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Research Article

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Chemical Constituents and Antimicrobial Activity of Sudanese Lagenaria siceraria Standley (Cucurbitaceae) Oil

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Abstract In this study, the constituents and antimicrobial activity of *Lagenaria siceraria* oil. have been investigated. *Lagenaria siceraria* **Standley** is a common fruit vegetable in the family Cucurbitaceae. The fruit has many uses in ethnomedicine. Fruit is used as diuretic, immunosuppressant, cardio-protective and cardio-tonic. *Lagenaria siceraria* possesses antioxidant, hypolipidemic and hepatoprotective properties. GC- MS analysis of *Lagenaria siceraria* oil revealed the presence of three major constituents: linoleic acid ethyl ester (57.96%); 9,12-octadecenoic acid methyl ester (19.56%) and hexdecanoic acid (10.15% %). The oil was screened for antimicrobial activity against five standard human pathogens by using the paper disc diffusion method. The oil showed moderate activity against *Pseudomonas aeruginosa*.

Keywords *Lagenaria siceraria,* Oil, Constituents, Antimicrobial Activity

Introduction

Lagenaria siceraria **Standley** is a common fruit vegetable in the family Cucurbitaceae. The fruit has many uses in ethnomedicine, it is also valued as a nutrient. Fruit is considered as a good source of carotene, vitamin C and B complex [1-2].

Preliminary phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, and steroids [3-4]. The plant also contains polyphenols, cucurbitacins and fibre [5]. Fruit is used as diuretic, immunosuppressant, cardio-protective and cardio-tonic [6,7].

It has been reported that *Lagenaria siceraria* possesses antioxidant [8,9], antidepressant [10] and hepatoprotective properties [11]. Seeds which contain leganin and a ribosome - inactivating protein has anti- HIV, antiproliferative and antitumor potential [12]. Seeds are also used traditionally against dropsy and intestinal worms Seeds are also valuable nutrient containing amino acids, minerals and vitamins [13,14].

Stem, leaves, fruit and seeds are used in herbal medicine against jaundice, diabetes, piles, colitis, ulcer, hypertension, congestive cardiac failure and skin infections. Leave juice is used in ethnomedicine against baldness [15,16].

Materials and Methods

Materials

Plant material

The seeds of *Lagenaria siceraria* were collected from a forest reserve around Nyala western Sudan. The plant was identified and authenticated by direct comparison with a herbarium sample. The plant material was shade - dried at room temperature and finally powdered.

Microbial isolates

Gram +ve: *Bacillus subtilis* and *Staphylococcus aureus*; **Gram –ve:** *Escherichia coli* and *Pseudomonas aeruginosa*; **fungal strain:** *Candida albicans.*

Positive controls

i. Ampicillin ii) gentamicin and iii) clotrimazole.

Media

-Muller –Hinton agar for bacteria; Sabouraud dextrose agar (oxoid, England) is used as media for fungal growth.

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

Powdered seeds of *Lagenaria siceraria* (400g) were exhaustively extracted with n-hexane by maceration. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4 ^{o}C for further work.

GC-MS analysis

The studied oil was analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μm, thickness) was used. Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program is presented in Table 1, while other chromatographic conditions are depicted in Table 2.

Table 1: Oven temperature program

Rate	Temperature (^{o}C)	Hold Time (min.$^{-1}$)
-	150.0	1.00
4.00	300.0	0.00

Table 2: Chromatographic conditions

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	- 1.0

Antimicrobial Assay

The paper disc diffusion method was used to screen the antimicrobial activity of the oil and performed by using Mueller Hinton agar (MHA). 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 (Whitman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of test sample. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured and recorded as average of two replicates.

Results and Discussion

The GC-MS analysis of *Lagenaria siceraria* oil showed 9 components dominated by fatty acids (Table 3). The total ions chromatograms is shown in Fig. 1.

