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

GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *ALLIUM CEPA* L. FIXED OIL

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Article Received on 27/05/2017 **Article Revised on 16/06/2017** **Article Accepted on 06/07/2017**

ABSTRACT

The present study was undertaken to identify and quantify the chemical constituents of seed oils from two cultivated varieties of *Allium cepa* – red onion and white onion and to evaluate their potential antimicrobial activity. GC-MS analysis showed 27 components for red onion. Major constituents are: 9,12-octadecadienoic acid methyl ester(42.45%), 9-octadecenoic acid methyl ester(19.44%), hexadecanoic acid(11.33%), cyclpentaneoctanoic acid methyl ester(6.82%) and methyl stearate(5.06%). The white onion gave 24 constituents being dominated by: 9,12-octadecadienoic acid methyl ester(55.29%), 9-octadecenoic acid methyl ester(16.07%), hexadecanoic acid(12.70%) and methyl stearate(4.91%). The antibacterial activity of the oils was assessed via cup plate agar diffusion assay against six standard human pathogens. The oil from red onion showed different antimicrobial responses against test organisms. It showed excellent activity against the bacterial strains: *Staphylococcus aureus* and *Bacillus subtilis*. It also exhibited significant activity against the yeast *Candida albicans*. However, it was inactive against *Pseudomonas aeruginosa*, *Escherichia coli* and the fungal species *Aspergillus niger*. White onion oil showed excellent activity against *Escherichia coli*. It also showed significant activity against *Staphylococcus aureus* and *Bacillus subtilis*.

KEYWORDS: *Allium cepa*, Fixed oil, GC-MS, Antimicrobial activity.

INTRODUCTION

The genus Allium is a large genus comprising more than 700 species.[1] *Allium cepa* (onion) comes in the first rank among edible Allium followed by *Allium sativum* (garlic).Onion has been cultivated for thousands of years. It is a known flavoring agent beside its medicinal uses. For a long time onion has been used in traditional medicine of different communities.The Chinese Pharmacopeia indicates the use of onion for asthma, cough, angina pectoris, dyspnea and tenesmus.[2]

Fresh bulb juice is used externally for boils and bronchitis and internally as emmenogogue, for asthma,renal malfunction and urinary disorders.[3] *Allium cepa* is also used for acute catarrhal inflammation.[4] Onion rubbed in the skin is claimed to promote hair growth.[5]

It has been shown that the consumption of onion may prevent tumor promotion, cardiovascular diseases, and age – dependent changes in blood vessels. Such effects seems to be associated with the antioxidant properties of onion which contains antioxidant flavonoids - quercetin and kaempferol.[2,6] The radical scavenging potential of onion is also responsible for its antiallergic and anti-inflammatory properties.[7,8] Aqueous bulb extract was active on *Asperillus flavus*. Aflatoxin B-1 production was inhibited by 44.80%.[3] In some *in vitro* studies, aqueous extract of bulb showed significant activity against the bacterial strains; *Escherichia coli* and *Streptococcus* species.[9,10] Fresh bulb administered orally to model animals exhibited activity against allergen-induced bronchial asthma.[11] Sharma and Sharma demonstrated[12,13] that onion bulb decreased serum cholesterol and plasma fibrinogen levels in model animals.

In continuation of our interest in the constituents of plants used in Sudanese system of medicine, this study was carried out to investigate the constituents of fixed oil from two cultivated varieties of *Allium cepa*(white and red onion) grown in Sudan.

MATERIALS AND METHODS

Plant material

Allium cepa seeds (red and white varieties) were collected from – Omdurman, Sudan.The plants were 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; 0.25 µm, thickness) was used for GC-MS analysis.

Test organisms

Allium cepa oils were screened for antibacterial and antifungal activities using the standard microorganisms shown in Table (1).

Table 1: Test organisms.

