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CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *ARISTOLOCHIA BRACTEOLATE* LAM. (ARISTOLOCIACEAE) OIL

Abdel Karim M.[1*], Elham A.[1], Alla M.[2] and Mai Mekki[1]

[1]Sudan University of Science and Technology, Faculty of Science.
[2]Karar University, General Science Dept.

Corresponding Author: Abdel Karim M.
Sudan University of Science and Technology, Faculty of Science.

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ABSTRACT

The objective of the present study is to investigate the constituents and antimicrobial activity of *Aristolochia bracteolata* oil. Twelve components were detected by GC-MS analysis. Major constituents are 9-octadecenoic acid methyl ester (52.96%), hexadecanoic acid methyl ester(28.36%) and methyl stearate(6.68%). The antimicrobial activity of the oil was evaluated by using disc diffusion bioassay against five standard human pathogens (Gram positive: *Staphylococcus aureus* and *Bacillus subtitis*; Gram negative: *Escherichs coli* and *Pseudomonas aeruginosa* and the fungal species *Candida albicans*). *Aristolochia bracteolata* oil showed moderate activity against *Escherichs coli*, *Staphylococcus aureus* and *Candida albicans*. However, the oil was inactive *Pseudomonas aeruginosa* and *Bacillus subtilis*.

KEYWORDS: Aristolochia bracteolate, Oil, GC-MS Analysis, Antimicrobial activity.

INTRODUCTION

Medicinal plants for thousands of years, played a vital role in human life. These plants comprise many bioactive molecules with diverse pharmacological effects. Hence they may treat a wide array of human disorders and serve as a renewed and tremendous source of new drug leads.[1]

Aristolochia bracteolate Lam. is a perennial climber in the family Aristolociaceae which comprise more than 500 species.[2] This plant which is distributed in Africa, Asia and south America, is a potential medicinal plant which is widely used in traditional system of medicine.[3] Preliminary phytochemical screening revealed the presence of tannins, saponins, flavonoids, alkaloids and terpenoids.[4]

Aristolochia bracteolate is a leading antimalarial plant.[5] It is also used traditionally against dysentery, hypertension, diabetes, fever and scorpion bite.[3] *Aristolochia bracteolate* reportedly possesses antimicrobial.[5-10] antiarthritis,[11,12] antioxidant,[13] anti-inflammatory, antihyperglycaemic and antihyperlipidemic properties.[14]

MATERIAL AND METHODS

Plant material

The seeds of *Aristolochia bracteolate* were collected from Kordofan-Sudan. The plant was authenticated by The Medicinal and Aromatic Plants Research Institute-Sudan.

Test organisms

Aristolochia bracteolate oil was investigated for antimicrobial activity using the standard microorganisms shown in Table 1.

Table 1: Test organisms.

No.	Microorganism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginesa*	G-ve
4	*Escherichia coli*	G-ve
5	*Candida albicans*	Fungi

Equipments

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).

Methods

Extraction of oil

Aristolochia bracteolate seeds (250g) were macerated with hexane at room temperature. Removal of the solvent under reduced pressure gave the oil.

GC-MS analysis

Aristolochia bracteolate oil was analyzed by gas chromatography- mass spectrometry. A Shimadzo GC-MS-QP2010 ultra instrument was used. Helium was used as carrier gas. Oven temperature program is given in Table 2, while other chromatography conditions are displayed in Table 3.

Table 2: Oven temperature program.

Rate	Temperature	Hold time (min^{-1})
-	60	0.00
10	300	3.00

Table 3: Chromatography conditions.

Column oven temperature	50.0c°
Injection temperature	300.00
Injection mode	Split
Flow control mode	Pressure
Pressure	100.00KPa
Total flow	50.0 ml/min
Column flow	1.61 ml/min
Linear velocity	46.3cm/sec
Purge flow	3.0 ml/min
Split ratio	-1.0

Testing of antimicrobial susceptibility

The paper disc diffusion method was used to screen the antibacterial activity of the studied oil and performed by using Mueller Hinton Agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines.[15] Bacterial suspension was diluted with sterile physiological solution to 10^8 cfu/ml (Turbidity= McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6mm in diameter) were placed on the surface of the MHA and soaked with 20µl of a solution of test sample. The inoculated plates were incubated at 37°C for 24 hours in the inverted position. The diameters (mm) of the inhibition zones were measured as average of two replicates.

