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IDENTIFICATION OF CHEMICAL CONSTITUENTS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *CITRULLUS COLOCYNTHIS* SEEDS OIL

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ABSTRACT

This study aimed to extract seeds oil of *Citrullus colocynthis* and to identify the chemical constituents of the medically important species *Citrullus colocynthis* seeds oil and to evaluate its potential antimicrobial activity. The seeds were collected from El-Obeid locality, North Kordofan State December (2018). The extraction of seeds oil was carried out at Standards and Metrology Laboratories in (El-Obeid) then analyzed at National Research Center in (Khartoum). 17 components of *Citrullus colocynthis* seeds oil extract were detected by Gas Chromatography-Mass spectroscopy (GC-MS) method. The results showed that the major components are: 9, 12-octadecanoic acid methyl ester (54.52 %); hexadecanoic acid methyl ester (15.39%); methyl stearate (14.24%) and 9-octadecanoic acid methyl ester (12.53%). The antimicrobial activity of the extracted seeds oil was evaluated using diffusion assay against Gram positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, and Fungus *Candida albicans*. The oil extract showed that highly activity against Fungus *Candida albicans* at concentration 100mg/ml and poor activity at concentration 75mg/ml but no distinct inhibition zone was revealed at both concentration 50mg/ml and 25mg/ml. For four types of bacteria at concentration 100mg/ml extracted seeds oil was partially active against four types of bacteria. More over at concentration 75mg/ml partially activity was exhibited against *Bacillus subtilis*, *Pseudomonas aeruginosa*, but poor activity was shown against *Escherichia coli* and *Staphylococcus aureus*. So far *Bacillus subtilis* showed partially activity at concentration 50mg/ml and 25mg/ml but poor activity was exhibited against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The antioxidant activity of the extracted oil was evaluated by using the standard 2, 2-diphenyl-1-picrylhydrazyl (DPPH) 0.5 ml. The antioxidant activity of the extracted oil was 39 ± 0.8 . This study Recommend to test Antimicrobial activity of this plant against other types of bacteria and fungus and Analysis of other parts of *Citrullus colocynthis*.

KEYWORDS

Citrullus colocynthis, GC-MS, antimicrobial and antioxidant activity.

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INTRODUCTION

The chemical ingredient of plants is attractive for the discovery of therapeutic agents and in discovering the real value of folklore remedies. Medicinal plants hold organic compounds which offer definite physiological action on the human

body and these bioactive materials includes tannins, alkaloids, carbohydrates, triterpenoids, steroids and flavonoids^{1,2}. These compounds are produced from primary or rather secondary metabolism of a live organism. Secondary metabolism was chemically and taxonomically very varied compounds with unclear role. They are extensively used in the human treatment, veterinary, agriculture, scientific research and innumerable other disciplines³. A huge number of photochemicals belong to numerous chemical classes have been shown to have inhibitory influences on all kinds of microorganisms⁴. Plant products have been division of phytomedicine as time immemorial. This can be resulting from plant leaves, flowers, roots, barks, fruits and seeds⁵. A variety of preparations shaped with medicinal plants comprise decoction, emulsion, pooms, liniments, electro actives and powdered⁶. The medicinal plants are evenly used in the beauty, perfumery and food industries⁷. The pharmaceutical manufacturing first extracts the active components prior to being used in the industry of drugs. Hence, there is the opportunity of finding the development of drugs in the medicinal plants⁸.

Citullus colocynthis have the conventional use in medication for cancer, carcinoma, endothelium, leukemia, tumors of the liver and spleen, even the eye. A decoction of the entire plant, made with juice of fennel is said to help indurations of the liver. Roots may also be applied as a purgative against as cites for jaundice, urinary diseases, rheumatism and forsake poison. This plant is accessible in the southern coastal areas of the Bay of Bengal. Conventional screening techniques have been used to investigate the pharmacological influences of photochemical compounds. *Citrullus colocynthis* are commonly known as the colocynth, bitter apple, bitter cucumber. It is an arid region vinyl plant native to the Mediterranean basin and Asia. It is similar to a frequent watermelon vine, but holds, small, hard fruit with a bitter pulp. *Citullus colocynthis* are broadly used in folk medicine for centuries and as a power source also. E.g. Oil seed and bio fuel. The leaves are diuretic and applied in

the treatment of jaundice and asthma. The root is helpful in inflammation of the breasts, amenorrhea, rheumatism, joint pains and is used externally in ophthalmic and uterine pains⁹. The study aimed to Extract and Identify *Citrullus colocynthis* seeds oil and to assess its antimicrobial and antioxidant effect of it.

