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Research Article

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Constituents and Antimicrobial Activity of Sudanese *Cordia africana* Oil

Abdel Karim M.[1]*, Afaf A.[2], Shaza A.[1]

[1]Sudan University of Science and Technology, Faculty of Science, Sudan
[2]Jazan University, Samtah University College, Saudi Arabia
[3]Omdurman Islamic University, Faculty of Pharmacy, Sudan

Abstract *Cordia Africana* is considered as a good source of herbal medicine, food (fruit is edible), firewood and bee forage. The plant is used traditionally against stomach-ache, tooth-ache, wounds and cough. In this study *Cordia Africana* oil has been investigated by GC-MS analysis. Furthermore, the oil has been assessed for its antimicrobial activity. The GC-MS analysis revealed the presence of 27 components. Major constituents are: a) 9,12-octadecadienoic acid (Z,Z)-, methyl ester (37.02%) (b)-hexadecanoic acid, methyl ester (18.02%) (c)-methyl stearate (10.01%) (d)- docosanoic acid, methyl ester (6.83%) (e)- 9-octadecenoic acid (Z)-, methyl ester (6.18%) and (f) eicosanoic acid, methyl ester (5.77%). The antimicrobial potential of the oil has been evaluated using the agar diffusion bioassay. The oil showed moderate activity against Gram positive *Staphylococcus aureus* and Gram negative *Pseudomonas aeruginosa.*

Keywords *Cordia africana* GC-MS analysis, Antimicrobial Activity

Introduction

Cordia africana Lam. (Synonym: *Cordia abyssinica*) is a small to medium-sized tree in the family Boraginaceae which comprises about 21 genera and 110 species [1]. *Cordia africana* grows up to 4-15m in height. The plant is widely distributed in east and south Africa [2]. *Cordia Africana* has a common occurrence in western Sudan where it is locally known as "Teak or Gombail" [3-5]. Wood is moderately hard and durable wood and serves as raw material for making high quality furniture and household materials [6]. *Cordia Africana* is considered as a good source of herbal medicine, food (fruit is edible), firewood and bee forage [7-9]. The plant is used traditionally against stomach-ache, tooth-ache, wounds and cough [10].

Materials and Methods

Plant materials

Seeds of *Cordai Africana* were collected from Kordofan (western Sudan) and authenticated by the Medicinal and Aromatic Plants Research Institute.

Instruments

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

Test of organisms

The following standard microorganisms were used to assess the antimicrobial potency of the oil*: Bacillus subtilis* (Gram (+ve), *Staphylococcus aureus* (Gram (+ve), *Pseudomonas aeroginosa* (Gram –ve), *Escherichia coli* (Gram – ve) and the fungal species *Candida albicans*

Methods

Extraction of oil

Powdered plant material (350g) was extracted with n-hexane using shaker extractor apparatus for 24h. Solvent was evaporated under reduced pressure to yield the oil.

Testing for antibacterial activity

The cup-plate agar diffusion method was used, with some minor modifications, to assess the antibacterial activity of the oil. Nutrient agar was used as medium for bacterial culture .One ml of the standardized bacterial stock suspension 10^8 to 10^9 CFU/ml was mixed with 100 ml of molten sterile nutrient agar which was maintained at 45°C. Aliquots (20ml) of the inoculated nutrient agar were distributed into sterile Petri-dishes. Two cups (10 mm in diameter) were cut into the seeded medium using a sterile cork borer (No. 4) and agar discs were removed. The wells were filled with 0.1 ml of test sample, and allowed to diffuse a room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 h. Two replicates were carried out for the test sample against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured and averaged as indicators of activity.

The same method was adopted for fungal species, but instead of nutrient agar, Sabouraud dextrose agar was used. The seeded medium was incubated for 48 hours at 25°C.

Results and Discussion

***Cordia africana* oil**

The total ion chromatograms of *Cordia Africana* oil is shown in Fig. 1 and the constituents of the oil are depicted in Table 1. The GC-MS analysis revealed the presence of 27 components.

