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Monechma ciliatum Oil : GC-MS Analysis and Antimicrobial Activity

Abdel Karim M.[1(*)] , Faiza , I.[2] and Inas ,O.[3]

1. Sudan University of Science and Technology, Faculty of Science.
2. International University of Africa, Faculty of Science.
4. University of Bahri, Faculty of Applied and Industrial Chemistry.

Abstract

Monechma ciliatum is an important medicinal plant used in traditional medicine in the treatment of a wide range of diseases. In Sudanese system of medicine it is used by local healers against common cold and asthma.In this study the fixed oil of *Monechma ciliatum* was extracted by maceration and its constituents were identified and quantified by GC-MS analysis. The antimicrobial potential of the oil was evaluated via the disc diffusion bioassay against six standard human pathogens. The oil showed significant antifungal activity against the test fungi . It also showed activity against *Staphylococcus aureus.* However , it was partially active against *Escherichia coli* and *Pseudomonas aeruginosa.* The oil was inactive against *Bacillus subtilis.*

Keyword: *Monechma ciliatum* , Fixed Oil , GC-MS analysis , Antimicrobial Activity.

Introduction

Monechma ciliatum –locally known as "Black Mahlab" – is an annual , almost glabrous herb reaching 1-2 ft in height(Andrews,1956). The plant is native to India and tropical Africa(Wichens,1976). It is distributed in Senegal, Sudan, Cameron, Malawi and Zambia(Wichens,1976). In "Nuba Mountains" of Sudan , natives use the seeds against asthma and common cold , it is also used as laxative. In Botswana , the herb is used by local healers to treat kidney and liver troubles and to kill pain(Inga and Stengard,1989).

Phytochemical screening of leaves showed the presence of alkaloids, flavonoids, tannins , triterpens, glycosides and anthraquinones (Uguru et.al.,1998). In another study , the methanolic extract of leaves demonstrated antimicrobial activity against a panel of human pathogens(Afaf et.al.,2015). Manal(2008) investigated the antimicrobial activity and pharmacological properties of *Monechma ciliatum* and testified that the chloroform fraction of leaves possesses significant antimicrobial activity against *S.aureus* . Ayoub and David(1984) claimed that the herb has anticancer constituents.

Various fractions of leaves were evaluated for oxytocic activity in uterine preparations . The methanolic extract contracted the non-pregnant uterus in model animals(Uguru et.al.,1995) and it seems that the plant contract the uterus via many mechanisms.

Also it was demonstrated that the methanolic extract of *Monechma ciliatum* effectively regulated the expression of LDLR and HMGCR genes influencing the cholesterol metabolism in HepG2 cells(Abdalbasit et.al.,2010).

Materials and Methods

Materials

Plant material

Monechma ciliatum seeds were purchased from the local market-Khartoum and authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum-Sudan.

Test organisms

Table 1 shows the standard microorganisms used in antimicrobial assay.

Table 1: Test organisms

Ser. No	Micro organism	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

Monechma ciliatum seeds (400g) were milled into fine powder and exhaustively extracted with n-hexane by maceration.The solvent was removed *in vacuo* to afford the oil. Alcoholic sodium hydroxide and alcoholic sulphuric acid were used for oil esterification.

GC-MS analysis

The oil was studied by gas chromatography – mass spectrometry using a QP2010 Ultra Instrument(Shimadzo) with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness). Analytical grade Helium (99.99 %) was used as carrier gas. Oven temperature program is shown below:

Rate: - ; **Temperature** :150^0 ; **Hold Time** (min^{-1}) : 1.00

Rate : 4.00 ; **Temperature** :3 00^0 ; **Hold Time** (min^{-1}) : 0.00

Other chromatographic conditions are tabulated below:

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

Antimirobial sensitivity test

(38g) of powdered agar were allowed to soak in distilled water for ten minutes. The medium was heated in a water bath to dissolve, swirled to mix and sterilized by autoclaving for 15 minutes at 121°C.Then cooled at 47° , mixed well and poured into sterile Petri dishes.

Disc diffusion bioassay

(20 ml) aliquots of molten agar were distributed into sterile Petri dishes. (0.1ml) of the standardized bacterial stock suspension(10^8-10^9 colony-forming units/ml) were soaked on agar medium plates

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using sterile cotton swab. Filter paper discs (6mm diameter) were sterilized and soaked in test sample solution and then placed on the surface of the test bacteria plates. Plates were incubated for 24h and the, diameter of inhibition zones were measured in triplicates and averaged. DMSO was used as negative contol ,while standard antimicrobial chemotherapeutics were used as positive control . The same process was adopted for antifungal activity, but potato dextrose agar was used instead of nutrient agar and incubation was extended for 72h.

