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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *CUCUMIS MELO* VAR. *FLEXUOUSAS* L. (CUCURBITACEAE) FIXED OIL

Dr. Abdel Karim M.[1]*, Inas A.[2] and Hind A.[1]

[1]Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.
[2]Omdurman Islamic University, Faculty of Pharmacy.

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

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ABSTRACT

This study was carried out to investigate the chemical constituents of *Cucumis melo* var. *flexuousas* L seed oil and to assess its antimicrobial activity. The GC-MS analysis revealed the presence of 22 constituents dominated by: 9,12-Z,Z-octadecadienoic acid methyl ester (48.08%),9-Z-octadecenoic acid methyl ester(18.05%%), hexadecanoic acid methyl ester(15.96%), methyl stearate(11.18%). *Cucumis Melo Var Flexuosus* oil was assessed for antimicrobial activity against five standard human pathogens. The oil showed excellent activity against *Escherichia coli* at test concentration. It also showed good activity against *Bacillus subtilis*. However, It exhibited partial activity against *Pseudomonas aeruginosa and Staphylococcus aureus*.The oil also displayed weak anticandidal potency.

KEYWORDS: *Cucumis melo* var. *flexuousas*, Fixed oil, GC-MS, Antimicrobial activity.

INTRODUCTION

The genus *Cucumis* includes cucumber and melon.Though the native habitat of melon and cucumis remained obscure for a long time, now there is some evidence that the native habitat is Asia.[1]

Cucumis melo var. *flexuousas* L. – also known as Armenian cucumber- grows up to 76-91cm.The plant is cultivated for its economic value as a vegatable.However, it is not known as a truly wild location.[2] The fruits, seeds and oil of this species are edible. Seeds contain 12.5-31.1% oil. The fruit is stomachic[3] and is used traditionally as skin moisturizer.[4] Flowers are emetic and expectorant. Seeds are vermifuge, febrifuge and digestive. Root is emetic and diuretic.[3]

The chemical composition and pharmacological effects of several Cucumis species have been reported.The aqueous and ethanol extracts of Cucumis melo var agrestis fruits were assessed for antioxidant activity. Both extracts showed significant activity towards free radicals.[5] Also the methanol extract of fruits exhibited marked free radical scavenging capacity.[6] In the paw edema model, the methanolic extract of fruits inhibited edema in a dose-dependant manner. The same extract showed analgesic effect in acetic acid –induced writhing model.[6]

Some Charentais melon cultivars were investigated for their volatiles content.[7] Also the volatiles in the skin and pulp of *Cucumis melo* var. *dudain* were studied[8].An ester (3-methylthiopropionic acid ethyl ester) with potential medical benefits was identified in a bioassay-guided fractionation scheme of *Cucumis melo* var. *conomon*.[9]

MATERIALS AND METHODS

Materials

Plant material

Seeds of *Cucumis melo var. flexuousas* were collected from Khartoum state(Sudan) and authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum-Sudan.

Instruments

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

Test organisms

Cucumis melo var. flexuousas oil was screened for antimicrobial activity using the Gram positive: *Bacillus subtilis* and *Escherichia coli*; Gram negative, *Pseudomonas aeroginosa, Escherichia coli* and the fungal species *Candida albicans.*

Methods

Extraction of oil from seeds of *Cucumis Melo* Var. *Flexuosus*

Powdered seeds of *Cucumis Melo* Var. *Flexuosus* (300g) were macerated with n-hexane.The solvent was removed under reduced pressure to give the oil.

GC-MS analysis

Analysis of *Cucumis Melo* Var. *Flexuosus* was done by gas chromatography – mass spectrometry on a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness). Chromatographic conditions are tabulated below.

Table 1: Oven temperature program.

Rate	Temperature(oC)	Hold Time (min.-1)
-	150.0	1.00
4.00	300.0	0.00

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

Testing for antibacterial activity

The cup-plate agar diffusion method was adopted to assess the antimicrobial activity of the oil.Mueller-Hinton agar and Sabouraud dextrose agar were the media used for microbial growth and they were prepared according to manufacture instructions.

Briefly, (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. Cups(10 mm in diameter) were cut using sterile cork borer (No 4), each cup was designed for one of the test solutions. Separate Petri dishes were designed for standard antibacterial chemotherapeutics.

The agar discs were removed. Cups were filled with 0.1 ml of test sample 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 compounds and the standard antibacterial chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in two replicates and averaged.

For antifungal activity Sabouraud dextrose agar was used as growth medium for fungi and incubation continued for 72h at 35°C.

RESULTS AND DISCUSSION

GC-MS analysis of *Cucumis Melo Var Flexuosus* fixed oil was carried out. Identification of the constituents was based on the MS library (NIST). 22 constituents were detected(Table 3).The typical total ion chromatogram is displayed Fig.(1).