MATERIAL AND METHODS

Plant material

Seeds of *Citrullus colocynthis* collected in December (2018) from Snout Village north El-Obeid North Kordofan state Sudan. The plant was authenticated by a plant taxonomist at the Department of Botany Faculty of Science University of Kordofan Sudan. The Seeds of *Citrullus colocynthis shade* dried and coarsely powered separately by hammer mill.

Instruments

GC-MS analysis was conducted on a Shimadzo GC-MSQP2010 Ultra instrument with a RTX-5MS column (30m length; 0.25mm diameter; 0.25µm thickness). And anti-oxidant activity was measured on spectrophotometer.

Test organisms:

Citrullus colocynthis oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in Table No.1.

Methods

Extraction of oil

240g of dried seeds powder were macerated in 200-400 ml mixture of methanol and Chloroform respectively at room temperature for three days and filtered after that left for slow solvent evaporation. The extract product used for further analysis.

Sample Preparation

2ml of the sample was mixed thoroughly with 7ml of alcoholic sodium hydroxide that was prepared by dissolving 2g in 100 ml methanol. 7 ml from alcoholic sulfuric acid (1ml H₂SO₄ to 100 ml methanol) was then added. The mixture was then shacked for 5 minutes. The content of the test tube was left to stand overnight. 1ml of super saturated sodium chloride was then added and the contents being shaken. 2ml of normal hexane was added and

the contents were shaken thoroughly for three minutes. Then the n-hexane layer (the upper layer of the test tube) was taken using disposable syringe. 5µl from the n-hexane extract was diluted with 5ml of diethyl ether. Then the mixture was filtered through syringe filter 0.45 µm and dried with 1g of anhydrous sodium sulphate as drying agent and 1µl of the diluted sample was injected in the GC-MS instrument.

GC-MS analysis

The qualitative and quantitative analysis of the sample was carried out by using GC-MS technique model (GCMS-QP2010-Ultra) from Japan Shimadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60°C with rate 10°C/min to 300°C as final temperature degree with 3 minutes hold time, the injection port temperature was 300°C, the ion source temperature was 200°C and the interface temperature was 250°C. The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 27 minutes. Identification of components for the sample was achieved by comparing their retention index and mass fragmentation patterns with those available in the library, the National Institute of Standards and Technology (NIST)¹⁰.

Antioxidant assay

The DPPH radical scavenging was determined according to the method of Shimada *et al.* (1992), with some modification. In 96-wells plate, the test samples were allowed to react with 2.2 Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated

control group. All tests and analysis were run in triplicate¹¹.

***In vitro* antimicrobial assay**

Preparation of bacterial suspensions

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

The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10⁸-10⁹ 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 obtained¹².

Preparation of fungal suspension:

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used¹².

Testing of antibacterial susceptibility

Disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton Agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory

Standards Guidelines Bacterial suspension was diluted with sterile physiological solution to 10^8 UCF/ml (turbidity = McFarland standard 0.5). One hundred micro liters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculums 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 μ l of a solution of each plant extracts. The inoculated plates were incubated at 37°C for 24h in the inverted position. The diameters (mm) of the inhibition zones were measured¹².

RESULTS AND DISCUSSION

GC-MS analysis of *Citrullus Colocynthis* seeds oil

GC-MS analysis of *Citrullus Colocynthis* 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 17 constituents (Table No.2). The typical total ion of the following constituents were detected in the chromatogram (Figure No.1).