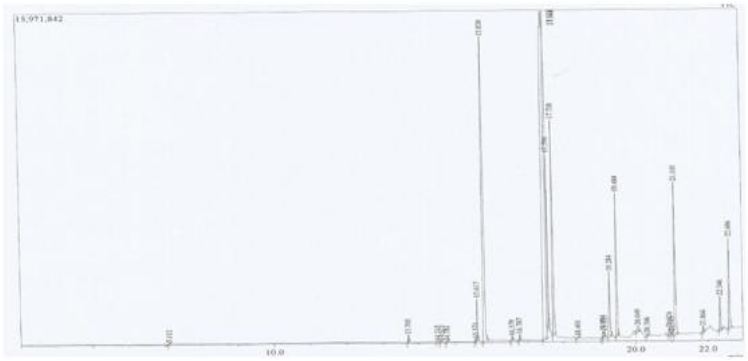

Figure 1: Total ion chromatograms of Cordia Africana

Table 1: Constituents of *Cordia Africana* oil

No.	R. Time	Area%	Name
1	7.112	0.05	Alpha-Terpineol
2	13.705	0.24	Methyl tetradecanoate
3	14.515	0.03	4-Octadecenoic acid methyl ester
4	14.621	0.04	Cis-5-Dodecenoic acid methyl ester
5	14.781	0.03	Pentadecanoic acid methyl ester
6	15.571	0.11	Methyl hexadec-9-enoate
7	15.617	1.47	9-Hexadecenoic acid methyl ester
8	15.830	18.02	Hexadecanoic acid methyl ester

9	16.579	0.19	Cis-10-Heptadecenoic acid methyl ester
10	16.787	0.22	Heptadecanoic acid methyl ester
11	17.523	37.02	9,12-Octadecadienoic acid methyl ester
12	17.568	6.18	9-Octadecenoic acid (Z)-methyl ester
13	17.590	3.63	9-Octadecenoic acid methyl ester(E)-
14	17.738	10.01	Methyl stearate
15	18.401	0.12	Cis-10-Nonadecenoic acid methyl ester
16	19.084	0.27	Cyclopropaneoctanoic acid methyl ester
17	19.284	0.21	9,12-Octadecdienyl chloride
18	19.284	3.24	Cis-11-Eicosenoic acid methyl ester
19	19.484	5.77	Eicosanoic acid methyl ester
20	20.049	0.40	Stigmast-7-en-3-ol
21	20.306	0.11	Heneicosanoic acid methyl ester
22	20.926	0.24	13-Docosenoic acid methyl ester
23	2.981	0.10	Methyl 11-docosenoate
24	21.105	6.83	Docosanoic acid methyl ester
25	21.866	0.27	Tricosanoic acid methyl ester
26	22.346	1.36	hexatricontane
27	22.606	3.86	Tetrcosanoic acid methyl ester
		100.00	

The following components were detected as major constituents:

a) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (37.02%)

Fig. 2 shows the mass spectrum of the 9,12-octadecadienoic acid (Z,Z)-, methyl ester. The peak at m/z294, with retention time 17.523, corresponds $M^+[C_{19}H_{34}O_2]^+$. The signal at m/z 263 is due to loss of a methoxyl.

b)-Hexadecanoic acid, methyl ester (18.02%)

Fig. 3 shows the mass spectrum of the hexadecanoic acid methyl ester. The peak at m/z 270 (RT: 15.830) accounts for the molecular ion: $M^+[C_{17}H_{34}O_2]^+$, while the signal at m/z 239 is due to loss of a methoxyl group.

c)-Methyl stearate (10.01%)

The mass spectrum of methyl stearate is shown in Fig. 4.The signal at m/z 298(RT: 17.738) is due to**:** $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z 267 is due to loss of a methoxyl.

d)- Docosanoic acid, methyl ester (6.83%)

The mass spectrum of docosanoic acid methyl ester is presented in Fig. 5. The signal at m/z 354 (RT: 21.105) is due to: $M^+[C_{23}H_{46}O_2]^+$. The signal at m/z 323 is due to loss of a methoxyl group.

e)- 9-Octadecenoic acid (Z)-, methyl ester (6.18%)

The mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is displayed in Fig. 6. The peak at m/z 296 (RT: 17.568) is due to the molecular ion: $M^+[C_{19}H_{36}O_2]^+$.

The signal at m/z 265 is attributed to loss of a methoxyl function.

