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Research Article

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GC-MS Analysis and Antimicrobial Activity of Sudanese *Acacia polyacantha* Willd. (Fabaceae) Seed Oil

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Abstract The present study was carried out to characterize the constituents of *Acacia polycantha* seed oil and to assess its antimicrobial activity. Thirty two components were detected by GC-MS analysis being dominated by: 9,12-octadecadienoic acid methyl ester (27.95%), methyl stearate (17.13%), hexadecanoic acid methyl ester (13.30%), 9-octadecanoic acid(Z) methyl ester (9.57%). The antimicrobial activity of the oil was evaluated using the diffusion assay against: Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseudomonasa aeruginosa* and the yeast *Candida albicans*. The oil showed moderate activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus.* However, it failed to exhibit activity against other test organisms.

Keywords *Acacia polyacantha*, Oil, GC-MS, Antimicrobial activity

Introduction

Acacia (Fabaceae) is a large genus comprising around 1350 species. Most Acacia species are rich in bioactive molecules including flavonoids and other phenolics [1]. Some Acacia species are used traditionally as antiinflammmatory, antidiabetic, antidiarrhoeic antimicrobial and as hypotensive [2,3]. The medicinally important species – *Acacia nilotica* – is used in Sudanese system of medicine against malaria, diabetes, wounds and intestinal worms [4-7] . Another Acacia species-*Acacia seyal* – is used against kidney disorders [8]. The antioxidant properties of *Acacia auricoliformis* has been reported [9].

Acacia polyacantha Willd. is an erect, deciduous tree distributed along tropical Africa extending from Gambia to Ethiopia , Kenya and Zimbabwe [10]. The plant is used traditionally against gastrointestinal disorders [10].

Materials and Methods

Plant material

Seeds of *Acacia polycantha* were collected from a forest reserve around Fula (western Sudan) and authenticated by direct comparison with a herbarium sample.

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

Acacia polycantha oil was screened for antibacterial and antifungal activity using the standard microorganisms: *Bacillus subtilis, Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Escherichia coli* and the fungal species *Candida albicans*.

Methods

Extraction of oil

Powdered seeds of *Acacia polycantha* (400g) were macerated with n-hexane. The solvent was removed *in vauo* to give the oil.

GC-MS analysis

The constituents of *Acacia polycantha* oil were investigated by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length; 0.25mm diameter; 0.25 µm, thickness). Chromatographic conditions are as follows: column oven temperature: 150.0 ^{o}C; injection temperature: 300.0 ^{o}C; injection mode: split; flow mode: linear velocity; pressure: 139KPa; total flow: 50.0ml/min; column flow: 1.54ml/sec.; linear velocity: 47.2cm/sec.; purge flow: 3.0 ml/min.; split ratio:-1.0. Oven temperature program is presented below:

Rate	Temperature (^{o}C)	Hold Time (min.$^{-1}$)
1.00	150.0	-
0.00	300.0	4.00

***In vitro* antimicrobial assay**

Muller Hinton agar and Sabouraud dextrose agars were used for bacterial and fungal cultures respectively. The disc diffusion method was used to determine the antimicrobial activity of the oil. Fresh cultures of microorganisms grown for 24 h were used and diluted to 10^{-1} with sterile physiological saline solution (0.85% NaCl). 100 µl of test microorganisms containing 2.0×10^{6} colony forming units (CFU/ml) for bacteria were inoculated on the surface of agar plates. Sterile discs with a diameter of 6 mm were placed onto each agar plate containing microorganisms. Then the test solution was dropped onto discs under sterile conditions and incubated at 37 °C for 24 h.(for bacteria), for fungi the incubation continued for 3 days at 25^{o}C. After incubation, the diameters of inhibition zones were measured in millimetres. All experiments were repeated two times. ampicillin, gentamicin and clotrimazole were used as positive controls ,while DMSO was used as negative control. Control discs were tested on the same microorganisms under the same conditions.

Results and Discussion

The GC-MS analysis

The studied oil was analyzed by GC-MS and the identification of the constituents was accomplished by comparison of retention times and through the MS library (NIST). The GC-MS analysis of the studied oil revealed the presence of 32 constituents (Table 2). The following constituents were detected in the chromatogram as major constituents:

9,12-Octadecadienoic acid methyl ester(27.95%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig. 1. The peak at m/z 294, which appeared at R.T. 17.514 in total ion chromatogram, corresponds $M^{+}[C_{19}H_{34}O_2]^{+}$. The signal at m/z263 is due to loss of a methoxyl function.

Methyl stearate(17.13%)

Fig. 2 shows the mass spectrum of methyl stearate. The signal at m/z 298(R.T. 17.757) corresponds $M^{+}[C_{19}H_{38}O_2]^{+}$, while the peak at m/z 267 accounts for loss of a methoxyl.