RESULT AND DISCUSSION

GC-MS analysis of *Aristolochia bracteolate* oil showed the presence of 12 components (Table 4). The typical total chromatogram (TIC) is shown in Fig. 1.

Table 4: Chemical constituents of*Aristolochia bracteolate* oil.

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.202	0.67
2.	9-Hexadecenoic acid, methyl ester, (Z)-	16.211	0.24
3.	Hexadecanoic acid, methyl ester	16.412	28.36
4.	cis-10-Heptadecenoic acid, methyl ester	17.231	0.10
5.	Heptadecanoic acid, methyl ester	17.442	0.15
6.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.160	3.86
7.	9-Octadecenoic acid (Z)-, methyl ester	18.222	52.96
8.	9-Octadecenoic acid, methyl ester, (E)-	18.255	2.66
9.	Methyl stearate	18.426	6.68
10.	cis-11-Eicosenoic acid, methyl ester	20.075	0.82
11.	Eicosanoic acid, methyl ester	20.278	2.16
12.	Docosanoic acid, methyl ester	21.984	1.34

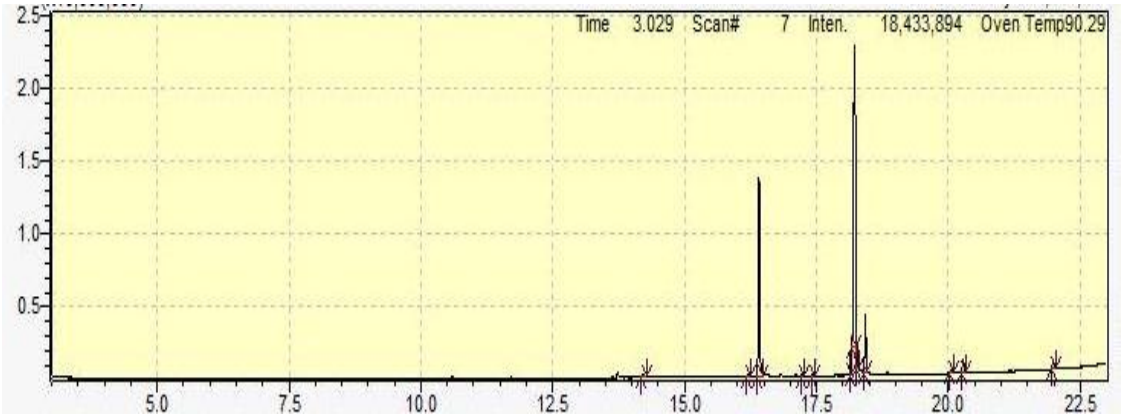

Fig. 1: Typical total ion chromatograms (TIC).

Major components of the oil are

i) 9-Octadecenoic acid (Z)-, methyl ester (52.96%).
ii) Hexadecanoic acid, methyl ester (28.36%).
iii) Methyl stearate (6.68%)

The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig. 2. The signal at m/z296 (RT.18.222) corresponds M^+ $[C_{19}H_{36}O_2]^+$.The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 3. The peak at m/z 270 (RT.16.412) is due to M^+ $[C_{17}H_{32}O_2]^+$. Fig.4 shows the mass spectrum of methyl stearate. The signal at m/z 298 (R.T.18.426) corresponds $M^+[C_{19}H_{38}O_2]^+$

