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Full Length Research Paper

Spectral analysis and anti-bacterial activity of methanolic fruit extract of *Citrullus colocynthis* using gas chromatography-mass spectrometry

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Phytochemicals are chemical compounds often referred to as secondary metabolites. Thirty three bioactive phytochemical compounds were identified in the methanolic extract of *Citrullus colocynthis*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight and molecular formula. Gas chromatography-mass spectrometry (GC-MS) analysis of *C. colocynthis* revealed the existence of the methyl 6-oxoheptanoate, hexanoic acid, 2-isopropyl-2-methyl-5-oxo-, methyl ester, dodecanoic acid, 3-hydroxy, benzofuran,2,3-dihydro, 1,1-Cyclopropanedimethanol, 2-methyl-α-phenyl, 1,1-cyclopropanedimethanol, 2-methyl-α-phenyl, 12,15-octadecadiynoic acid, methyl ester, (5ß)pregnane-3,20ß-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutan, 3-(N,N-dimethyllaurylammonio)propanesulfonate, 2H-1-benzopyran-3,4-diol,2-(3,4-dimethoxyphenyl)-3,4dihydro-6-met, 11,13-dihydroxy-tetradec-5-ynoic acid, methyl ester, Cyclopenta[1,3]cyclopropa[1,2]cycloheptan-3(3aH)-one,1,2,3b,6,7, 4-(2,4,4-trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one, 1-tetradecanamine,N,N-dimethyl, α-D-glucopyranoside, O-α-D-glucopyranosyl-(1,fwdarw.3)-ß-D-fructo, acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-, 9-octadecenamide,(z)-, butyrophenone,2´,3,4´,6´-tetramethyl-, ethyl 5,8,11,14,-eicosatetraenoate, 9,12,15-octadecatrienoic acid, 2,3,-dihydroxypropyl ester, (Z,Z,Z)-, 1H-cyclopropa[3,4]benz[1,2-e]ezulene -5,7b,9,9a 476.241018tetrol,1a,1b,4,4a, 9,12,15-octadecatrienoic acid, 9,10-Secocholesta -5,7,10(19)-triene-3,24,25,-triol,(3ß,5Z,7E)-, 9,12,15-Octadecatrienoic acid,2,3-dihydroxypropyl ester, (Z,Z,Z)-, triazido-(1,2,3,4,5-pentamethylcyclopenta-2,4-dienyl)-german, ethyl iso-allocholate, α-N-Normethadol, Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, phthalic acid , decyl oct-3-yl ester, 1,2-Benzenedicarboxylic acid , bis(8-methylnonyl)ester, phthalic acid, di(6-ethyl-3-octyl)ester, y-tocopherol, 1,4-ethanonaphthalene -6,9(4H)-dione,1,4a,5,8a-tetrahydro-4,5,7,10 and vitamin E. Methanolic extract of bioactive compounds of *C. colocynthis* was assayed for *in vitro* antibacterial activity against *Proteus mirabilis, Escherichia coli*, *Pseudomonas aerogenosa, P. mirabilis, Staphylococcus aureus* and *Klebsiella pneumonia* by using the diffusion method in agar. The zone of inhibition was compared with different standard antibiotics. The diameters of inhibition zones ranged from 4.91±0.260 to 1.03±0.200 mm for all treatments.

Key words: Gas chromatography-mass spectrometry, phytochemicals, *Citrullus colocynthis*.

INTRODUCTION

Secondary metabolites play an important role as source of new compounds in pharmaceutical industry and need special consideration. Biotechnological approaches, specifically plant tissue culture plays a vital role in search for alternatives to production of desirable medicinal compounds from plants (Lamson and Brignall, 2000; Rao and Ravishankar, 2002; Rusak et al., 2002; Scalbert et al., 2005; Karuppusamy, 2009). *Citrullus colocynthis* is the plant which produces colocynth apples and is very similar to the common watermelon vine. The colocynth apples are small fruit pulp that taste bitter. The plant bears solitary sterile flowers and branched tendrils. It has a large, fleshy perennial root, which sends out slender, tough, angular, scabrid vine-like stems. The leaves are angular, lobed and, as already stated. *C. colocynthis* (L.) Schard. is an Iranian medicinal plant that has traditionally been used as an abortifacient and to treat constipation, oedema, bacterial infections, cancer and diabetes (Delazar et al., 2006). The capacity for plant cell, tissue and organ cultures to produce and accumulate many of the same valuable chemical compounds as the parent plant in nature, has been recognized almost since the inception of *in vitro* technology. The strong and growing demand in today's market place for natural, renewable products has refocused attention on *in vitro* plant materials as potential factories for secondary phytochemical products (Karuppusamy, 2009; Al-Tameme et al., 2015a; Hameed et al., 2015a). Colocynthin is soluble in water and alcohol, but insoluble in benzol, benzin, carbon disulfide and ether. Dilute acids resolve it into dextrose and tasteless colocynthein, acetic acid being likewise formed (Delazar et al., 2006). Walz obtained from an alcoholic extract of colocynth, an ether-soluble crystalline and tasteless substance insoluble in water, which he called colocynthin (Memon et al., 2003).

