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Research Article

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Constituents and Antimicrobial Activity of Sudanese *Albizia lebbeck* (Apiaceae) Oil

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Abstract This study was carried out to investigate the constituents and antimicrobial activity of *Albizia lebbeck* oil. This plant has a wide array of uses in phytotherapy. Leaves, bark, flowers and seeds are all used in traditional system of medicine. *Albizia lebbeck* is used as antinflammatory, anti-asthmatic, antiseptic and anti-dysenteric. GC-MS analysis of *Albizia lebbeck* oil showed the presence of major constituents : 9,12-octadecenoic acid methyl ester (28.20%); 9-octadecenoic acid methyl ester(18.08%); hexdecanoic acid (11.86% %) and methyl stearate (9.19%). The oil was assessed for antimicrobial activity against five standard human pathogens using the cup plate agar diffusion bioassay. The oil showed weak activity against *Escherichia coli. Pseudomonas aeruginosa* and the fungal species *Candida albicans.*

Keywords *Albizia lebbeck,* Oil, Constituents, Antimicrobial Activity

Introduction

Albizia lebbeck is a multipurpose fast – growing, perennial legume in the family Apiaceae [1- 4]. The plant has diverse uses in ethnomedicine beside some industrial applications [5]. Leaves, bark, flowers and seeds are all used in traditional system of medicine [5]. *Albizia lebbeck* is used as antinflammatory, anti-asthmatic, antiseptic and anti-dysenteric [6]. The plant is also used against allergic rhinitis, lung problems, gingivitis, wounds, gonorrhea, ringworms, leucorrhoea and abdominal tumors [2,5-8].

The bark is bitter, astringent and tonic. It is used against infections, eczema, bronchitis, skin diseases, scabies, piles, deafness and syphilis [2,5,9,10]. The leaves are antiprotozoal, antifertility, antiseptic and antimicrobial. Leaves are used traditionally for trauma and tuberculosis [11]. The flowers are also used in traditional medicine against chronic cough, asthma, leprosy, chronic catarrh, seminal weakness, spermatorrhea and snake bite [3,,5,12,13]. Seed is a natural remedy for diarrhea, gonorrhea and piles. Seeds are brain tonic, astringent and aphrodisiac [3,10].

Materials and Methods

Materials

Plant Material

Albizia lebbeck seeds were collected from Damazin- Sudan. The plant was authenticated by direct comparison with reference herbarium sample.

Instruments

GC-MS analysis 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 *Albizia lebbeck* oil was screened for antimicrobial activity using the standard microorganisms: *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeroginosa, Escherichia coli* and *Candida albicans.*

Methods

Extraction of Oil

Powdered seeds of *Albizia lebbeck* (400g) were macerated with n-hexane at room temperature. The solvent was removed under reduced pressure to give the oil. The oil was esterified by alcoholic sodium hydroxide and alcoholic sulphuric acid.

GC-MS Analysis

The extracted 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 as follows: **column oven temperature**: 150.0^{o}C; **injection temperature** : 300.0^{o}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.

Antimicrobial Assay

Preparation of bacterial and fungal suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^8-10^9 colony forming units per ml. Serial dilutions of the stock suspension were made in sterile normal saline in tubes and (0.02 ml) of the appropriate dilutions were transferred onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours. Fungal cultures were maintained on Sabouraud dextrose agar incubated at 25°C for 72h.

Testing for antimicrobial activity

(2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was then left to settle and in each of these plates cups (10 mm in diameter) were cut using sterile cork borer (No 4). The agar discs were removed. The cups were filled with sample (0.1 ml) and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured and recorded as an average of two replicates.

The same procedure mentioned above was applied during the antifungal assay using Sabouraud dextrose agar.

Results and Discussion

Albizia lebbeck oil was studied by GC-MS. The analysis revealed the presence of 23 components as shown in Table 1. The total ions chromatogram is presented in Fig. 1.

