



CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *IPOMOEA SINENSIS* (CONVOLVULACEAE) OIL

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ABSTRACT

Ipomoea is a large and diverse genus in the family Convolvulaceae with more than 600 species mainly distributed through tropical and subtropical regions. Ipomoea species have a long history of traditional uses. Some of these species are nutritionally important. In this study the oil from *Ipomoea sinensis* was studied by GC-MS and the antimicrobial activity was evaluated. The GC-MS analysis showed twenty constituents. Major constituents are: 9,12-octadecadienoic acid methyl ester (32.29%), hexadecanoic acid methyl ester (25.27%), methyl stearate (14.64%) and 9-octadecenoic acid methyl ester (7.32%). In the cup plate agar diffusion assay, the studied oil showed moderate activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

KEYWORDS: *Ipomoea sinensis*, Oil, GC-MS Analysis, Antimicrobial Activity.

INTRODUCTION

Ipomoea is a large and diverse genus in the family Convolvulaceae with more than 600 species mainly distributed through tropical and subtropical regions.^[1-5]

Ipomoea sinensis is an annual herb with numerous stems in the family Convolvulaceae. Ipomoea species have a long history of traditional uses. Some of these species are nutritionally important. *Ipomoea batatas* is cultivated worldwide for its nutritional value.^[6,7] *Ipomoea equatica* –which is rich in carotenoids- is used as foodstuff in some Asian countries.^[8-10] Several *Ipomoea* species are used in phytotherapy against a wide array of human disorders.^[8,9,11-17] *Ipomoea asarifolia* is used against itch while *Ipomoea batata* is a natural remedy for asthma, tumors of mouth and throat. Leaves are astringent, demulcent, laxative and tonic.^[11,12] *Ipomoea cairica* is used in the treatment of inflammations and Rheumatism.^[17]

MATERIAL AND METHODS

Plant material

Seeds of *Ipomoea sinensis* were collected from Folla, western Sudan. The plant was authenticated by The Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan.

Instruments

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

Test organisms

The antimicrobial potential of *Ipomoea sinensis* oil was estimated by the cup plate agar diffusion bioassay using the standard microorganisms: *Bacillus subtilis* (G+ve), *Staphylococcus aureus* (G+ve), *Pseudomonas aeruginosa* (G-ve), *Escherichia coli* (G-ve) and the yeast *Candida albicans*.

Methods

Extraction of oil

Powdered seeds of *Ipomoea sinensis* (250g) were macerated with n-hexane at room temperature for 48hr. The solvent was removed under reduced pressure giving the oil.

GC-MS analysis

The oil was analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument. Chromatographic conditions are outlined below.

Table 1: Oven temperature program.

Rate	Hold Time (min. ⁻¹)	Temperature(°C)
-0	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
Rate	4/min
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.
Split ratio	- 1.0

Antimicrobial activity

Mueller Hinton agar and Sabouraud dextrose agar were used as media for bacterial and fungal cultures respectively. The antimicrobial activity was performed according to the method described by Jasmine et.al.^[18]

Agar Petri dishes maintained at 45°C in a water bath were seeded with an overnight culture (1ml) of

bacteria(10^7 - 10^8 cfu/ml). Wells (8mm in diameter) were cut on the seeded agar via a sterile cork borer. The cups were filled with (0.1ml) of the test solution and the Petri dishes were left to settle and then incubated for 24 h. at 37°C. The assay was carried out in duplicates. After incubation the diameters of inhibition zones were measured and averaged as indicator of activity. The same procedure was adopted for antifungal activity, but Sabouraud dextrose agar was used instead of Mueller Hinton agar and incubation was continued for three days at 25°C.

RESULTS AND DISCUSSION

The oil from *Ipomoea sinensis* has been investigated. The oil of this species was extracted and studied by GC-MS. Furthermore, the oil has been assessed for antimicrobial activity via the agar diffusion bioassay against five standard pathogenic microbes. Identification of the constituents was accomplished by consulting the MS library (NIST) and also by matching retentions times with the database of the library.

