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# GC-MS ANALYSIS AND BIOLOGICAL ACTIVITY OF SUDANESE *CITRULLUS LANATUS* L.(CUCURBITACEAE) FIXED OIL

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## ABSTRACT

The present study was designed to investigate the chemical constituents of *Citrullus lanatus* seed oil and to evaluate its potential antimicrobial activity. 25 components were detected by GC-MS analysis. Major constituents are: 9,12-octadecadienoic acid(48.47%), hexadecanoic acid(17.51%),), 9-octadecenoic acid (15.10%), and methyl stearate(12.96%). The antimicrobial activity of the oil was evaluated via cup plate agar diffusion assay against six standard human pathogens(Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseudomonasa aeruginosa* and the fungi *Candida albicans* and *Aspergillus niger*). The oil showed different antimicrobial responses against test organisms. It was partially active against *Eschericha coli*, *Pseudomonas aeruginosa* and the fungus *Candida albicans*, but it exhibited significant activity against *Staphylococcus aureus*.

**KEYWORDS**: *Citrullus lanatus*, Fixed oil, GC-MS, Antimicrobial activity.

## INTRODUCTION

The family Cucurbitaceae is a large family including about 95 genera and 950-980 species.[1] Many species belonging to this family are used in traditional medicine. This family includes: gourds, melons, cucumbers, pumpkins and squashes. Cucurbitaceae is distributed in tropical and subtropical regions[2] and many plants belonging to this family are cultivated worldwide for their economic value.

*Citrullus lanatus* L.(Cucurbitaceae) is a prostrate or climbing annual with several herbaceous, rather firm and stout stems up to 3 m long.[3] Watermelon(*Citrullus lanatus*) which is water-rich(92% of total weight) has been used for centuries in ethnomedicine.[4] Fruits are diuretic, expectorant, stomachic, strengthening, aphrodisiac, astringent to the bowels and blood purifier. Fruit is said to allay thirst, cures biliousness. It is good for sore eyes, scabies, itches and is a brain tonic.[5] The plant is also claimed to be anthelmintic, anticancer, antibacterial, demulcent and is said to treat dropsy and renal stones.[4] In Sudanese traditional medicine *Citrullus lanatus* is used for burns, swellings, rheumatism, gout and as laxative.[6] It is also used against jaundice, bed wetting and erectile dysfunction,[7,8] Fruit rind is used to treat diabetes and alcoholic poisoning. Seeds contain alkaloids, flavanoids, tannins, amino acids, carbohydrates, cardioglycosides, terpenoids,steroids, carotenoids, oils and fats.[6] Seeds are used as demulcent, diuretic, tonic, vermifuge and hypotensive . Seeds oil is reported to paralyze roundworms and tapeworms.[6 5]

*Citrullus lanatus* is a rich source of many vitamins including: A (3%) and B complex(B1, B2, B3, B5, B6 and B9), which ranges between 1-3%. It also contains Vitamin C (14%). In addition the plant is rich in essential amino acids like arginine, glutamine and aspartic acid.[9] In disc diffusion bioassay, seed extracts showed evidence of antibacterial properties[10] against a panel of human pathogens including *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Bacillus cereus*. Also the antimicrobial potential of *citrullus colocynthis* and *citrullus vulgaris* was studied and significant activities were reported.[11] The antimicrobial effects of *Citrullus lanatus* extracts can provide evidence for the ethnomedical usage of this plant in the treatment of some human disordrs such as gastrointestinal infection, diarrhea, respiratory and skin diseases. The antimicrobial potency could be attributed to bioactive constituents(flavonoid, steroids, alkaloids, saponins. etc). *Citrullus lanatus* oil significantly inhibited ear edema, granuloma hyperplasia and paw edema in model animals.[12] It significantly lifted pain threshold on mouse hot-plate responses and reduced their writhing time.[13] Methanolic extract of *Citrullus lanatus* seeds showed antioxidant potential and was evaluated, *in vivo*, for anti-ulcerogenic activity.[13]

*Citrullus lanatus* extracts showed activity against *G. lamblia*, and could be investigated as a lead for a novel treatment of giardiasis.[14] In model animal studies, adminstration of

Citrulline, a constituent of *Citrullus lanatus*, improved endothelium-dependent vasorelaxation and decreased aortic medial thickening.[15]

Using Eddy's hot plate method, the analgesic potential of peels aqueous extract was evaluated and promising results were obtained.[16] *Citrullus lanatus* seed extract is reported to possess good antioxidant and anti-ulcer activity.[17] Some studies demonstrated potential laxative properties for aqueous fruit pulp extract.[18] *In vivo* studies indicated that administration of seed methanoic extract significantly reduced the prostate size ($P< 0.05$).[9,19]

## MATERIALS AND METHODS

### Plant material

Fruits of *Citrullus lanatus* were purchased from the local market – Omdurman, Sudan.The plant was kindly authenticated by 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

*Citrullus lanatus* 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 oil from *Citrullus lanatus*

Powdered shade-dried seeds of *Citrullus lanatus* (300g) were exhaustively macerated with n-hexane at room temperature for 48h. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4°C for further manipulation.

