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Research Article

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Chemical Constituents and Antimicrobial Activity of Sudanese *Lucaena leucocephala* (Fabaceae) Oil

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Abstract *Leucaenia leucocephala* is native to southern Mexico, northern and central America. *Leucaenia leucocephala* is a multipurpose tree providing food, medicine, shade and firewood. In this study *Leucaenia leucocephala* oil was analyzed by GC-MS. Thirty constituents were detected being dominated by: 9,12-octadecadienoic acid-z,z- methyl ester (37.29%); hexadecanoic acid methyl ester (18.17%). The oil was evaluated for antimicrobial activity against five standard microorganisms using the disc diffusion method. The studied oil showed moderate activity against *Staphylococcus aureus*. It also exhibited partial activity against *Pseudomonas aeruginosa*.

Keywords *Lucaena leucocephala*, Oil, GC-MS analysis, Antimicrobial Activity

Introduction

Leucaenia leucocephala L. is an evergreen shrub in the family Fabaceae. The plant can reach 5-20m in height [1,2]. *Leucaenia leucocephala* is native to southern Mexico and northern and central America. However, the plant is diffused through the continents [3]. *Eucaenia leucocephala* is a multipurpose tree providing food, medicine, shade and firewood [4-7]. Young leaves and pods are edible, though some caution is advised since leaves contain mimosene- an amino acid- which can be harmful in large quantities [8,9], roasted seeds are emollient [10]. A decoction of bark and root is used as abortefacient [8]. *Leucaenia leucocephala* has been used traditionally as anthelmintic, antimicrobial, antidiabetic, antihistaminic, antitumor and hepatoprotective [11]. Phytochemical screening of leaves revealed the presence of cardiac glycosides, alkaloids, flavonoids, tannins and saponins [4]. Bioactivity- guided studies indicated anthelmintic, antidiabetic and antiproliferative activities [4]. It has been reported that the leaves and seed extracts possess antioxidant activity [12]. These extracts affected renal function by reducing total protein and albumin. Leaves and seed possess antidiabetic effect [13,14].

Materials and Methods

Plant material

Lucaena leucocephala seeds were collected from a forest reserve around Damazin, Sudan. The plant was authenticated by the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan.

GC-MS analysis

GC-MS analysis of the oil was conducted on a Shimadzo GC-MS-QP2010 Ultra instrument with RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μm, thickness).

Test organisms

The oil from *Lucaena leucocephala* was screened for antimicrobial activity using the standard microorganisms shown in Table (1).

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	Candida albicans	fungi

Extraction of oil

Powdered shade-dried seeds of *Lucaena leucocephala* (350g) were exhaustively extracted with n-hexane at room temperature. The solvent was removed under reduced pressure to give the oil.The oil was esterified as follows :the oil(2ml) was placed in a test tube and 7ml of alcoholic sodium hydroxide were added followed by 7ml of alcoholic sulphuric acid. The tube was stoppered and shaken vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added, then (2ml) of normal hexane were added and the tube was vigorously shaken for five minutes. The hexane layer was then separated. (5µl) of the hexane extract were mixed with 5ml diethyl ether . The solution was filtered and the filtrate(1µl) was injected in the GC-MS vial.

GC- MS analysis of the oil

Lucaena leucocephala oil was analyzed by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument. Helium was used as carrier gas. Chromatographic conditions are presented below:

Oven temperature program

Rate : --- ; Tempt. , 150.0^{0}C ; Hold time(min.$^{-1}$) ,1.00

Rate : 4.00 ; Tempt. , 300.0^{0}C ; Hold time(min.$^{-1}$) ,0.00

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.
Spilt ratio	- 1.0

Antimicrobial assay

Mueller Hinton and Sabouraud dextrose agars were the media used for the growth of bacteria and fungi respectively. The media was prepared according to the manufacturer instructions. Microbial cultures (5.0x10^{7}cfu/ml) were streaked on the surface of the solid medium contained in Petri dishes. Filter paper discs (Oxid, 6mm) were placed on the surface of the inoculated agar and then impregnated with 100mg/ml of test sample. For bacteria, the plates were incubated at 37^{o}C for 24h, while for fungi incubation continued for 72h at 25^{o}C. The assay was carried out in duplicates and the diameters of inhibition zones were measured and averaged as indicator of activity.

Results and Discussion

GC-MS analysis of *Lucaena leucocephala* oil was performed. Thirty constituents were detected. The constituents of the oil are presented in Table 3. Identification of the constituents was based on retention times and MS library data. Major constituents are briefly discussed below:

The mass spectrum of 9,12-octadecadienoic acid-z,z- methyl ester (37.29%) is shown in Fig. 1. The peak at m/z294, which appeared at RT 17,519 in total ions chromatograms corresponds $M^+[C_{19}H_{34}O_2]$. The signal at m/z 263 is due to loss of a methoxyl.