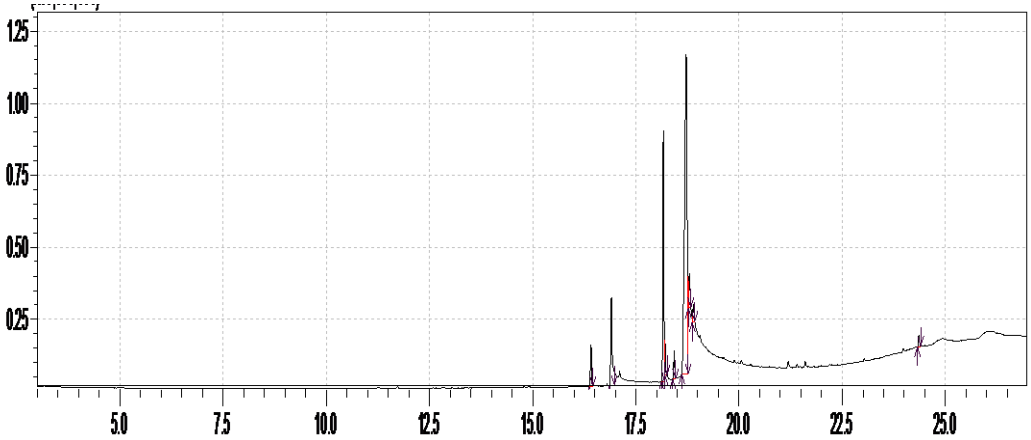

Figure 1: Total ions chromatograms of the oil

Table 3: Constituents of the oil

S. No.	Name	Ret. Time	Area%
1	Hexadecanoic acid, methyl ester	16.411	3.39
2	n-Hexadecanoic acid	16.908	10.15
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.164	19.56
4	9-Octadecenoic acid (Z)-, methyl ester	18.205	1.43
5	Methyl stearate	18.428	2.02
6	Linoleic acid ethyl ester	18.716	57.96
7	9,11-Octadecadienoic acid, methyl ester, (E,E)-	18.797	3.15
8	Octadecanoic acid	18.891	1.41
9	Squalene	24.354	0.93

The oil was dominated by:

i)- Linoleic acid ethyl ester (57.96%).

ii)- 9,12-Octadecadienoic acid methyl ester (19.56%).

iii)- Hexadecanoic acid methyl ester (10.15%).

The mass spectrum of linoleic acid ethyl ester is illustrated in Fig. 2. The signal at m/z 308 (RT.18.716) is due to the molecular ion: $[C_{20}H_{36}O_2]$. Figure 3 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294 (RT. 18.164) corresponds: M^+ $[C_{19}H_{34}O_2]^+$. The mass spectrum of hexadecanoic acid methyl ester is presented in Figure 4. The peak at m/z 270 (RT.16.908) is due to M^+ $[C_{17}H_{32}O_2]^+$.

