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Constituents and antimicrobial activity of sudanese *Proboscidea Louisianica* (Mill.) thell (Martyniaceae) oil

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Abstract
Proboscidea louisianica (Mill.) Thell is a spreading annual plant. Young fruits are edible. Seed oil contains: among others-linoleic. oleic and palmitic acids beside some sterols. The claws of the mature seed pods are traditionally used against rheumatic pain. In this study the constituents of *Proboscidea louisianica* seed oil have been characterized by GC-MS and the antimicrobial activity of the oil has been assessed. Fifteen components have been detected by GC-MS analysis. Major constituents are: 9,12-octadecadienoic acid methyl ester(43.99%), 9-ctadecenoic acid (Z) methyl ester(25.40%), hexadecanoic acid methyl ester(12.01%) and methyl stearate(8.89%),The antimicrobial activity of the oil was evaluated using the diffusion assay against: Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseudomonasa aeruginos*a and the yeast *Candida albicans*. The oil showed moderate anticandidal activity. It also exhibited weak activity against *Staphylococcus aureus*. However, it failed to exhibit activity against other test organisms.

Keywords: *Proboscidea louisianica*, oil, gc-ms, antimicrobial activity

1. Introduction
For thousands of years humans used medicinal plants against a wide spectrum of human disorders. Recently there have been a renewed interest in the constituents of medicinal plants and their impact on human physiology. Many secondary metabolites (alkaloids, steroids, flavonoids…etc) are endowed with potential biological activities. Research in the field of phytochemistry and pharmacology may provide valuable data that could serve in drug discovery and drug development bearing in mind the global concern of microbial multi-drug resistance. A considerable percentage of the marketed medicines are derived directly of indirectly from plants. In developing countries, where modern drugs are beyond affordability, herbal medicine plays a vital role in primary healthcare.
Proboscidea louisianica (Mill.) Thell is a spreading annual plant in the family Martyniaceae. The plant is covered with trichomes tipped by droplets of oil [1, 2]. *Proboscidea louisianica* is native to northern Mexico and southwestern United States. Young fruits are edible [3, 4]. Seed oil contains – among others-linoleic, oleic and palmitic acids beside some sterols. The constituents of the essential oil has been reported [5, 6]. This volatile oil seems to have allelopathic effect on cotton plants [7, 8]. The claws of the mature seed pods are traditionally used against rheumatic pain [9].

Materials and Methods
Plant material
The seeds of *Proboscidea louisianica* (Mill.) Thell were collected from a forest reserve around Damazin-Sudan.The plant was authenticated by the Medicinal and Aromatic Plants Research Institute,Khartoum, Sudan. The freshly collected plant material was dried under shade at room temperature and powdered.

Materials for antimicrobial screening
- **Bacterial strains**

Bacillus subtilis NCTC 8236 (Gram + ve bacteria).
Staphylococcus aureus ATCC 25923(Gram +ve Bacteria).
Escherichia coli ATCC 25922(Gram -ve bacteria**)**
Pseudomonas aeruginosa ATCC 27853 (Gram -ve bacteria)

- **Fungal strains**

Candida albicans ATCC7596

- **Standards**

-Ampicilin
-gentamicin
-clotrimazole

Methods
GC-MS analysis
Proboscidea louisianica (Mill.) Thell oil was extracted by maceration.Volatiles of the oil were determined via A Gillent Technologies 7890A Gas Chromatography (GC) system coupled with mass spectrometry (MS) detector. The esterified oil was prepared at 100mg/ml via dilution in solvent and was injected into the system. Blank analysis was also performed. The chromatographic settings are; injection source: GC auto sampler and thermal separation probe (TSP); injection volume: 1 µL (sample); injection mode: splitless and split ratio 1:5 and oven temperature: initial 35 °C, increased to 180 °C (6 °C/min), held 5 min, increased to 230 °C (1°C/min) and held 20 min. Other settings; column: non-polar capillary DB-1 of 100% dimethyl-polysiloxane (30 m x 0.53 mm id, film thickness 0.25 µm); carrier gas: helium (1 ml/min); ionization energy: 70 eV; front inlet pressure: 6.78 psi, oven equilibrium time: 3 min; MS source and MS quad temperature: 350^0, 290^0, 250^0, 230^0, and 150^0. The constituents of the oil were characterized via the National Institute of Standards and Technology (NIST)

Library Chem-Station software.