(f) Eicosanoic acid, methyl ester (5.77%)

Fig. 7 shows the mass spectrum of the eicosanoic acid methyl ester. The peak at m/z 326 (RT: 19.484) corresponds $M^+[C_{21}H_{42}O_2]^+$, while the signal at m/z 295 accounts for loss of a methoxyl.

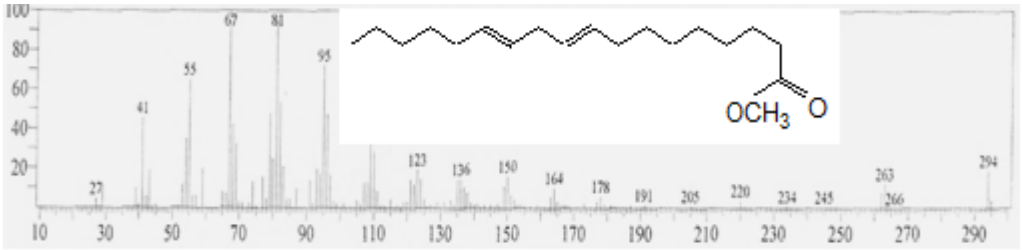

Figure 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester

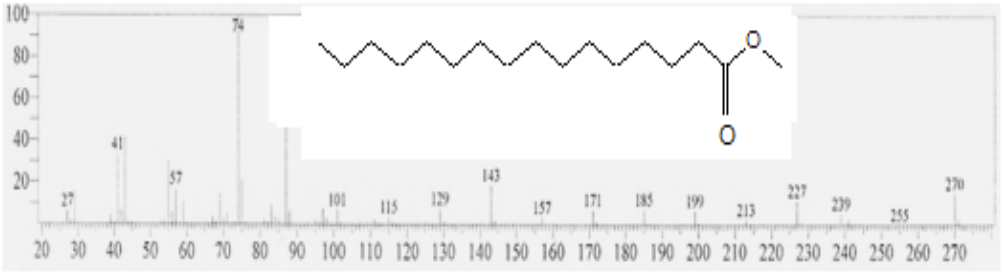

Figure 3: Mass spectrum of hexadecanoic acid methyl ester

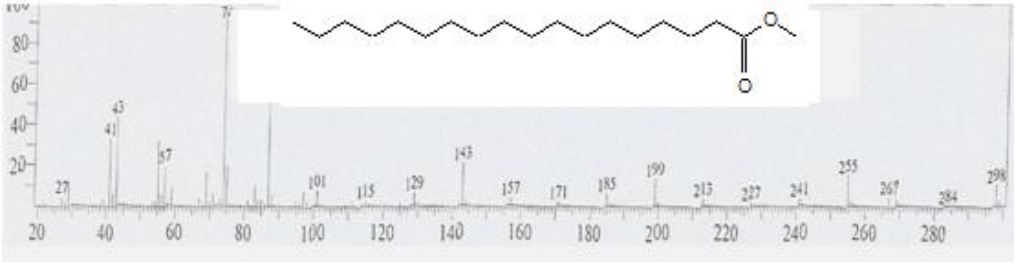

Figure 4: Mass spectrum of methyl stearate

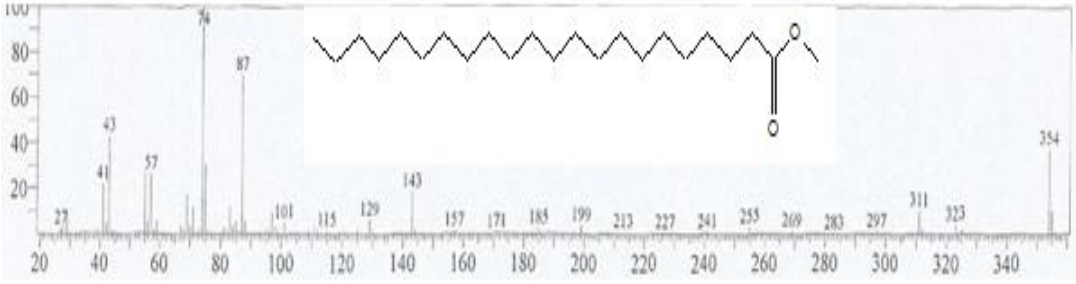

Figure 5: Mass spectrum docosanoic acid, methyl ester

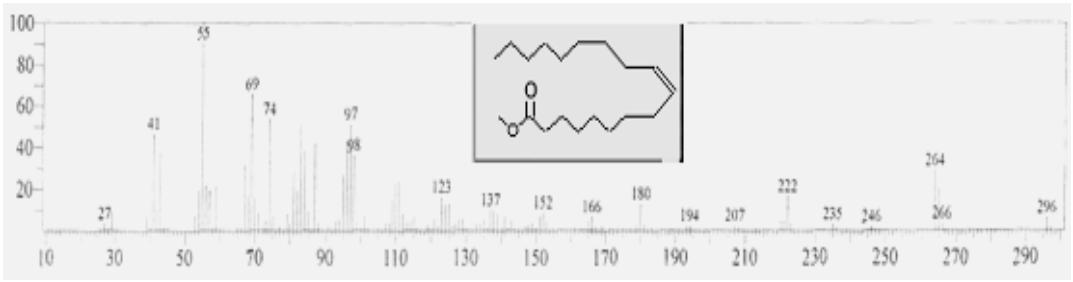

Figure 6: mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

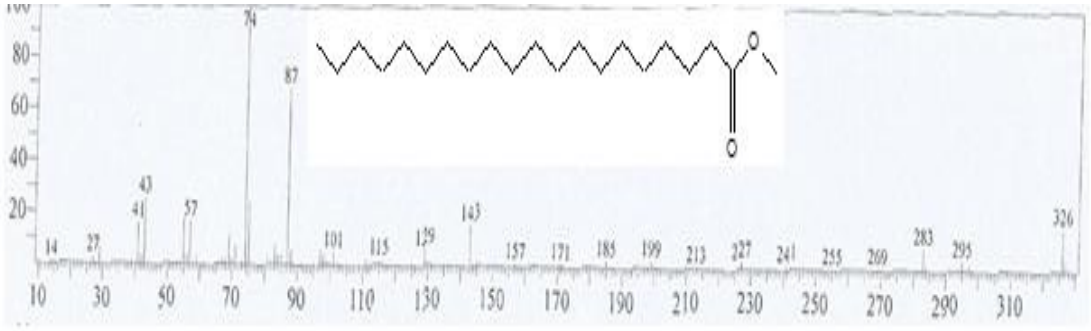

Figure 7: Mass spectrum eicosanoic acid methyl ester

Antimicrobial activity

The oil was screened for antimicrobial activity against five standard microorganisms (Table 2). The results are depicted in Table 3. The oil showed moderate activity against Gram positive *Staphylococcus aureus* and Gram negative *Pseudomonas aeruginosa*. Ampicilin, gentamicin and clotrimazole were used as positive controls (Table 4).