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

Methods

Extraction of *Allium cepa* oil

Red and white onion seeds (400g) were separately macerated with n-hexane at room temperature for 48h..The solvent was removed under reduced pressure to give the oils. For GC-MS analysis, the oil was esterified via a methanolic solution of NaOH and a methanolic solution of sulphuric acid. The oven temperature program is displayed below, while other chromatographic conditions are shown in Table 2.

Rate	Temperature	Hold time (min^{-1})
-	60.0	0.00
10.00	300.0	0.00

Table 2: Chromatographic conditions.

Column oven temperature	1300.0 °C
Injection temperature	280.0 °C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	93.1KPa
Total flow	50.0ml/ min
Column flow	1.50ml/sec
Linear velocity.	44.7cm/sec
Purge flow	3.0ml/min.
Spilt ratio	: - 1.0

Antimicrobial assay

Bacterial and fungal suspensions

Aliquots of 24 hours broth culture of the test micoorganisms were distributed aseptically onto nutrient agar slopes and incubated at 37°C for 24 hours. After harvesting the bacterial growth, it was washed off with sterile normal saline, and then suspended in (100 ml) of normal saline to afford 108-109 colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

Fungal cultures were maintained on potato dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing for antimicrobial activity

(2ml) of the standardized bacterial stock suspension were mixed with (200 ml) of sterile molten nutrient agar being maintained at 45°C. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. Plates were divided into two halves, two cups in each half (10 mm in diameter) were cut using cork borer (No 4). Each one of the halves was designed for one of the test solutions.The agar discs were removed, alternate cups were filled with (0.1 ml) samples of each test solution and allowed to diffuse at room temperature for two hours. The plates were then incubated at 37°C for 24 hours.

The above procedure was repeated for different concentrations of the test solutions and the standard chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in duplicates and averaged.

RESULTS AND DISCUSSION

Allium cepa: Red Onion

GC-MS analysis of red onion fixed oil

Red onion oil was analyzed by GC-MS and identification of constituents was accomplished by comparison with the MS library (NIST) and also via the observed fragmentation pattern, a 90-95% match was observed.

The GC-MS analysis revealed the presence of 27 components (Table 3).The typical total ion chromatograms (TIC) is depicted in Fig.1.

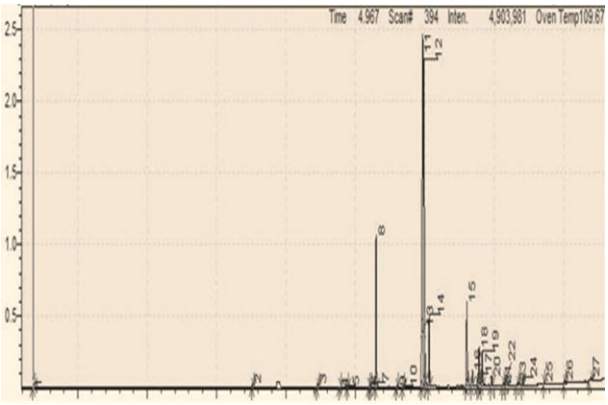

Fig. 1: Typical total ion chromatograms (TIC).

Table 3: Contituents of *Allium cepa* oil.

