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CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *VANGUERIA MADAGASCARINSIS* (RUBIACEAE) OIL

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ABSTRACT

This study was designed to investigate the constituents of *Vangueria madagascarinsis* seed oil and to assess its antimicrobial activity. GC-MS analysis of *Vangueria madagascariensis* oil was performed. Nineteen constituents were detected. Main constituents are: 9,12-octadecadienoic acid-z,z- methyl ester (53.68%), hexadecanoic acid methyl ester (15.43%), 9-octadecenoic acid methyl ester(12.89%) and methyl stearate(10.23%). The antimicrobial activity of the oil was assessed against five standard human pathogens: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonasa aeruginosa* and the fungal species *Candida albicans*. *Vangueria madagascarinsis* oil showed significant activity against *Pseudomonas aeruginosa* and moderate activity against *Escherichia coli* and the yeast *Candida albicans*. The oil also exhibited weak activity against *Staphylococcus aureus*. However, it was inactive against *Bacillus subtilis.*

KEYWORDS: Vangueria madagascarinsis, Oil, GC-MS Analysis, Antimicrobial Activity.

INTRODUCTION

During the last few decades the control of infectious diseases and multi-drug resistance became a global concern.[1] It seems that pathogens developed a new resistance mechanism that rendered the new generation of antibiotics ineffective.[2] Medicinal plants with their long history in ethnomedicine recently attracted the attention of researchers as leads for drug discovery and drug development.[3,4]

Vangueria is a genus of flowering plants in the family Rubiaceae. The genus contains over 50 species distributed in Africa.[5] The fruits of *Vangueria infausta*, are consumed by humans.[6] The roots and leaves are used by traditional healers.[4] Leaves of *Vangueria spinosa* are used traditionally as antimicrobial.[7] Leaf extracts of *Vangueria spinosa* were screened for antibacterial activity. The ethyl acetate fraction was significantly active.[7]

Vangueria madagascarinsis – also known as Spanish tamarind and tamarind of Indies.[8] is a plant of many medicinal attributes. The plant is cultivated in some African and Asian countries for its medicinal and nutritional value.[10,11] Roots and bark are used in traditional medicine. In some African countries an extract from the roots is used to treat worm infections.[6] It has been reported that the plant possesses antimicrobial properties.[12] The antioxidant and cytotoxic properties has also been documented.[13] However, Some *Vangueria* species - *V. latifolia*, *V. pygmaea*, *V. thamnus* - are known to be toxic.[14]

MATERIALS AND METHODS

Plant material

Seeds of *Vangueria madagascariensis* were collected from Damazin, Sudan and authenticated by the Medicinal and Aromatic Plants Research Institute, Sudan. The seeds were shade – dried at room temperature and powdered.

GC-MS analysis

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 oil from *Vangueria madagascariensis* seeds 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 *Vangueria madagascariensis* (300g) 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.

'Constitents of the oil

The studied 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^{o}C; Injection temperature: 300^{o}C; Rate 4/mim; Injection mode: Split; Flow control mode:Lnear velocity; Pressure : 139.3 KPa; Total flow: 50.0 ml/min; Column flow: 1-54ml/sec/; Linear velocity : 47.2 Cm / sec,; Purge flow : 3.0 ml/ min; Split ratio: -1.0.

Antimicrobial assay

Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungus respectively. The media were prepared according to manufacturer instructions.

Broth 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 fingi the plates were incubated at 25^{o}C for 3days. The assay was carried out in duplicates and the diameters of inhibition zone were measured and averaged as indicator of activity. Ampicilin, gentamicin and clotrimazole were used as positive controls and DMSO as negative control.

RESULTS AND DISCUSSION

GC-MS analysis of *Vangueria madagascariensis* oil was performed. Nineteen constituents were detected. The constituents of the oil are presented in Table 2 and the total ions chromatograms is shown in Fig.1. Identification of the constituents was based on retention times and MS library data.

