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Research Article

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Constituents and Antimicrobial Activity of *Annona senegalensis* (Annonaceae) Oil

Abdel Karim, M.[1]*, Bakhita, T.[1], Weam, M.A. Khojali[2]

[1]Sudan University of Science and Technology, Faculty of Science, Sudan
[2]University of Hail, Faculty of Pharmacy, Saudi Arabia

Abstract *Annona senegalensis* Persoon is a small tree or herb in the family Annonaceae. All parts of the plant find some applications in ethnomedicine. Leaves are used by traditional healers against tuberculosis, small pox and yellow fever. Root is used against snake bite, gastritis, sexual impotency, infectious diseases and erectile dysfunction. In this study *Annona senegalensis* oil was studied by GC-MS. The oil was also evaluated for antimicrobial activity. The GC-MS analysis showed 22 components dominated by: 9,12-octadecadienoic acid methyl ester (38.36%), 9-octadecenoic acid methyl ester (21.57%), hexadecanoic acid methyl ester (18.12%) and methyl stearate (14.47%). The oil showed good activity against *Pseudomonas aeruginosa* and moderate activity against *Escherichia coli* and *Candida albicans*.

Keywords *Annona senegalensis*, Oil, GC-MS Analysis, Antimicrobial Activity

Introduction

Annona senegalensis Persoon is a small tree or herb in the family Annonaceae. Usually the plant is 4-6m tall, but under favorable conditions it may reach 11 m in height. In the African continent *Annona senegalens* is occurs in forests, swamps, river banks and savannah woodland[1].All parts of the plant find some applications in ethnomedicine. Leaves are used by traditional healers against tuberculosis, small pox and yellow fever [2,3]. Root is used against snake bite, gastritis, sexual impotency, infectious diseases and erectile dysfunction [4-6]. Plant juice is a natural remedy for chicken pox [7]. The plant is also used as antidiabetic, antimalarial and as antidote for venomous bites [8-10]. Bark is used against open sores [11]. Phytochemical screening of *Annona senegalensis* revealed the presence of many bioactive components including flavonoids [12], tannins [13], alkaloids [14], saponins [15] and steroids [16] beside volatile oil [17]. *Annona senegalensis* is considered as a rich source of many nutrients. This plant contains minerals including Zn, Fe, Mg, K, Cu, Mn and Cr. It also contains ascorbic acid and some amino acids [18,19]. It has been reported that all parts of the plant contains volatile oil dominated by p-cymene (36.0%) and phellandrene (25%) [20]. Several pharmacological studies have been conducted on different parts of *Annona senegalensis*. The antimicrobial, anticonvulsant, antiinflammatory, antioxidant, analgesic and other biological activities of *Annona senegalens* is have been demonstrated [21-39].

Materials and Methods

Plant Material

Annona senegalensis seeds were collected from a forest reserve around Damazin, Sudan and authenticated by the Medicinal and Aromatic Plants Research Institute (Sudan).Seeds were shade–dried at room temperature and powdered.

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 *Annona senegalensis* was screened for antimicrobial activity using the standard microorganisms shown in Table 1.

Extraction of oil

Powdered shade-dried seeds of *Annona senegalensis* (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

Annona senegalensis oil was analyzed by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument. Helium was used as carrier gas.

- 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

Other chromatographic conditions are shown below:

Column oven temperature	: 150^{o}C
Injection temperature	: 300^{o}C
Injection mode	: Split
Flow control mode	: Linear velocity
Pressure	: 139.3KPa
Total flow	: 50.0ml/min
Column flow	: 1.54cm/sec.
Linear velocity	: 47.2cm/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 manufacture instructions. 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 24 h., while for fungi the plates were incubated at 25 ^{o}C for 3 days. The assay was carried out in duplicates and the diameters of inhibition zone were measured and averaged. Ampicilin, gentamicin and clotrimazole were used as positive control and DMSO as negative control.

Results and Discussion

Annona senegalensis oil was studied by GC-MS. The GC-MS analysis showed 22 constituents which were confirmed by the retention times and mass spectra fragmentation pattern. Major components are: (i)-9,12-Octadecadienoic acid methyl ester (38.36%) (ii)-9-Octadecenoic acid methyl ester (21.57%) (iii)- hexadecanoic acid methyl ester (18.12%) and (iv)- Methyl stearate (14.47%).

Table 1: Constituents of the oil

No.	Name	R. Time	Area %
1	L –Alpha Terineol	6.980	0.10
2	Methyl tetradecanoate	13.565	0.33
3	5-Octadecenoic acid methyl ester	14.480	0.01
4	Pentadecanoic acid methyl ester	14.640	0.05
5	7,10-Hexadecadienoic acid methyl ester	15.370	0.01
6	7-Hexdecenoic acid methyl ester	15.429	0.03
7	9-Hexadecenoic acid methyl ester	15.474	0.29
8	Hexadecanoic acid methyl ester	15.688	18.12
9	6-Octadecenoic acid methyl ester	16.436	0.17
10	Heptadecanoic acid methyl ester	16.645	0.29
11	9,12-Octadecadienoic acid methyl ester	17.384	38.36
12	9-Octadecenoic acid methyl ester	17.432	21.57
13	Methyl stearate	17.604	14.47
14	Cyclopentaneoctanoic acid methyl ester	18.938	1.48
15	9-Octadecenoic acid, 12-Hydroxy methyl ester	19.093	0.43
16	Cis-11-Eicosecoic acid methyl ester	19.136	0.35
17	Eicosanoic acid methyl ester	19.337	2.04
18	Phenol, 2,2-methylbis[6-1,1-dimethylene]	20.258	0.12
19	Docosanoic acid methyl ester	20.954	0.55
20	Tricosanoic acid methyl ester	21.718	0.11
21	Tetracosanoic acid methyl ester	22.456	0.36
22	Squalene	23.194	0.77
			100.00

