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Research Article

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Antimicrobial Activity and GC-MS analysis of Sudanese *Carthamus tinctorius* (Compositeae) Oil

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Abstract *Carthamus tinctorius* is an annual highly branched herb in the family Compositeae. The palnt has been used traditionally as purgative, analgesic and antipyretic. *Carthamus tinctorius* is also used by traditional healers against rheumatism, bronchitis, menstrual cramps and whooping cough. In this study the oil from *Carthamus tinctorius* was studied by GC-MS and the antimicrobial activity was evaluated. The GC-MS analysis showed twenty one constituents. Major constituents are: 9,12-octadecadienoic acid methyl ester (55.83%), hexadecanoic acid methyl ester (14.04%),9-octadecenoic acid methyl ester (9.32%), methyl stearate (8.82%). In the cup plate agar diffusion assay. The oil showed good antibacterial activity against *Pseudomonas aeruginosa.* It also showed partial activity against other test organism except *Escherichia coli*.

Keywords *Carthamus tinctorius*, Oil, GC-MS analysis, Antimicrobial Activity

Introduction

Carthamus tinctorius is an annual highly branched herb in the family Compositeae [1]. This plant is cultivated mainly for seed oil. The plant is also used for coloring foods and as a flavoring agent. Seed oil is used in cooking and cosmetics. *Carthamus tinctorius* thrives in arid climates like Sudan, Egypt and Southern Asia. The flower petals, which produce shades of colors, have previously been used as dyes [2]. Seed oil contains large amount of linoleic acid (70%) [3,4]. The plant has been used traditionally as purgative, analgesic and antipyretic [5]. *Carthamus tinctorius* is also used by traditional healers against rheumatism, bronchitis ,menstrual cramps and whooping cough [6]. Some local healers use *Carthamus tinctorius* against phlegmatic fever, melancholia, diabetes and dropsy [7-9]. Flowers of *Carthamus tinctorius* have been used against cardiovascular, cerebrovascular and gynecological complications [10]. Some research shed light on the therapeutic potential of the aqueous extract of *Carthamus tinctorius* for cardiovascular disease [10]. It has been reported that the aqueous extract has antihypertensive, antioxidant, anticoagulant, anticancer properties beside immunosuppressive and neuroprotective properties [10].

Material and Methods

Plant material

Seeds of *Carthamus tinctorius* were purchased from the local market, Khartoum, Sudan. The plant was authenticated by direct comparison with a reference herbarium sample.

Instruments

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

Test organisms

The antimicrobial potential of the studied oil was estimated by the cup plate agar diffusion bioassay using the standard microorganisms: *Bacillus subtilis* (G+ve), *Staphylococcus aureus* (G+ve), *Pseudomonas aeroginosa* (G-ve) , *Escherichia coli*(G-ve) and the yeast *Candida albicans.*

Methods Extraction of oil

Powdered seeds of *Carthamus tinctorius* (250g) were macerated with n-hexane at room temperature for 48hr. The solvent was removed under reduced pressure giving the oil.

GC-MS analysis

The oil was analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument. Chromatographic conditions are outlined in tables 1 and 2.

Table 1: Oven temperature program

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

Table 2: Chromatographic conditions

Column oven temperature	150.0^{o}C
Injection temperature	300.0^{o}C
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.
Spilt ratio	- 1.0

Antimicrobial activity

Mueller Hinton agar and Sabouraud dextrose agar were used as media for bacterial and fungal cultures respectively. The antimicrobial activity was performed using the cup plate agar diffusion bioassay. Agar Petri dishes maintained at 45^{o}C in a water bath were seeded with an overnight culture(1ml) of bacteria(10^{7}-10^{8} cfu/ml). Wells (8mm in diameter) were cut on the seeded agar via a sterile cork borer. The cups were filled with (0.1ml) of the test solution and the Petri dishes were left to settle and then incubated for 24 h. at 37^{o}C. The assay was carried out in duplicates. After incubation the diameters of inhibition zones were measured and averaged as indicator of activity. The same procedure was adopted for antifungal activity, but Sabouarud dextrose agar was used instead of Mueller Hinton agar and incubation was continued for three days at 25^{o}C.

Results and Discussion

GC-MS analysis

GC-MS analysis of *Carthamus tinclorius* oil was conducted and the identification of the constituents was initially accomplished by comparison of the retention times and consulting the MS library (NIST). Excellent matching was observed when comparing the mass spectra with the database on MS library. The GC-MS analysis of the studied oil revealed the presence of 21 components (Table 3). The typical total ion chromatograms (TIC) is shown in Figure 1.

