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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE LUFFA AEGYPTIACA (LINN.) M.ROEM FIXED OIL

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ABSTRACT

This study was planed to identify the constituents of *Luffa egyptiaca* fixed oil, which find many applications in ethnomedicine, and to evaluate its antimicrobial activity. *Luffa egyptiaca* oil was analyzed by GC-MS and identification of constituents was accomplished by comparison with the MS library (NIST) as well as the observed fragmentation pattern. The GC-MS analysis showed the presence of 17 components**.** Major constituents are: i) 9,12-octadecadienoic acid methyl ester(51.97%) , ii)9-Z-octadecenoic acid methyl ester(12.83%), iii) hexadecanoic acid methyl ester(11.84%), iv)methyl stearate(8.27%) , v)9,12-octadecadienoyl chloride(5.56%). The oil exhibited excellent activity against *Pseudomonas aeruginosa* and *Bacillus subtilis* in the range: 100-25mg/ml and 100-12.50mg/ml respectively. It also showed significant activity against *Escherichia coli* at 100-25mg/ml. At 100mg/ml, it exhibited significant activity against the yeast *Candida albicans.*

KEYWORDS: *Luffa egyptiaca* oil , GC-MS analysis, Antimicrobial activity.

INTRODUCTION

Extensive studies have been conducted to document scientific evidence for the traditional claims of the therapeutic efficacy of medicinal plants.[1-3] Furthermore, the issue of multidrug resistance triggered more research in plants to isolate and characterize new phytochemicals with pharmacological effects serving as leads for new drugs.

Medicinal plants are used throughout Africa to treat a wide spectrum of diseases. However, scientific data on the constituents of these plants is very scarce. Hence this study was planned to identify and quantify the constituents of *Luffa egyptiaca* oil, which find many applications in ethnomedicine, and to evaluate its antimicrobial activity.

Luffa aegyptiaca (Linn.) M. Roem. is widely distributed in Sudan and it is reported to originate from India.[4] *Luffa egyptiaca* is a climbing annual vine in the family Cucurbitaceae. Fruits are diuretic and lactogogue.[5-9] Fruits are also used against nephritis, dropsy and chronic bronchitis,[10-12] Some studies demonstrated that the plant posssses antimicrobial,[13,15] anthelmintic,[16,17] antiinflammatory[18] hepatoprotective[19,20] anticancer, [21-23] anaesthetic[24] and enzyme inhibition properties.

The plant is reported to contain: cucurbitacins[25,26] saponins[27] flavonoids[28,29] aminoacids[30] vitamins[31] beside some fatty acids.[31]

MATERIALS AND METHODS

Plant material

Luffa egyptiaca seeds were collected from Khartoum, Sudan.The plant was authenticated by The Institute of Aromatic and Medicinal Plants- Khartoum, Sudan.

Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness) was used for GC-MS analysis .

Test organisms

Luffa egyptiaca oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in Table (1).

Table 1: Test organisms.

Ser. No	Microorganism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
5	*Aspergillus niger*	fungi
6	*Candida albicans*	fungi

Methods

Extraction of Luffa egyptiaca oil

Luffa egyptiaca seeds (400g) were macerated with n-hexane at room temperature for 48h. The solvent was removed under reduced pressure to give the oil. For GC-MS analysis, the oil was esterified. The oven temperature program is displayed below, while other chromatographic conditions are shown in Table 2.

Rate	Temperature	Hold time(min^{-1})
--	60.0	0.00
10.00	300.0	0.00

Table 2: Chromatographic conditions.

Column oven temperature	60.0 °C
Injection temperature	280.0 °C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	93.1KPa
Total flow	50.0ml/ min
Column flow	1.50ml/sec
Linear velocity.	44.7cm/sec
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial assay

Preparation of bacterial suspensions

Diffusion method was the method used for screening the oil. Mueller Hinton and Saboraud dextrose agars were the media used as the growth media for the bacteria and the fungi respectively. The media were prepared according to the manufacturer's instructions.

Aliquots (1ml) of 24 hours broth culture of the test microorganisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. Bacterial growth was harvested and washed off with sterile normal saline , then it was suspended in (100 ml) of normal saline to afford about 108-109 colony forming units per ml. Average number of viable organism 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.(0.02 ml) of the appropriate dilutions were transferred onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature and then incubated at 37°C for 24 hours.

Fungal cultures were maintained on potato dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing for antibacterial activity

(2ml) of the standardized bacterial stock suspension were mixed with (200 ml) of sterile molten nutrient agar which was maintained at 45°C. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle. Each plate was divided into two halves. In each half two cups (10mm in diameter) were cut using sterile cork borer (No 4). Each half was designed for a test solution.

