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Research Article

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Constituents and Antimicrobial Activity of Sudanese *Acacia nilotica* Subsp. *Nilotica* (Fabaceae) Oil

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Abstract Worldwide, traditional medicine is still playing a vital role in primary health care and numerous pharmacological reports on the impact of bioactive phytochemicals on human physiology even potentiated the applications of medicinal plants.In this study, *Acacia nilotica* Subsp. *nilotica* oil has been analyzed by GC-MS. The analysis showed 26 components. Major constituents are : methyl-10-trans-12-cis-octadecadienoate(39.39%); 9-octadecenoic acid methyl ester(20.37%); hexadecanoic acid methyl ester(14.62%) and methyl stearate(11.25%). The antmicrobial activity of the oil has been assessed. At a concentration of 100mg/ml the oil showed significant activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* However , it showed no activity against *Escherichia coli, Candida albicans* and *Aspergillus niger.*

Keywords *Acacia nilotica* Subsp. *nilotica* , Oil , Constituents , Antimirobial 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 important Acacia species-*Acacia seyal* – is used against kidney disorders [8]. The antioxidant properties of *Acacia auricoliformis* has been reported [9]. The Acacia species *Acacia polyacantha* is used traditionally against gastrointestinal disorders [10].

Materials and Methods

Plant Material

Acacia nilotica seeds were collected from a forest reserve around Khartoum (Sudan) and authenticated by direct comparison with a herbarium sample.

Instruments

The oil of *Acacia nilotica* was studied 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).

Microorganisms

The antimicrobial assay was performed using the following standard microorganisms:

*Bacillus subtilis (*G+ve), *Staphylococcus aureus (*G+ve), *Pseudomonas aeroginosa* (G-ve), *Escherichia coli* (G-ve), *Aspergillus nige*r (fungus) and *Candida albicans* (fungus).

Extraction of oil

Dry powdered *Acacia nilotica* seeds (300g) were exhaustively extracted with *n*-hexane at room temperature for 72hr. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further work.

GC-MS analysis

The constituents of *Acacia nilotica* oil were investigated by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument. 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 Table 1:

Table 1: Oven temperature program

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

A**ntimicrobial activity**

The antimicrobial screening was performed by using the cup plate agar diffusion assay. Bacterial culture was maintained in nutrient agar while fungal culture was performed on Sabouraud dextrose agar. Wells (6 mm in diameter) were made in the seeded agar using sterile cork borer (No. 4). Test samples were added into wells of the seeded medium and then incubated for 24 hrs (at 37 ^{0}C) for bacteria and for 72 hrs at 25^{0}C for fungal species. The diameters of inhibition zones were measured as average of two replicates.

Constituents of *Acacia nilotica* oil

The oil of *Acacia nilotica* was studied by GC-MS which showed the presence of 26 constituents. The total ion chromatograms is displayed in Fig. 1 , while the different constituents of the oil are depicted in Table 2.

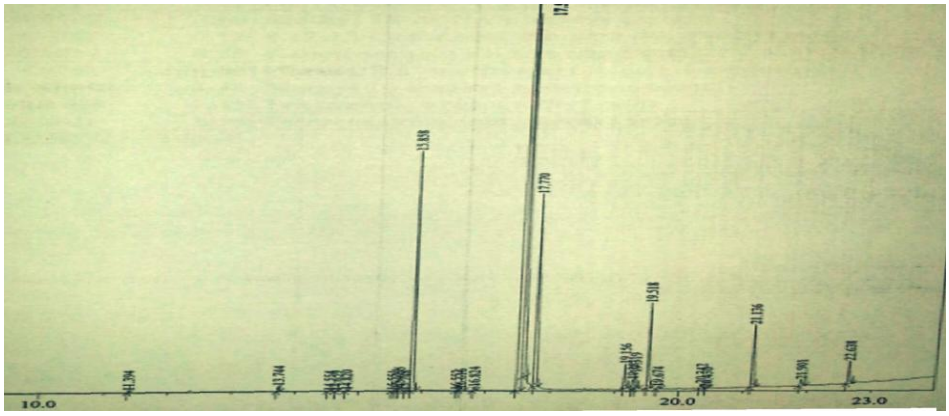


Figure 1: Total ions chromatograms

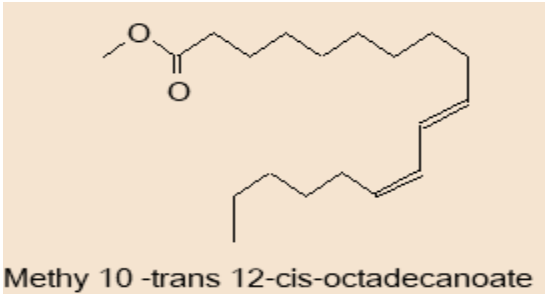
Table 2: Constituents of the oil

No.	Name	RT	Area %
1	Butylated hydroxytoluene	11.394	0.08
2	Methyl tetradecanoate	13.744	0.25
3	Cis-5-Docosecenoic acid methyl ester	14.554	0.01
4	5-Octadecenoic acid methyl ester	14.659	0.01
5	Pentadecanoic acid methyl ester	14.820	0.05