Hexadecanoic acid methyl ester(13.30%)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig. 3. The signal at m/z 270 (R.T. 15.827) corresponds $M^{+}[C_{17}H_{34}O_2]^{+}$. The signal at m/z239 is due to loss of a methoxyl.

9-Octadecenoic acid(Z) methyl ester (9.57%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig. 4. The peak at m/z 296, which appeared at R.T. 17.564 corresponds $M^+[C_{19}H_{36}O_2]^+$. The signal at m/z265 accounts for loss of a methoxyl function.

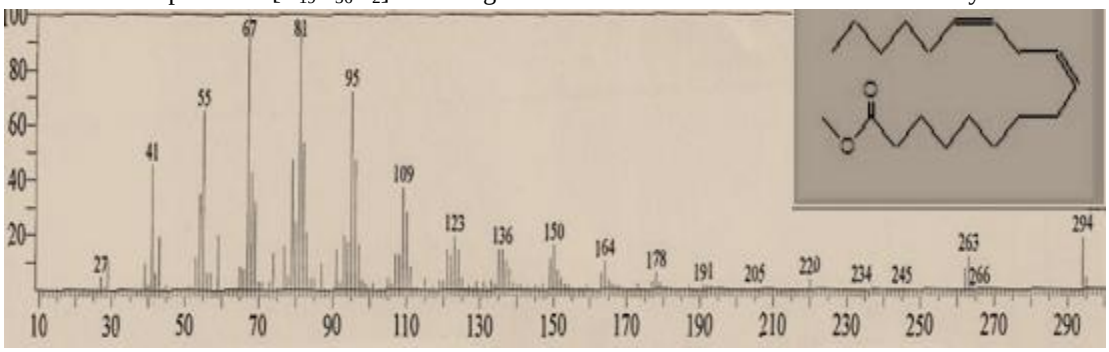

Figure 1: Mass spectrum of 9,12-octadecadienoic acid methyl ester

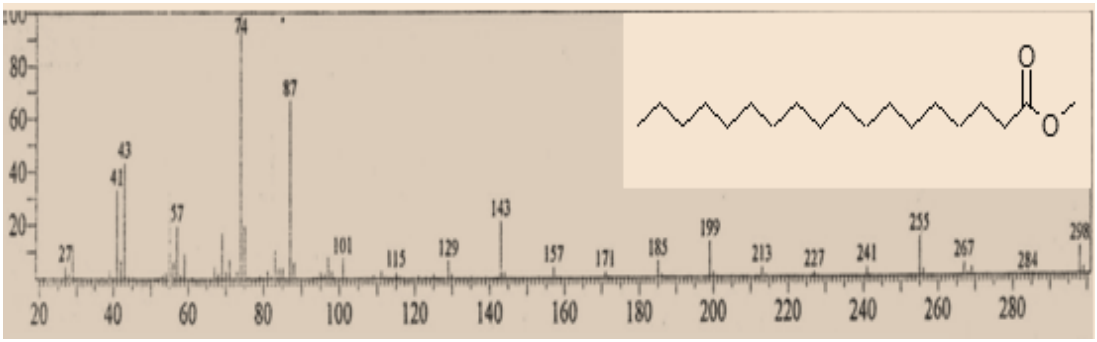

Figure 2: Mass spectrum of methyl stearate

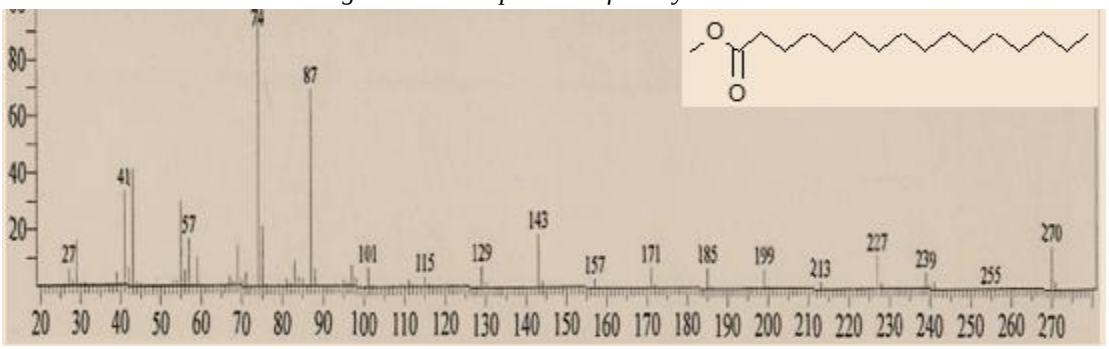

Figure 3: Mass spectrum of hexadecanoic acid methyl ester

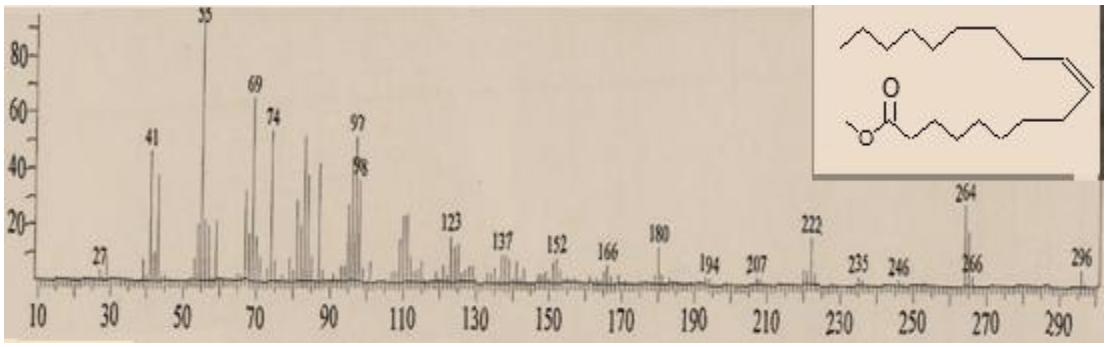

Figure 4: Mass spectrum of 9-octadecenoic acid methyl ester

Antimicrobial activity

The oil was screened for antimicrobial activity against five standard microorganisms. The average of the diameters of the growth inhibition zones are shown in Table (1). The results were interpreted in terms of the commonly used terms (<9mm: inative; 9-12mm:partially active; 13-18mm: active; >18mm: very active).

Table 1 : Antimicrobial activity of the oil

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	15	7	8	15	--
Ampicilin	40	30	15	--	--	--
Gentacycin	40	19	25	22	21	--
Clotrimazole	30	--	--	--	--	38

Sa.*: Staphylococcus aureus,* Ec.*: Escherichia coli,* Pa.: *Pseudomonas aeruginosa*, An.: *Aspergillus niger*, Ca.: *Candida albicans, Bs.: Bacillus subtilis*

The oil showed moderate activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. However, it failed to exhibit activity against other test organisms.