MATERIALS AND METHODS

Plant and extraction

Fresh plants were collected from local market in Hilla city, middle of Iraq. The plant was identified by Prof. Dr. Abdul-Kareem, Babylon University, Faculty of Science for Women. Fruits were thoroughly washed using deionized water, mopped with tissue paper and air-dried in a shade to prevent the decomposition of chemical constituents (Al-Tameme et al., 2015b; Hameed et al., 2015b). All seeds were separated manually from the pulp. The dried pulp of fruits was homogenized with a grinder (Muleinex) to fine powder before extraction. The pulp powder was extracted three times at room temperature with 100 ml of methanol for 6 h according to Halliwell and Gutteridge (1985) and Yoshikawa et al. (2007). Methanol-soluble portions were pooled from the 300 ml filtrate. The oven (45 to 50°C) dried ethanol extract (10 g) was dissolved in freshly prepared normal saline (0.9%) to a final stock solution (10 mg/ml).

Gas chromatography–mass spectrum (GC-MS) analysis

The GC-MS analysis of the plant extract was made in a 7890 Agilent A instrument under computer control at 70 eV. About 1 µl of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 min (Mohammed and Imad, 2013; Kareem et al., 2015). As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, the bigger the signal obtained which was then processed by a computer. The time from when the injection was made (Initial time) to when elution occurred is referred to as the retention time (RT). While the instrument was run, the computer generated a graph from the signal called chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the gas chromatography column into the detector (Imad et al., 2014a). The X-axis showed the RT and the Y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass (Imad et al., 2014b). The mass/charge (M/Z) ratio obtained was calibrated from the graph obtained, which was called as the mass spectrum graph which is the fingerprint of a molecule. Before analyzing the extract using GC-MS, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1 ml/min. The electron gun of mass detector liberated electrons having energy of about 70eV. The column employed here for the separation of components was Elite 1 (100% dimethyl poly siloxane). The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries (Imad et al., 2014c; Hamza et al., 2015).

Determination of antibacterial activity of crude bioactive compounds of *C. colocynthis*

The test pathogens (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli,* and *Staphylococcus aureus)* were swabbed in Muller Hinton agar plates. 60 µl of the plant extract was loaded on the bored wells (Hameed et al., 2015c; Hussein et al., 2015). The wells were bored in 0.5 cm in diameter. The plates were incubated at 37°C for 24 h and were examined. After the incubation, the diameter of inhibition zones around the discs was measured.

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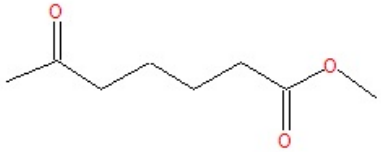
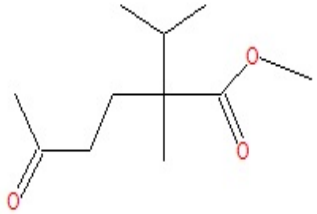
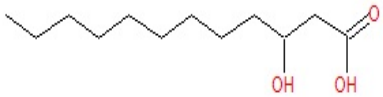
Table 1. Major phytochemical compounds identified in methanolic extract of *Citrullus colocynthis*.

S/N	Phytochemical compound	RT (min)	Formula	Molecular weight	Exact mass	Chemical structure	MS Fragment-ions	Pharmacological actions
1	Methyl 6-oxoheptanoate	4.929	$C_8H_{14}O_3$	158	158.094295		55,84,111,126,143	Anti-cancer activity
2	Hexanoic acid, 2-isopropyl-2-methyl-5-oxo-, methyl ester	6.016	$C_{11}H_{20}O_3$	200	200.141245		55,69,83,98,115,130,157,168,185	Antifungal activity
3	Dodecanoic acid, 3-hydroxy	6.303	$C_{12}H_{24}O_3$	216	216.1725445		55,69,83,96,112,123,138,151,180,200	New chemical compound

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at $P < 0.05$ using Duncan's multiple range test (by SPSS software) Version 9.1 (Jasim et al., 2015).