Constituents of oil

Twenty components have been detected by GC-MS analysis (Table 3). The typical total ion chromatograms (TIC) is presented in Fig 1.

Table 3: Constituents of the oil.

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.275	0.20
2.	Pentadecanoic acid, methyl ester	15.407	0.03
3.	9-Hexadecenoic acid, methyl ester, (Z)-	16.288	0.33
4.	Hexadecanoic acid, methyl ester	16.497	25.27
5.	cis-10-Heptadecenoic acid, methyl ester	17.306	0.09
6.	Heptadecanoic acid, methyl ester	17.519	0.22
7.	4,7,10-Hexadecatrienoic acid, methyl ester	18.097	0.62
8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.259	32.29
9.	9-Octadecenoic acid (Z)-, methyl ester	18.302	7.32
10.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.325	6.03
11.	Methyl stearate	18.511	14.64
12.	Cyclopropanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	19.957	0.45
13.	11,14-Eicosadienoic acid, methyl ester	19.995	0.44
14.	Heptadecanedioic acid, 9-oxo-, dimethyl ester	20.049	1.36
15.	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	20.121	1.04
16.	Eicosanoic acid, methyl ester	20.356	3.91
17.	13-Docosenoic acid, methyl ester, (Z)-	21.882	0.31
18.	Docosanoic acid, methyl ester	22.060	2.47
19.	Heneicosanoic acid, methyl ester	23.640	1.15
20.	.gamma.-Sitosterol	24.236	1.83

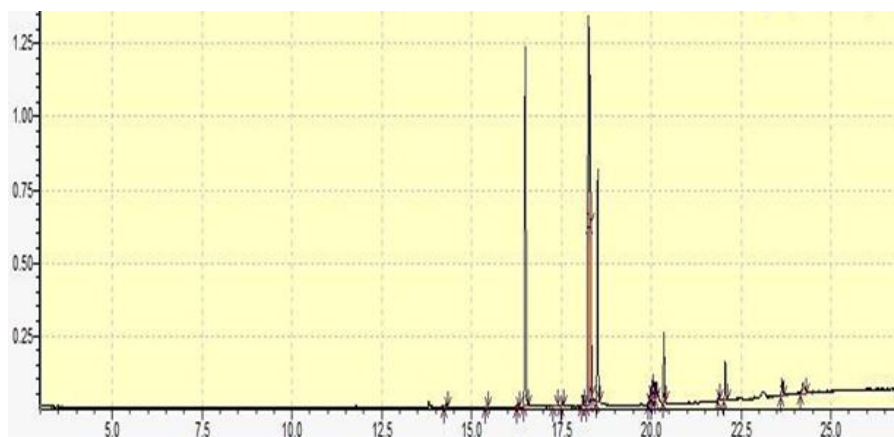


Fig. 1: Typical total ion chromatograms.

Major constituents are discussed below:

9,12-octadecadienoic acid methyl ester (32.29%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.2. The peak at m/z 294 (R.T. 18.259) coincides with $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z 263 is due to loss of a methoxyl.

Hexadecanoic acid methyl ester (25.27%),

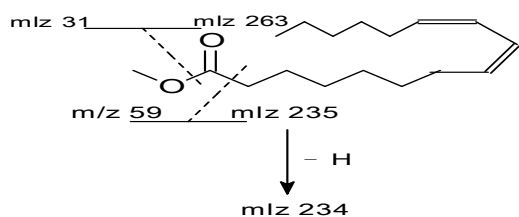
Fig. 3 presents the EI mass spectrum of hexadecanoic acid methyl ester. The peak at m/z 270 with retention time 16.497, is attributed to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m/z 239 is due to loss of methoxyl function.

