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RESEARCH ARTICLE

GC-MS Analysis and Antimicrobial Activity of Sudanese Cucumis melo L. (Cucrbitaceae) Fixed Oil

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ABSTRACT

The present study was designed to investigate the chemical constituents of Sudanese Cucumis melo seed oil and to evaluate its potential antimicrobial activity. 28 components were detected by GC-MS analysis. Major constituents are: methyl 10-trans,12-cis-octadecadienoate (49.28%) , 9-octadecenoic acid methyl ester(19.40%) , hexadecanoic acid methyl ester(15.92%) , methyl stearate(11.22%)

The antimicrobial activity of the oil was evaluated via cup plate agar diffusion bioassay against six standard human pathogens(Gram positive: Staphylococcus aureus and Bacillus subtilis; Gram negative : Escherichia coli and Pseudomonasa aeruginosa and the fungi Candida albicans and Aspergillus niger) . The oil showed different antimicrobial responses against test organisms. It gave significant activity against the fungus :Candida albicans and partial activity against Staphylococcus aureus.It seems that the oil is a lead for further optimization.

INTRODUCTION

The Cucrbitaceae family comprises hundreds of wild and cultivated varieties[1,2]. This family includes : gourds, melons , cucumbers, pumpkins and squashes. Cucurbitaceae is distributed in tropical and subtropical regions [3].

Plants of this family produce fruits with edible pericarp which could be important source of minerals , fiber, β- carotene(provitamin A) and vitamin C[3]. Seeds are diuretic ,beneficial for enlargement of prostate gland, chronic eczema.Fruits are tonic and laxative[4].The economic value of melons is quite substantial[4] .

Cucumis melo L. (melon) genotypes differ largely in morphological and biochemical traits and intraspecific classification of such variability has been difficult.Still most taxonomists rely on the work of Noudin[5]. Ninety one accessions of *Cucumis melo* were investigated for oil content and oil characteristics. Oil content ranged from 12.5-39.1%; iodine value 106.0-124% . GC analysis revealed the presence of linoleic , oleic , palmitic and stearic acids [6] .

Cucumis melo extract was evaluated for antioxidant, anti-inflammatory and analgesic properties. The extract inhibited the production of superoxide anion in a dose – dependant manner. It also showed anti-inflammatory and analgesic properties[7]. In another study, the therapeutic potential of traditionally used seeds were evaluated for antioxidant, analgesic and anti-inflammatory activities.Results showed potent anti-inflammatory, antioxidant and analgesic activity[8].

Yanty *et.al*. evaluated[9] the physicochemical characteristics of *Cucumis melo* seed oil. The oil was predominated with linoleic acid (69.0%) followed by oleic acid (16.8%) and palmitic acid (8.4%).

Materials and Methods

Plant material

The fruits of *Cucumis melo* were purchased from the local market – Omdurman, Sudan.The plant was authenticated by direct comparison with a herbarium sample.

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

Cucumis melo oil was 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*	fungus
6	*Candida albicans*	fungus

Methods

Extraction of oil from *Cucumis melo*

Dry-powdered seeds of *Cucumis melo* (300g) were macerated with n-hexane at room temperature for 48h..The solvent was removed under reduced pressure and the oil was kept in the fridge at 4°C for further manipulation.

Esterification of oil

A Methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in 100ml methanol. A stock solution of methanolic sulphuric acid was prepared by mixing (1ml)of concentrated sulphuric acid with (99ml) methanol.

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 n-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

Cucumis melo fixed oil was analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness)was used. Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program is given in Table 2, while other chromatographic conditions are depicted in Table 3.

Table 2: Oven temperature program

Rate	Temperature(C)	Hold time (mim.$^{-1}$)
-	60.0	0.00
10.00	300.0	0.00

Table 3: Chromatographic conditions

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

Antimicrobial assay

Preparation of bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in (100 ml) of normal saline to produce a suspension containing about 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.

Preparation of fungal suspensions

Fungal cultures were maintained on 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

The cup-plate agar diffusion method was adopted , with some minor modifications, to assess the

antimicrobial activity. (2ml) of the standardized bacterial stock suspension were mixed with (200 ml) of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4). Each one of the halves was designed for one of the test solutions. Separate Petri dishes were designed for standard antibacterial chemotherapeutics (ampicillin and gentamycin).