RESULTS AND DISCUSSION

Herbal medicine represents one of the most important fields of traditional medicine all over the world. Different extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effects (Cragg et al., 1997; Hameed et al., 2015d). GC-MS analysis of the compounds carried out in methanolic extract of *C. colocynthis* is shown in Table 1. The GC-MS chromatogram of the 33 peaks of the compounds detected are as shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *C. colocynthis* showed the presence of twenty major peaks and the components corresponding to the peaks were determined as follows: the first set up peak was determined to be methyl 6-oxoheptanoate (Figure 2). The second peak was indicated to be hexanoic acid, 2-isopropyl-2-methyl-5-oxo-, and methyl ester (Figure 3). The next peaks were considered to be dodecanoic acid, 3-hydroxy, benzofuran,2,3-dihydro, 1,1-cyclopropanedimethanol, 2-methyl-α-phenyl, 1,1 cyclopropanedimethanol, 2-methyl-α-phenyl, 12,15-Octadecadiynoic acid, methyl ester, (5ß)Pregnane-3,20ß-diol, 14α,18α-[4-methyl-3-oxo-(1- oxa-4-azabutan, 3-(N,N-Dimethyllaurylammonio) propanesulfonate, 2H-1-benzopyran-3,4-diol,2-(3,4-dimethoxyphenyl)-3,4dihydro-6-met, 11,13-dihydroxy-tetradec-5-ynoic acid, methyl ester, cyclopenta[1,3]cyclopropa[1,2]cycloheptan-3(3aH)-one,1,2,3b,6,7, 4-(2,4,4-trimethyl-cyclohexa-1,5-dienyl)- but-3-en-2-one, 1-Tetradecanamine,N,N-dimethyl, α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1,fwdarw.3)-ß-D-fructo, acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-, 9-

Table 1. Contd.

