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Research Article

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Constituents and Antimicrobial Activity of *Momordica charantia* Seed Oil

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Abstract *Momordica charantia* is an important medicinal plant used in traditional medicine in the treatment of a wide range of diseases. The plant was used against Infections, cancer, leukemia, and diabetes. The leaves and fruit have both been used occasionally to make teas and beer or to season soups in the western world. In this study *Momordica charantia* oil was studied by GC-MS .Major constituents of the oil are: 9,12-octadecadienoic acid methyl ester(47.64%), hexadecanoic acid, methyl ester (17.06%) and 9,12,15-octadecatrienoic acid methyl ester (13.29%). The oil was assessed for antimicrobial activity. At a concentration of 100mg/ml, the oil showed good activity against *Escherichia coli*.

Keywords *Momordica charantia*, Oil, GC-MS Analysis, Antimicrobial Activity

Introduction

Oils are extracted from the leaves, petals, stems, seed, and even the roots of the plants which generally contain volatile oils in different concentrations.

Numerous compounds that make up essential oils have been identified. Some of these are present in infinitesimal quantities [1,2].

Essential oils are natural volatile complex plant compounds, oily or liquid-like in nature and frequently characterized by strong fragrance [3,4], they have low solubility in water but are soluble in fats, alcohol and organic solvents . They are stored in specialized plant cells, usually oil cell or ducts, resin ducts, glands or trichomes (glandular hairs) [5,6] and many could be extracted from the leaves, flowers, buds, fruits, roots wood or bark of plants by variety of methods including solvent and supercritical fluid extraction, expression under pressure, fermentation or effleurage [3]. Steam or hydro-distillation are used predominantly for commercial production [4-7].

Momordica charantia is an annual climbing plant producing stems 5 meters or more long that scrambles over the ground or climb up into the surrounding vegetation, supporting itself by means of tendrils. The plant is an important market vegetable in southern and eastern Asia [8], it is also cultivated on a small scale in tropical America and in the southern part of the United States. The plant is also grown for medicinal purpose. *Momordica charantia* was traditionally used against Infections, cancer, leukemia, and diabetes [9-11]. The leaves and fruit have both been used occasionally to make teas and beer or to season soups in the Western world.

The fruit of the plant, which is known as the bitter melon. At least three different groups of constituents in bitter melon have been reported to have blood-sugar lowering actions of potential benefit in diabetes mellitus [12]. These include a mixture of steroidal saponins and alkaloids.

Materials and Methods

Materials

Plant Material

Momordica charantia 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

S. No	Micro organism	Type
1	*Staphylococcus aureus*	G+ve
2	*Escherichia coli*	G-ve
3	*Pseudomonas aeruginose*	G-ve
4	*Candida albicans*	fungus

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.

Methods

Extraction of oil from *Momordica charantia* seeds

Powdered shade-dried seeds of *Momordica charantia* (300g) were exhaustively extracted with *n*-hexane (soxhlet). The solvent was removed under reduced pressure giving the oil.

GC-MS analysis

Momordica charantia seed oil was analyzed by gas chromatography – mass spectrometry using A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness). The instrument was used to identify and quantify the components of the studied oil. Analytical grade Helium (99.99 %) was used [13].

Rate: -; **Temperature**: 1500; **Hold Time** (min-1): 1.00

Rate: 4.00; **Temperature**: 3000; **Hold Time** (min-1): 0.00

Other chromatographic conditions are tabulated below:

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

A (24) hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated for 24h at 37º C. Bacterial growth was washed off with 100 ml sterile normal saline giving approximately 108- 109 C.F.U/ ml. The average number of viable organisms per ml of the stock suspension was determined. Serial dilutions of the stock suspension were made in sterile normal saline solution (0.02 ml) volumes of the appropriate dilution were transferred onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature and then incubated at 37°C for 24 hours.

The fungal cultures were maintained on Sabouraud dextrose agar. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspension was stored in the refrigerator until used. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) soaked with a solution of each test sample was placed on the surface of the seeded agar. The inoculated plates were incubated at 37 °C for 24 h. The diameters (mm) of the inhibition zones were measured as average of two replicates[14].

Results and Discussion

Gas chromatography – mass spectrometry

The GC-MS analysis of *Momordica charantia* oil showed 21 constituents (Table 3). Fig. 1 shows the total ions chromatograms.