Antimicrobial assay

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 72h. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing for antimicrobial susceptibility

The paper disc diffusion method was used to screen the antibacterial activity of test sample and performed by using Mueller Hinton agar (MHA). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ 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, 6 mm in diameter) were placed on the surface of the MHA and soaked with 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.The same procedure was used for antifungal activity, but Sabouraud dextrose agar was used as medium for fungal culture and incubation continued for 72h at 25ºC.

Results and Discssion

Proboscidea louisianica oil was analyzed by GC-MS. 15 components have been detected.Total ions chromatograms is presented in Fig.1,while constiruents are illustrated in Table 1.

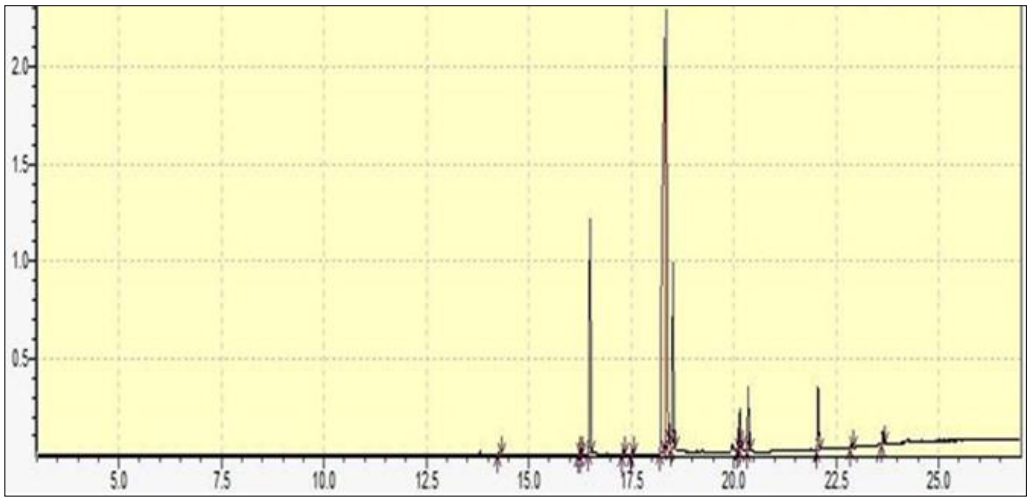

Fig 1: Total ions chromatograms

Table 1: Constituents of the oil

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.278	0.08
2.	7-Hexadecenoic acid, methyl ester, (Z)-	16.242	0.08
3.	9-Hexadecenoic acid, methyl ester, (Z)-	16.289	0.19
4.	Hexadecanoic acid, methyl ester	16.499	12.01
5.	cis-10-Heptadecenoic acid, methyl ester	17.308	0.12
6.	Heptadecanoic acid, methyl ester	17.521	0.14
7.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.310	43.99
8.	9-Octadecenoic acid (Z)-, methyl ester	18.360	25.40
9.	Methyl stearate	18.520	8.89
10.	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	20.130	0.84
11.	cis-13-Eicosenoic acid, methyl ester	20.154	1.78
12.	Eicosanoic acid, methyl ester	20.356	2.66
13.	Docosanoic acid, methyl ester	22.062	2.98
14.	Tricosanoic acid, methyl ester	22.867	0.16
15.	Tetracosanoic acid, methyl ester	23.641	0.68

Major constituents of the oil are discussed below:

1. 9,12-Octadecadienoic acid methyl ester(43.99%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.2.The peak at m/z 294, which appeared at R.T. 18.310 in total ion chromatogram, corresponds M^+ $[C_{19}H_{34}O_2]^+$. The peak at m/z 263 is due to loss of a methoxyl, while the fragment m/z 59 is due to loss of (CH_3-O-C=O).