As major constituents:

The mass spectrum of 9,12-octadecanoic acid methyl ester (54.52%) is shown in Figure No.2. The peak at m/z 294, which appeared at R.T. 17.114 in total ion chromatogram, corresponds $M^+[C_{19}H_{34}O_2]^+$. The signal at m/z263 corresponds to loss of a methoxyl function.

The Mass spectrum of hexadecanoic acid methyl ester (15.39%) is depicted in Figure No.3. The signal at m/z 270 (R.T. 15.401) corresponds $M^+[C_{17}H_{34}O_2]^+$. The signal at m/z 239 is due to loss of a methoxyl.

The mass spectrum of methyl stearate (14.24%) is depicted in Figure No.4. The signal at m/z 298(R.T. 17.323) corresponds $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z267 corresponds to loss of a methoxyl.

The mass spectrum of 9-octadecanoic acid methyl ester (12.53%) is displayed in Figure No.5. The peak at m/z 296, which appeared at R.T. 17.139 corresponds $M^+[C_{19}H_{36}O_2]^+$. The signal at m/z265 accounts for loss of a methoxyl function.

Antimicrobial activities of the extracts

Assessment of antibacterial activities of Oil seed *Citrullus colocynthis* extracts with different concentrations (100, 75, 50 and 25 mg/ml) were carried out against four types of bacteria, two gram positive (*Bacillus Subtilis* and *staphylococcus*) and two gram negative (*Escherichia coli* and *Pseudomonas*). The extracts also examined against one fungus (*Candida albicans*) with 100mg/ml concentration. The assessments of antimicrobial activities of the extracts against bacteria and fungus were shown in Table No.3.

Antioxidant activity

The results indicated that *Citrullus colocynthis* seed oil contain antioxidant compounds this is in agreement with previously reported studies¹³.

Table No.1: Test organisms

S.No	Microorganisms	Type
1	<i>Bacillus subtilis</i>	G+ve
2	<i>Staphylococcus aureus</i>	G+ve
3	<i>Escherichia coli</i>	G-ve
4	<i>Pseudomonas aeruginosa</i>	G-ve
5	<i>Candida albicans</i>	Fungous

Table No.2: Constituents of *Citrullus Colocynthis* seeds oil

S.No	Name	R.T	Area	Area%
1	Methyl tetradecanoate	13.291	487523	0.12
2	Pentadecanoic acid, methyl ester	14.360	177890	0.04
3	7-Hexadecenoic acid, methyl ester, (Z)-	15.148	61632	0.02
4	9-Hexadecenoic acid, methyl ester, (Z)-	15.191	202954	0.05
5	Hexadecanoic acid, methyl ester	15.401	62069781	15.39
6	Heptadecanoic acid, methyl ester	16.362	575456	0.14
7	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.114	219827304	54.52
8	9-Octadecenoic acid (Z)-, methyl ester	17.139	50531630	12.53
9	Methyl stearate	17.323	57406926	14.24
10	9,12,15-Octadecatrienoic acid, methyl ester	18.493	508601	0.13
11	Cis-11-Eicosenoic acid, methyl ester	18.867	1886824	0.47
12	Eicosanoic acid, methyl ester	19.067	4653808	1.15
13	Docosanoic acid, methyl ester	20.690	1726826	0.43
14	Tricosanoic acid, methyl ester	21.457	260325	0.06
15	Tetracosanoic acid, methyl ester	22.197	1372415	0.34
16	Squalene	22.918	947033	0.23
17	Hexacosanoic acid, methyl ester	23.604	572981	0.14

Table No.3: Antimicrobial activity of Oil seed *C. colocynthis* (inhibition zone in mm)

S.No	Conc mg/ml	E.c	Ps.a	B.s	S.a	C.a
1	100	13	15	15	16	30
2	75	12	13	13	10	12
3	50	12	12	13	10	-
4	25	10	10	13	10	-

Gram negative bacteria; E.c = *Escherichia coli*, Ps.a = *Pseudomonas aeruginosa*
 Gram positive bacteria; B.s = *Bacillus subtiles*, S.a = *Staphylococcus aureus*,
 Fungus; C.a = *Candida albicans*.