6	7 10-Hexadecadienoic acid methyl ester	15.511	0.02
7	11-Hexadecenoic acid methyl ester	15.611	0.11
8	Hexadecenoic acid methyl ester	15.655	0.16
9	13-Docosenoic acid methyl ester	15.742	0.02
10	Hexadecanoic acid methyl ester	15.858	14.62
11	9 12-Hexadecdienoic acid methyl ester	16.552	0.02
12	Cis-10-Heptadecenoic acid methyl ester	16.616	0.10
13	Heptadecanoic acid methyl ester	16.824	0.17
14	Methyl 10-trans 12-cis-octadecadienoate	17.539	39.39
15	9-Octadecenoic acid methyl ester	17.579	20.37
16	Methyl stearate	17.770	11.25
17	9 12-Octadecadienoic (z z) acid methyl ester	19.156	1.27
18	Oxaraneoctanoic acid 3-octyl methyl ester	19.281	0.54
19	Cis-11-Eicosenoic acid methyl ester	19.319	0.81
20	Methyl 18-methylnonadecanoate	19.518	4.68
21	9 12 15-Octadecatrienoc acid methyl ester	19.674	0.12
22	Heneicosanoic acid methyl ester	20.342	0.25
23	Phenol 2 2`-methylene(6-1 1-dimethyl)	20.430	0.04
24	Methyl 20-methylheneicosanoate	21.136	3.53
25	Tricosanoic acid methyl ester	21.901	0.43
26	Tetracosanoic acid methyl ester	22.638	1.48
			100%

The following major components were detected:

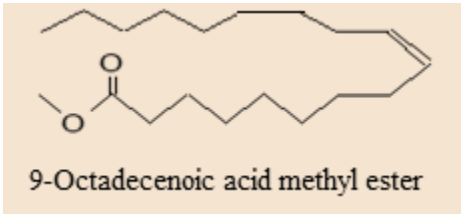
i-Methyl 10-trans-12-cis-octadecadienoate(39. 39%)



Methy 10 -trans 12-cis-octadecanoate

The EI mass spectrum of methyl 10-trans-12-cis-octadecadienoate is shown in Fig. 2. The peak at m/z 294, which appeared at R.T. 17.539 in total ion chromatogram, corresponds $M^+[C_{19}H_{34}O_2]$. The signal at m/z 263 is due to loss of a methoxyl group.

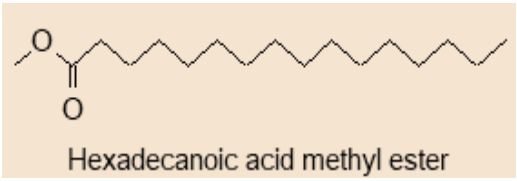
ii- 9-Octadecenoic acid methyl ester(20.37%)



9-Octadecenoic acid methyl ester

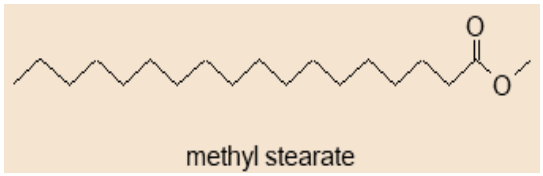
The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is displayed in Fig. 3. The peak at m/z 296, which appeared at R.T. 17.579 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$. The peak at m/z 263 corresponds to loss of a methoxyl function.

iii- Hexadecanoic acid methyl ester (14.62%)



The EI mass spectrum of hexadecanoic acid methyl ester is shown in Fig. 4. The peak at m/z 270, which appeared at R.T. 15.858 in total ion chromatogram, corresponds to $M^+[C_{17}H_{34}O_2]^+$. The peak at m/z 239 corresponds to loss of a methoxyl function.

iv- Methyl stearate (11.25%)



The mass spectrum of methyl stearate is displayed in Fig. 5. The peak at m/z 298, which appeared at R.T. 17.770 in total ion chromatogram, corresponds to $M^+[C_{19}H_{38}O_2]^+$. The peak at m/z 267 corresponds to loss of a methoxyl function.