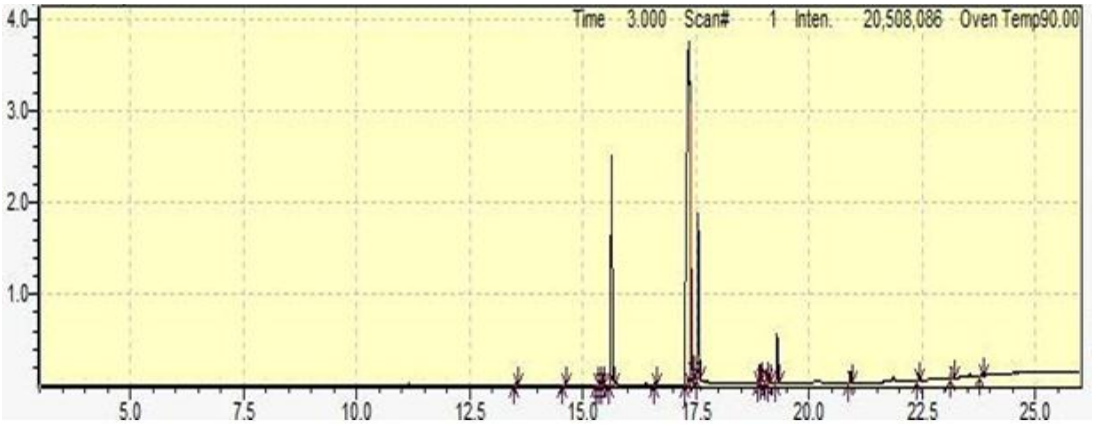

Fig. 1: Total ions chromatograms.

Table 2: Constituents of the oil.

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	13.531	0.11
2.	Pentadecanoic acid, methyl ester	14.603	0.05
3.	7,10-Hexadecadienoic acid, methyl ester	15.333	0.03
4.	7-Hexadecenoic acid, methyl ester, (Z)-	15.394	0.08
5.	9-Hexadecenoic acid, methyl ester, (Z)-	15.435	0.40
6.	Hexadecanoic acid, methyl ester	15.644	15.43
7.	Heptadecanoic acid, methyl ester	16.607	0.18

8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.355	53.68
9.	9-Octadecenoic acid (Z)-, methyl ester	17.383	12.89
10.	Methyl stearate	17.556	10.23
11.	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	18.900	0.46
12.	9,12-Octadecadienoyl chloride, (Z,Z)-	18.937	0.73
13.	cis-11,14-Eicosadienoic acid, methyl ester	19.066	0.45
14.	cis-11-Eicosenoic acid, methyl ester	19.099	0.71
15.	Eicosanoic acid, methyl ester	19.298	2.72
16.	Docosanoic acid, methyl ester	20.919	0.60
17.	Tetracosanoic acid, methyl ester	22.422	0.43
18.	Squalene	23.158	0.70
19.	Hexacosanoic acid, methyl ester	23.823	0.12

Major constituents are briefly discussed below

The mass spectrum of 9,12-octadecadienoic acid-z,z-methyl ester(53.68%) is shown in Fig.2. The peak at m/z294, which appeared at RT 17.355 in total ions chromatograms corresponds $M^+[C_{19}H_{34}O_2]$. The signal at m/z263 is due to loss of a methoxyl.

Fig.3 shows the mass spectrum of hexadecanoic acid methyl ester (15.43%). The molecular ion $M^+[C_{17}H_{34}O_2]$ appeared at m/z270(RT,15.644). The signal at m/z 239 accounts for loss of a methoxyl.

The mass spectrum of 9-octadecanoic acid methyl ester (12.89%) is presented in Fig.4.The signal at m/z296 (RT, 17.383) is due to the molecular ion $M^+[C_{19}H_{36}O_2]^+$. The peak at m/z266 is due to loss of a methoxyl.

Fig.5 shows the mass spectrum of methyl stearate(10.23%). The peak at m/z298 which appeared at RT 17.556 corresponds $M^+[C_{19}H_{38}O_2]^+$, while the signal at m/z267 corresponds to loss of a methoxyl.

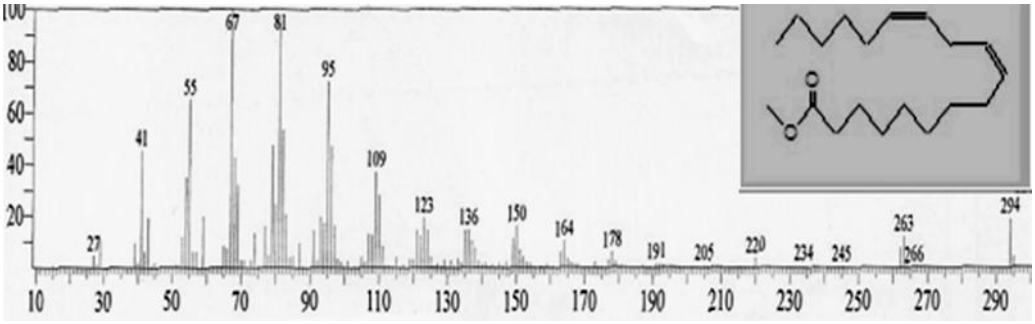

Fig. 2: Mass spectrum of 9,12-octadecadienoic.