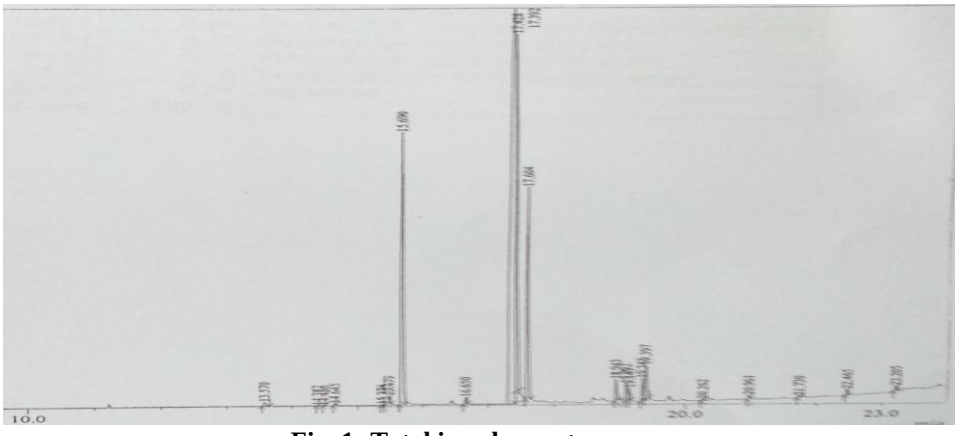

Fig. 1: Total ion chromatograms.

Table 3: Contituents of *Cucumis Melo Var Flexuosus* oil.

ak#	R.Time	Area	Area%	Name
1	13.570	524824	0.20	Methyl tetradecanoate
2	14.382	32839	0.01	cis-5-Dodecenoic acid, methyl ester
3	14.486	43712	0.02	5-Octadecenoic acid, methyl ester
4	14.645	219576	0.08	Pentadecanoic acid, methyl ester
5	15.374	88446	0.03	7,10-Hexadecadienoic acid, methyl ester
6	15.433	148432	0.06	7-Hexadecenoic acid, methyl ester, (Z)-
7	15.479	876033	0.33	9-Hexadecenoic acid, methyl ester, (Z)-
8	15.690	42472437	15.96	Hexadecanoic acid, methyl ester
9	16.650	776841	0.29	Heptadecanoic acid, methyl ester
10	17.392	127957677	48.08	9,12-Octadecadienoic acid (Z,Z)-, methyl
11	17.428	48043578	18.05	9-Octadecenoic acid (Z)-, methyl ester
12	17.604	29744195	11.18	Methyl stearate
13	18.943	2457377	0.92	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),
14	19.092	2043770	0.77	9-Octadecenoic acid, 12-hydroxy-, methyl
15	19.143	1303379	0.49	cis-11-Eicosenoic acid, methyl ester
16	19.342	2761768	1.04	Eicosanoic acid, methyl ester
17	19.397	4298664	1.62	PGH1, methyl ester
18	20.262	172461	0.06	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
19	20.961	454404	0.17	Docosanoic acid, methyl ester
20	21.730	298642	0.11	Tricosanoic acid, methyl ester
21	22.465	748550	0.28	Tetracosanoic acid, methyl ester
22	23.205	655029	0.25	Squalene
		266122634	100.00	

The following compounds were detected as major constituents:

9,12-Z,Z-Octadecadienoic acid methyl ester (48.08%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is displayed in Fig.2.The peak at m/z294(R.T. 17.392 -in total ion chromatogram) corresponds $M^{+}[C_{19}H_{34}O_2]^{+\cdot}$The signal at m/z263 corresponds to loss of a methoxyl function.

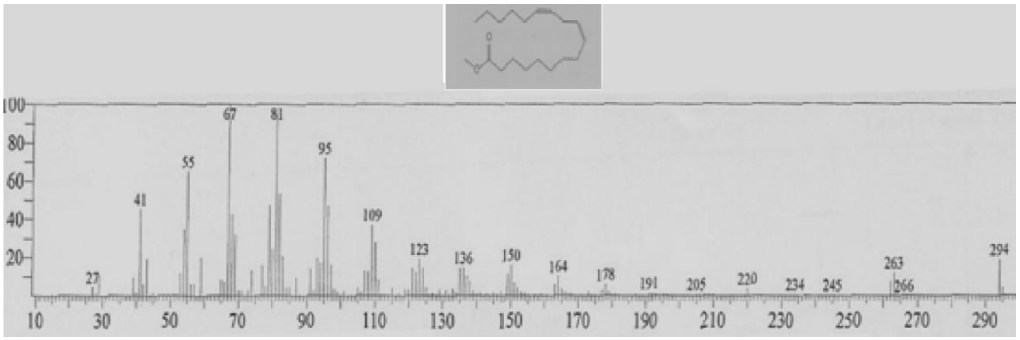

Fig. 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester.

9-Z-Octadecenoic acid methyl ester(18.05%%)

Fig. 3 shows the EI mass spectrum of 9-octadecenoic acid methyl ester.The peak at m/z 296, which appeared at R.T. 17.42 in total ion chromatogram, corresponds $M^{+}[C_{19}H_{36}O_2]^{+\cdot}$ while the peak at m/z266 accounts for loss of a methoxyl.