2. 9-Octadecenoic acid methyl ester (25.40%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig.3.The peak at m/z 296, which appeared at R.T. 18.360 corresponds $M^+[C_{19}H_{36}O_2]^+$. The signal at m/z265 accounts for loss of a methoxyl function.

3. Hexadecanoic acid methyl ester (12.01%)

The EI mass spectrum of hexadecanoic acid methyl ester is shown in Fig. 4.The peak at m/z 270, which appeared at R.T. 16.499 in total ion chromatogram, corresponds to $M^+[C_{17}H_{34}O_2]^+$. The peak at m/z239 corresponds to loss of a methoxyl.

4. Methyl stereate (8.89%)

The mass spectrum of methyl stereate is presented in Fig.5.The signal at m/z298(RT.18.520)is due to the molecular ion:$M^+[C_{19}H_{38}O_2]$, while the signal at m/z267 accounts for loss of a methoxyl.

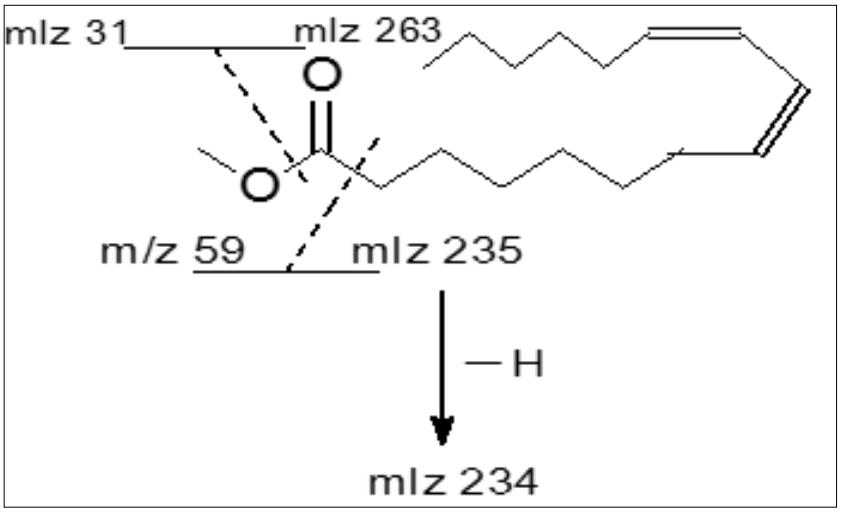

Fig 1: Major fragmentation of 9, 12-octadecadienoic acid methyl ester

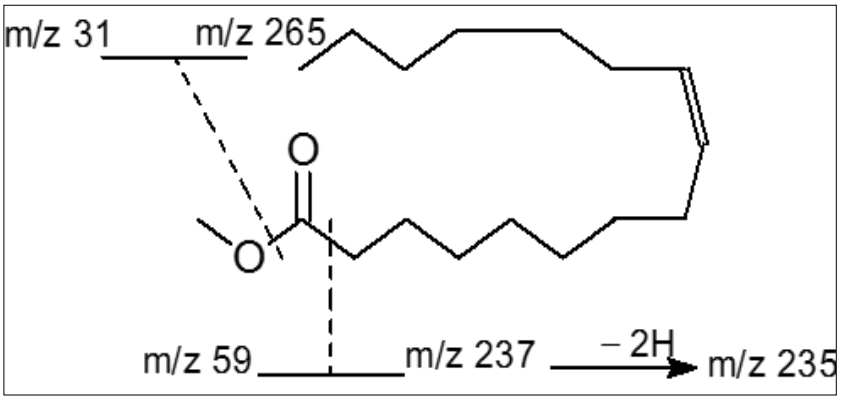