Major components of the oil are:

i-9,12-Octadecadienoic acid methyl ester (38.36%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig. 1. The peak at m/z 294, which appeared at R.T. 17.384 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$. The peak at m/z 263 corresponds to loss of a methoxyl function.

ii-9-Octadecenoic acid methyl ester (21.57%)

Fig.2 shows the mass spectrum of 9-octadecenoic acid methyl ester. The peak at m/z 282 (RT., 17.432) accounts for: $M^+[C_{18}H_{34}O_2]^+$

iii- Hexadecanoic acid methyl ester (18.12%)

Fig.3 shows the mass spectrum of hexadecanoic acid methyl. The peak m/z 270 (R.T. 15.688) was detected in the spectrum. It corresponds $M^+[C_{17}H_{34}O_2]^+$. The peak at m/z 239 is due to loss of a methoxyl.

iv- Methyl stearate (14.47%).

The EI mass spectrum of methyl stearate is displayed in Fig. 4. The peak at m/z 298 with R.T. 17.604 is due to $M^+[C_{19}H_{38}O_2]^+$, while the signal at m/z 267 corresponds to loss of a methoxyl group.

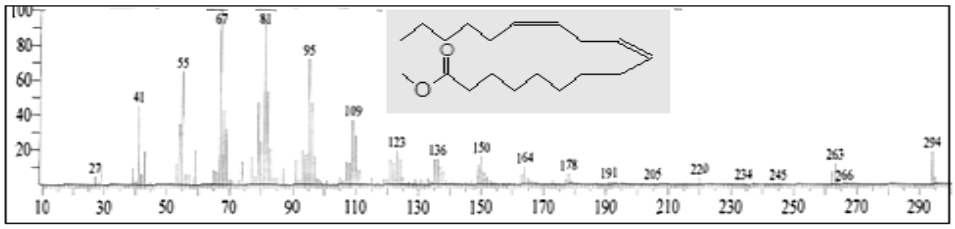

Figure 1: Mass spectrum of 9,12-octadecanoic acid methyl ester

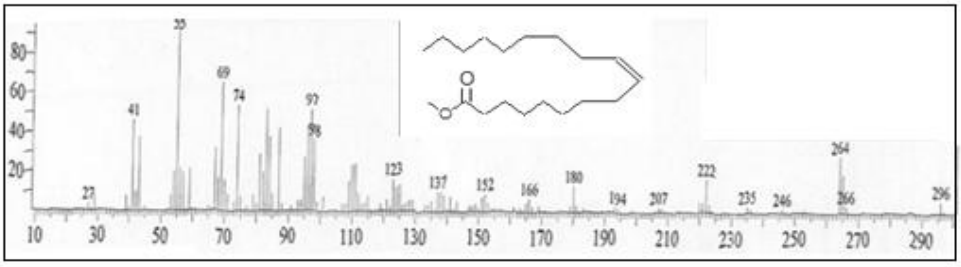

Figure 2: Mass spectrum of 9-octadecenoic acid methyl ester

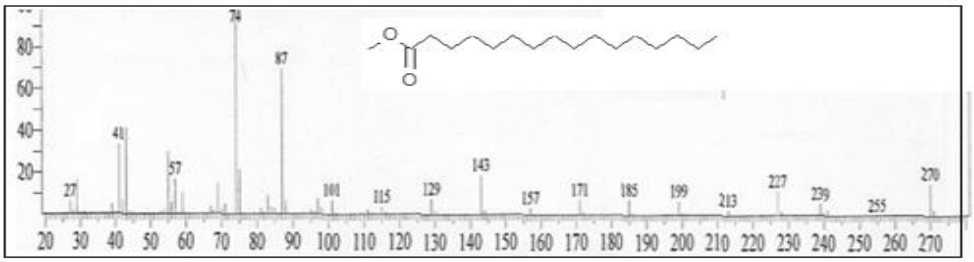

Figure 3: Mass spectrum of hexadecanoic acid methyl ester

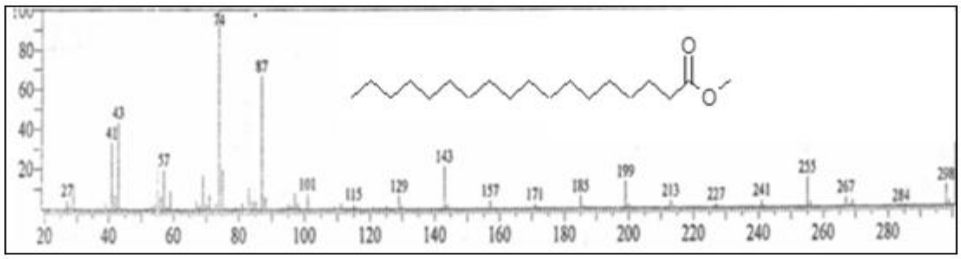

Figure 4: Mass spectrum of methyl stearate

Antimicrobial activity

Annona senegalensis oil was evaluated for antimicrobial activity against five standard microorganisms using disc diffusion method. 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 studied oil showed significant activity against *Pseudomonas aeruginosa* and moderate activity against *Escherichia coli* and *Candida albicans*.

Table 2: Inhibition zones (mm/mg sample)

Type	Sa	Bs	Ec	Ps	Ca
Oil (100mg/ml)	13	--	14	16	14
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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