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

LACTUCA SATIVA OIL: GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY

Prof. Abdel Karim M.[1]*, Asma M.H.[2] and Inas O.[3]

[1]Sudan University of Science and Technology, Faculty of Science.
[2]University of Nyala, Faculty of Education.
[4]University of Bahri, Faculty of Applied and Industrial Chemistry.

***Corresponding Author: Prof. Abdel Karim M.**
Sudan University of Science and Technology, Faculty of Science.

Article Received on 12/03/2017 **Article Revised on 01/04/2017** **Article Accepted on 22/04/2017**

ABSTRACT

Lettuce (*Lactuca sativa*) is an important leafy vegetable globally cultivated for its economic value. This study was carried out to identify and quantify the constituents of Sudanese lettuce seed oil. The GC-MS analysis of Lettuce seed oil revealed the presence of the following major components: methyl 10-trans,12-cis-octadecadienoate (54.18%), hexadecanoic acid methyl ester (13.71%), 9-octadecenoic acid methyl ester(11.41%) and methyl stereate(5.36%). The antimicrobial activity was accomplished via disc diffusion bioassay against six standard pathogens. The oil showed activity against all test organisms. Significant activity against *Pseudomonas aeruginosa* was observed. Also the oil showed excellent activity against *Escherichia coli*, and the fungal species *Candida albicans*.

KEYWORDS: *Lactuca sativa*, Seed Oil, GC-MS, Antimicrobial Activity.

INTRODUCTION

Lactuca sativa (Lettuce), which is cultivated worldwide for its economic value, is occupying an important position among leafy vegetables. Luttice belongs to the family Asteraceae (formerly called Compositeae).[1,2] Lettuce is an annual glabrous herb reaching 30-100cm in height.[3,4] Luttice comprises seven main cultivars, usually termed morphotypes. The plant is included in salad and vetatables curries and some varieties are cooked.[5,6] *Lactuca sativa* is rich in vitamins C and E as well as carotene.[5,7,8]

Sid et.al.[9] demonstrated that the oil from seed possesses analgesic, sedative, and anticonvulsant properties, while Roman et.al.[10] claimed that the plant has hypoglycemic activity. It has been reported that the leaf methanolic extract exhibited significant radical scavenging capacity both in model animals and *in vitro*.[11]

MATERIALS AND METHODS

Materials

Plant material

Lactuca sativa seeds were purchased from the local market-Khartoum and authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum-Sudan.

Test organisms

The standard microorganisms used for assessing antimicrobial activity are depicted 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	*Aspergillus niger*	fungus
6	*Candida albicans*	fungus

Methods

Extraction of oil

Powdered seeds of *Lactuca sativa* (300g) were extracted with n-hexane (soxhlet).The solvent was removed *in vacuo* to afford the oil. The oil was esterified using a methanolic solution of sodium hydroxide (2g of sodium hydroxide in 100ml methanol) and methanolic sulphuric acid (1ml of concentrated sulphuric acid in 99ml methanol).

GC-MS analysis

The extracted oil was analyzed by gas chromatography – mass spectrometry using a QP2010 Ultra Instrument(Shimadzo) with a RTX-5MS column (30m,length; 0.25mm diameter; 0.25 µm, thickness).

Analytical grade Helium (99.99 %) was used as carrier gas. Oven temperature program is shown below:
Rate: -; Temperature:150^0; Hold Time (min^{-1}): 1.00
Rate: 4.00; Temperature:3 00^0; Hold Time (min^{-1}): 0.00
Other chromatographic conditions are tabulated below:

Table 2: Chromatographic conditions.

Column oven temperature	150.0°C
Injection temperature	300.0°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

Antimirobial sensitivity test

Thirty eight grams of powdered agar were dispersed in 1 liter of distilled water and allowed to soak for ten minutes. The medium was heated in a water bath to dissolve, swirled to mix and sterilized by autoclaving for 15 minutes at 121°C.Then cooled at 47°, mixed well and poured into sterile Petri dishes.

Disc diffusion bioassay

Twenty ml aliquots of molten agar were distributed into sterile Petri dishes. (0.1ml) of the standardized bacterial stock suspension(10^8-10^9 colony-forming units/ml) were soaked on agar medium plates using sterile cotton swab. Sterilized filter paper discs (6mm diameter) were soaked in test sample solution and then placed on the surface of the test bacteria plates. Plates were incubated for 24h. Following incubation the, diameter of inhibition zones were measured in triplicates and averaged. Standard antimicrobial chemotherapeutics were used as positive control,while DMSO was used as negative control.