The agar discs were removed, alternate cups were filled with(0.1 ml) samples of each test solution using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.

Results and Discussion

GC-MS analysis of *Cucumis melo* fixed oil

GC-MS analysis of *Cucumis melo* oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern. Comparison of the mass spectra with the database on MS library revealed about 90-95% match.

Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 28 components(Table 4).The typical total ion chromatograms (TIC) is depicted in Fig.1.

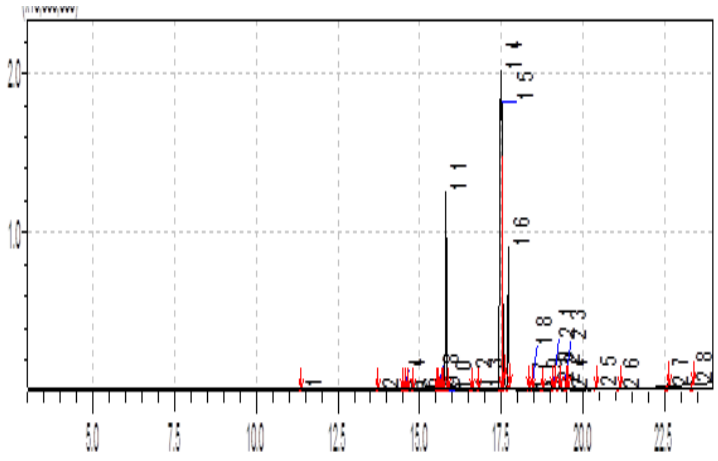

Fig.1:Typical total ion chromatograms

Some important constituents are discussed below:

Methyl 10-trans,12-cis-octadecadienoate (49.28%)

The EI mass spectrum of methyl 10-trans,12-cis-octadecadienoate is shown in Fig 2, the peak at m/z 294,which appeared at RT 17.497 in total ion chromatogram (TIC) correspond M^+ $\{C_{19}H_{34}O_2\}$.The peak at m/z 263 correspond to loss of methoxyl function.

Table 4: Contituents of *Cucumis melo* oil

Peak#	R.Time	Area	Area%	Name
1	11.341	253369	0.16	Butylated Hydroxytoluene
2	13.693	281916	0.17	Methyl tetradecanoate
3	14.470	26761	0.02	Tricosanoic acid, 10,14,18,22-tetramethyl-, m
4	14.505	27109	0.02	5-Octadecenoic acid, methyl ester
5	14.608	21509	0.01	cis-5-Dodecenoic acid, methyl ester
6	14.766	150571	0.09	Pentadecanoic acid, methyl ester
7	15.497	58127	0.04	Methyl 9,12-heptadecadienoate
8	15.556	84747	0.05	Methyl hexadec-9-enoate
9	15.601	496512	0.30	9-Hexadecenoic acid, methyl ester, (Z)-
10	15.695	12771	0.01	7-Hexadecenoic acid, methyl ester, (Z)-
11	15.804	25962526	15.92	Hexadecanoic acid, methyl ester
12	16.562	197993	0.12	10,13-Eicosadienoic acid, methyl ester
13	16.772	383377	0.24	Heptadecanoic acid, methyl ester
14	17.497	80362616	49.28	Methyl 10-trans,12-cis-octadecadienoate
15	17.530	31626283	19.40	9-Octadecenoic acid, methyl ester, (E)-
16	17.717	18293070	11.22	Methyl stearate
17	18.314	37268	0.02	9,12-Octadecadienoic acid, methyl ester
18	18.424	41111	0.03	cis-10-Nonadecenoic acid, methyl ester
19	18.713	150831	0.09	Methyl 9.cis.,11.trans.t,13.trans.-octadecatrie
20	19.062	972220	0.60	9,12-Octadecadienoic acid, methyl ester
21	19.094	212755	0.13	10,13-Octadecadienoic acid, methyl ester
22	19.265	703269	0.43	cis-11-Eicosenoic acid, methyl ester
23	19.462	1410551	0.87	Methyl 18-methylnonadecanoate
24	19.506	222457	0.14	Cyclopropanebutanoic acid, 2-[(2-[(2-pentylc
25	20.374	268471	0.16	Phenol, 2,2'-methylenebis[6-(1,1-dimethylet
26	21.083	223820	0.14	Methyl 20-methyl-heneicosanoate
27	22.585	262413	0.16	Tetracosanoic acid, methyl ester
28	23.329	314167	0.19	Squalene
		163058590	100.00	

9-Octadecenoic acid methyl ester(19.40%)

Fig. 3 shows the EI mass spectrum of 9-octadecenoic acid methyl ester.The peak at m/z 296, which appeared at R.T. 17.530 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$, while the peak at m/z266 accounts for loss of a methoxyl function.