Agar discs were removed, alternate cups were filled with (0.1 ml) samples of each test solution and allowed to diffuse at room temperature for two hours. The plates were then incubated at 37^{o}C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured in duplicates and averaged.

RESULTS AND DISCUSSION

GC-MS analysis of *Luffa egyptiaca* oil

Luffa egyptiaca oil was analyzed by GC-MS and identification of constituents was accomplished by comparison with the MS library (NIST). The observed fragmentation pattern was also discussed. The GC-MS analysis showed the presence of 17 components (Table 3).The typical total ion chromatograms (TIC) is depicted in Fig.1.

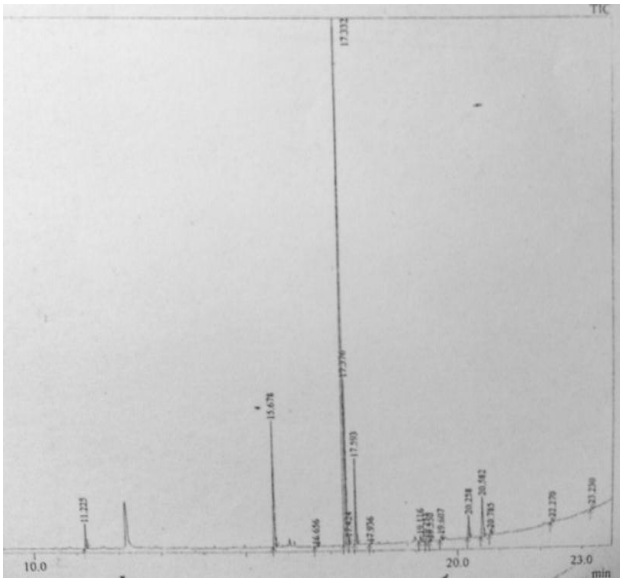

Fig. 1: The typical total ion chromatograms.

Table 3: Constituents of *Luffa egyptiaca* oil.

Peak#	R.Time	Area	Area%	Name
1	11.225	304696	2.27	Butylated Hydroxytoluene
2	15.678	1585303	11.84	Hexadecanoic acid, methyl ester
3	16.656	22395	0.17	Hexadecanoic acid, 14-methyl-,methyl este
4	17.332	6961031	51.97	9,12-Octadecadienoic acid (Z,Z)-, methyl e
5	17.376	1717988	12.83	9-Octadecenoic acid (Z)-, methyl ester
6	17.424	61829	0.46	9-Octadecenoic acid, methyl ester, (E)-
7	17.593	1107392	8.27	Methyl stearate
8	17.936	15929	0.12	8,11-Octadecadienoic acid, methyl ester
9	19.116	110621	0.83	Heptadecanoic acid, heptadecyl ester
10	19.254	56387	0.42	Linoleic acid ethyl ester
11	19.350	39124	0.29	Methyl 18-methylnonadecanoate
12	19.607	101325	0.76	9,12-Octadecadienoic acid, ethyl ester
13	20.258	312869	2.34	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
14	20.582	745341	5.56	9,12-Octadecadienoyl chloride, (Z,Z)-
15	20.785	74739	0.56	l-(+)-Ascorbic acid 2,6-dihexadecanoate
16	22.270	100138	0.75	7,10-Octadecadienoic acid, methyl ester
17	23.230	77667	0.58	Squalene
		13394774	100.00	

Major components are

1. 9,12-Octadecadienoic acid methyl ester (51.97%)

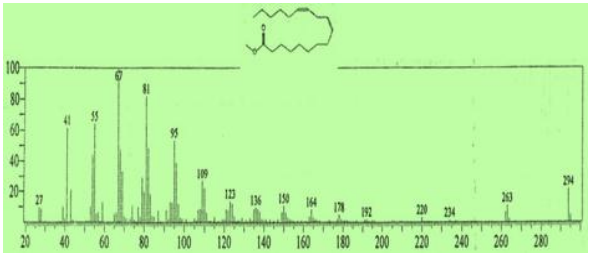

Fig. 2: Mass spectrum of 9, 12-octadecadienoic acid methyl ester.

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. 17.332 in total ion chromatogram, corresponds $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z263 corresponds to loss of a methoxyl function.