Peak#	R.Time	Area	Area%	Name
1	3.407	33749	0.02	Hexanoic acid, methyl ester
2	11.288	316619	0.19	Butylated Hydroxytoluene
3	13.642	437849	0.26	Methyl tetradecanoate
4	14.452	74571	0.04	5-Octadecenoic acid, methyl ester
5	14.717	171488	0.10	Pentadecanoic acid, methyl ester
6	15.506	165233	0.10	9-Hexadecenoic acid, methyl ester, (Z)-
7	15.551	625010	0.37	11-Hexadecenoic acid, methyl ester
8	15.749	19149990	11.33	Hexadecanoic acid, methyl ester
9	16.512	219281	0.13	cis-10-Heptadecenoic acid, methyl ester
10	16.720	181782	0.11	Heptadecanoic acid, methyl ester
11	17.430	71762304	42.45	9,12-Octadecadienoic acid (Z,Z)-, methyl e
12	17.475	32863215	19.44	9-Octadecenoic acid (Z)-, methyl ester
13	17.504	3741060	2.21	9-Octadecenoic acid ,methyl ester, (E)-
14	17.661	8558009	5.06	Methyl stearate
15	19.017	11531144	6.82	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethyl
16	19.216	2175602	1.29	11-Eicosenoic acid, methyl ester
17	19.414	1518038	0.90	Eicosanoic acid, methyl ester
18	19.460	4828791	2.86	Methyl 7,11,14-eicosatrienoate
19	19.570	4184176	2.48	6,9-Octadecadienoic acid, methyl ester
20	19.906	1199399	0.71	9,12,15-Octadecatrienoic acid, methyl este
21	20.325	166427	0.10	Phenol, 2,2'-methylenebis[6-(1,1-dimethyle
22	20.460	2145002	1.27	Methyl 5,13-docosadienoate
23	20.858	355927	0.21	13-Docosenoic acid, methyl ester, (Z)-
24	21.033	980084	0.58	Methyl 20-methyl-heneicosanoate
25	21.797	216549	0.13	Tricosanoic acid, methyl ester
26	22.536	387921	0.23	Tetracosanoic acid, methyl ester
27	23.495	1068165	0.63	Cholest-7-en-3-ol, 4-methyl-, (3.beta.,4.alpl
		169057385	100.00	

Some important constituents are discussed below:

9,12-Octadecadienoic acid methyl ester (42.45%)

Fig. 2 shows the EI mass spectrum of 9,12-octadecadienoic acid methyl ester.The peak at m/z294, which appeared at R.T. 17.430 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^{+\cdot}$The peak at m/z263 corresponds to loss of a methoxyl function.

9-Octadecenoic acid methyl ester(19.44%)

Fig. 3 shows the EI mass spectrum of 9-octadecenoic acid methyl ester.The peak at m/z 296, which appeared at R.T. 17.475 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^{+\cdot}$ while the peak at m/z266 accounts for loss of a methoxyl function**.**

Hexadecanoic acid methyl ester(11.33%)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.4.The peak at m/z 270, which appeared at R.T. 15.749 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m/z239 is attributed to loss of a methoxyl function.

Cyclpropaneoctanoic acid methyl ester(6.82%)

The Mass spectrum of cyclpropaneoctanoic acid methyl ester is depicted in Fig. 5. The peak at m/z 270, which appeared at R.T.19.017 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m/z239 is attributed to loss of a methoxyl function.

Methyl stearate(5.06%)

Mass spectrum of methyl stearate is shown in Fig. 6.The peak at m/z 298, which appeared at R.T. 17.661 corresponds to $M^+[C_{19}H_{38}O_2]^{+\cdot}$The peak at m/z267 corresponds to loss of a methoxyl function.

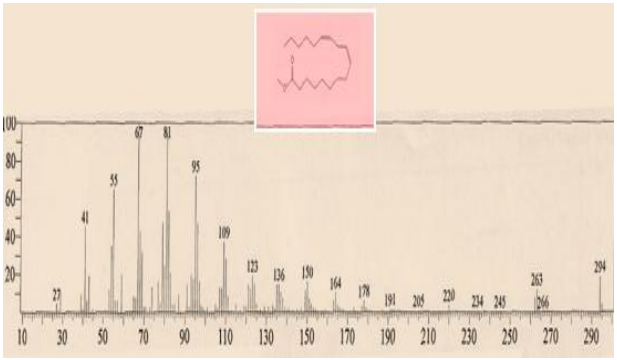

Fig. 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester.

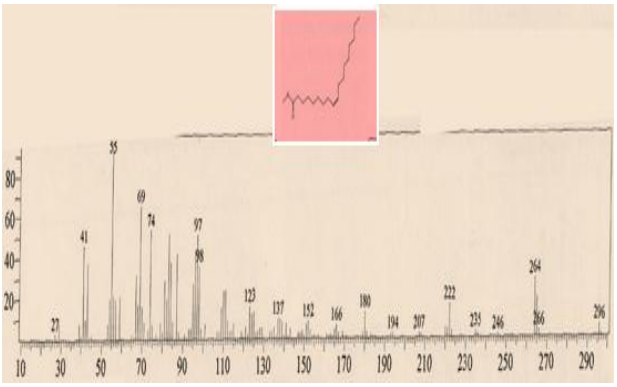

Fig. 3: Mass spectrum of 9-octadecenoic acid methyl ester.