Table 2: Test organisms

No	Micro organism	Type	Source
1	*Bacillus subtillus*	G+ve	ATCC* 2836
2	*Staphylococcus aureus*	G+ve	ATCC* 29213
3	*Pseudomonas aeroginosa*	G-ve	NCTC* 27853
4	*Escherichia coli*	G-ve	ATCC* 25922
5	*Candida albicans*	fungi	ATCC* 7596

* NCTC. National collection of type culture, Colindale, England

*ATCC. American type culture collection, Maryland, USA

Table 3: Inhibition zones (mm)

Sample	Sa.	Bs.	Ec.	Ps.	Ca.
Oil (100mg/ml)	15	--	7	15	12

* B.S. = *Bacillus subtilis*, S.a. =*Staphylococcus aureus*, E.c. = *Escherichia coli*, P.a. = *Pseudomonas aeruginosa*, C.a.= *Candida albicans* ; Result: >18 mm: Sensitive, 13 to 18 mm: moderate: 9-12 ,partially active: : < 9 , inactive.

Table 4: Inhibitory effect of standard drugs

Drug	Sa	Bs	Ec	Ps	Ca
Ampicilin (40mg/ml)	30	15	--	--	--
Gentamicin (40mg/ml)	19	25	22	21	--
Clotrimazole (30mg/ml)	--	--	--	--	38

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