



## GC-MS analysis and antimicrobial activity of Sudanese *Citrullus colocynthis* schard (Cucurbitaceae) fixed oil

\*<sup>1</sup> Abdel Karim M, <sup>2</sup> Taiser A, <sup>3</sup> Khalid MS

<sup>1,2</sup> Department of Chemistry, Faculty of Science, Sudan University of Science and Technology, Khartoum, Sudan

<sup>3</sup> Faculty of Pharmacy, International University of Africa, Zambia

### Abstract

The present study was carried out to investigate constituents of the medicinally important *Citrullus colocynthis* seed oil and to assess its antimicrobial activity. Forty one components were detected by GC-MS analysis. Major components are: 9,12-octadecanoic acid methyl ester(39.30%); hexadecanoic acid methyl ester(15.82%); methyl stearate(13.92%) and 9-octadecanoic acid methyl ester(12.51%).

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 *Pseudomonas aeruginosa* and the yeast *Candida albicans*. The oil showed activity against *Escherichia coli* and *Bacillus subtilis*, but it was partially active against *Staphylococcus aureus*.

**Keywords:** *Citrullus colocynthis*, fixed oil, GC-MS, antimicrobial activity

### Introduction

*Citrullus colocynthis* Schard is a perennial creeping herb in the family Cucurbitaceae native to tropical Africa and Asia <sup>[1]</sup>. The plant has spherical fruits being yellow when ripened <sup>[2]</sup>.

In moderate doses, the plant is diuretic and catalartic. However, in high doses it is a gastrointestinal irritant and emetic. The plant is used against fever, intestinal parasites, dropsy, constipation and cerebral congestion <sup>[3]</sup>. *Citrullus colocynthis* has antitumor properties due to the presence of  $\beta$ -substance), colocynthin (a resin) and colocynthin. Seeds contain albuminoids and a fixed oil <sup>[5, 6, 7]</sup>.

Different extracts of *Citrullus colocynthis* showed promising antibacterial potential <sup>[8]</sup>. Immature fruits showed significant antiinflammatory activity <sup>[9]</sup>. Different extracts of *Citrullus colocynthis* showed anticandidal and antibacterial activities. Lowest activity was *colocynthis* demonstrated excellent free radical scavenging capacity <sup>[11]</sup>.

In animal model studies, root aqueous extract showed significant reduction in blood sugar levels. It also improved the biochemical parameters in alloxan – induced diabetes <sup>[12]</sup>.

In some in vivo studies, it was reported that different root and stem extracts displayed analgesic and antiinflammatory activities without acute toxicity <sup>[13]</sup>.

The hypoglycemic activity of *Citrullus colocynthis* ethanol

extract was studied in vivo. Serum cholesterol, phospholipids and triglycerides levels were significantly reduced <sup>[14]</sup>. In another study, the effect of *Citrullus colocynthis* on fertility and reproductive system was reported. Long term supplementation of *Citrullus colocynthis* to female models caused diverse effects in fertility and reproductive system <sup>[15]</sup>.

### Materials and Methods

#### Materials

##### Plant material

Seeds of *Citrullus colocynthis* were purchased from the local market-Khartoum (Sudan) and authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum-Sudan.

#### Instruments

GC-MS analysis was conducted on a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25  $\mu$ m, thickness).

#### Test organisms

*Citrullus colocynthis* oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in Table (1).

**Table 1:** Test organisms

Ser. No	Micro organism	Type
1	<i>Bacillus subtilis</i>	G+ve
2	<i>Staphylococcus aureus</i>	G+ve
3	<i>Pseudomonas aeruginosa</i>	G-ve
4	<i>Escherichia coli</i>	G-ve
6	<i>Candida albicans</i>	fungus

## Methods

### Phytochemical Screening

*Citrullus colocynthis* fruits were screened for secondary metabolites using the method described by Harborne [16].

### Extraction of oil

Powdered shade-dried seeds of *Citrullus colocynthis*(500g) were exhaustively extracted with n-hexane at room temperature. The solvent was removed *in vacuo* and the oil was

kept in the fridge at 4°C for further manipulation.

### GC-MS analysis

The oil of *Citrullus colocynthis* was analyzed by gas chromatography – mass spectrometry using A Shimadzu GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length; 0.25mm diameter; 0.25 μm, thickness). Chromatographic conditions are depicted in Tables (2) and (3).

**Table 2:** Chromatographic conditions

Column oven temperature	150.0°C
Injection temperature	300.0°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

**Table 3:** Oven temperature program

Rate	Temperature (°C)	Hold Time (min. <sup>-1</sup> )
-	150.0	1.00
4.00	300.0	0.00

### In vitro antimicrobial assay

The disc diffusion method was used to determine the antimicrobial activity of the oil. Fresh cultures of microorganisms grown for 24 h were used and diluted to 10<sup>-1</sup> with sterile physiological saline solution (0.85% NaCl). 100 μl of test microorganisms containing 2.0×10<sup>6</sup> colony forming units (CFU/ml) for bacteria were inoculated on the surface of Muller Hinton Agar plates. Sterile discs with a diameter of 6 mm were placed onto each agar plate containing microorganisms. Then the test solution was dropped onto discs under sterile conditions and incubated at 37 °C for 24 h. After incubation, the diameters of inhibition zones were measured in millimetres. All experiments were repeated three times. ampicillin, gentamycin and clotrimazole were used as positive controls, while DMSO was used as negative control. Control discs were tested on the same microorganisms under the same conditions.