2. 9-Z-Octadecenoic acid methyl ester (12.83%)

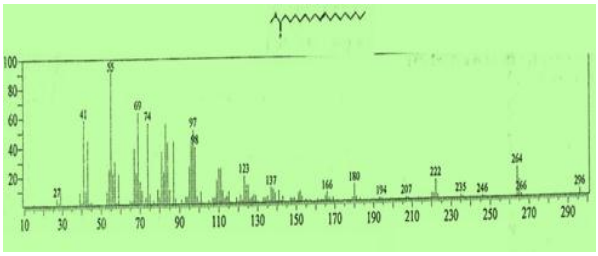

Fig. 3: Mass spectrum of 9-octadecenoic acid methyl ester.

Fig. 3 displays the mass spectrum of 9-octadecenoic acid methyl ester. The peak at m/z 296(R.T. 17.376) corresponds $M^+[C_{19}H_{36}O_2]^+$, while the signal at m/z265 is attributed to loss of a methoxyl group.

3. Hexadecanoic acid methyl ester (11.84%)

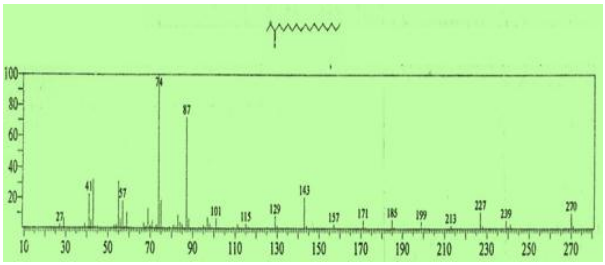

Fig. 4: Mass spectrum of hexadecanoic acid methyl ester.

Fig. 4 shows the mass spectrum of hexadecanoic acid methyl ester. The signal at m/z 270(R.T. 15.678) corresponds $M^+[C_{17}H_{34}O_2]^+$ .The peak at m/z239 is due to loss of a methoxyl.

4. Methyl stearate (8.27%)

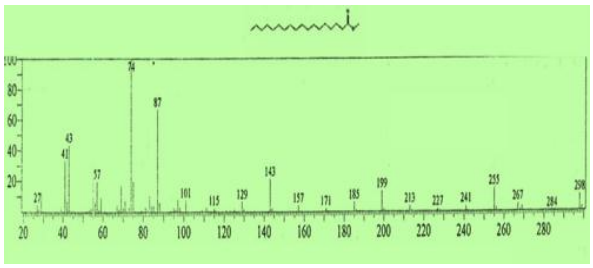

Fig. 5: Mass spectrum of methyl stearate.

The EI mass spectrum of methyl stearate is shown in Fig. 5.The signal at m/z 298, which appeared at R.T.17.593 - in total ion chromatogram- corresponds $M^+[C_{19}H_{38}O_2]^+$.The peak at m/z267 accounts for loss of a methoxyl group.

5. 9, 12-Octadecadienoyl chloride (5.56%)

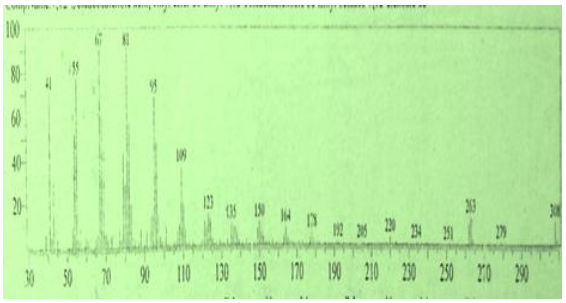

Fig. 6: Mass spectrum of 9, 12-octadecadienoyl chloride.

The mass spectrum of 9, 12-octadecadienoyl chloride is shown in Fig.6.The peak at m/z 298(R.T. 20.582)

corresponds $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z267 is due to loss of a methoxyl function.

Antimicrobial activity

The oil was screened for antimicrobial activity against six standard microorganisms. The average of the diameters of the growth inhibition zones are shown in Table (4) .The results were interpreted in terms of the commonly used terms (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (5) and (6) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 4: Antibacterial activity of *Luffa egyptiaca* oil.

Drug	Conc. mg/ml	Ec	Ps	Sa	Bs	Ca	An
L. egyptiaca oil	100	15	20	13	16	15	12
	50	15	17	13	16	12	12
	25	15	17	13	16	12	12
	12.5	13	15	12	16	11	11
	6.25	12	12	10	15	10	9

Table 5: Antibacterial activity of standard chemotherapeutic agents.

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	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 6: 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*
- M.D.I.Z: Mean diameter or growth inhibition zone (mm). Average or two replicates

The oil showed excellent activity against *Pseudomonas aeruginosa* and *Bacillus subtilis* in the range: 100-25mg/ml and 100-12.50mg/ml respectively. It also showed significant activity against *Escherichia coli* at 100-25mg/ml. At 100mg/ml, it exhibited significant activity against the yeast *Candida albicans.*

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