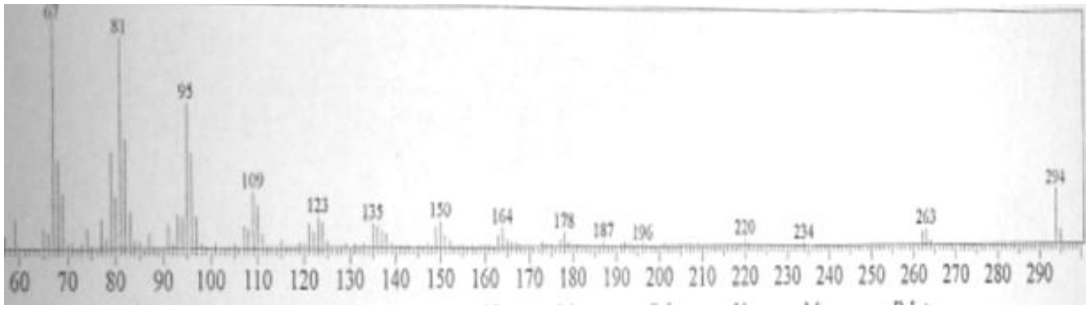


Figure 2: Mass spectrum of methyl 10-trans-12-cis-octadecadienoate

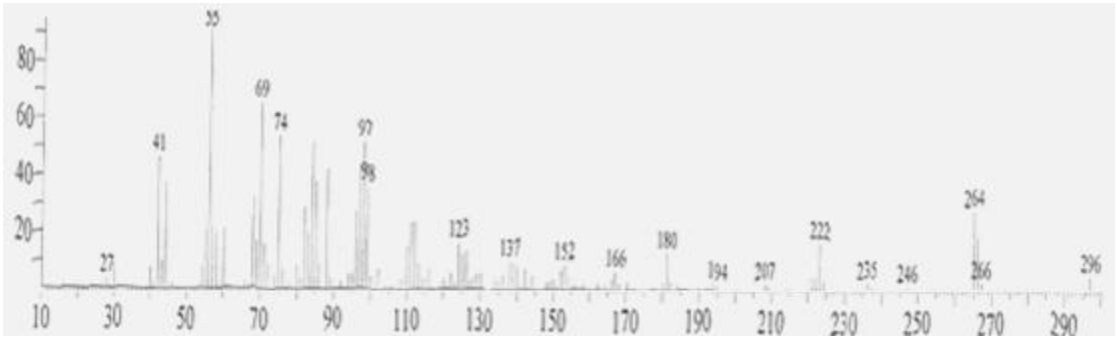


Figure 3: Mass spectrum of 9-octadecenoic acid methyl ester

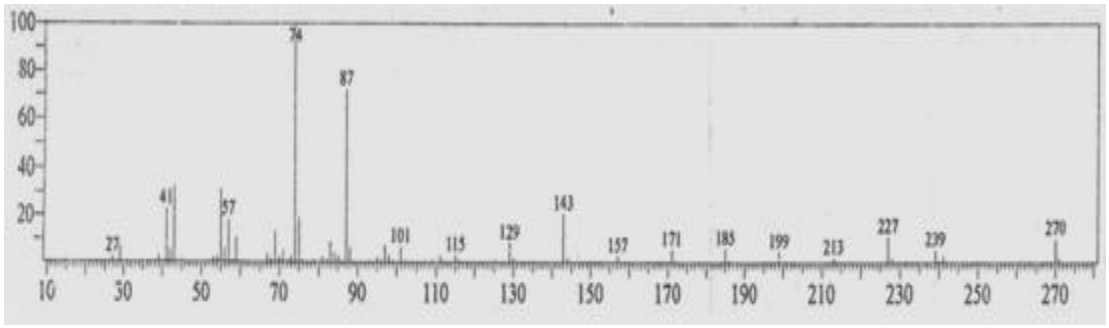


Figure 4: Mass spectrum of hexadecanoic methyl ester

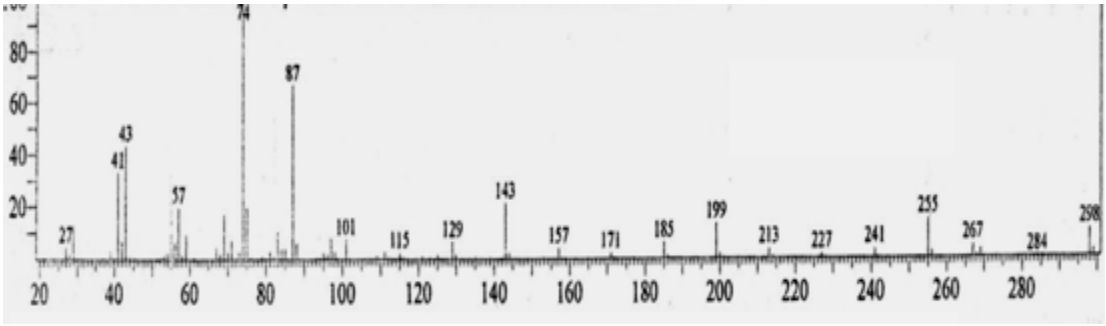


Figure 5: Mass spectrum of methyl stearate

Antimicrobial Activity

Acacia nilotica oil was evaluated for antimicrobial activity *via* the cup plate agar diffusion bioassay. The average of the diameters of the growth of inhibition zones are shown in Table 3. The results were interpreted in terms of the commonly used terms (<9mm: inactive; 9-12mm: partially active; 13-18mm: active;>18mm: very active)

Table 3: Inhibition diameter (mm) of *Acacia nilotica* seed oil

Sample	Conc. (mg/ml)	Ec	Ps	Sa	Bs	Ca	An
Acacia nilotica oil	100	-	18	19	21	-	-

Ec.*: Escherichia coli;* Pa.: *Pseudomonas aeruginosa;* An.: *Aspergillus niger;* Ca.: *Candida albicans;*
Bs.: Bacillus subtilis

The oil showed significant activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* However, it showed no activity against *Escherichia coli, Candida albicans* and *Aspergillus niger.*

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