## Results and Discussion

### Phytochemical screening

Phytochemical screening of *Citrullus Colocynthis* fruit pulp gave positive reactions for: sterols, flavonoids, tannins alkaloids and saponins.

### The GC-MS analysis

GC-MS analysis of *Citrullus Colocynthis* fixed oil was conducted and the identification of the constituents was accomplished by comparison with the MS library (NIST)

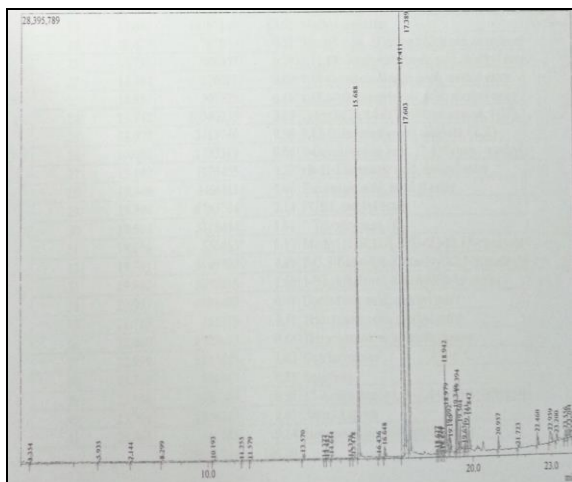
### Constituents of oil

The GC-MS analysis of the studied oil revealed the presence of 41 constituents (Table 4).The typical total ion

chromatogram (TIC) of hexane extract is shown in Fig.1.

**Table 4:** Constituents of *Citrullus Colocynthis* oil

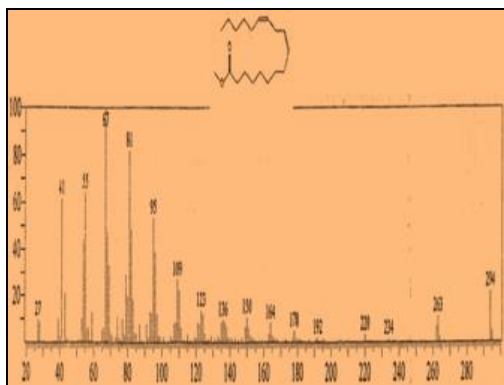
pk#	R.Time	Area	Area%	Name
1	3.354	75263	0.02	Hexanoic acid, methyl ester
2	5.935	259934	0.08	Octanoic acid, methyl ester
3	7.144	86403	0.03	Acetic acid, octyl ester
4	8.299	226442	0.07	Anethole
5	10.193	278808	0.09	Nonanoic acid, 9-oxo-, methyl ester
6	11.255	22164	0.01	Dodecanoic acid, methyl ester
7	11.579	37571	0.01	Nonanedioic acid, dimethyl ester
8	13.570	595623	0.19	Methyl tetradecanoate
9	14.377	40580	0.01	5-Octadecenoic acid, methyl ester
10	14.482	71933	0.02	4-Octadecenoic acid, methyl ester
11	14.644	352523	0.11	Pentadecanoic acid, methyl ester
12	15.375	47127	0.01	7,10-Hexadecadienoic acid, methyl ester
13	15.478	420823	0.13	9-Hexadecenoic acid, methyl ester, (Z)-
14	15.688	50320634	15.82	Hexadecanoic acid, methyl ester
15	16.436	303977	0.10	9,12-Octadecadienoic acid, methyl ester
16	16.648	1197837	0.38	Heptadecanoic acid, methyl ester
17	17.389	125012736	39.30	9,12-Octadecadienoic acid (Z,Z)-, methyl
18	17.411	39777863	12.51	9-Octadecenoic acid (Z)-, methyl ester
19	17.603	44267149	13.92	Methyl stearate
20	18.627	707030	0.22	Methyl 9.cis.,11.trans.t,13.trans.-octadeca
21	18.738	908557	0.29	9,12,15-Octadecatrienoic acid, methyl est
22	18.794	276017	0.09	7,10-Octadecadienoic acid, methyl ester
23	18.857	507529	0.16	8,11,14-Docosatrienoic acid, methyl ester
24	18.942	10340235	3.25	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),
25	18.979	3113740	0.98	9,12-Octadecadienoyl chloride, (Z,Z)-
26	19.092	2733519	0.86	9-Octadecenoic acid, 12-hydroxy-, methy
27	19.140	1175455	0.37	cis-11-Eicosenoic acid, methyl ester
28	19.340	4466111	1.40	Eicosanoic acid, methyl ester
29	19.394	6793704	2.14	PGH1, methyl ester
30	19.504	3215444	1.01	7-Tetradecenal, (Z)-
31	19.656	556463	0.17	Methyl (11R,12R,13S)-(Z)-12,13-epoxy-1
32	19.741	3149706	0.99	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethyl
33	19.842	5095416	1.60	1-Naphthalenol, decahydro-4a-methyl-
34	20.957	1508408	0.47	Docosanoic acid, methyl ester
35	21.723	361373	0.11	Tricosanoic acid, methyl ester
36	22.460	1540013	0.48	Tetracosanoic acid, methyl ester
37	22.959	3237649	1.02	Dotriacontane
38	23.200	986867	0.31	Squalene
39	23.556	1580305	0.50	17-Aldrostanone, 3-(3,4-dimethylpheny
40	23.704	1996383	0.63	.beta.-Amyrin
41	23.863	441985	0.14	Hexacosanoic acid, methyl ester
		318087299	100.00	



