy. Seeds are traditionally used against rheumatism, vomiting and jaundice.[2-4] Seeds mixed with *moringa olefera* is a remedy for spleen and liver diseases.[5] Some *Brassica juncea* preparations have been used as diuretic, liver – bile stimulant and laxative.[6] Seeds are used by local healers against abscesses, cold, rheumatism and lumbago.[7] A Paste made from seeds is used as treatment for arthritis, backache, stye, paralysis and edema of lungs and live. Seeds are also used as stimulant and emmenagogue.[7,14] Diverse pharmacological activities have been associated with seeds including hypoglycemic,[8] anxiolytic,[12] antidiabetic,[10,13] antioxi-ant[9]and hepatoprotective activities. It has been reported that the seeds and leaves may reduce the risk of heart attack and diabetic heart diseases.[15,16] The biological activity of some phytochemicals isolated from *Brassica juncea* has been explored. The antioxidant activity of some isorhamnetin and kaempferol conjugates has been reported.[17,18] Two constituents of *Brassica juncea* – sinapine and sinapic acid – exhibited antioxidant, anxiolytic and cognition – improving activities.[19-23] The antifungal activity of some isothiocyanates isolated from *Brassica juncea* has been documented.[24-26]

MATERIALS AND METHODS

Plant Material

Brassica juncea seeds were purchased from the local market- Khartoum (Sudan) and authenticated by direct comparison with a reference herbarium sample.

Instruments

Brassica juncea oil was studied by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length ; 0.25mm diameter ; 0.25 µm, thickness).

Microorganism

The antimicrobial assay was performed using the following standard microorganisms:

*Bacillus subtilis (*G+ve) *Staphylococcus aureus(*G+ve), *Pseudomonas aeroginosa* (G-ve) ,*Escherichia coli* (G-ve) and *Candida albicans* (fungus).

Extraction of oil

Brassica juncea seeds (300g) were exhaustively extracted with n-hexane at room temperature for 72hr.The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further work.

GC-MS analysis

The constituents of *Brassica juncea* oil were investigated by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument .Chromatographic conditions are as follows: column oven temperature :150.0^{o}C ; injection temperature:300.0^{o}C ;injection mode : split; flow mode: linear velocity; pressure:139KPa; total flow: 50.0ml/min ; column flow:1.54ml/sec. ; linear velocity: 47.2cm/sec. ;purge flow:3.0 ml/min. ; split ratio: -1.0. Oven temperature program is presented Table 1.

Table 1: Oven temperature program.

Rate	Temperature(^{o}C)	Hold Time (min.$^{-1}$)
-	150.0	1.00
4.00	300.0	0.00

A**ntimicrobial activity**

The antimicrobial screening was performed by using the cup plate agar diffusion assay . Bacterial culture was maintained in nutrient agar while fungal culture was performed on Sabouraud dextrose agar. Wells (6 mm in diameter) were made in the seeded agar using sterile cork borer (No. 4).Test samples were added into wells of the seeded medium and then incubated for 24 hrs.(at 37^{0}C)-for bacteria and for 72 hrs at25^{0}C for fungal species. The diameters of inhibition zones were measured as average of two replicates.

RESULTS AND DISCUSSION

Brassica juncea oil was analyzed by GC-MS. The analysis showed 15 constituents (Table 2). Major constituents of the oil are:

i-13-Docosenic acid methyl ester(43.61%)
ii-9,12-Octadecadienoic acid methyl ester(17.50%).
iii-9,12,15-Octadectrienoic acid methyl ester(12.49%).
iv-9-hexadecenoic acid methyl ester(8.11).
v- cis-13-Eicosenoic acid methyl ester(7.83%)

i)-13-Docosenoic acid methyl ester (43.61%)

The mass spectrum of 13- docosenoic acid, methyl ester is shown in Fig.1. The peak at m/z 352, which appeared at R.T. 20.433 in total ion chromatogram, corresponds M $^{+}$ $[C_{23}H_{44}O_2]$ $^{+}$. The peak at m/z 322 corresponds to loss of methoxyl function.

ii-9,12-Octadecadienoic acid methyl ester (17.50%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.2. The peak at m/z 294, which appeared at R.T. 16.952 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{34}O_2]^{+}$ .The peak at m/z263 corresponds to loss of a methoxyl function.

iii) 9,12,15-Octadecatrienoic acid methyl ester (12.49%)