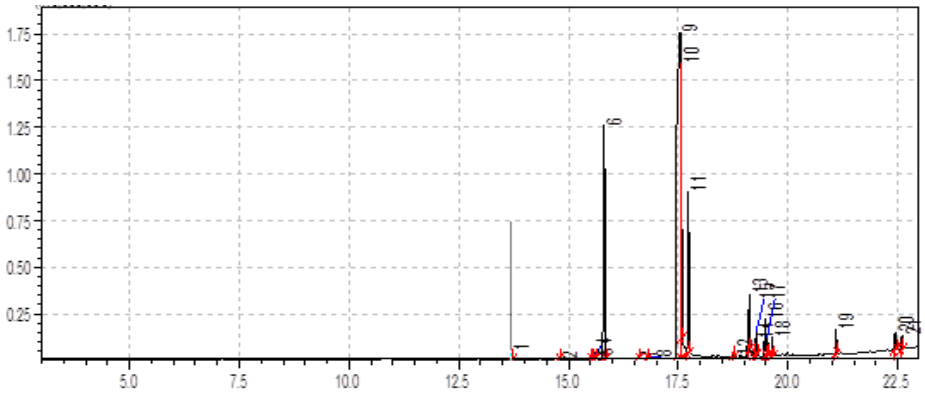

Figure 1: Chromatograms of Carthamus tinclorius oil

Table 3: Constituents of *Carthamus tinclorius* oil

No.	Name	Ret. Time	Area%
1	Methyl tetradecanoate	13.701	0.40
2	Pentadecanoic acid, methyl ester	14.777	0.06
3	7,10-Hexadecadienoic acid, methyl ester	15.506	0.02
4	7-Hexadecenoic acid, methyl ester, (Z)-	15.566	0.08
5	9-Hexadecenoic acid, methyl ester, (Z)-	15.611	0.13
6	Hexadecanoic acid, methyl ester	15.814	14.04
7	cis-11,14-Eicosadienoic acid, methyl ester	16.573	0.11
8	Heptadecanoic acid, methyl ester	16.783	0.11
9	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.543	55.83
10	9-Octadecenoic acid (Z)-, methyl ester	17.565	9.32
11	Methyl stearate	17.733	8.82
12	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.762	0.12
13	Tridecanedial	19.119	2.48
14	Oxiraneoctanoic acid, 3-octyl-, methyl ester	19.245	0.85
15	cis-13-Eicosenoic acid, methyl ester	19.279	1.07
16	Eicosanoic acid, methyl ester	19.477	1.79
17	PGH1, methyl ester	19.531	0.92
18	1-Naphthalenol, decahydro-4a-methyl-	19.642	1.01
19	Docosanoic acid, methyl ester	21.098	1.15
20	15-Tetracosenoic acid, methyl ester, (Z)-	22.449	0.97
21	Tetracosanoic acid, methyl ester	22.602	0.72

The GC-MS analysis revealed the following major constituents:

i) 9,12-Octadecadienoic acid methyl ester (55.82%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Figure 2. The peak at m/z294 (R.T. 17.543) coincides with $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z263 is due to loss of a methoxyl.

ii) Hexadecanoic acid methyl ester (14.04%)

Figure 3 shows the mass spectrum of hexadecanoic acid methyl. The peak m/z 270 (R.T., 15.814) was detected in the spectrum. It corresponds $M^+[C_{17}H_{34}O_2]^+$. The peak at m/z 239 is due to loss of a methoxyl.

iii) 9-Octadecenoic acid methyl ester (9.32%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig. 4. The peak at m/z 296 (R.T., 17.565) corresponds $M^+[C_{19}H_{36}O_2]^+$, while the signal at m/z266 is attributed to loss of a methoxyl.

iv) Methyl stearate (8.82%)

The EI mass spectrum of methyl stearate is displayed in Figure 5. The peak at m/z 298 with R.T. 17.733 is due to $M^+[C_{19}H_{38}O_2]^+$, while the signal at m/z 267 corresponds to loss of a methoxyl group.