**Esterification of oil**

A Methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in 100ml methanol. A stock solution of methanolic sulphuric acid was prepared by mixing (1ml) of concentrated sulphuric acid with (99ml) methanol.

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 shaked vigorously for five minutes and then left overnight (2ml) of supersaturated sodium chloride were added, then (2ml) of n- hexane were added and the tube was vigorously shaked 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**

*Citrullus lanatus* fixed 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 given in Table 2, while other chromatographic conditions are depicted in Table 3.

**Table 2: Oven temperature program.**

| Rate | Temperature(C) | Hold time (mim.$^{-1}$) |
|---|---|---|
| - | 60.0 | 0.00 |
| 10.00 | 300.0 | 0.00 |

**Table 3: Chromatographic conditions.**

| | |
|---|---|
| Column oven temperature | 1300.0 °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**

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in (100 ml) of normal saline to produce a suspension containing about 108-109 colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The 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 in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

**Preparation of fungal suspensions**

Fungal cultures were maintained on 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**

The cup-plate agar diffusion method was adopted, with some minor modifications, to assess the 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 in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the test solutions. Separate Petri dishes were designed for standard antibacterial chemotherapeutics (ampicillin and gentamycin). The agar discs were removed, alternate cups were filled with(0.1 ml) samples of each test solution using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours.

The above procedure was repeated for different concentrations of the test solutions and the standard chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.

## RESULTS AND DISCUSSION

### GC-MS analysis of *Citrullus lanatus* fixed oil

Identification of oil constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern. Comparison of the mass spectra with the database on MS library revealed about 90-95% match.

### Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 25 components (Table 4). The typical total ion chromatograms (TIC) is depicted in Fig.1.