Fig.3 shows the mass spectrum of 9,12,15-octadecatrienoic acid methyl ester.The molecular ion $M^{+}[C_{19}H_{32}O_2]^{+}$ appeared at m/z 292 (R.T.17.033) in total ion chromatogram.The fragment m/z261 is due to loss of a methoxyl.

iv)9-Hexadecenoic acid methyl ester (8.11%)

The mass spectrum of 9-hexadecenoic acid methyl ester s displayed in Fig.4.The molecular ion $M^{+}[C_{19}H_{34}O_2]$ appeared at m/z268(RT.17.012).

v) cis-13-Eicosenoic acid methyl ester (7.83%)

The EI mass spectrum of cis-13-Eicosenoic acid methyl ester is shown in Fig. 5.The peak at m/z324, which appeared at R.T. 18.776 in total ion chromatogram, corresponds to $M^{+}[C_{21}H_{40}O_2]^{+}$ .The peak at m/z293 corresponds to loss of a methoxyl function.

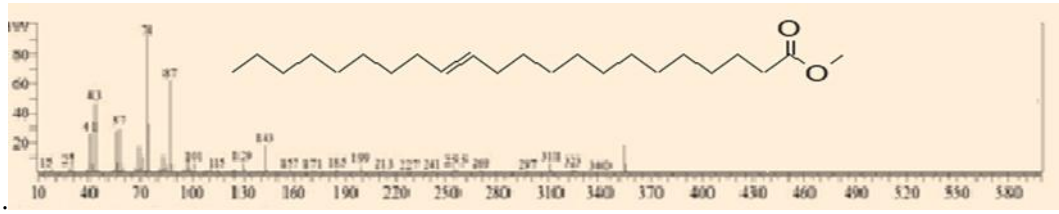

Fig. 1: Mass spectrum of 13- docosenoic acid, methyl ester.

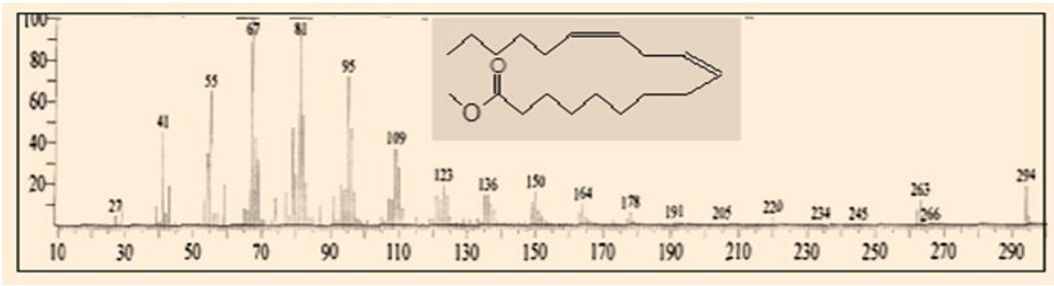

Fig. 2: Mass spectrum of 9,12-octadecanoic acid methyl ester.

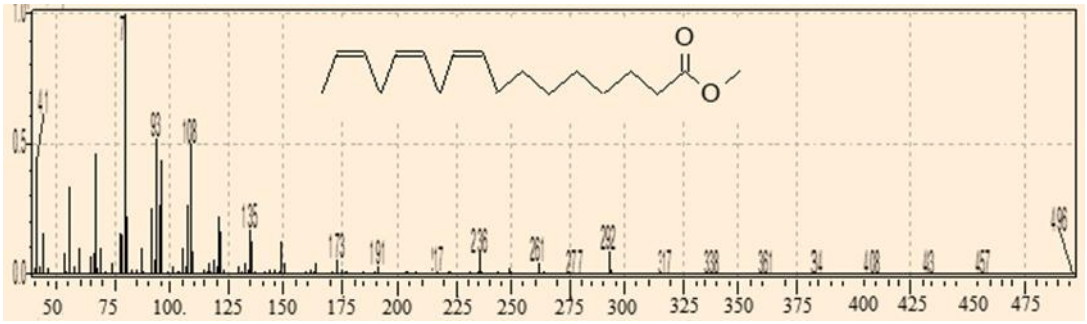

Fig. 3: Mass spectrum of 9,12,15-Octadecatrienoic acid methyl ester.