Fig**.** 2 shows the mass spectrum of hexadecanoic acid methyl ester (18.17%). The molecular ion $M^+[C_{17}H_{34}O_2]$ appeared at m/z 270 (RT,15.830). The signal at m/z 239 accounts for loss of a methoxyl.

The EI mass spectrum of eicosanoic acid methyl ester(6.46%) is shown in Fig. 3. The peak at m/z 326, which appeared at R.T. 19.487 accounts for the molecular ion : $M^+[C_{21}H_{42}O_2]^+$. The peak at m/z 295 corresponds to loss of a methoxyl function.

The mass spectrum of 9-octadecenoic acid methyl ester(6.36%) is presented in Fig. 4. The signal at m/z 296 (RT,17.555) is due to the molecular ion $M^+[C_{19}H_{36}O_2]^+$. The peak at m/z 266 is due to loss of a methoxyl.

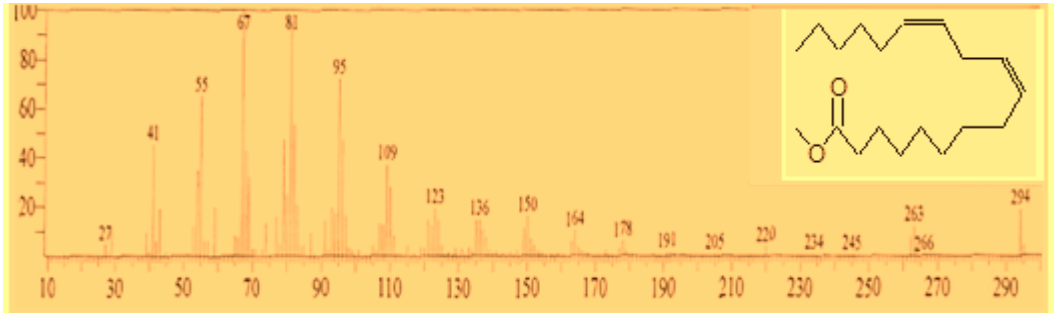

Figure 1: Mass spectrum of 9,12-octadecadienoic acid methyl ester

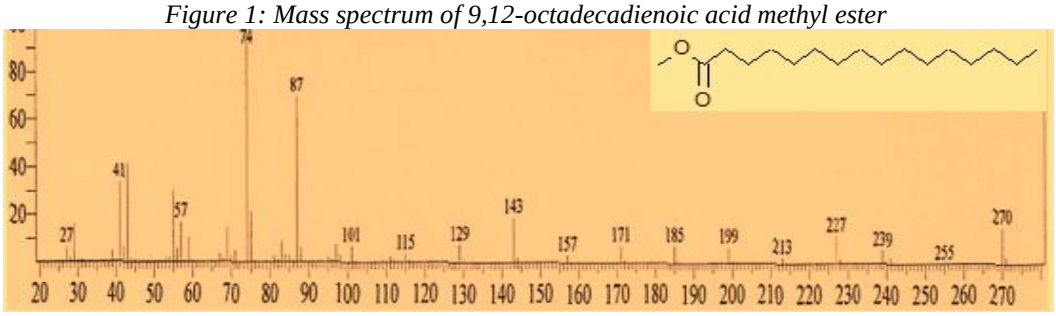

Figure 2: Mass spectrum of hexadecanoic acid methyl ester

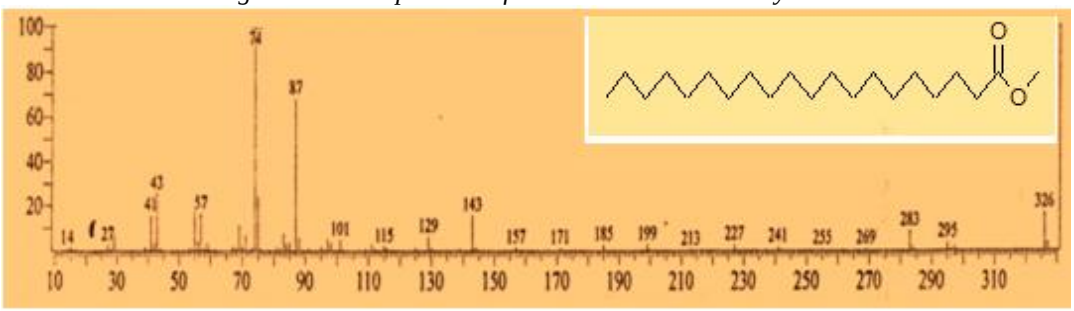

Figure 3: Mass spectrum of eicosanoic acid methyl ester

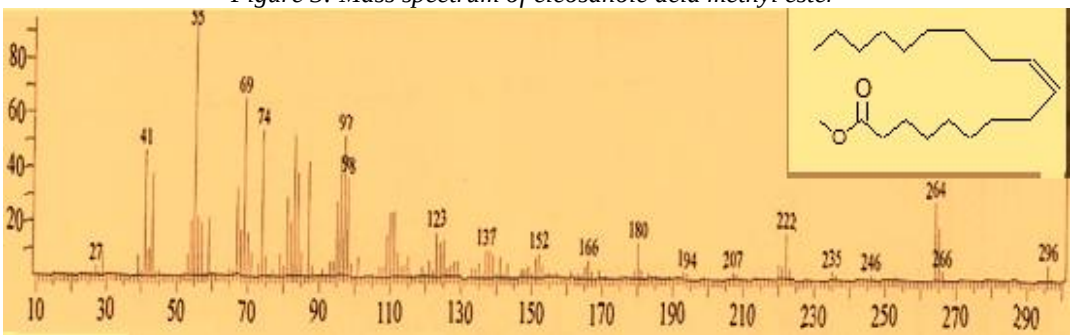

Figure 4: Mass spectrum of 9-octadecenoic acid methyl ester

Antimicrobial assay

The oil was evaluated for antimicrobial activity against five standard microorganisms using the disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table (2). Results were interpreted as follows: (>9mm: inative;9-12mm:partially active;13-18mm: active;<18mm:very active). Ampicilin, gentamicin and clotrimazole were used as positive controls. The studied oil showed moderate activity against *Staphylococcus aureus*. It also exhibited partial activity against *Pseudomonas aeruginosa*.

Table 2: Antimicrobial activity of oil

Sample	Sa	Bs	Ec	Ps	Ca.
Oil (100mg/ml)	15	--	--	12	7