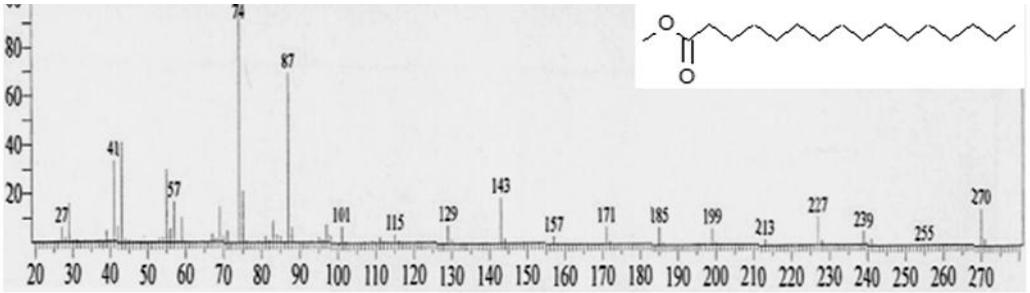

Fig. 3: Mass spectrum of hexadecanoic acid methyl ester.

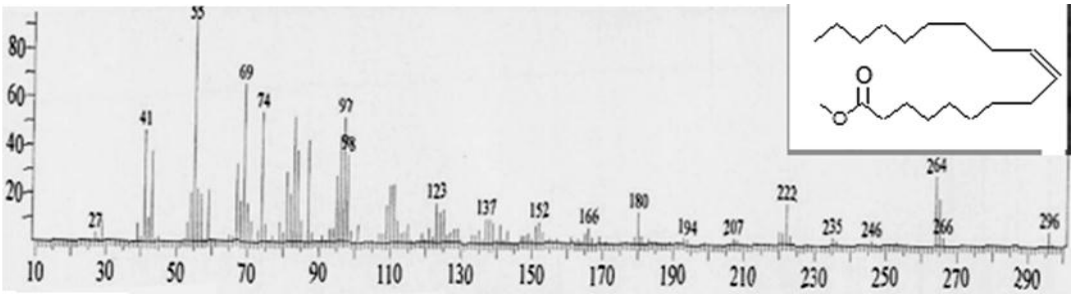

Fig. 4: Mass spectrum of 9-octadecenoic acid methyl ester.

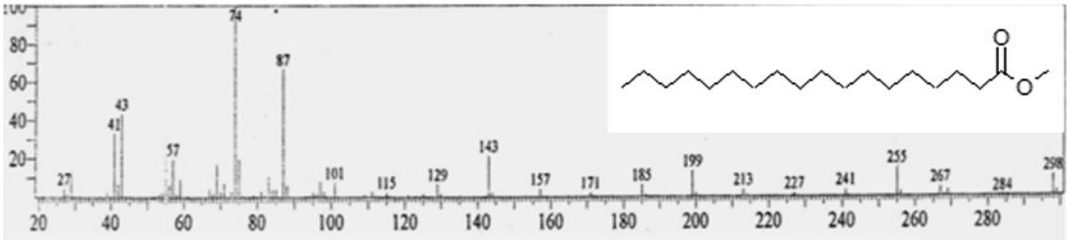

Fig. 4: Mass spectrum of methyl stearate.

Antimicrobial assay

The oil was evaluated for antimicrobial activity against standard microorganisms using disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table (3).

Table 3: Antimicrobial activity of *Vangueria madagascari* oil.

Sample	Sa	Bs	Ec	Ps	Ca.
Oil (100mg/ml)	10	--	15	17	14
Ampicilin (40mg/ml)	30	15	--	--	--
Gentamicin (40mg/ml)	19	25	22	21	--
Clotrimazole (30mg/ml)	--	--	--	--	38

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 significant activity against *Pseudomonas aeruginosa* and moderate activity against *Escherichia coli* and the yeast Candida albicans. The oil also exhibited weak activity against *Staphylococcus aureus*. However, it was inactive against *Bacillus subtilis*.

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

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