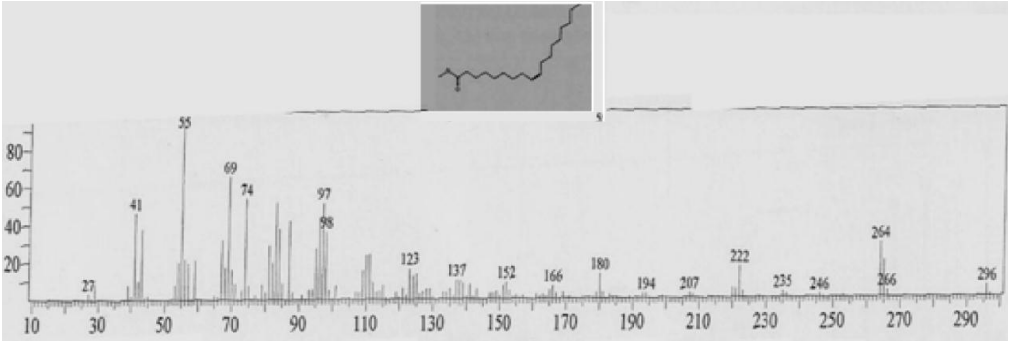

Fig. 3: Mass spectrum of 9-octadecenoic acid methyl ester.

Hexadecanoic acid methyl ester(15.96%)

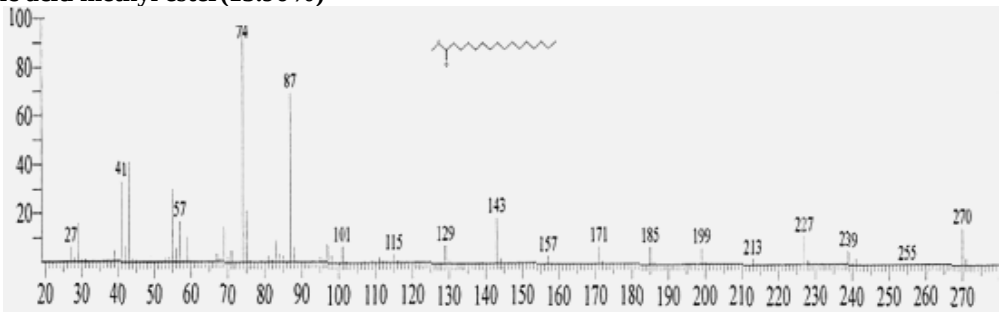

Fig. 4: Mass spectrum of hexadecanoic acid methyl ester.

The mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.4.The peak at m/z 270 (R.T.15.690) corresponds $M^{+}[C_{17}H_{34}O_2]^{+\cdot}$The signal at m/z239 corresponds to loss of a methoxyl.

Methyl stearate(11.18%)

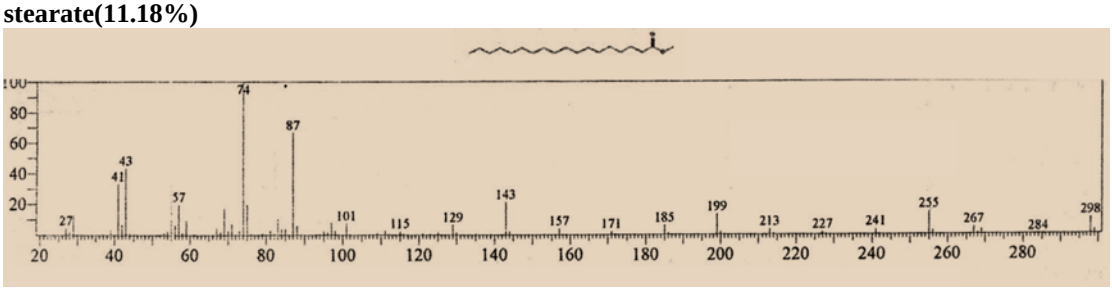

Fig.5 : Mass spectrum of methyl stearate

Fig. 5 shows the mass spectrum of methyl stearate. The signal at m/z 298(R.T.17.604) corresponds $M^{+}[C_{19}H_{38}O_2]^{+\cdot}$ while the peak at m/z267 corresponds to loss of a methoxyl group.

Antibacterial activity

Cucumis Melo Var Flexuosus oil was assessed for antimicrobial activity against five standard human pathogens. The diameters of the growth of inhibition zones are shown in Table (4). Results were interpreted as follows: (<9mm: inative; 9-12mm: partially active; 13-18mm: active; >18mm: very active). Tables (5) and (6) show the antibacterial and antifungal activities of standard drugs respectively.

Table 4: Antibacterial activity of *Cucumis Melo Var Flexuosus*oil.

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	10	14	16	12	10

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 Escherichia coli*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

The oil showed excellent activity against *Escherichia coli* at test concentration. It also showed good activity against *Bacillus subtilis*. However, It exhibited partial activity against *Pseudomonas aeruginosa and Staphylococcus aureus.*The oil also displayed weak anticandidal potency.

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