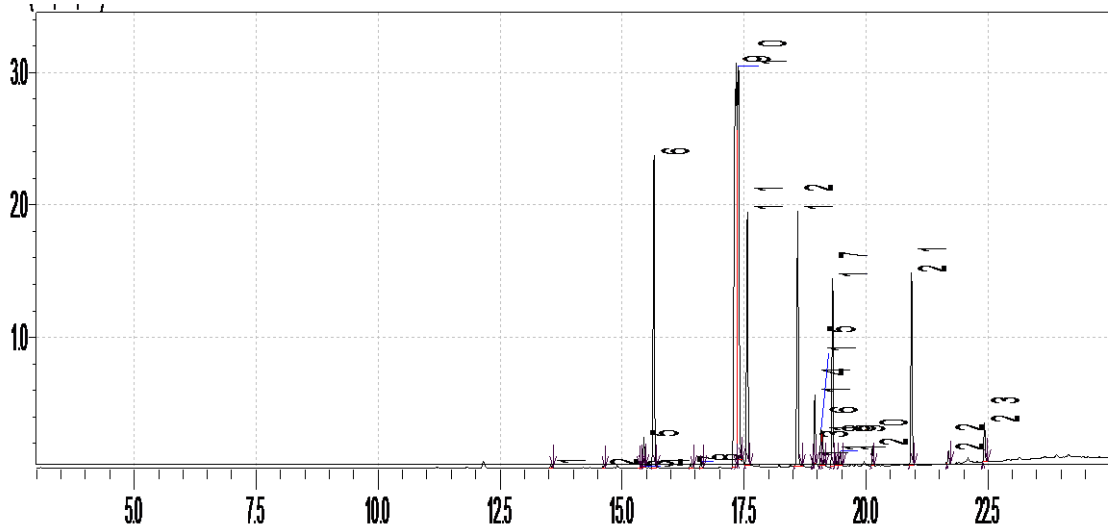

Figure 1: Total ions chromatograms

Table 1: Constituents of *Albizia lebbeck* oil

S. No.	Name	Ret.Time	Area%
1	Methyl tetradecanoate	13.537	0.11
2	Pentadecanoic acid, methyl ester	14.612	0.07
3	7,10-Hexadecadienoic acid, methyl ester	15.339	0.01
4	7-Hexadecenoic acid, methyl ester, (Z)-	15.410	0.04
5	9-Hexadecenoic acid, methyl ester, (Z)-	15.445	0.83
6	Hexadecanoic acid, methyl ester	15.654	11.86
7	cis-10-Heptadecenoic acid, methyl ester	16.405	0.15
8	Heptadecanoic acid, methyl ester	16.616	0.20
9	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.343	28.20
10	9-Octadecenoic acid (Z)-, methyl ester	17.380	18.08
11	Methyl stearate	17.566	9.19
12	Methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate	18.599	10.05
13	6,9-Octadecadienoic acid, methyl ester	18.910	0.26
14	Cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl)methyl]-, methyl ester	18.948	2.17
15	cis-11-Eicosenoic acid, methyl ester	19.081	1.34
16	cis-13-Eicosenoic acid, methyl ester	19.105	0.90
17	Eicosanoic acid, methyl ester	19.313	6.52
18	PGH1, methyl ester	19.361	0.43
19	1-Naphthalenol, decahydro-4a-methyl-	19.470	0.50
20	Heneicosanoic acid, methyl ester	20.132	0.59
21	Docosanoic acid, methyl ester	20.933	6.88
22	Tricosanoic acid, methyl ester	21.689	0.39
23	Tetracosanoic acid, methyl ester	22.426	1.23

The following compounds were detected in the chromatogram as major constituents:

i) 9 12-Octadecenoic acid methyl ester (28.20%)
ii) 9-Octadecenoic acid methyl ester (18.08%).
iii) Hexdecanoic acid (11.86 %).
iv) Methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate (10.05%)
v) Methyl stearate (9.19%).

Fig. 2 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294 (RT. 17.343) corresponds M^+ $[C_{19}H_{34}O_2]^+$. The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig. 3. The signal at m/z 296 (RT.17.380) corresponds M^+ $[C_{19}H_{36}O_2]^+$.

The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 4. The peak at m/z 270 (RT.15.654) is due to M^+ $[C_{17}H_{32}O_2]^+$. The mass spectrum of methyl 9. cis., 11.trans.t,13.trans.-octadecatrienoate is illustrated in Fig. 5.The peak at m/z 292 (RT.18.599) accounts for the molecular ion: $M^+[C_{19}H_{32}O_2]^+$. Fig.6 shows the mass spectrum of methyl stearate. The signal at m/z 298 (R.T.17.566) corresponds M+$[C_{19}H_{38}O_2]$+.