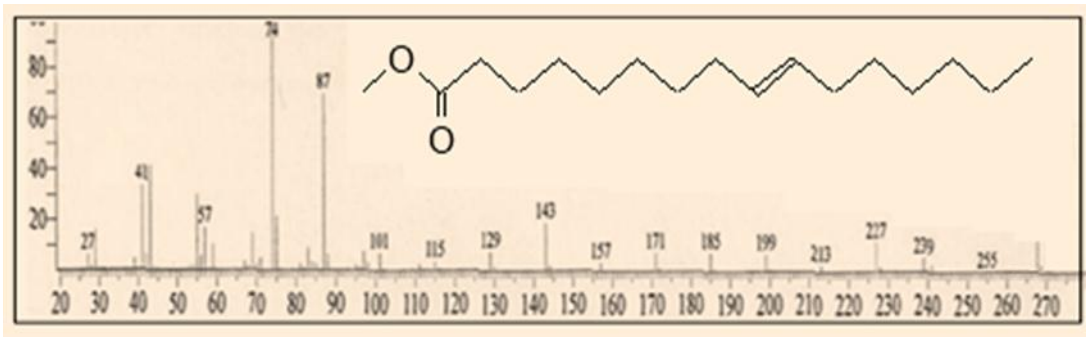

Fig. 4: Mass spectrum of 9-hexadecenoic acid methyl ester.

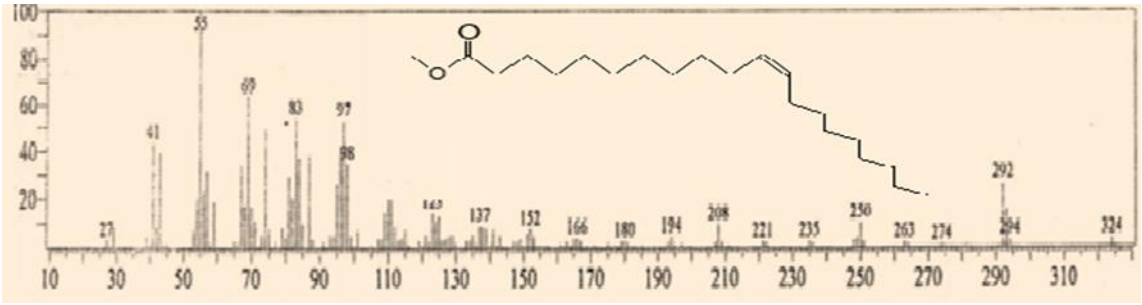

Fig. 5: Mass spectrum of Cis-11-Eicosenoic acid methyl ester.

Table 2: Constituents of the oil.

No.	Name	RT	Area %
1	9-Hexadecenoic acid methyls ester(Z)	15.120	0.06
2	Hexadecanoic acid methyls ester	15.309	2.43
3	9 12-Octadecadienoic acid methyl ester	16.952	17.50
4	9-Hexadecenoic acid methyls ester(Z Z)	7.012	8.11
5	9 12 15-Octadectrienoic acid methyl ester	17.033	12.49
6	Methyl stearate	17.222	0.92
7	11 14 17-Eicosatrienoic acid methyl ester	18.634	0.38
8	Cis-13-Eicosenoic acid methyl ester	18.776	7.83
9	Cis-11-Eicosenoic acid methyl ester	18.829	1.40
10	Eicosanoic acid methyl ester	18.975	0.71
11	13-Docosenic acid methyl ester	20.433	43.61
12	8 11 14-Docosatrienoic acid methyl ester	20.474	0.96
13	Docosanoic acid methyl ester	20.595	0.65
14	15 –Tetracosenoic acid methyl ester	21.940	2.33
15	Tetracosanoic acid methyl ester	22.096	0.61
			100%

Antimicrobial activity

The studied oil was assessed for antimicrobial activity via the cup plate agar diffusion bioassay using five standard human pathogens. The average of the diameters of the growth of inhibition zones are shown in Table 3 .The results were interpreted.

In commonly used terms (<9mm: inative; 9-12mm: partially active;13-18mm: active;>18mm:very active) Tables (4) and (5) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 3: Inhibition diameters (mm) of the oil.

Drug	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca
corochorus olitorius oil	100	10	9	--	10	--

Table 4: Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm).

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	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 5: Antifungal activity of standard chemotherapeutic agent.

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*
- *Bs.: Bacillus subtilis*
- Ca.: *Candida albicans*

The oil showed partial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*.

However, it failed to exhibit activity against *Staphylococcus aureus* and *Candida albicans*.

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