Results and discussion

The total ion chromatogram is shown in Fig. 1. A Tabulation of oil constituents is given in Table 3.

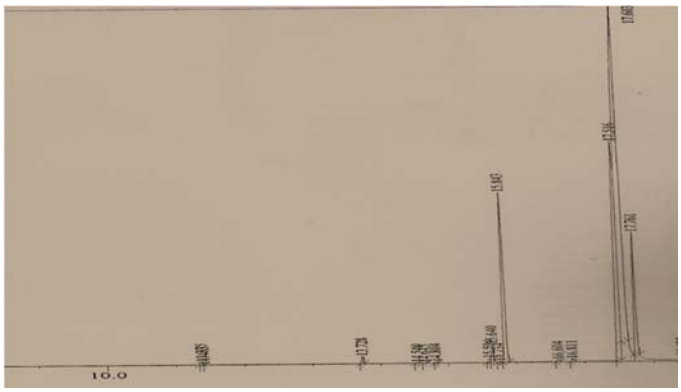

Fig.1: Total ion Chromatograms

Table 3: Constituents of *Monechma ciliatum* seed oil

Peak#	R.Time	Area	Area%	Name
1	11.375	263375	0.08	Butylated Hydroxytoluene
2	11.405	20784	0.01	Dodecanoic acid, methyl ester
3	13.728	733415	0.23	Methyl tetradecanoate
4	14.540	87636	0.03	5-Octadecenoic acid, methyl ester
5	14.645	27802	0.01	6-Octadecenoic acid, methyl ester
6	14.804	117914	0.04	Pentadecanoic acid, methyl ester
7	15.594	451087	0.14	Methyl hexadec-9-enoate
8	15.640	1701793	0.53	14-methylpentadec-9-enoic acid methyl es
9	15.734	68064	0.02	9-Hexadecenoic acid, methyl ester, (Z)-
10	15.843	22565958	7.08	Hexadecanoic acid, methyl ester
11	16.604	228486	0.07	cis-10-Heptadecenoic acid, methyl ester
12	16.811	294252	0.09	Heptadecanoic acid, methyl ester
13	17.516	41971945	13.16	9,12-Octadecadienoic acid (Z,Z)-, methyl
14	17.603	84948827	26.64	9-Octadecenoic acid (Z)-, methyl ester
15	17.761	14728755	4.62	Methyl stearate
16	18.425	458112	0.14	cis-10-Nonadecenoic acid, methyl ester
17	18.646	203612	0.06	Nonadecanoic acid, methyl ester
18	19.144	122447	0.04	10,13-Octadecadienoic acid, methyl ester
19	19.328	35243819	11.05	11-Eicosenoic acid, methyl ester
20	19.531	35515180	11.14	Eicosanoic acid, methyl ester
21	20.054	3873362	1.21	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-
22	20.145	1859376	0.58	Stigmast-8(14)-en-3.beta.-ol
23	20.330	646347	0.20	Heneicosanoic acid, methyl ester
24	20.952	10966599	3.44	13-Docosenoic acid, methyl ester
25	21.148	32767714	10.28	Methyl 20-methyl-heneicosanoate
26	21.889	807156	0.25	Tricosanoic acid, methyl ester
27	22.645	27855344	8.74	Tetracosanoic acid, methyl ester
28	23.341	298036	0.09	Methyl 22-methyl-tetracosanoate
		318827197	100.00	

Major constituents of the oil are discussed below:

i) 9-Octadecenoic acid methyl ester(26.64%)

At retention time (17.603) , the molecular ion M^+ $(C_{19}H_{36}O_2)^+$ of 9-octadecenoic acid methyl ester appeared(Fig. 2) at m/z 296. The peak at m/z 264 is due to loss of methoxyl function.

ii)9,12-octadecadienoic acid methyl ester(13.16%)

The EI mass spectrum of 9,12-octadecanoic acid methyl ester is depicted in Fig.3 .The molecular ion , $M^+[C_{19}H_{34}O_2]^+$ appeared at m/z 294 (R.T. 17.516) in total ion chromatogram.The peak at m/z263 is due to loss of a methoxyl.

iii)Eicosanoic acid methyl ester(11.14%)

The EI mass spectrum of eicosanoic acid methyl ester is displayed in Fig. 4.The signal at m/z326 ,with retention time 19.531 , corresponds $M^+[C_{21}H_{42}O_2]^+$ .The peak at m/z295 accounts for loss of a methoxyl function

iv)11-Eicosenoic acid methyl ester(11.05%)