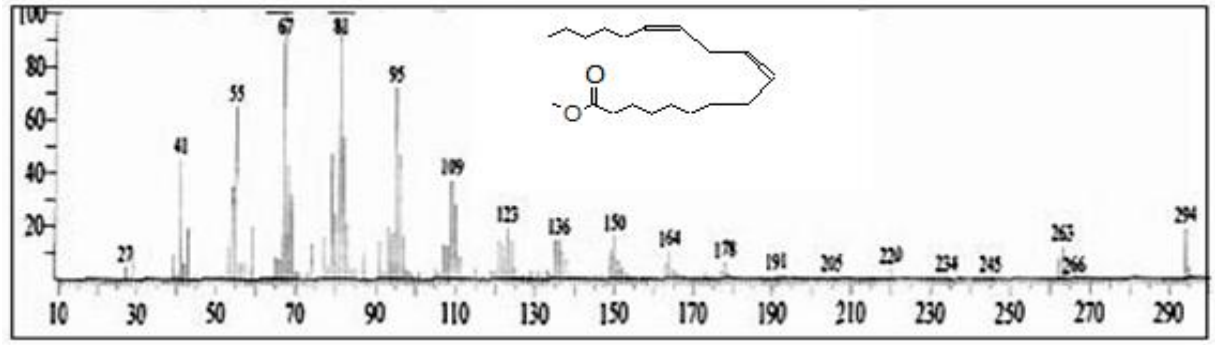

Figure 2: Mass spectrum of 9,12-octadecadienoic acid

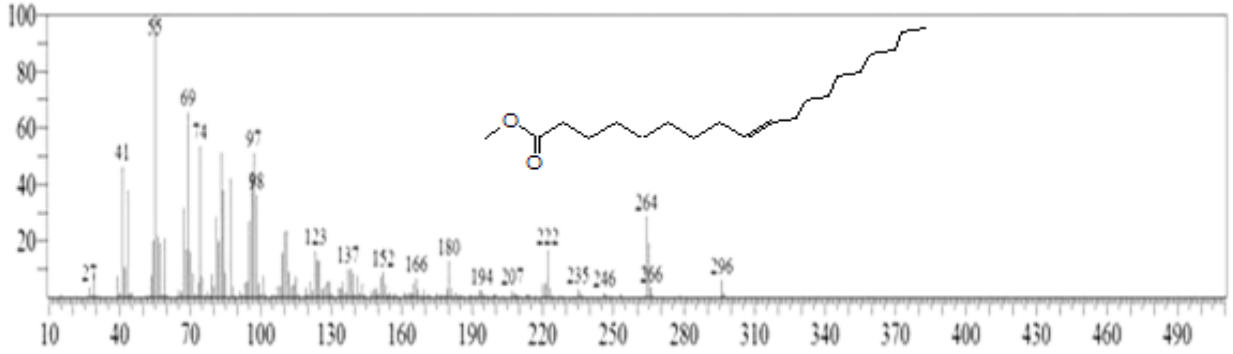

Figure 3: Mass spectrum for 9-octadecenoic acid[z]-, methyl ester

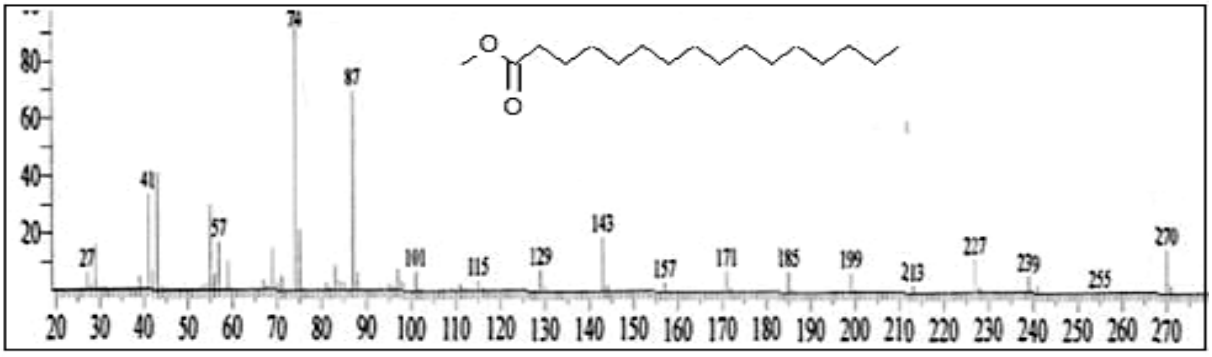

Figure 4: Mass spectrum of hexadecanoic acid methyl ester

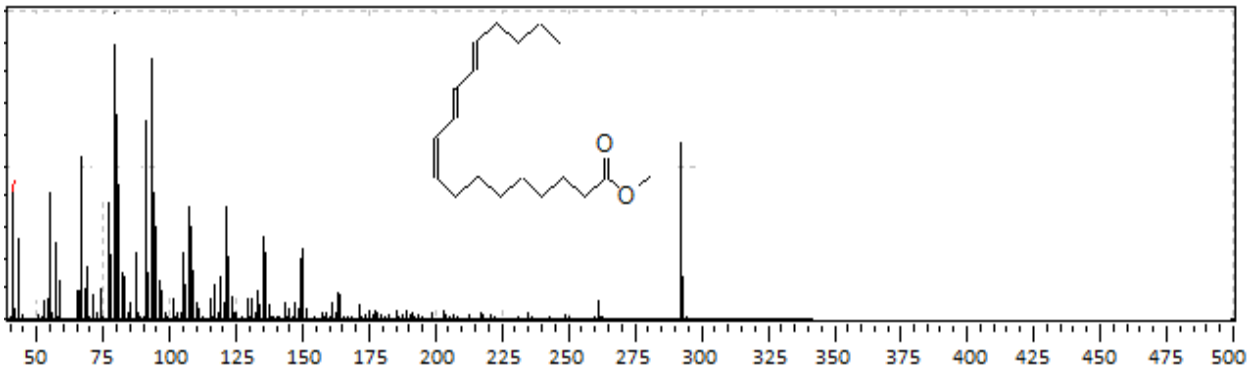

Figure 5: Mass spectrum of methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate

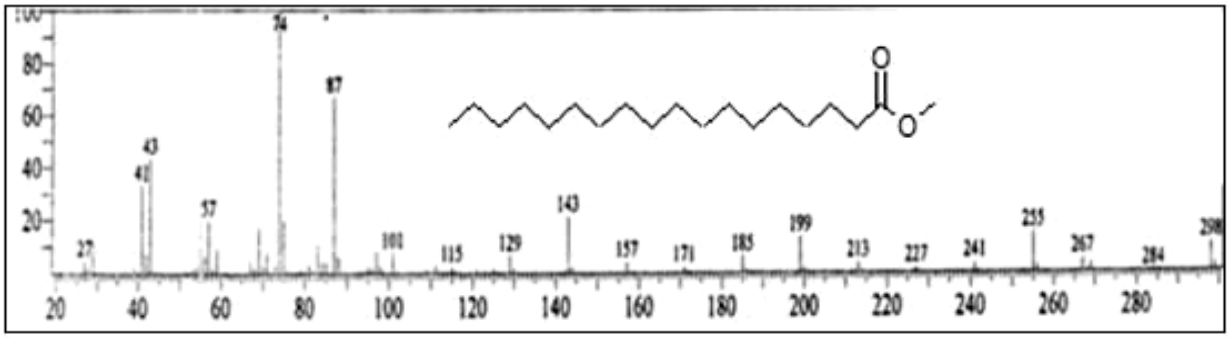

Figure 6: Mass spectrum of methyl stearate

Antimicrobial assay

Albizia lebbeck oil was evaluated for its antimicrobial activity against five standard human pathogens. The average of the diameters of the growth inhibition zones are presented in Table (2). Results were interpreted in conventional terms: (<9mm: inative; 9-12mm: partially active; 13-18mm: active; >18mm: very active). Ampicilin, gentamicin and clotrimazole were used as positive controls. The oil showed weak activity against *Escherichia coli. Pseudomonas aeruginosa* and the fungal species *Candida albicans.*

Table 2: Inhibition zones (mm/mg sample)

Type	Sa	Bs	Ec	Ps	Ca
Oil(100mg/ml)	--	--	13	10	10
Ampicilin(40mg/ml)	30	15	--	--	--
Gentacycin(40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

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

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