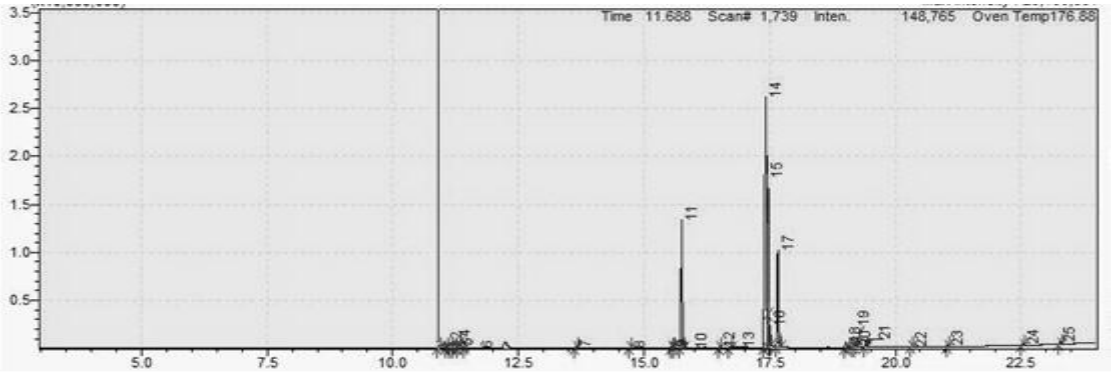


**Fig.1: Typical total ion chromatograms (TIC)**

**Table 4: Contituents of *Citrullus lanatus* oil.**

| Peak# | R.Time | Area | Area% | Name |
|---|---|---|---|---|
| 1 | 10.913 | 90821 | 0.06 | Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-met |
| 2 | 11.065 | 121485 | 0.08 | .beta.-curcumene |
| 3 | 11.170 | 33633 | 0.02 | .alpha.-Farnesene |
| 4 | 11.231 | 46897 | 0.03 | .beta.-Bisabolene |
| 5 | 11.291 | 382995 | 0.27 | Butylated Hydroxytoluene |
| 6 | 11.435 | 66608 | 0.05 | Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6- |
| 7 | 13.644 | 168170 | 0.12 | Methyl tetradecanoate |
| 8 | 14.719 | 83161 | 0.06 | Pentadecanoic acid, methyl ester |
| 9 | 15.552 | 232549 | 0.16 | 9-Hexadecenoic acid, methyl ester, (Z)- |
| 10 | 15.591 | 47588 | 0.03 | 9,12-Octadecadienoic acid, methyl ester |
| 11 | 15.751 | 25076411 | 17.51 | Hexadecanoic acid, methyl ester |
| 12 | 16.513 | 110126 | 0.08 | 8,11,14-Docosatrienoic acid, methyl ester |
| 13 | 16.723 | 294124 | 0.21 | Heptadecanoic acid, methyl ester |
| 14 | 17.428 | 69398249 | 48.47 | 9,12-Octadecadienoic acid (Z,Z)-, methyl e |
| 15 | 17.463 | 21619036 | 15.10 | 9-Octadecenoic acid (Z)-, methyl ester |
| 16 | 17.498 | 2600045 | 1.82 | 9-Octadecenoic acid, methyl ester, (E)- |
| 17 | 17.664 | 18554535 | 12.96 | Methyl stearate |
| 18 | 19.014 | 1321581 | 0.92 | 7,10-Octadecadienoic acid, methyl ester |
| 19 | 19.165 | 199027 | 0.14 | 9-Octadecenoic acid, 12-hydroxy-, methyl |
| 20 | 19.217 | 358373 | 0.25 | cis-11-Eicosenoic acid, methyl ester |
| 21 | 19.415 | 1408694 | 0.98 | Methyl 18-methylnonadecanoate |
| 22 | 20.326 | 113251 | 0.08 | Phenol, 2,2'-methylenebis[6-(1,1-dimethyle |
| 23 | 21.036 | 249362 | 0.17 | Methyl 20-methyl-heneicosanoate |
| 24 | 22.538 | 259366 | 0.18 | Tetracosanoic acid, methyl ester |
| 25 | 23.282 | 352689 | 0.25 | Squalene |
| | | 143188776 | 100.00 | |

Some important constituents are discussed below.

**9,12-Octadecadienoic acid methyl ester (48.47%)**

Fig. 2 shows the EI mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z294, which appeared at R.T. 17.42 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$. The peak at m/z263 corresponds to loss of a methoxyl function.

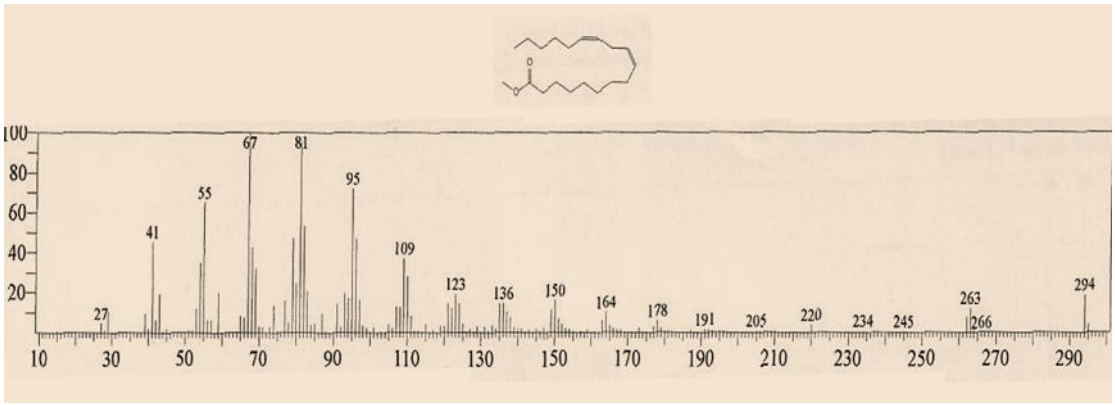


**Fig. 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester.**

**Hexadecanoic acid methyl ester(17.51%)**

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig. 3.The peak at m/z 270, which appeared at R.T. 15.751 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m/z239 is attributed to loss of a methoxyl function.

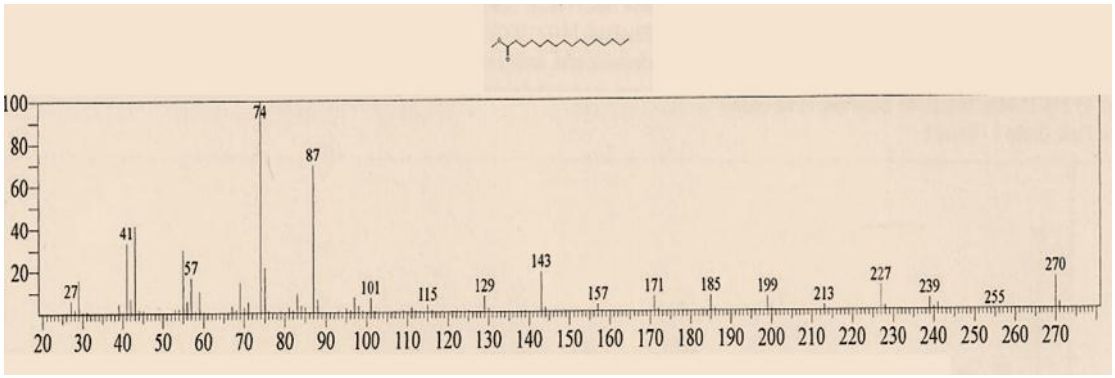


**Fig. 3: Mass spectrum of hexadecanoic acid methyl ester.**

**9-Octadecenoic acid methyl ester(15.10%)**

Fig. 4 shows the EI mass spectrum of 9-octadecenoic acid methyl ester. The peak at m/z 296, which appeared at R.T. 17.46 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$, while the peak at m/z266 accounts for loss of a methoxyl function.