Ampicilin (40mg/ml)	30	15	--	--	--
Gentamycin (40mg/ml)	19	25	22	21	--
Clotrimazole (30mg/ml)	--	--	--	--	38

Sa.*: Staphylococcus aureus;* Ec.*: Escherichia coli;* Pa.: *Pseudomonas aeruginosa*; Bs.*: Bacillus subtilis;* Ca.: *Candida albicans*

Table 3: Constituents of the oil

No.	Name	Ret.time	Area %
1	Alpha-Terpeneol	7.112	0.06
2	1H-Cyclprop[e]azulene,1a,2,3,4,4a,56,7]	10.047	0.02
3	Methyl tetradecaanoate	13.706	0.10
4	5-Octadecenoic acid ,methyl ester	14.518	0.03
5	Cis-5-Dodecenoic acid methyl ester	14.621	0.2
6	Pentadecanoic acid methyl ester	14.783	1.48
7	9-Hexadecenoic acid methyl ester(Z)	15.618	0.77
8	Hexadecanoic acid methyl ester	15.830	18.17
9	Methyl -9,12-Heptadecadienoate	16.579	0.24
10	Cis-10-Heptaddecenoic acid methyl ester	16.578	0.20
11	Heptadecanoic acid methyl ester	16.788	0.27
12	9.12-Octadecadienoic acid (Z) methyl ester	17.519	37.29
13	9-Octadecenoic acid (Z) methyl ester	17.555	6.36
14	9-Octadecenoic acid(E) methyl ester	17.651	0.36
15	Phytol	17.647	0.41
16	Methyl stearate	18.294	0.41
17	Methyl 9-cis-,11-trans-octadecadienoate	18.622	0.09
18	Trans-Generylgereiol	18.396	0.11
19	Nonadecanoic acid methyl ester	18.604	0.04
20	9,12-Octadecadienoyl chloride (Z,Z)-	19.123	0.74
21	Oxiraneoctanoic acid methyl ester	19.248	0.27
22	Cie-11-Eicosenoic acid methyl ester	19.284	0.61
23	Eicosanoic acid , methyl ester	19.487	6.46
24	PGHI , methyl ester	19.537	1.17
25	Methyl 13,16-docosadienoate	19.649	0.18
26	Heneicosanoic acid methyl ester	20.308	0.38
27	Docosanoic acid methyl ester	21.106	5.78
28	Tricosanoic acid PGHI , methyl ester	21.867	0.59
29	9.19-Cyclolanost-24-en-3-ol-(3-beta)-	22.497	0.43
30	Tetracosanoic acid methyl ester	22.608	3.26

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