The same process was adopted for antifungal activity, but potato dextrose agar was used instead of nutrient agar and incubation was continued for 72h.

RESULTS AND DISCUSSION

The total ion chromatogram is shown in Fig. 1. A Tabulation of oil constituents is given in Table 3.

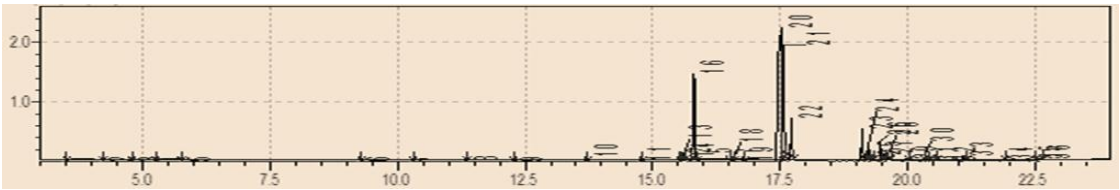

Fig.1: Total ion Cheromatograms.

Table 3: Constituents of *Lactus sativa* oil.

Peak#	R.Time	Area	Area%	Name
1	3.456	25099	0.01	Hexanoic acid, methyl ester
2	4.183	14014	0.01	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-m
3	4.755	13787	0.01	Benzene, 1-methyl-3-(1-methylethyl)-
4	5.213	38138	0.02	.gamma.-Terpinene
5	5.735	10837	0.00	1,6-Octadien-3-ol, 3,7-dimethyl-
6	9.241	51380	0.02	3-Cyclohexene-1-methanol, .alpha.,.alpl
7	10.276	35786	0.01	Caryophyllene
8	11.339	240740	0.10	Butylated Hydroxytoluene
9	12.250	54816	0.02	Heptadecane
10	13.692	1175291	0.49	Methyl tetradecanoate
11	14.767	265960	0.11	Pentadecanoic acid, methyl ester
12	15.503	73469	0.03	Hexadecane
13	15.557	108758	0.05	9-Hexadecenoic acid, methyl ester, (Z)-
14	15.601	1197857	0.50	cis-10-Nonadecenoic acid, methyl ester
15	15.695	54118	0.02	7-Hexadecenoic acid, methyl ester, (Z)-
16	15.809	32721483	13.71	Hexadecanoic acid, methyl ester
17	16.490	44927	0.02	Eicosane
18	16.564	277372	0.12	cis-10-Heptadecenoic acid, methyl ester
19	16.772	291690	0.12	Heptadecanoic acid, methyl ester
20	17.530	129308646	54.18	Methyl 10-trans,12-cis-octadecadienoate
21	17.558	27239639	11.41	9-Octadecenoic acid, methyl ester, (E)-
22	17.720	12803481	5.36	Methyl stearate
23	19.108	11865091	4.97	Tridecanedial
24	19.227	4353069	1.82	Oxiraneoctanoic acid, 3-octyl-, methyl e
25	19.264	1903521	0.80	11-Eicosenoic acid, methyl ester
26	19.465	5983948	2.51	Eicosanoic acid, methyl ester
27	19.511	2112012	0.88	Methyl eicos-11-en-14-ynoate
28	19.620	1896016	0.79	9,12,15-Octadecatrienoic acid, methyl es
29	19.955	725885	0.30	8,11,14-Eicosatrienoic acid, methyl ester
30	20.290	80300	0.03	Heneicosanoic acid, methyl ester
31	20.374	141721	0.06	Phenol, 2,2'-methylenebis[6-(1,1-dimeth
32	20.491	402511	0.17	Naphthalene, decahydro-2,3-dimethyl-
33	21.082	1690110	0.71	Methyl 20-methyl-heneicosanoate
34	21.849	212561	0.09	Tricosanoic acid, methyl ester
35	22.433	489851	0.21	15-Tetracosenoic acid, methyl ester, (Z)-
36	22.586	766886	0.32	Tetracosanoic acid, methyl ester
		238670770	100.00	

Major constituents of the oil are discussed below:

i) Methyl 10-trans,12-cis-octadecadienoate (54.18%)

Fig. 2 shows the mass spectrum of methyl 10-trans-12-cis-octadecadienoate. The signal at m/z 294(RT 17.530) corresponds M^+ $\{C_{19}H_{34}O_2\}^{+\cdot}$ while the peak at m/z 263 accounts for loss of methoxyl.