Methyl stearate (14.64%)

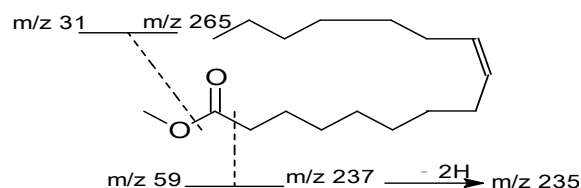
The EI mass spectrum of methyl stearate is shown in Fig. 4. The peak at m/z 298 which appeared at R.T. 18.511 corresponds to $M^+[C_{19}H_{38}O_2]^+$. The peak at m/z 267 accounts for loss of methoxyl.

9-Octadecenoic acid methyl ester (7.32%)

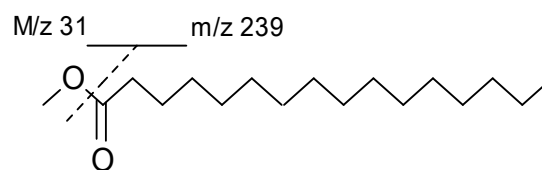
The mass spectrum of 9-octadecenoic acid methyl ester is depicted in Fig. 5. The signal at m/z 296 which appeared at R. T 18.302 is due to $M^+[C_{19}H_{36}O_2]^+$, while the signal which appeared at m/z 265 is due to loss of methoxyl.



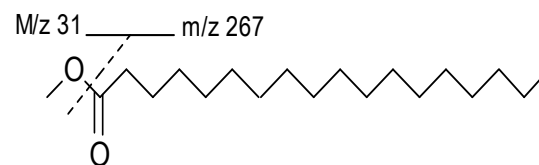
Major fragmentation of 9,12-octadecadienoic acid methyl ester



Major fragmentation of 9-octadecenoic acid methyl ester.



Major fragmentation of hexadecanoic acid methyl ester



A Major fragmentation of methyl stearate

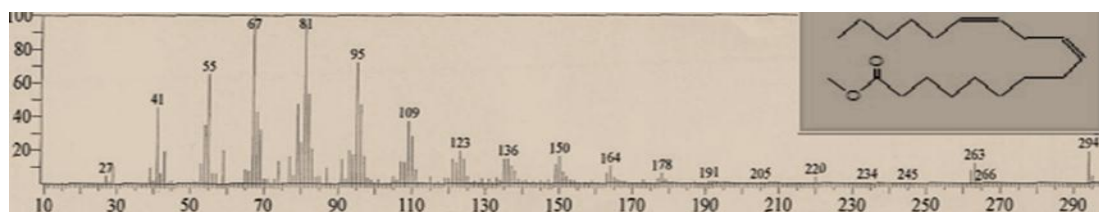


Fig. 2: Mass spectrum of 9,12-octadecanoic acid methyl ester.

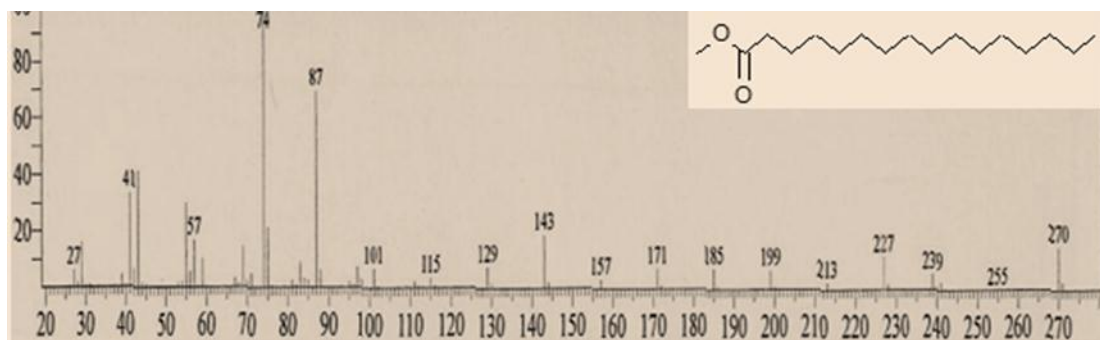


Fig. 3: Mass spectrum of hexadecanoic acid methyl ester.