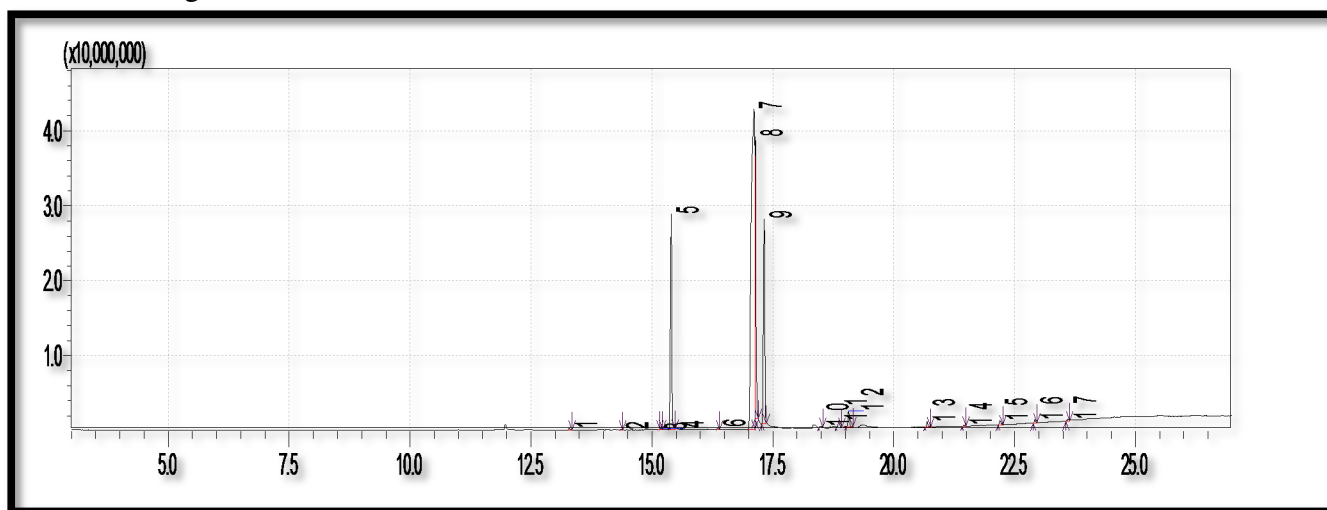


Figure No.1: Chromatograms of *Citrullus Colocynthis* oil

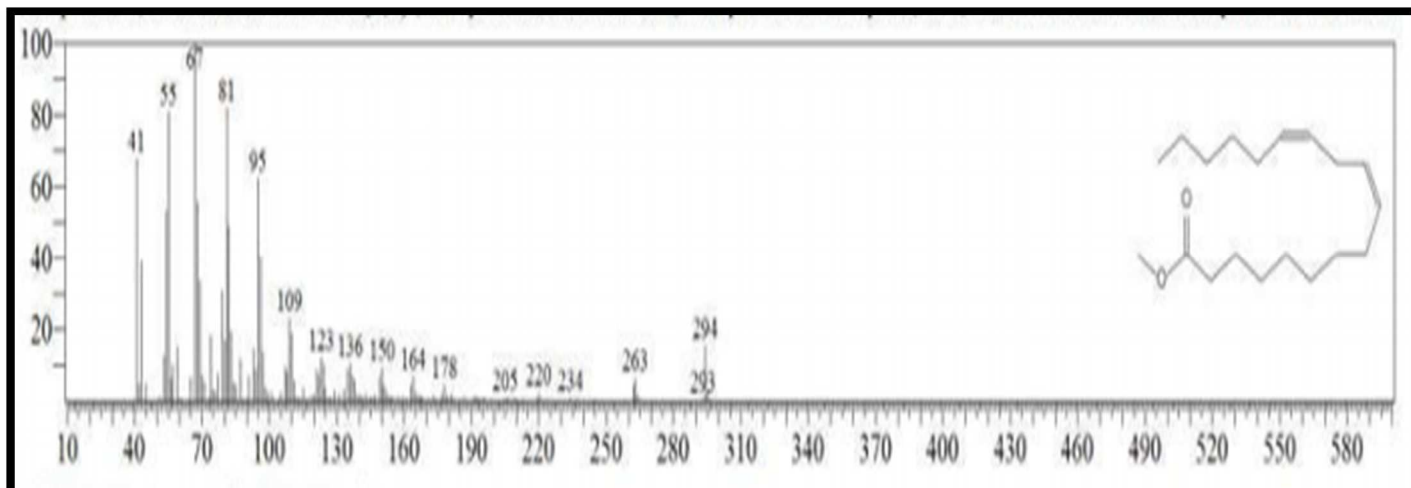


Figure No.2: Mass spectrum of 9. 12-Octadecanoic acid methyl ester

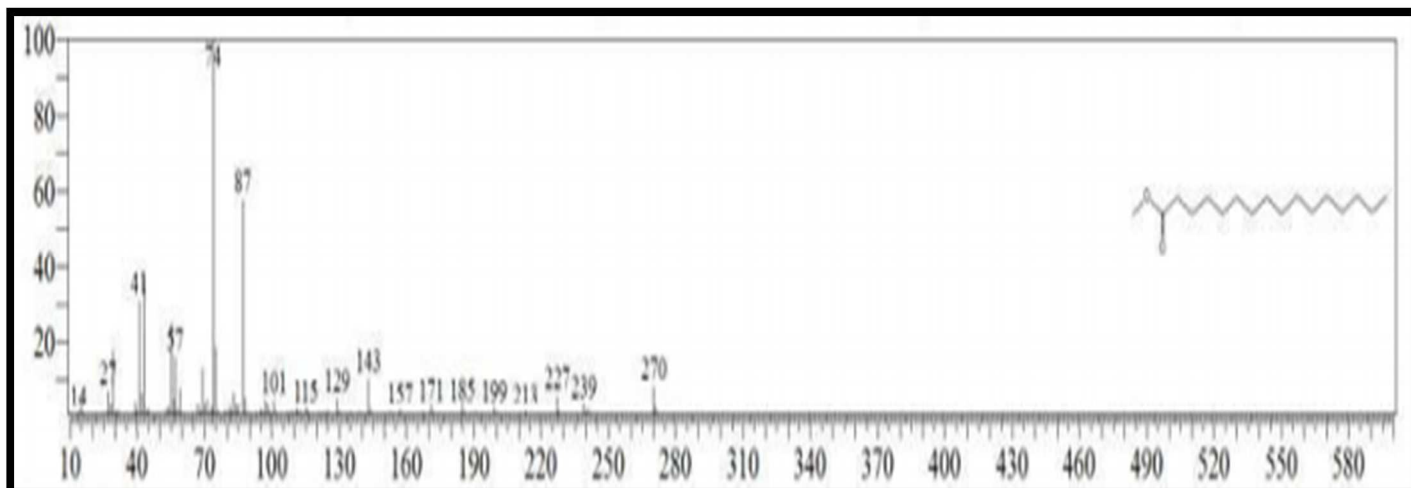


Figure No.3: Mass spectrum of Hexadecanoic acid methyl ester

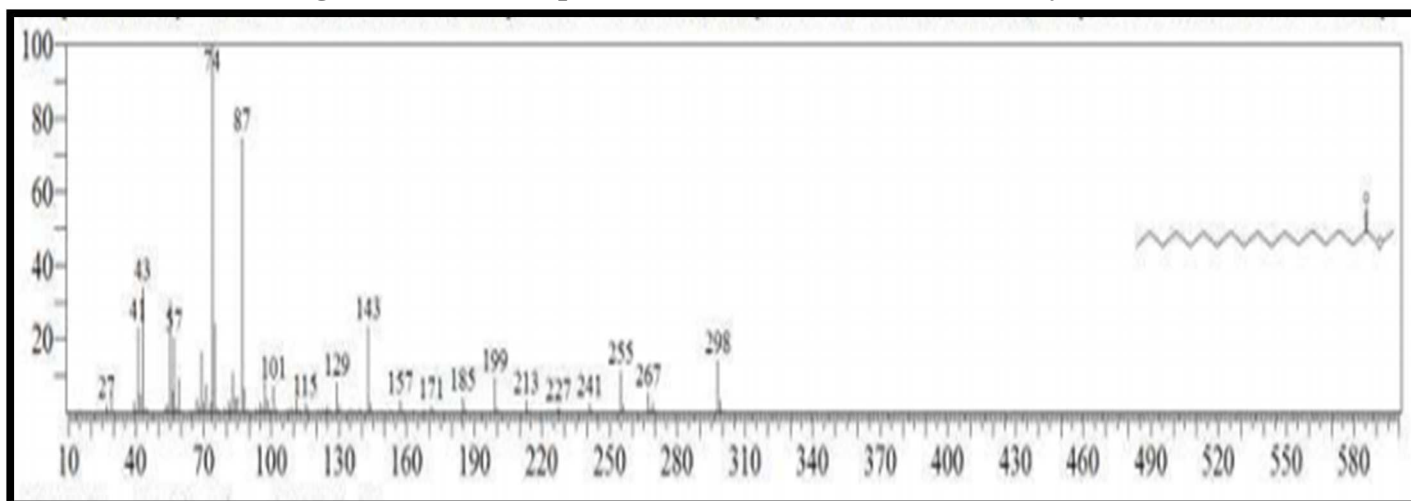