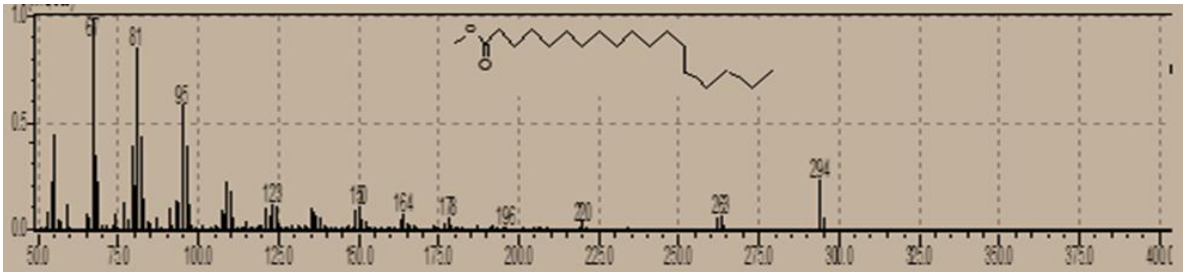

Fig 2: Mass spectrum of methyl 10-trans,12-cis-octadecadienoate.

ii) Hexadecanoic acid methyl ester (13.71%)

The molecular ion M^+ $(C_{17}H_{34}O_2)^+$ for hexadecanoic acid appeared at m/z270(RT, 15.809). The peak at m/z 239 is due to loss of a methoxy(Fig.3).

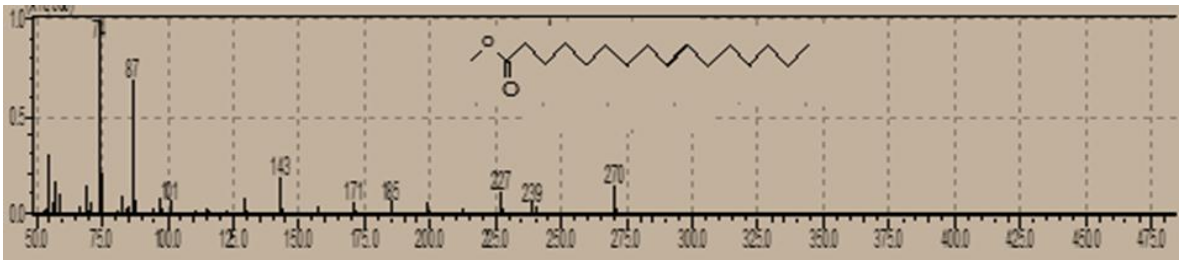

Fig 3: mass spectrum of hexadecanoic acid (methyl ester).

iii) 9-Octadecenoic acid methyl ester (11.41%)

The EI mass spectrum of 9-octadecenoic acid methyl ester is shown in Fig. 4. The signal at m/z 296(RT 17.558) correspond M^+ $(C_{19}H_{36}O_2)^+$. The peak at m/z 264 is due to loss of methoxyl function.

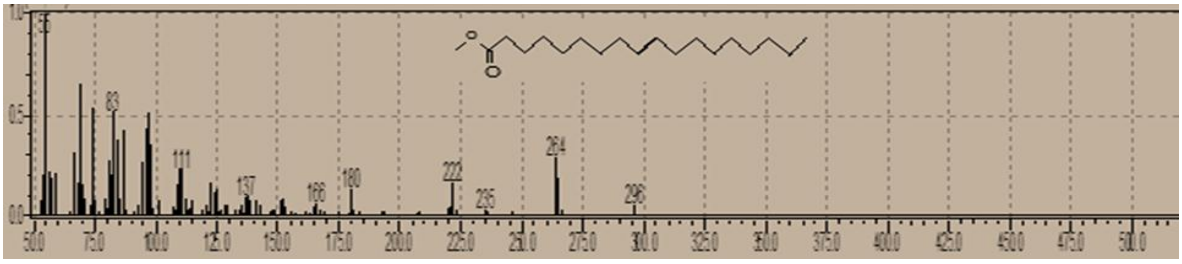

Fig 4: Mass spectrum of 9-octadecenoic acid methyl ester.

iv) Methyl stereate(5.36%)

The mass spectrum of methyl stereate is displayed in Fig.5. The peak at m/z298(RT 17.720) corresponds M^+ $(C_{19}H_{38}O_2)^{+\cdot}$ while the signal at m/z267 accounts for loss of a methoxyl.