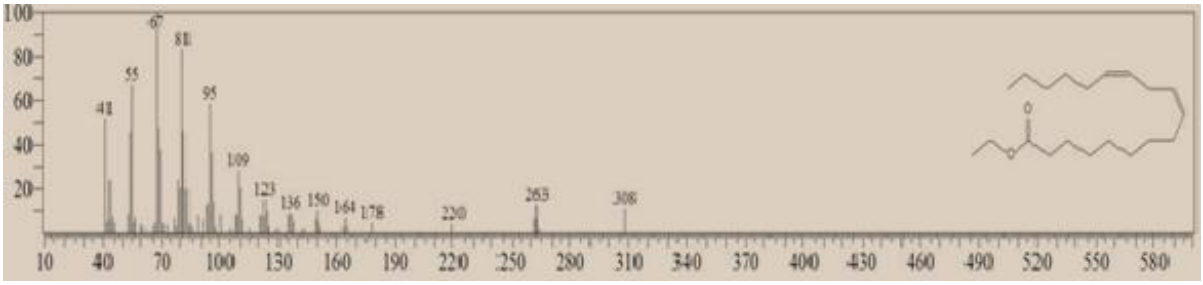

Figure 2: Linoleic acid ethyl ester

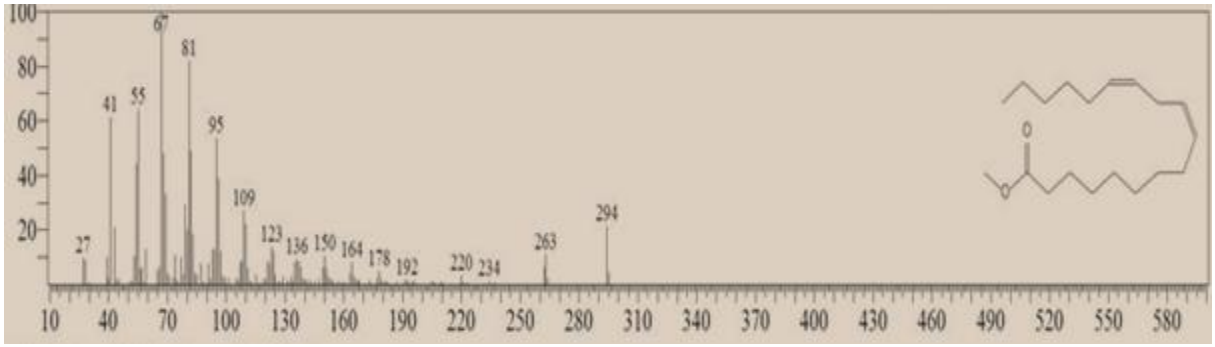

Figure 3: Mass spectrum of 9,12-Octadecadienoic acid methyl ester

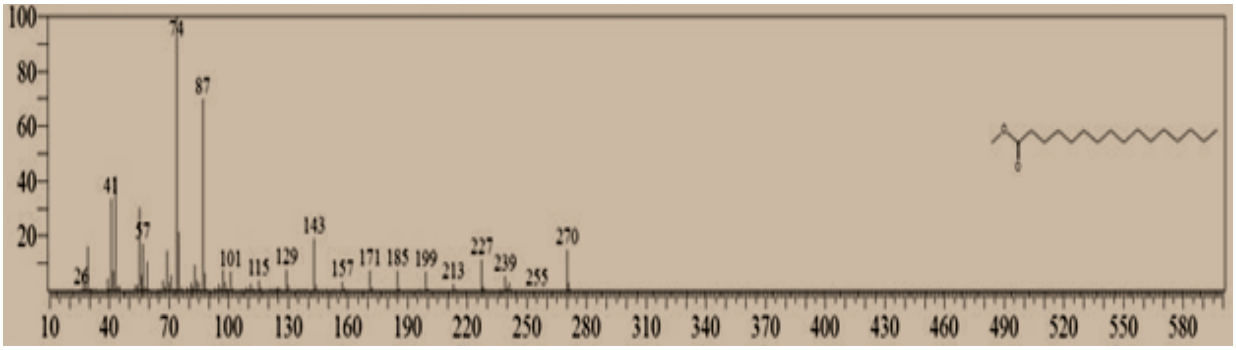

Fig.4 : Mass spectrum of hexadecanoic acid methyl ester

Antimicrobial Activity

The oil from seeds of *Lagenaria siceraria* was screened for antimicrobial activity against five standard microorganisms. The average of the diameters of the growth inhibition zones are depicted in Table (4). 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 oil showed moderate activity against *Pseudomonas aeruginosa* beside weak activity against *Staphylococcus aureus* and *Bacillus subtilis.*

Table 4: Inhibition zones (mm/mg sample)

Type	Sa	Bs	Ec	Ps	Ca
Oil (100mg/ml)	12	10	--	15	--
Ampicilin (40mg/ml)	30	15	--	--	--
Gentamicin (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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