The mass spectrum of Cis-11-eicosenoic acid methyl ester is shown in Fig.5 .The peak at m/z324(R.T. 19.328) corresponds to $M^+[C_{21}H_{40}O_2]^+$ .The peak at m/z293 corresponds to loss of a methoxyl function.

v)Methyl 20-methy-hexeicosanoate(10.28%)

The molecular ion$(C_{23}H_{46}O_2)^+$ for methyl 20-methy-hexeicosanoate appeared at m/z354(Fig.6) with retention time 21.148. The loss of a methoxyl is demonstrated by m/z323..

vi)Tetracosanoic acid methyl ester(8.74%)

The signal at m/z382(Fig.7) corresponds the molecular ion$(C_{25}\ H_{50}\ O_2)^+$ for tetracosanoic acid methyl ester(RT,22.645) , while the peak at m/z351 is due to loss of a methoxyl group.

vii)Hexadecanoic acid methyl ester (7.08%)

The molecular ion M^+ $(C_{17}H_{34}O_2)^+$ for hexadecanoic acid appeared at m/z270(RT, 15.843). The peak at m/z 239 is due to loss of a methoxy(Fig.8).

viii) Methyl stereate(4.62%)

The mass spectrum of methyl stereate is displayed in Fig.9 . The peak at m/z298(RT 17.761) corresponds M^+ $(C_{19}H_{38}O_2)^+$, while the signal at m/z267 accounts for loss of a methoxyl