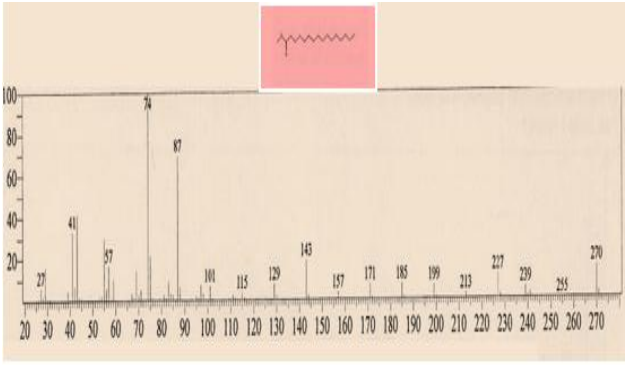

Fig. 4: Mass spectrum of hexadecanoic acid methyl ester.

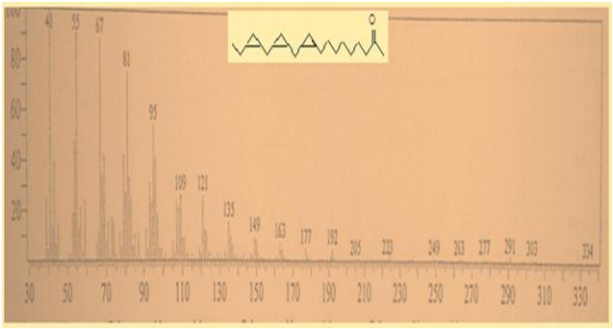

Fig. 5: Mass spectrum of cyclpropaneoctanoic acid methyl ester.

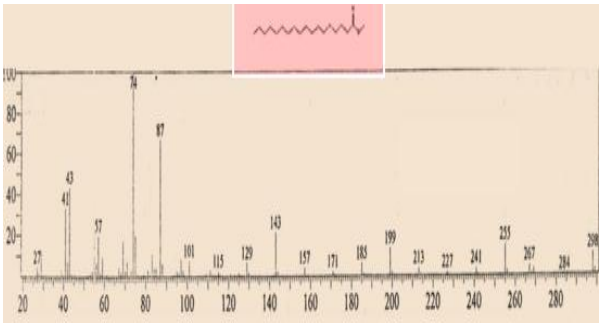

Fig. 6: Mass spectrum of methyl stearate.

Antimicrobial activity

Using cup plate agar diffusion bioassay,the oil was evaluated for antimicrobial activity against six standard human pathogens. Diameters of the growth of inhibition zones are given in Table (4).The results were interpreted in terms of the commonly used terms (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active).Tables (5) and (6) display the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 4: Antimicrobial activity of red onion oil.

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca	An
Oil	100	18	18	-	-	19	-

Table 5: Antibacterial activity of standard chemotherapeutic agents.

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 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*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

The oil from red onion showed excellent activity against the bacterial strains: *Staphylococcus aureus* and *Bacillus subtilis*. It also exhibited significant activity against the yeast *Candida albicans*(Table 4). However, it was inactive against *Pseudomonas aeruginosa, Escherichia coli* and the fungal species *Aspergillus niger*.

Allium Cepa: Variety Texas early yellow (White Onion)

GC-MS analysis

The total ion chromatogram for white onion seed oil is shown in Fig. 7 and the constituents of the oil are displayed in Table 7.