Figure No.4: Mass spectrum of Methyl stearate

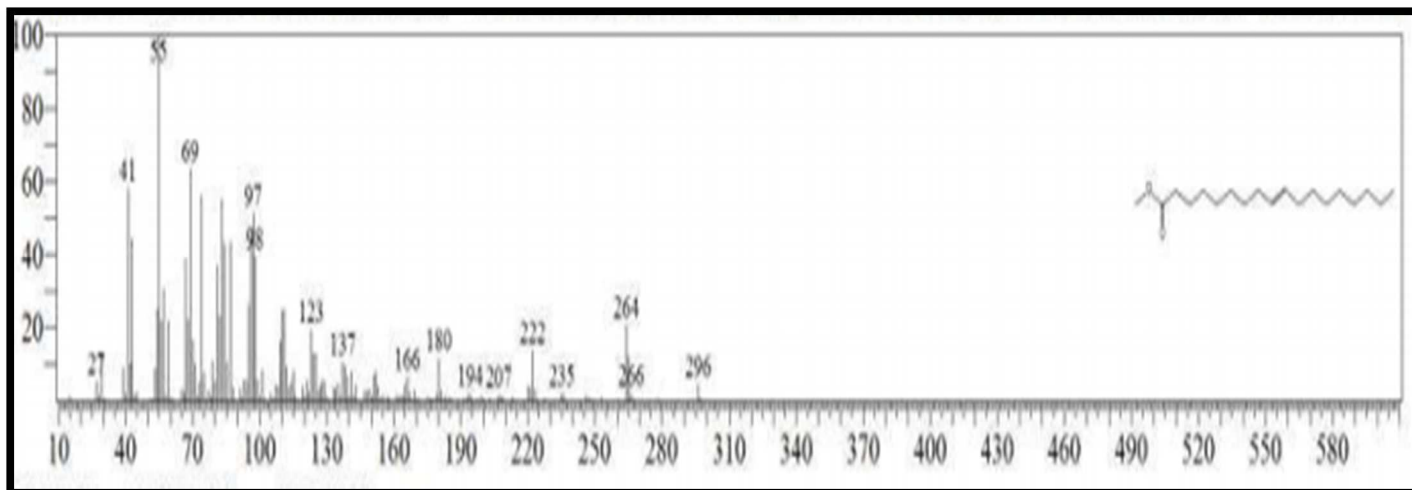


Figure No.5: Mass spectrum of 9-Octadecanoic acid methyl ester

CONCLUSION

In the present investigation, the overall results from the GC-MS analysis of *Citullus colocynthis* seed oil showed 17 components the major components are: 9, 12-octadecanoic acid methyl ester (54.52 %); hexadecanoic acid methyl ester (15.39%); methyl stearate (14.24%) and 9-octadecanoic acid methyl ester (12.53%). And the seed oil has potential antimicrobial and antioxidant activity.

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CONFLICT OF INTEREST

There is no conflict of interest.

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