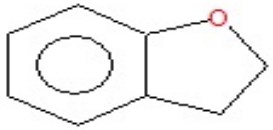
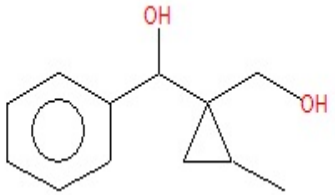
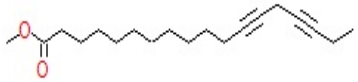
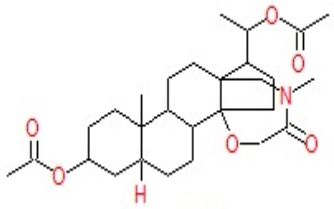
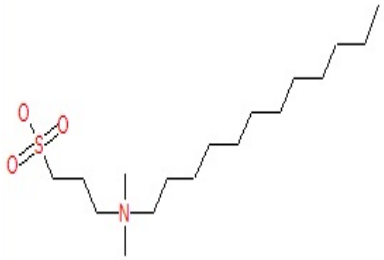
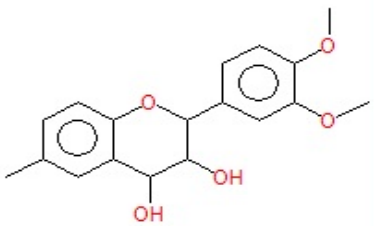
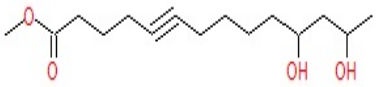
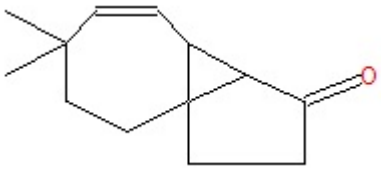
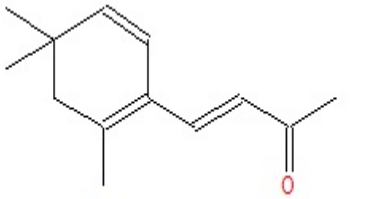
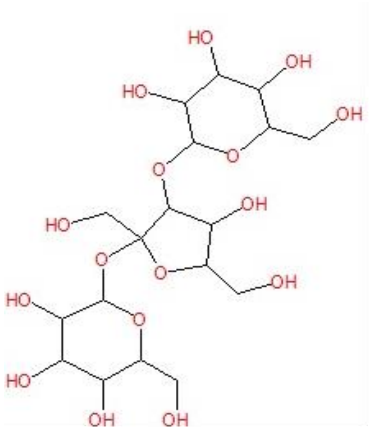
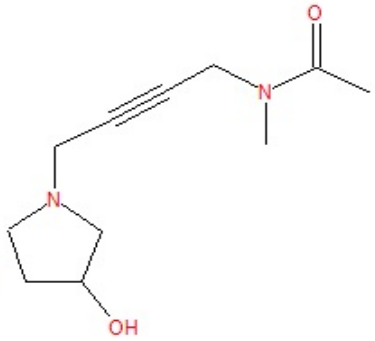
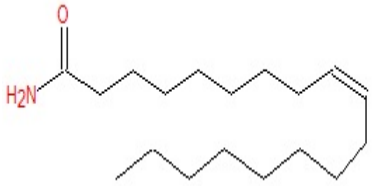
4	Benzofuran,2,3-dihydro	6.686	C_8H_8O	120	120.0575147		51,63,77,91,105,120	Analgesic and anti-inflammatory
5	1,1-Cyclopropanedimethanol, 2-methyl-α-phenyl-	8.368	$C_{12}H_{16}O_2$	192	192.115029		51,79,91,105,132150,174, 192	Immunosuppressanand anti-tumor
6	12,15-Octadecadiynoic acid, methyl ester	9.816	$C_{19}H_{30}O_2$	290	290.22458		55,74,91,106,119,133,147, 178,205,259,290	New chemical compound
7	(5ß)Pregnane-3,20ß-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutan	10.050	$C_{28}H_{43}NO_6$	489	489.309038		57,73,133,161,267,328,36 0,399,459	New chemical compound
8	3-(N,N-Dimethyllaurylammonio)propanesulfonate	10.234	$C_{17}H_{37}NO_3$	335	335.249414		58,69,84,97,115,128,140,1 52,165,179,194,213	Anti-scaling agents

Table 1. Contd.

9	2H-1-Benzopyran-3,4-diol,2-(3,4-dimethoxyphenyl)-3,4dihydro-6-met	11.035	$C_{18}H_{20}O_5$	316	316.131073		119,137,151,165,180	New chemical compound
10	11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester	11.344	$C_{15}H_{26}O_4$	270	270.18311		55,79,91,108,121,143,179,211,255	New chemical compound
11	Cyclopenta[1,3]cyclopropa[1,2]cycloheptan-3(3aH)-one,1,2,3b,6,7	11.653	$C_{13}H_{18}O$	190	190.135765		78,91,119,133,147,162,190	New chemical compound
12	4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one	11.784	$C_{13}H_{18}O$	190	190.135765		77,91,119,147,175,190	New chemical compound
13	1-Tetradecanamine,N,N-dimethyl-	12.562	$C_{16}H_{35}N$	241	241.27695		58,69,84,95,114,128,142,156,170,184,198,212,226,241	New chemical compound

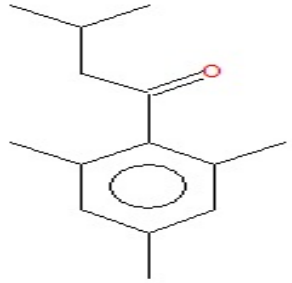
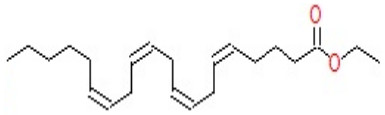
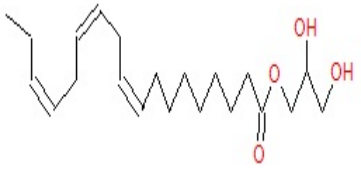
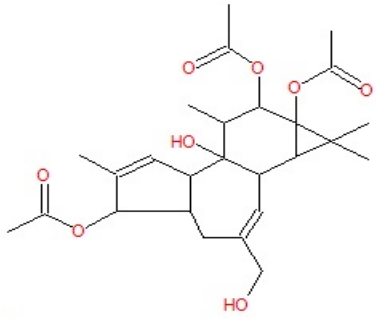
octadecenamide,(z)-, butyrophenone,2´,3,4´,6´-tetramethyl-, ethyl 5,8,11,14,-eicosatetraenoate, 9,12,15-octadecatrienoic acid, 2,3,-dihydroxypropyl ester, (Z,Z,Z)-, 1H cyclopropa[3,4]benz[1,2-e]ezulene -5,7b,9,9a 476.241018 tetrol,1a,1b,4,4a, 9,12,15-

Table 1. Contd.