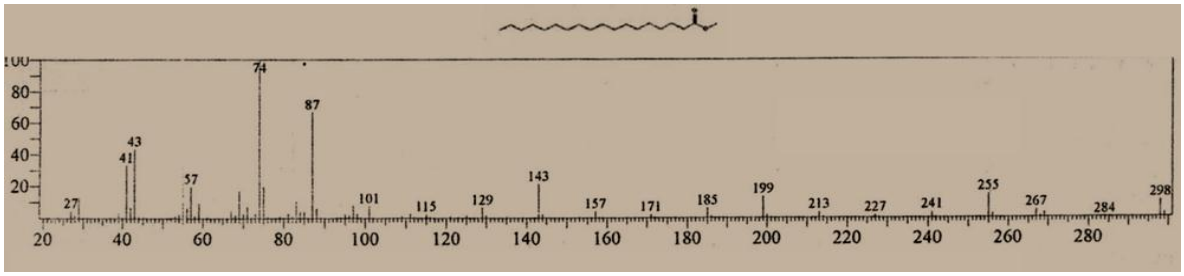

Fig 5: Mass spectrum of methyl stereate.

Antimicrobial assay

The oil was assessed for antimicrobial activity against six standard organisms. Diameters of the growth inhibition zones are displayed in Table 4.The results were interpreted according to the common terms: (<9mm: inative; 9-12mm:partially active; 13-18mm: active;>18mm: very active).Tables (5) and (6) show the antimicrobial activity of standard drugs.

Table 4: Antibacterial activity of Lactus sativa oil:M.D.I.Z (mm).

Drug	Conc. (mg/ml)	Ec	Ps	Sa	Bs	Ca	An
Lactus sativa oil	100	17	20	16	16	17	15

Table 5: 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 6: Antifungal activity of standard chemotherapeutic agents against standard fungi.

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*

The oil showed activity against all test organisms. Significant activity against *Pseudomonas aeruginosa* was observed. Also the oil showed excellent activity against *Escherichia coli*, and the fungal species *Candida albicans*(Table 4).

REFERENCES

1. Judd,W.S., Campbell, C.S., Kellogg, E.A., Stevens,P.F. "Plant Systematics: A Phylogenetic Approach" Sinauer Associates, Sunderland MA, 1999.
2. Funk,V.,Bayer,R.I, keeley,s., Chan,R., Watson,l.,,B., Schiling, E.,Panero, J.L.,Baldwin, B.Garcia-Jacas, N., Susanna,A.,jansen, R.K., *Biologiske Skrifter*, 2005; 55: 343.
3. Dostal,I.(ed), Nova Kvetena CSSR, 2 dil. Praha Academic, 1114, 1989.
4. Rubatzky,V.E., yamaguchim, M., " World Vegetables"(1997), new York, Chapman and hall.E. 2007.
5. Anonymus,D. " The Wealth of India", PID, CSIR, new Delhi, P12, 1962.
6. Lebeda,A., Ryder,E.J., Grube,R., Dolezalova,I., Kristkova,E. "Lettuce(Asteraceae lactuca spp.", In: Singh,R.J.(ed),Genetic Resources,Chromosome Engineering and Crop Improvement, Vegetable Crops, boca Raton, CRC Press, tailor and Francis Group, 2007; 3: 377.
7. Kirtikar,K.R. and Basu,B.D." Indian Medicinal Plants", 1987; Vol.I and II, 2nd Ed.,1438.
8. Nadkharni,K.M. " Indian Material Media" Popular Prakashan Pvt.Ltd., Bombay, 1976; 719.
9. Sid,S.A, EL-Kashef,H., ElMazes,M.M. and Slam,O., *Fitoterapia*, 1996, 67: 215.
10. Roman,R.R., Flores,S.H. and Alarcon,A.F., *J.Ethnophamacology*, 1995; 48: 25.
11. Munish, G., Chanchal,G., Palok,K. and Suresh,B.,*Ancient Science of Life*, 2004; XXVI(1): 6.
12. Mou,B.; Luttice, In:Prohens,J. and Nuez,F.(eds): Handbook of Plant Breeding; Vegetables I New York,Springer Scince, 2008; 75.