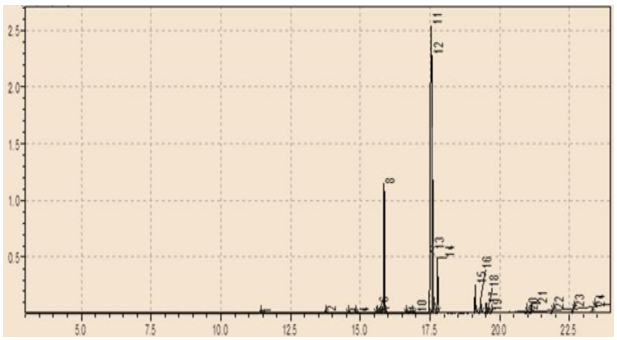

Fig. 7: Total ion chromatograms.

Table 7: Constituents of white onion seed oil.

Peak#	R.Time	Area	Area%	Name
1	11.394	68651	0.04	Butylated Hydroxytoluene
2	13.743	314213	0.19	Methyl tetradecanoate
3	14.554	56099	0.03	cis-5-Dodecenoic acid, methyl ester
4	14.817	146226	0.09	Pentadecanoic acid, methyl ester
5	15.549	16256	0.01	7,10-Hexadecadienoic acid, methyl ester
6	15.610	117178	0.07	cis-10-Nonadecenoic acid, methyl ester
7	15.654	578983	0.36	9-Hexadecenoic acid, methyl ester, (Z)-
8	15.849	20584142	12.70	Hexadecanoic acid, methyl ester
9	16.615	224501	0.14	7-Hexadecenoic acid, methyl ester, (Z)-
10	16.822	138646	0.09	Heptadecanoic acid, methyl ester
11	17.543	89577834	55.29	9,12-Octadecadienoic acid (Z,Z)-, methyl e
12	17.579	26035926	16.07	9-Octadecenoic acid (Z)-, methyl ester
13	17.605	5237654	3.23	9-Octadecenoic acid, methyl ester, (E)-
14	17.762	7960780	4.91	Methyl stearate
15	19.116	4638187	2.86	9-Octadecynoic acid, methyl ester
16	19.316	2309491	1.43	11-Eicosenoic acid, methyl ester
17	19.514	1438018	0.89	Methyl 18-methylnonadecanoate
18	19.560	212969	0.13	9,12,15-Octadecatrienoic acid, methyl este
19	19.672	185644	0.11	7,10,13-Eicosatrienoic acid, methyl ester
20	20.959	231466	0.14	cis-10-Nonadecenoic acid, methyl ester
21	21.133	1270433	0.78	Methyl 20-methyl-heneicosanoate
22	21.898	184898	0.11	Tricosanoic acid, methyl ester
23	22.635	350957	0.22	Tetracosanoic acid, methyl ester
24	23.380	140293	0.09	Squalene
		162019445	100.00	

The following compounds were detected as major constituents of the oil:

i) 9,12-Octadecadienoic acid methyl ester(55.29%), RT,17.543.
ii) 9-Z-Octadecenoic acid methyl ester (16.07%), RT,17.579.
iii) Hexanoic acid methyl ester (12.70%), RT,15.849.
iv) Methyl stearate (4.91%), RT,17.762.

Antimicrobial activity

Using the cup plate agar diffusion assay, the oil was screened for its antimicrobial activity against the standard microorganisms shown in Table (1).The diameters of the growth inhibition zones are given in Table (8).

Table 8: Antimicrobial activity of white onion seed oil.

Type	Conc.mg/ml	Sa	Bs	Ec	Ps	Ca	An
Oil	100	16	16	26	-	11	10

White onion oil is very active against *Escherichia coli*. It also showed excellent activity against *Staphylococcus aureus* and *Bacillus subtilis*. However, the oil was inactive against *Pseudomonas aeruginosa*. Though, the red variety gave excellent antifungal activity against the yeast *Candida albicans*, the white onion merely showed a partial activity. Noteworthy is the different antifungal responses of the two onion varieties towards *Aspergillus niger*. Whilst the red variety was completely inactive against *Aspergillus niger*, the white onion demonstrated partial activity towards this fungus.

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