14	α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1,fwdarw.3)-ß-D-fructo	13.209	$C_{18}H_{32}O_{16}$	504	504.169035		60,73,85,97,113,126,145,191	Anti-microbial and anti-spasmodic
15	Acetamide,N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	13.672	$C_{11}H_{18}N_2O$	210	210.136827		56,68,124,192	New chemical compound
16	9-Octadecenamide,(z)-	15.429	$C_{18}H_{35}NO$	281	281.271864		59,72,83,114,184,212,264,281	Anti-inflammatory activity, antibacterial activity

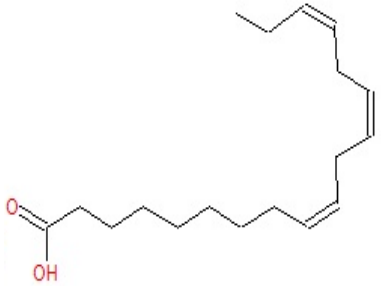
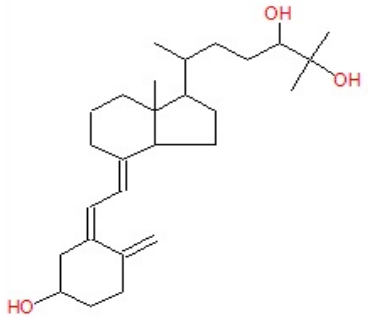
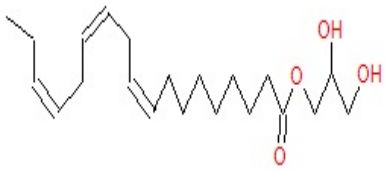
octadecatrienoic acid, 9,10-secocholesta -5,7,10(19)-triene-3,24,25,-triol,(3ß,5Z,7E),9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-, triazido-(1,2,3,4,5 pentamethylcyclopenta-2,4-dienyl)-german, ethyl iso-allocholate, α-N-Normethadol, octadecanoic

Table 1. Contd.

17	Butyrophenone,2´,3,4´,6´-tetramethyl-	16.236	$C_{14}H_{20}O$	204	204.151415		51,91,119,147,171,204	New chemical compound
18	Ethyl 5,8,11,14,-eicosatetraenoate	16.207	$C_{22}H_{36}O_2$	332	332.27153		55,67,79,91,119,150,175,217,275,332	New chemical compound
19	9,12,15-Octadecatrienoic acid, 2,3,-dihydroxypropyl ester, (Z,Z,Z)-	16.493	$C_{21}H_{36}O_4$	352	352.26136		57,67,79,95,109,155,173,232,261,291,321,352	Anti-inflammatory and CNS depressant activity
20	1H-Cyclopropa[3,4]benz[1,2-e]azulene -5,7b,9,9a 476.241018tetrol,1a,1b,4,4a	16.779	$C_{26}H_{36}O_8$	4476	476.241018		55,69,131,200,296,324,356,416,476	New chemical compound

acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, phthalic acid, decyl oct-3-yl ester, 1,2-Benzenedicarboxylic acid, bis(8-methylnonyl)ester, phthalic acid, di(6-ethyl-3 octyl)ester, y-tocopherol, 1,4-ethanonaphthalene -6,9(4H)-dione,1,4a,5,8a-tetrahydro-4,5,7,10 and vitamin E (Figures 4 to 34). In this study, five clinical pathogens were selected for antibacterial activity, namely, *S. aureus, K. pneumoniae, P. aeroginosa, E. coli,* and *Proteus mirabilis*. The maximum zone formation against *P. aeroginosa* was from 4.91±0.260 to 1.03±0.200 (Table 2).

Table 1. Contd.