Table 2: Constituents of the oil

No.	Name	Ret. time	Area %
1	Alpha-Terpeneol	7.112	0.04
2	Dodecanoic acid	11.386	0.01
3	Methyl tetradecaanoate	13.705	0.08
4	Cis-5-Docenenoic acid ,methyl ester	14.517	0.01
5	Pentadecanoic methyl ester	14.780	0.03
6	Cis-10-Nonadecenoic acid methyl ester	15.616	1.48
7	9-Hexadecenoic acid methyl ester	15.710	0.08
8	Hexadecenoic acid methyl ester	15.827	13.30
9	Cis-10-Heptadecenoic acid Hexadecenoic acid methyl ester	16.579	0.24
10	Heptadecanoic acid Hexadecenoic acid methyl ester	16.787	0.37
11	9,12-Octadecadienoic acid methyl ester	17.514	27.95
12	9-Octadecenoic acid (Z) methyl ester	17.564	9.57
13	9-Octadecenoic acid (E) methyl ester	17.590	3.08
14	Phytol	17.651	0.36
15	Methyl stearate	17.757	17.13
16	Trans-Geranylgeraniol	18.392	0.15
17	Nonadecanoic acid methyl ester	18.622	0.15
18	Gama-Linolenic acid methyl ester	18.764	0.07
19	Methyl-5,11,14-eicosatrienoic acid methyl ester	19.086	0.60
20	Tridecanedial	19.127	4.71
21	Oxiraneoctanoic acid methyl ester	19.248	2.86
22	Cie-11-Eicosenoic acid methyl ester	19.285	0.72
23	Eicosanoic acid PGHI , methyl ester	19.491	7.91
24	PGHI , methyl ester	19.540	1.19
25	1-Naphthalenol decahydro-4a- , PGHI , methyl ester	19.648	1.49
26	Tricyclo[20.8..0.(7.16)]triacontane	19.982	0.23
27	Stigmast-7-en-3-ol,(3-beta-,5-alpha.,24S)	20.046	0.29
28	Heneicosanoic acid PGHI , methyl ester	20.307	0.23
29	Phenol,2,2`-methylene-bis[6-(1,1-dimethyl)	20.405	0.06
30	Methyl 20-methyl-heneicosanoate	21.105	3.52
31	Tricosanoic acid PGHI , methyl ester	21.868	0.46
32	Tetracosanoic acid PGHI , methyl ester	22.608	2.63

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