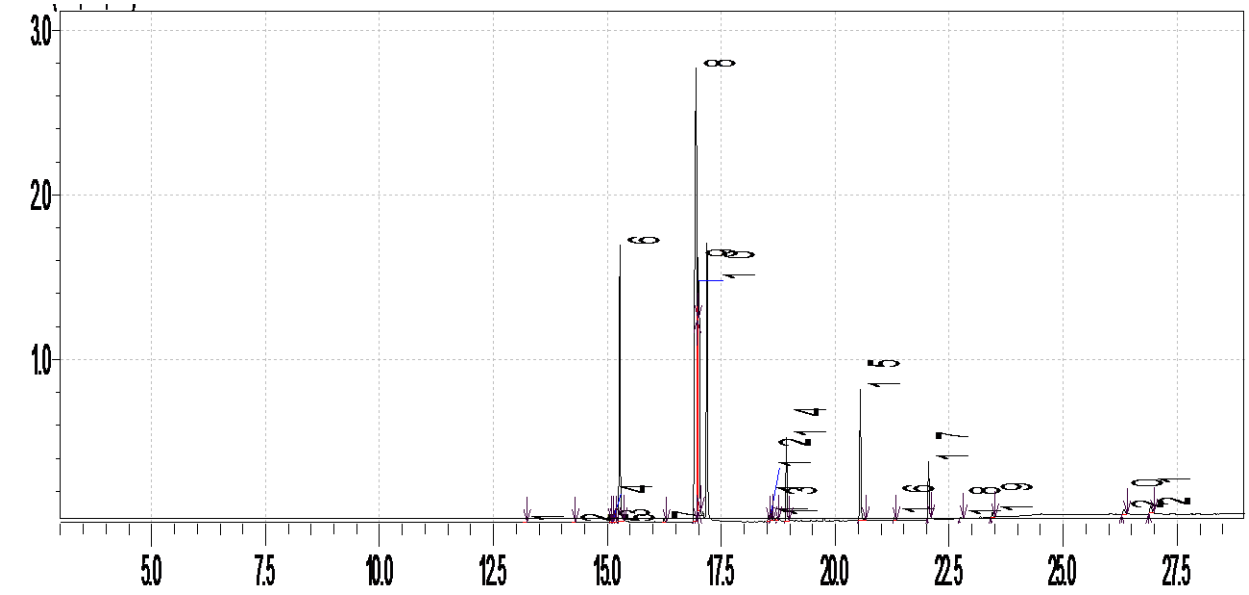

Figure 1: Total ions chromatograms

Major components of the oil are:

i)-9,12-Octadecadienoic acid methyl ester (47.64%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in (Fig. 2). The peak at m/z 294, which appeared at R.T. 16.948 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$. The peak at m/z 263 corresponds to loss of a methoxyl function.

ii)-Hexadecanoic acid, methyl ester (17.06%)

Fig. 3 shows the mass spectrum of hexadecanoic acid methyl ester. The molecular ion: $M+[C_{17}H_{34}O_2]+$ appeared at m/z 270 (R.T.15.272). The fragment at m/z 239 is due to loss of a methoxyl function.

iii)-9,12,15-Octadecatrienoic acid methyl ester (13.29%)

Fig. 4 shows the mass spectrum of 9,12,15-octadecatrienoic acid methyl ester. The molecular ion: $M^+[C_{19}H_{32}O_2]^+$ appeared at m/z 292 at (R.T.17.010) .The fragment at m/z261 is due to loss of a methoxyl.