21	9,12,15-Octadecatrienoic acid	17.008	$C_{18}H_{30}O_2$	278	278.22458		55,67,79,93,108,121,135,222,249,278	Anti-inflammatory and anti-atherogenic properties
22	9,10-Secocholesta -5,7,10(19)-triene-3,24,25,-triol,(3ß,5Z,7E)-	17.912	$C_{18}H_{36}O_2$	416	416.329044		55,69,91,118,136,158,176,207,221,253,383,416	New chemical compound
23	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	17.294	$C_{27}H_{44}O_3$	352	352.26136		57,67,79,95,109,155,173,232,261,291,321,352	New chemical compound

Several active chemical constituents of *C. colocynthis* plant were recorded. They are grouped as alkaloids, flavonoids, saponins, tannins, carbohydrates, glycosides and essential oils. Plant based natural constituents can be derived from any part of the plant like stems, leaves, flowers, roots, fruits and seeds (Gordon and David, 2001). A number of plant secondary metabolites, including flavonoids and curcubitacins have previously been reported from *C. colocynthis* (Seger et al., 2005). It has been used in herbal treatment of diabetes (Karim et al., 2011), edema, bacterial infection and cancer. The aqueous pulp extract of the fruit is used for kidney, liver functions treatment (Rahbar and Nabipour, 2010).

Conclusion

From the results obtained in this study, it could be

Table 1. Contd.

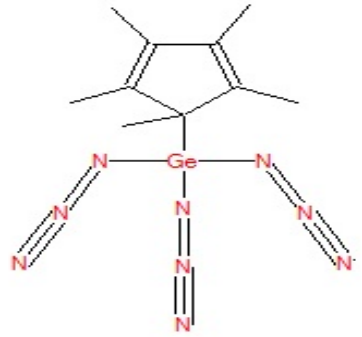
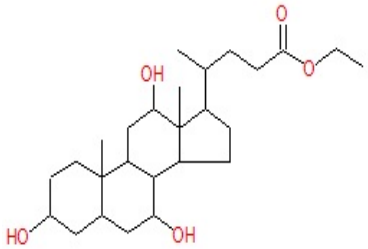
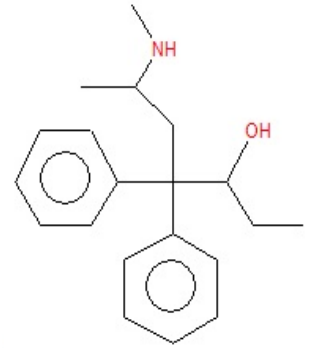
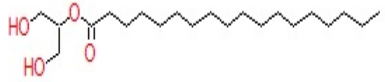
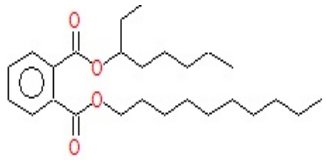
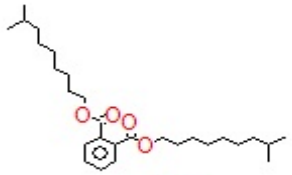
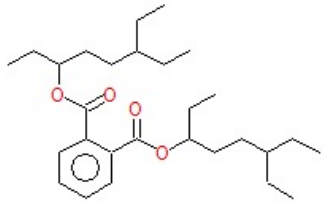
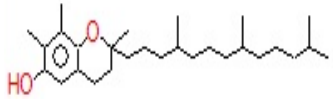
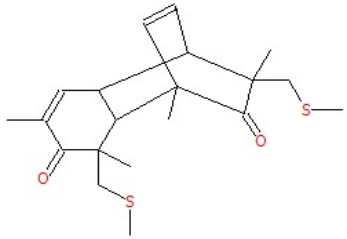
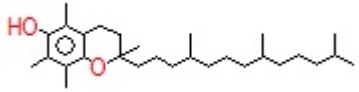
24	Triazido-(1,2,3,4,5-pentamethylcyclopenta-2,4-dienyl)-german	18.485	$C_{10}H_{15}GeN_9$	335	335.06622		53,91,105,119,134,148,177,207,235,267,335	Anti-inflammatory
25	Ethyl iso-allocholate	18.742	$C_{26}H_{44}O_5$	436	436.318874		55,69,81,95,213,253,400,418	Anti microbial agents and anti-inflammatory activity.
26	α-N-Normethadol	18.456	$C_{20}H_{27}NO$	297	297.209265		58,77,91,115,134,165,178,208,239,277,297	Antioxidant activity.
27	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	21.695	$C_{21}H_{42}O_4$	358	358.30831		57,73,98,134,185,267,284,327,358	Antimicrobial and anti-inflammatory.

Table 1. Contd.