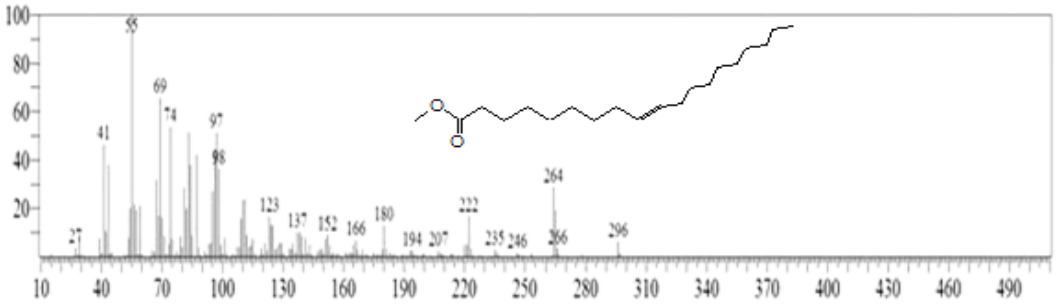

Fig. 2: Mass spectrum for 9-octadecenoic acid[z]-,methyl ester.

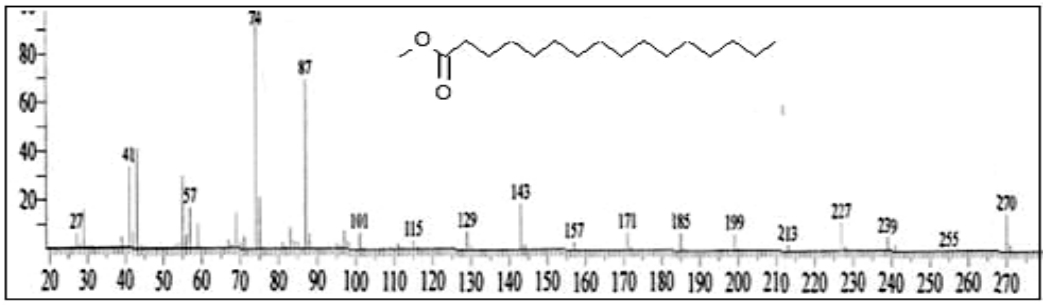

Fig. 3: Mass spectrum of hexadecanoic acid methyl ester.

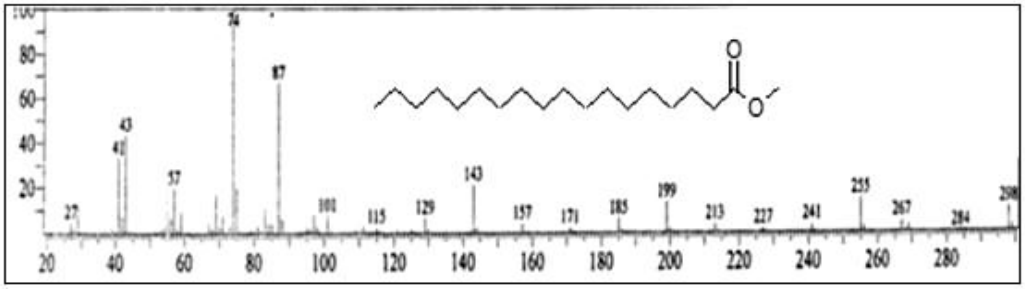

Fig. 4: Mass spectrum of methyl stearate.

Antimicrobial assay

The paper disc diffusion method was used to screen the antimicrobial potential of *Aristolochia bracteolata* oil against five standard human pathogens. The average of the diameters of the growth of inhibition zones are presented in Table (5). The oil showed moderate activity against *Escherichs coli*, *Staphylococcus aureus* and the fungal species *Candida albicans*. However, the oil was inactive against *Pseudomonas aeruginosa* and *Bacillus subtilis*.

Table 5: The antimicrobial activity of *Aristolochia bracteolate*oil.

Type	Sa	Bs	Ec	Ps	Ca
Oil(100mg/ml)	15	--	16	--	15
Ampicilin(40mg/ml)	30	15	--	--	--
Gentacycin(40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

(<9mm: inactive, 9-12mm: partially active, 13-18mm: active: > 18 mm very active).
Ec: *Escherichs coli*.
Ps: *Pseudomonas aeruginosa*.
Sa: *Staphylococcus aureus*.
Bs: *Bacillus subtilis*.
Ca: *Candida albicans*.

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