Fig 2: Major fragmentation of 9-octadecenoic acid methyl ester

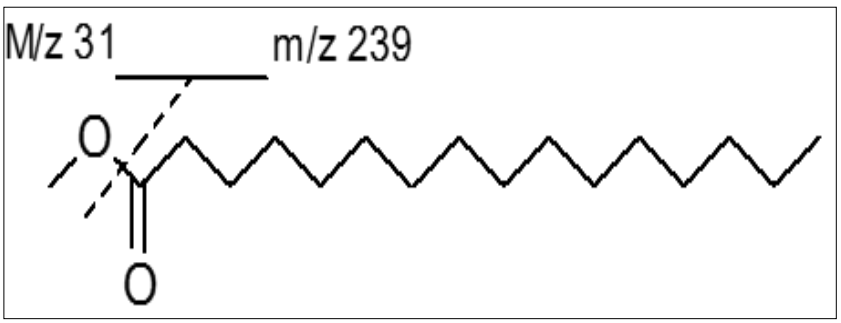

Fig 3: Major fragmentation of hexadecanoic acid methyl ester

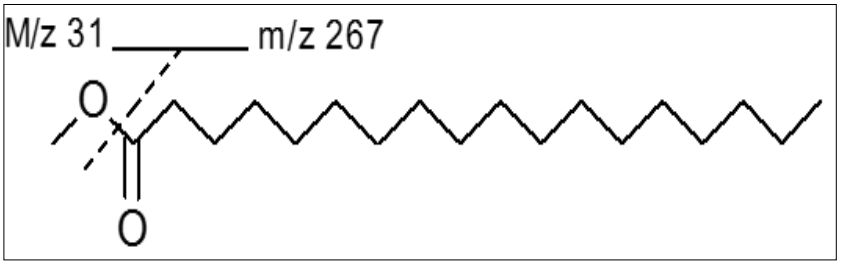

Fig 4: A Major fragmentation of methyl stereate

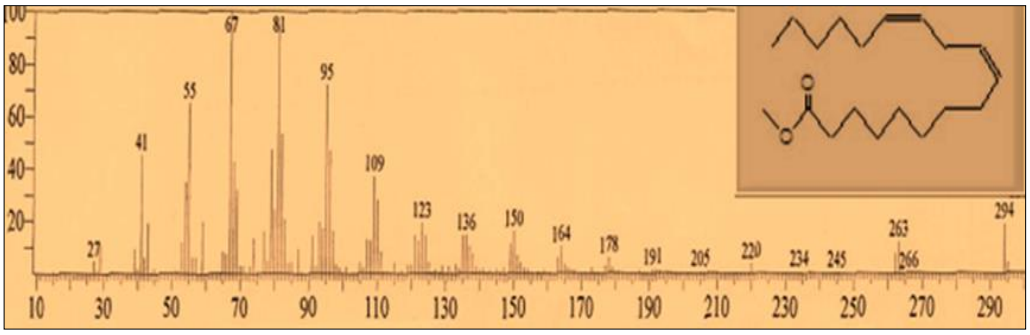

Fig 2: Mass spectrum of 9, 12-octadecanoic acid methyl ester

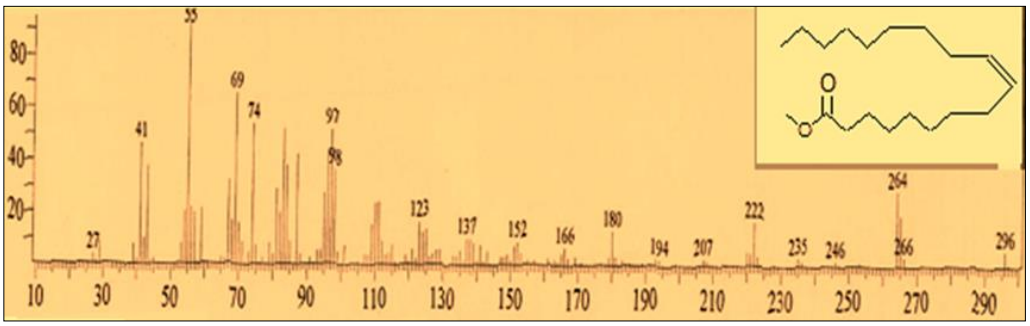

Fig 3: Mass spectrum of 9-octadecenoic acid methyl ester

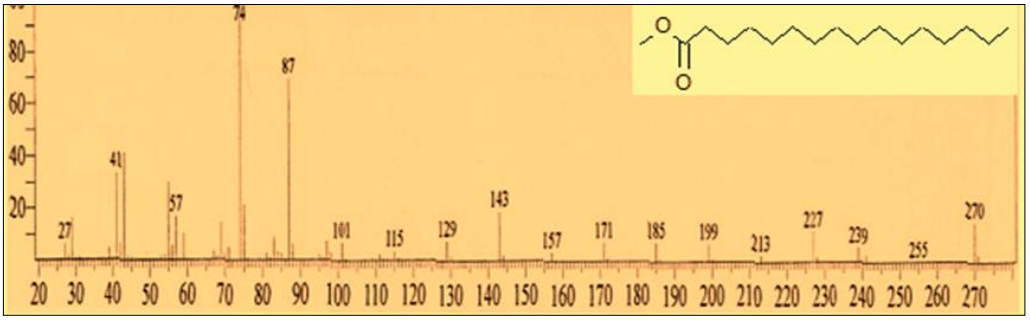

Fig 4: Mass spectrum of hexadecanoic acid methyl ester

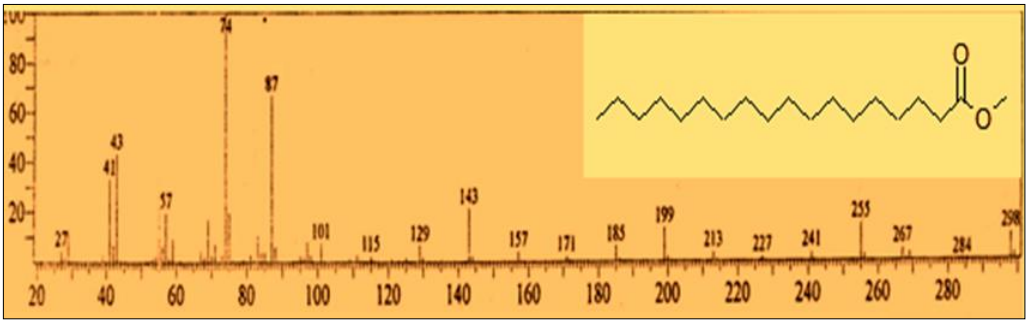

Fig 5: Mass spectrum of methyl stearate

Antimicrobial assay

The disc diffusion bioassay was used to screen the antimicrobial activity of the oil against five standard human pathogens. The average of the diameters of the growth of

inhibition zones are shown Table (2). The results were interpreted as follows: (<9mm: inative; 9-12mm: partially active;13-18mm: active; >18mm:very active). Tables (3) and (4) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively. *Proboscidea louisianica* oil showed moderate anticandidal activity.It also exhibited weak activity against *Staphylococcus aureus.* However, it failed to exhibit activity against other test organisms.

Table 2: Antibacterial activity of *Proboscidea louisianica* oil

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	11	-	-	-	15

Table 3: Antibacterial activity of standard chemotherapeutic agents

Drug	Conc.(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 4: Antifungal activity of standard chemotherapeutic agent

Drug	Conc.(mg/ml)	An	Ca
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Sa: Staphylococcus aureus
Ec: Escherichia coli
Pa: Pseudomonas aeruginosa
An: Aspergillus Niger
Ca: Candida albicans
Bs: Bacillus subtilis

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