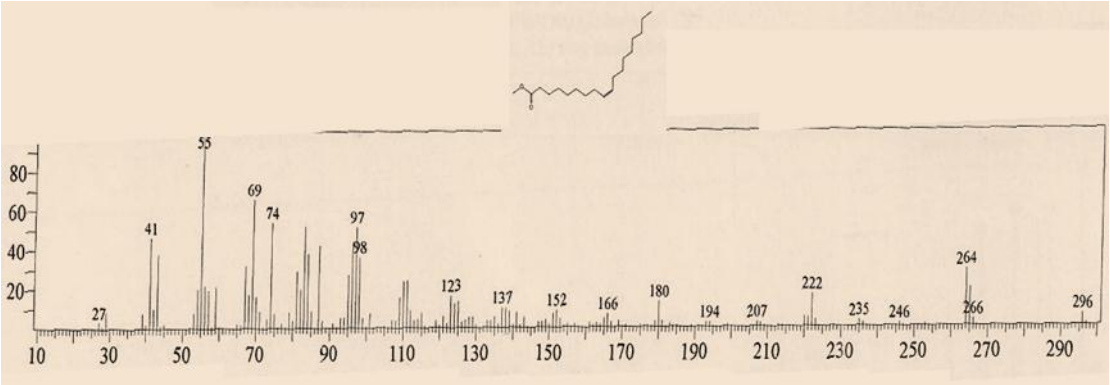


**Fig. 4: Mass spectrum of 9-octadecenoic acid methyl ester.**

**Methyl stearate(12.96%)**

Mass spectrum of methyl stearate is shown in Fig. 5.The peak at m/z 298, which appeared at R.T. 17.66 corresponds to $M^{+}[C_{19}H_{38}O_2]^{+}$. The peak at m/z267 corresponds to loss of a methoxyl function.

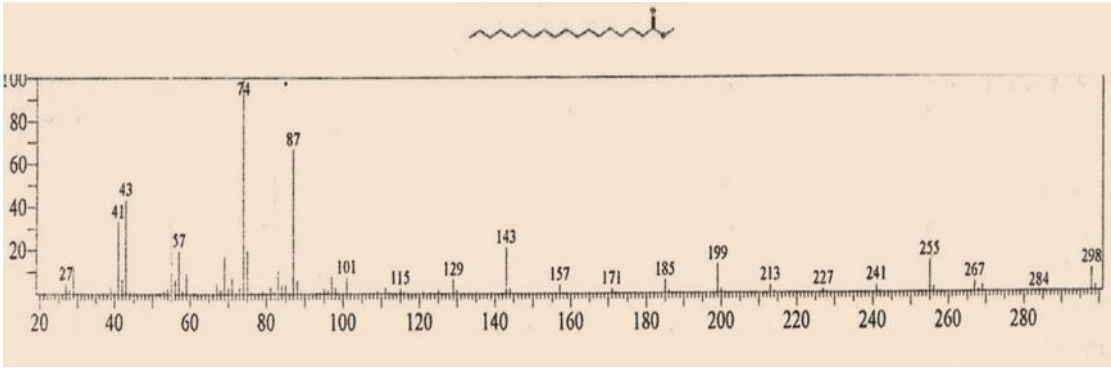


**Fig. 5: Mass spectrum of methyl stearate.**

**Antimicrobial activity**

In cup plate agar diffusion assay, the oil was screened for antimicrobial activity against six standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table (5). The results were interpreted in terms of the commonly used terms (<9mm: inative; 9-12mm: partially active; 13-18mm: active;>18mm:very active). Tables (6) and (7) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

**Table 5: Antibacterial activity of *Citrullus lanatus* oil.**

| Type | Conc.(mg/ml) | Sa | Bs | Ec | Ps | Ca | An |
|---|---|---|---|---|---|---|---|
| Oil | 100 | 16 | 8 | 12 | 10 | 10 | 7 |

**Table 6: 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 7: 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*

The oil was partially active against *Escherichia coli*, *Pseudomonas aeruginosa* and the fungus *Candida albicans*, but it exhibited significant activity against *Staphylococcus aureus*.

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