**Fig.1:** Chromatograms of *Citrullus Colocynthis* seed oil

The following constituents were detected in the chromatogram as major constituents:

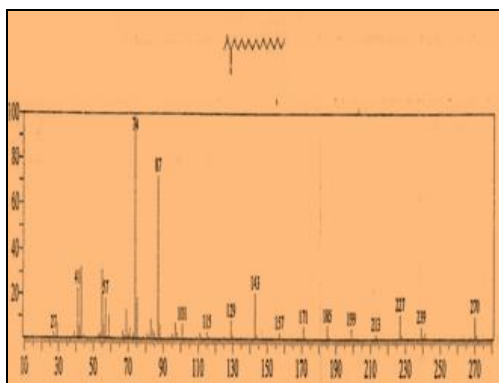
**9. 12-Octadecanoic acid methyl ester (39.30%)**



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

The EI mass spectrum of 9,12-octadecanoic acid methyl ester is shown in Fig. 2. The peak at m/z 294, which appeared at R.T. 17.389 in total ion chromatogram, corresponds  $M^+[C_{19}H_{34}O_2]^+$ . The signal at m/z263 corresponds to loss of a methoxyl function.

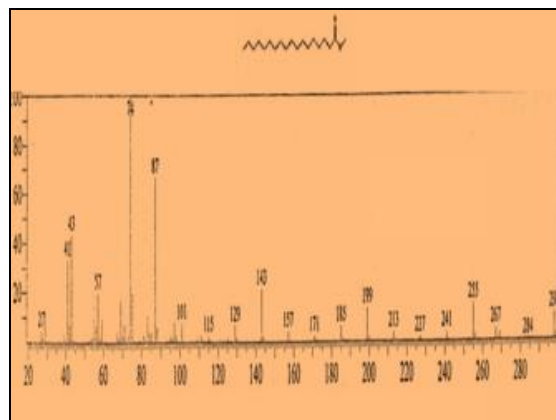
**Hexadecanoic acid methyl ester(15.82%)**



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

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.3. The signal at m/z 270 (R.T. 15.688) corresponds  $M^+[C_{17}H_{34}O_2]^+$ . The signal at m/z239 is due to loss of a methoxyl.

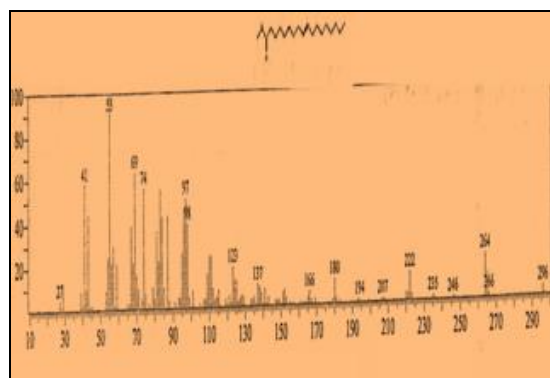
**Methyl stearate (13.92%)**



**Fig 4:** Mass spectrum of methyl stearate

Fig. 4 shows the mass spectrum of methyl stearate. The signal at m/z 298(R.T. 17.603) corresponds  $M^+[C_{19}H_{38}O_2]^+$  while the peak at m/z267 corresponds to loss of a methoxyl.

**9-Octadecanoic acid methyl ester (12.51%)**



**Fig 5:** Mass spectrum of 9-octadecanoic acid methyl ester

The mass spectrum of 9-octadecanoic acid methyl ester is displayed in Fig. 5. The peak at m/z 296, which appeared at R.T. 17.411 corresponds  $M^+[C_{19}H_{36}O_2]^+$ . The signal at m/z265 accounts for loss of a methoxyl function.

**Antimicrobial activity**

The oil was screened for antimicrobial activity against five standard microorganisms. The average of the diameters of the growth inhibition zones are shown in Table (5). The results were interpreted in terms of the commonly used terms (<9mm: inactive;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:** Antimicrobial activity of *Citrullus Colocynthis* oil

Drug	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca
<i>Citrullus Colocynthis</i> oil	100	12	8	10	12	-

**Table 6:** Antibacterial activity of standard drugs

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 7:** Antifungal activity of standard drug

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 showed activity against *Escherichia coli* and *Bacillus subtilis*, but it was partially active against *Staphylococcus aureus*.

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