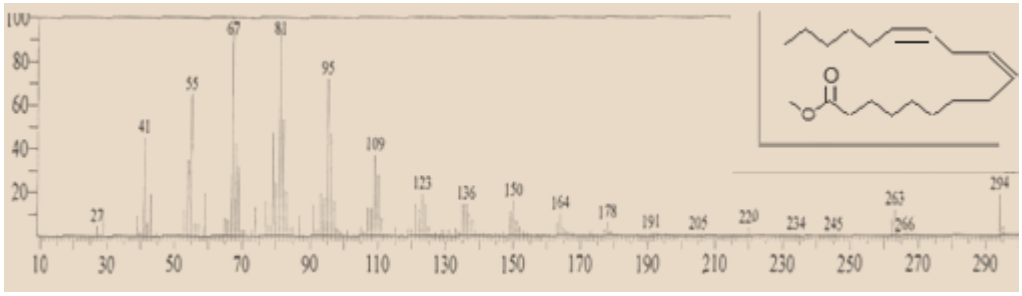

Figure 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester

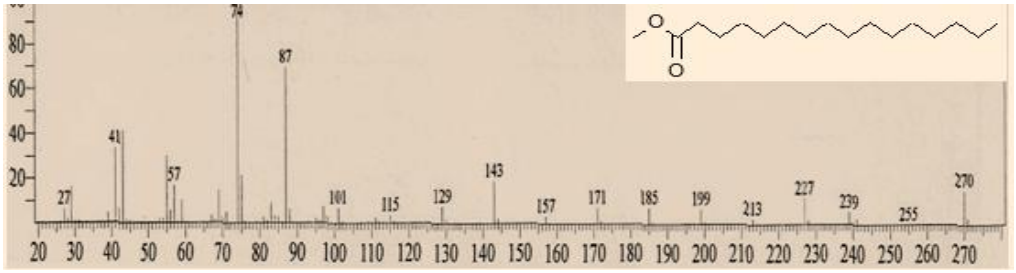

Figure 3: Mass spectrum of hexadecanoic acid methyl ester

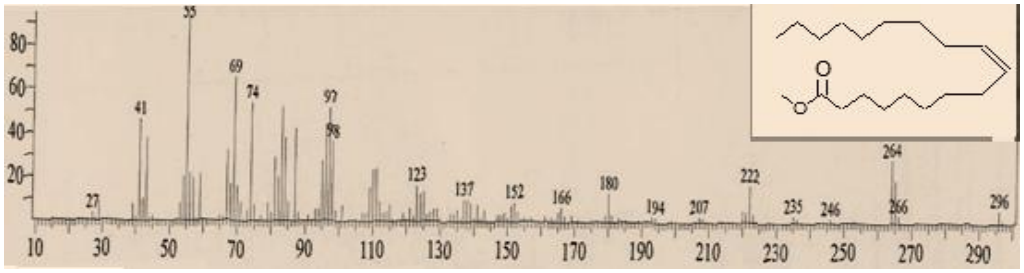

Figure 4: Mass spectrum of 9-octadecenoic acid methyl ester

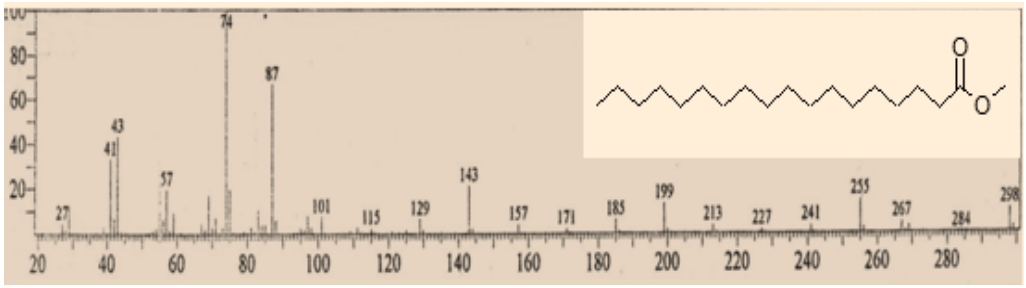

Figure 5 : Mass spectrum of methyl stearate

Antimicrobial activity

Carthamus tinclorius seed oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table 4.The results were interpreted in commonly used terms (<9mm: inative; 9-12mm: partially active; 13-18mm: active; >18mm: very active). Ampicilin, gentamicin and clotrimazole were used as positive controls. The oil showed good antibacterial activity against *Pseudomonas aeruginosa.*It also showed partial activity against other test organism except *Escherichia coli*.

Table 4: Inhibition zones of (mm/mg sample)

Sample	Ec	Pa	Sa	Bs	Ca
Carthamus tinclorius (100mg/ml)	-	15	12	12	13
Ampicilin (40mg/ml)			30	15	
Gentamicin (40mg/ml)	22	21	19	25	-
Clotrimazole (30mg/ml)	--	--	--	--	38

Sa.: *Staphylococcus aureus*; Bs.: *Bacillus subtilis*; Ec.: *Escherichia coli*; Pa.: *Pseudomonas aeroginosa*; Ca.: *Candida albicans*.

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