28	Phthalic acid , decyl oct-3-yl ester	22.650	$C_{26}H_{24}O_4$	418	418.30831		57,104,149,167,193,251,307	Anti-hyper cholesterol
29	1,2-Benzenedicarboxylic acid, bis(8-methylnonyl)ester	23.245	$C_{28}H_{46}O_4$	446	446.33961		71,99,149,167,193,228,289,307,321,361,389,417	Preventing many diseases such as anti-(hypertension, cholesterol)
30	Phthalic acid , di(6-ethyl-3-octyl)ester	24.487	$C_{28}H_{46}O_4$	446	446.33961		57,84,111,149,167,307	Biological activities like antimicrobial, antifungal and antioxidant activity
31	y-Tocopherol	25.340	$C_{28}H_{48}O_2$	416	416.36543		57,107,135,151,191,205,232,260,288,316,344,372	Antioxidant activity
32	1,4-Ethanonaphthalene -6,9(4H)-dione,1,4a,5,8a-tetrahydro-4,5,7,10	25.443	$C_{20}H_{28}O_2S_2$	364	364.153072		61,77,91,105,135,149,187,255,289,364	New chemical compound
33	Vitamin E	26.530	$C_{29}H_{50}O_2$	430	430.38108		57,69,91,121,165,205,246,274,302,330,358,386,430	Antioxidant activity

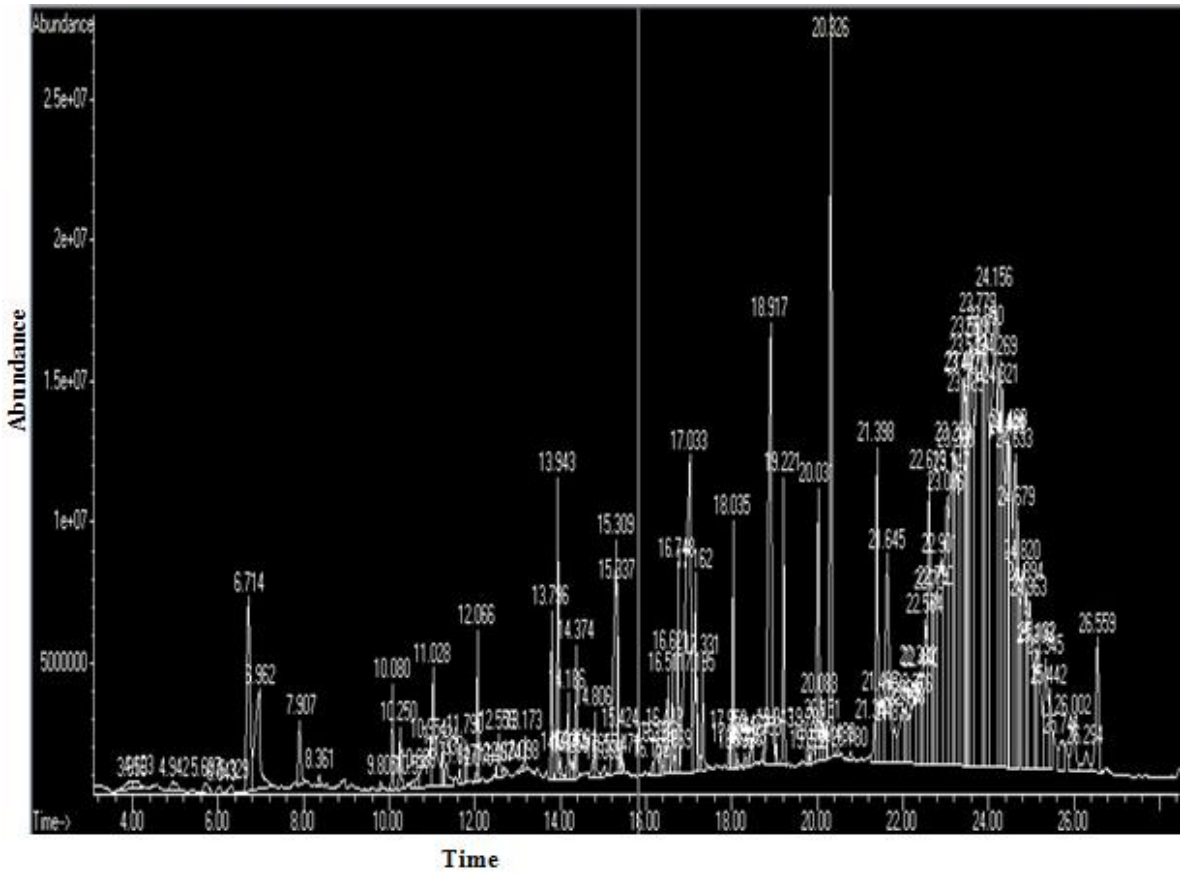


Figure 1. GC-MS profile of *Citrullus colocynthis*.