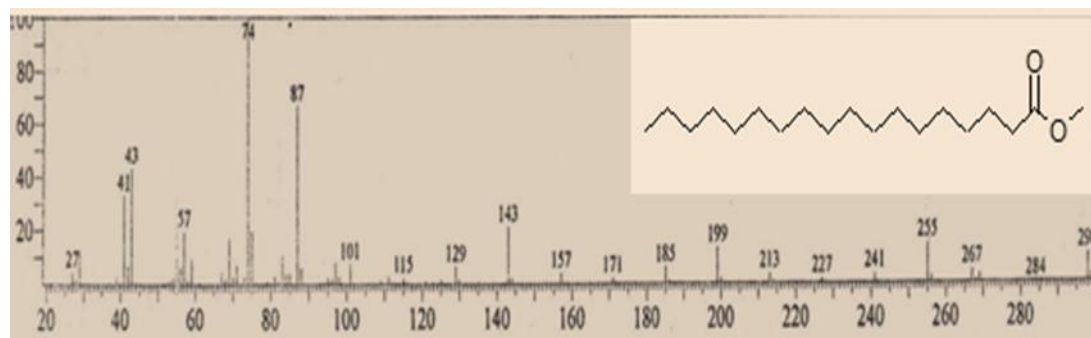


Fig. 4: Mass spectrum of methyl stearate.

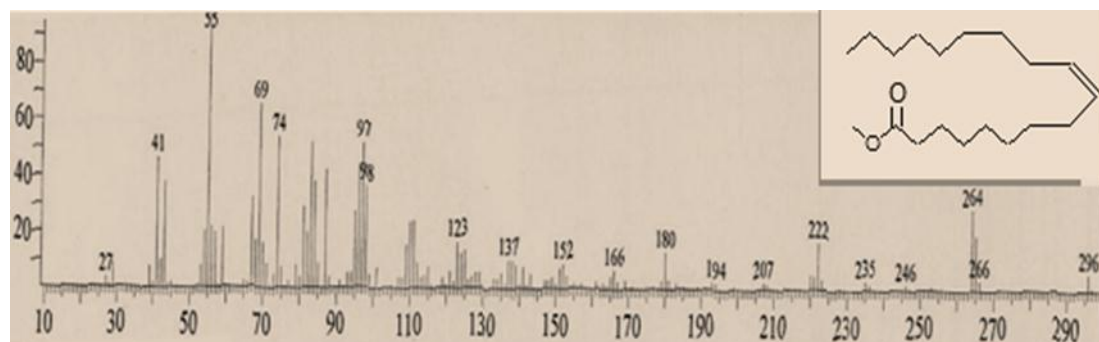


Fig. 5: Mass spectrum of 9-octadecenoic acid methyl ester.

Antimicrobial activity

In the cup plate agar diffusion bioassay, the studied oil was assessed for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table 4. The results were interpreted in term of commonly used

terms : <9mm: inactive; 9-12mm partially active; 13-18: active;>18mm: very active. Ampicilin, gentamicin and clotrimazole were used as positive controls. The studied oil showed moderate activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albican*.

Table 4: Diameters of inhibition zones (mm).

Type	Ec.	Ps.	Sa.	Bs.	Ca.
Oil (100mg/ml)	16	13	15	--	16
Ampicilin					
40 (mg/ml)	--	--	30	15	--
20 (mg/ml)	--	--	25	14	--
10 (mg/ml)	--	--	15	11	--
Gentamicin					
40 (mg/ml)	22	21	19	25	--
20 (mg/ml)	18	15	18	22	--
10 (mg/ml)	15	12	14	17	--

Table 6: Antifungal activity of standard drug.

Drug	Conc.(mg/ml)	An	Ca
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Sa: *Staphylococcus aureus*.

Ec: *Escherichia coli*.

Ps: *Pseudomonas aeruginosa*.

Bs: *Bacillus subtilis*.

Ca: *Candida albicans*.

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