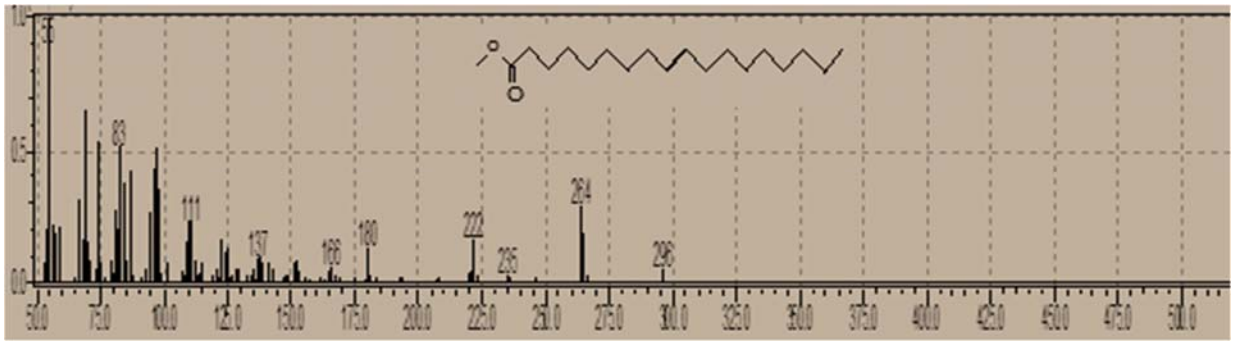

Fig. 2 ; Mass spectrum of 9-octadecenoic acid methyl ester

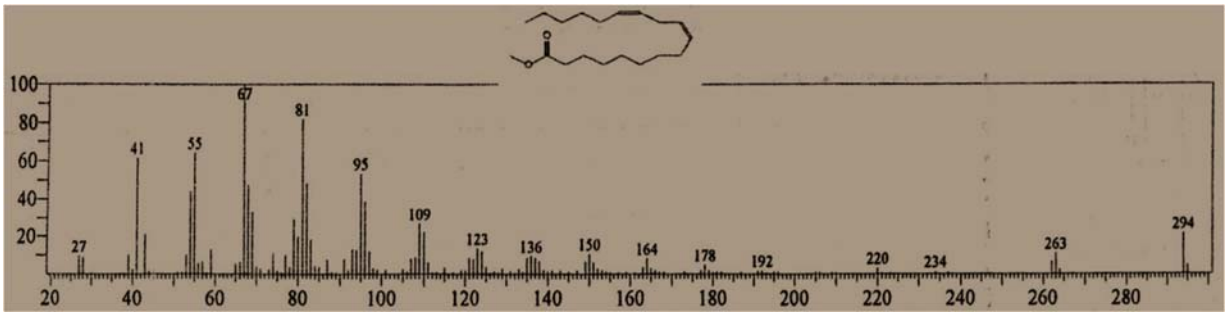

Fig.3 : Mass spectrum of 9,12-octadecanoic acid methyl ester

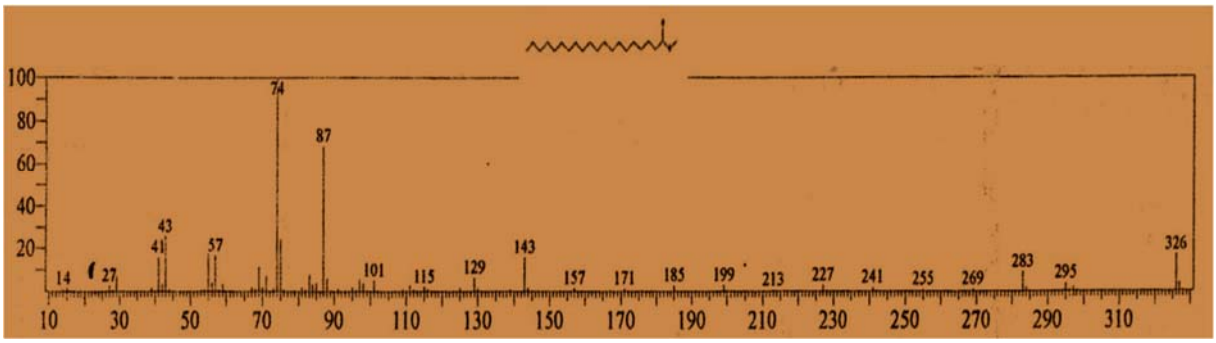

Fig. 4: Mass spectrum of eicosanoic acid methyl ester

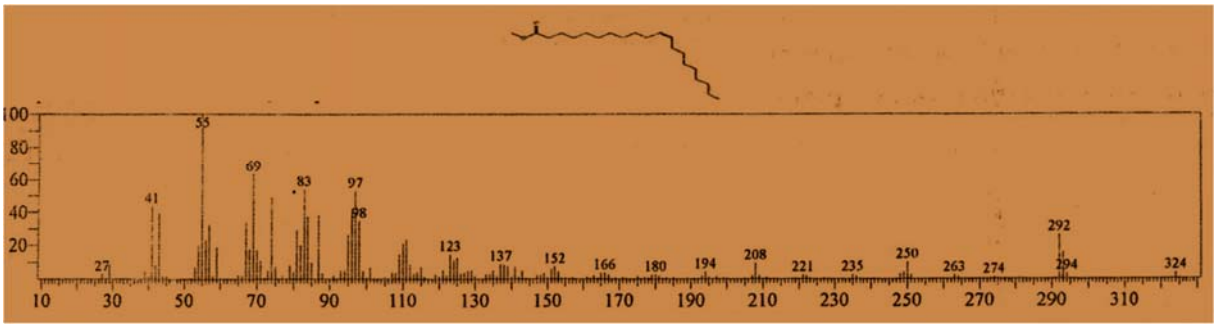

Fig.5 : Mass spectrum of 11-eicosenoic acid methyl ester

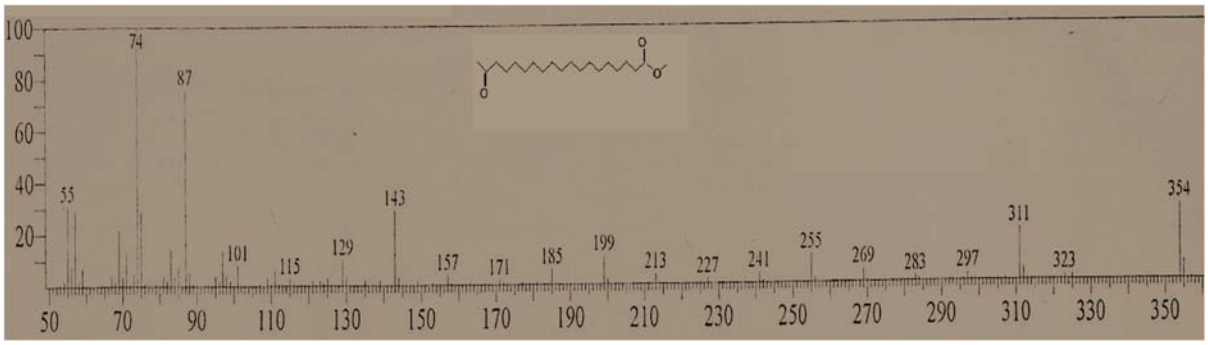

Fig.6 : Mass spectrum of methyl 20-methy-hexeicosanoate

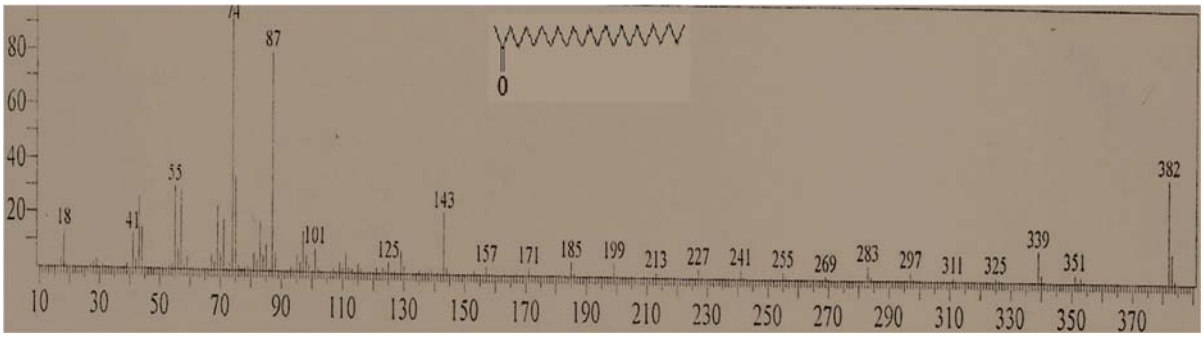

Fig.7 : Mass spectrum of tetracosanoic acid methyl ester

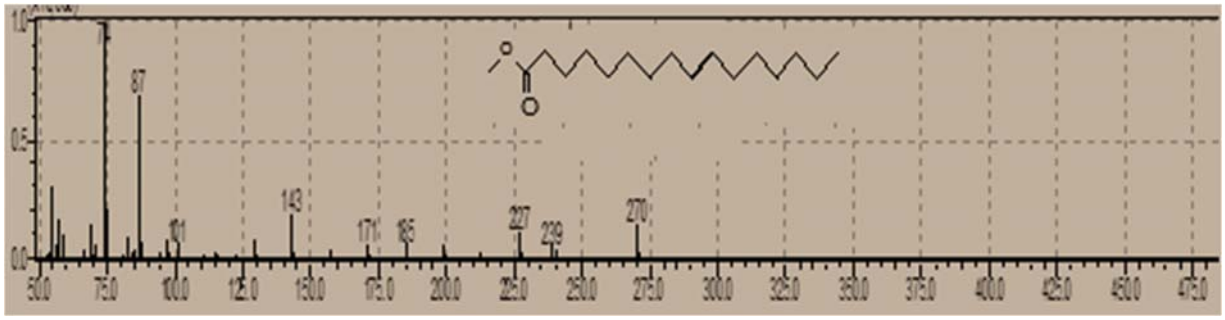

Fig.8; mass spectrum of hexadecanoic acid(methyl ester)

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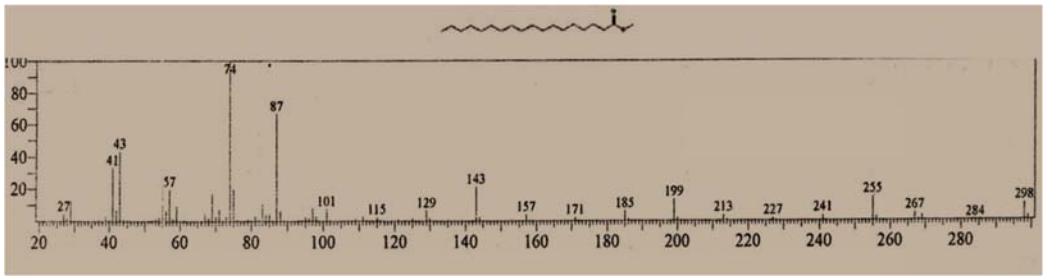

Fig.9: Mass spectrum of methyl stereate

Antimicrobial assay

The oil was assessed for antimicrobial activity against six standard organisms. Diameters of the growth inhibition zones are displayed in Table 4 .The results were interpreted according to the common terms : (<9mm: inative; 9-12mm:partially active; 13-18mm: active;>18mm: very active) .Tables (5) and (6) show the antimicrobial activity of standard drugs.

Table 4 : Antibacterial activity of *Monechma ciliatum* oil :M.D.I.Z (mm)

Drug	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca	An
Monechma ciliatum oil	100	12	11	14	--	15	16

Table 5 : Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	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 showed significant antifungal activity against the test fungi . It also showed activity against *Staphylococcus aureus.* However , it was partially active against *Escherichia coli* and *Pseudomonas aeruginosa.* The oil was inactive against *Bacillus subtilis*.This oil could serve as a lead for further optimization.

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