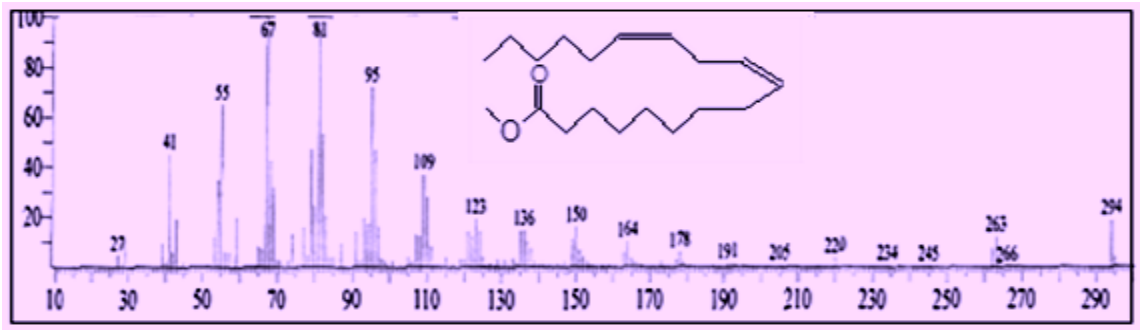

Figure 2: Mass spectrum of 9,12-octadecanoic acid methyl ester

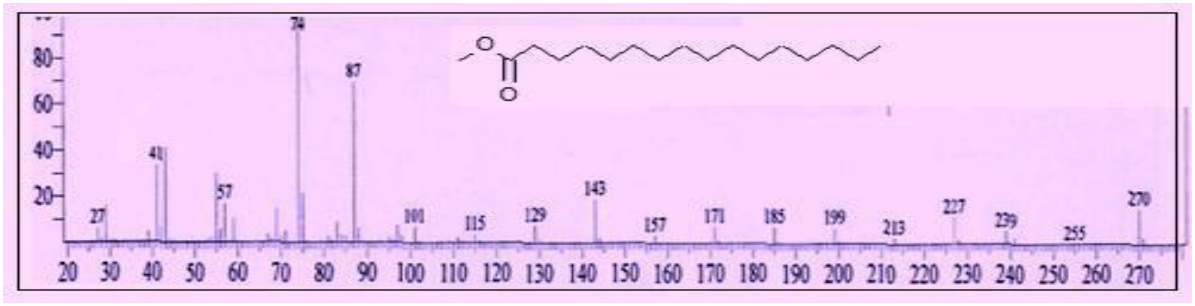

Figure 3:Mass spectrum of hexadecanoicacidmethyl ester

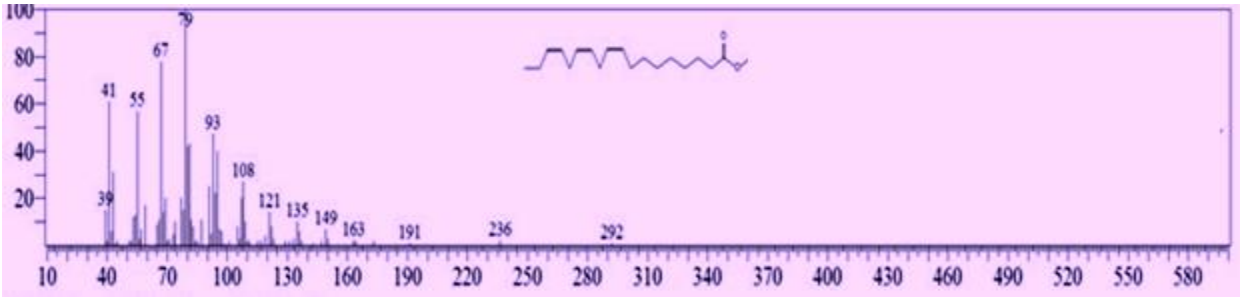

Figure 4: Mass spectrum of 9,12,15-octadecatrienoic acid methyl ester

Table 3: Constituents of the oil

S. No.	Name	Ret. Time	Area%
1	Methyl tetradecanoate	13.179	0.05
2	Pentadecanoic acid, methyl ester	14.245	0.03
3	9-Hexadecenoic acid, methyl ester, (Z)-	15.070	0.27
4	9,12-Hexadecadienoic acid, methyl ester	15.111	0.04
5	trans-13-Octadecenoic acid, methyl ester	15.163	0.03
6	Hexadecanoic acid, methyl ester	15.272	17.06
7	Heptadecanoic acid, methyl ester	16.240	0.21
8	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.948	47.64
9	9-Octadecenoic acid (Z)-, methyl ester	16.980	1.79
10	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	17.010	13.29
11	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	18.534	0.45
12	11,14,17-Eicosatrienoic acid, methyl ester	18.589	0.46

13	cis-11-Eicosenoic acid, methyl ester	18.724	0.20
14	Eicosanoic acid, methyl ester	18.926	4.68
15	Docosanoic acid, methyl ester	20.547	8.18
16	Tricosanoic acid, methyl ester	21.307	0.36
17	Tetracosanoic acid, methyl ester	22.044	3.36
18	Pentacosanoic acid, methyl ester	22.756	0.16
19	Hexacosanoic acid, methyl ester	23.444	0.38
20	Stigmasterol	26.331	0.60
21	.gamma.-Sitosterol	26.920	0.76

Antimicrobial Activity

The antimicrobial activity of the oil was examined against Gram positive bacteria: *staphylococcus aureus*, Gram negative bacteria *Escherichia coli, Pseudomonas aeruginosa* and the fungus *candida albicans*. The obtained results are compared with reference drugs (ampicilin, gentamicin and clotrimazole) as presented in Table 4. The oil exhibited good activity against *Escherichia coli*

Table 4: Inhibition zones (mm/mgsample)

Type	Sa	Ec	Ps	Ca
Oil (100mg/ml)	11	14	12	12
Ampicilin (40mg/ml)	30	--	--	--
Gentamicin (40mg/ml)	19	22	21	--
Clotrimazole	--	--	--	38

<9mm: Inactive; 9-12mm: partially active; 13-18mm: active;> 18mm: very active

Sa.: *Staphylococcus aureus*; Ec.: *Escherichia coli*; Pa.: *Pseudomonas aeruginosa*; Ca.: *Candida albicans*

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