Oleic acid (9-octadecenoic acid) is a common monounsaturated fat in human diet. It may be responsible for the hypotensive potential of olive oil[10] .

Oleic acid finds some applications in soap industry and it is used in small amounts as excipient in pharmaceutical industries. It is also used as soldening flux in stained glass work. Oleic acid is employed as emollient[11]. The consumption of

oleate in olive oil has been associated with decreased risk of breast cancer[12] .

Hexadecanoic acid methyl ester(15.92%)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig. 4.The peak at m/z 270, which appeared at R.T. 15.804 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m/z239 is attributed to loss of a methoxyl function.

Hexadecanoic acid (palmitic acid) is a saturated fatty acid .It is wide-spread in plants and humans . This acid is produced first during the synthesis of fatty acids[13] and is considered as precursor of long-chain fatty acids. Palmitic acid is a major lipid component of human breast milk[14,15].The acid finds applications in soaps and cosmetics industries. It is also used in food industry .

Methyl stearate(11.22%)

Mass spectrum of methyl stearate is shown in Fig. 5.The peak at m/z 298, which appeared at R.T. 17.717 corresponds to $M^+[C_{19}H_{38}O_2]^+$ .The peak at m/z267 corresponds to loss of a methoxyl function.

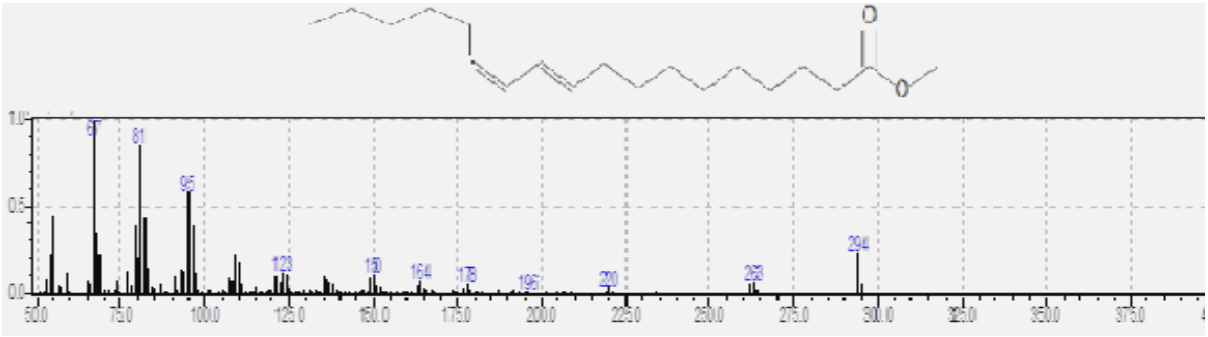

Fig.2 Mass spectrum of methyl 10-trans,12-cis-octadecadienoate

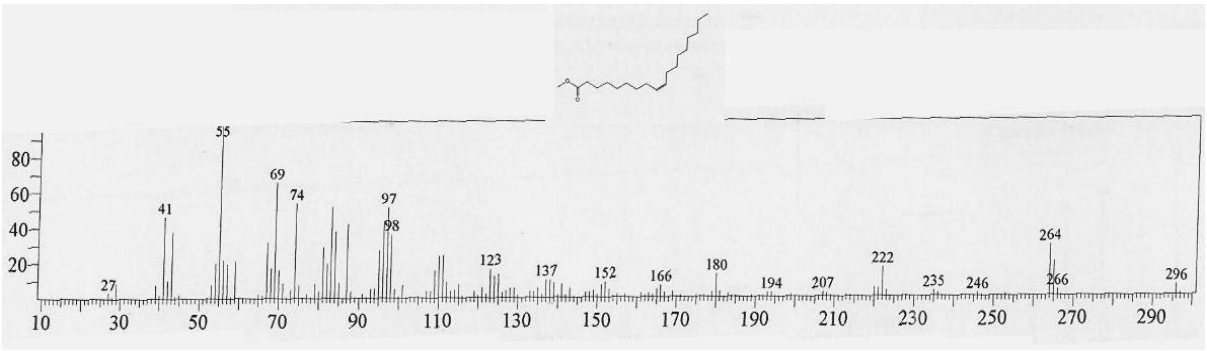

Fig. 3: Mass spectrum of 9-octadecenoic acid methyl ester

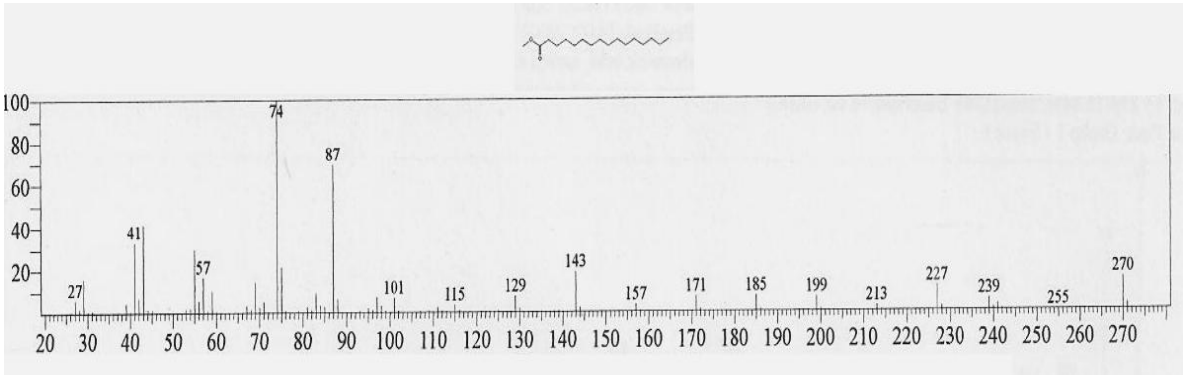

Fig. 4: Mass spectrum of hexadecanoic acid methyl ester

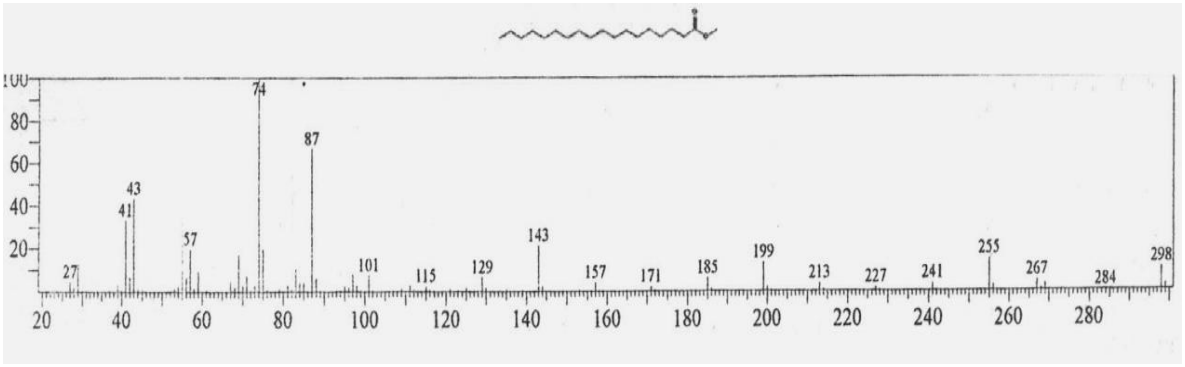

Fig. 5: Mass spectrum of methyl stearat

Antibacterial activity

In cup plate agar diffusion assay ,the oil was screened for antimicrobial activity against six standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table (5) .The results were interpreted in terms of the commonly used terms (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (6) and (7) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 5 : Antimicrobial activity of *Cucumis melo* oil

Type	Conc.(mg/ml)	**Sa**	Bs	Ec	Ps	Ca	An
Oil	100	12	8	-	-	19	8

Table 6 : 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 7 : 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 was partially active against *Staphylococcus aureus* , but it exhibited significant activity against the fungus: *Candida albicans*.

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