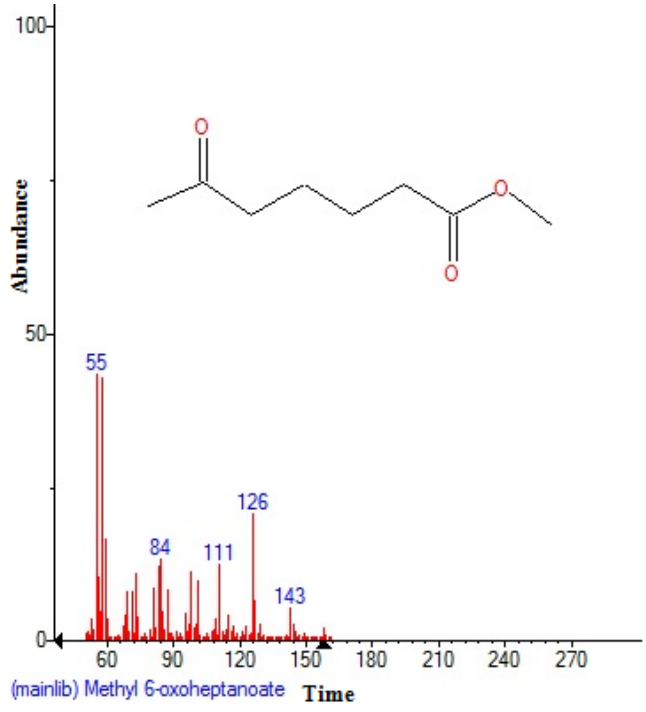


Figure 2. Structure of methyl 6-oxoheptanoate present in the *Citrullus colocynthis* by using GC-MS analysis

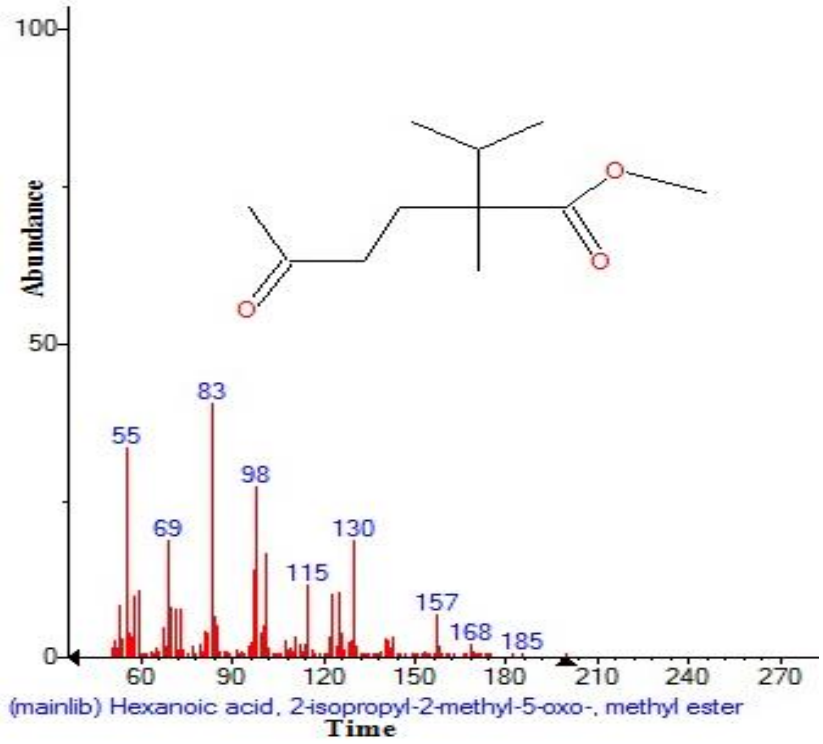


Figure 3. Structure of hexanoic acid, 2-isopropyl-2-methyl-5-oxo-, methyl ester present in the *Citrullus colocynthis* by using GC-MS analysis.

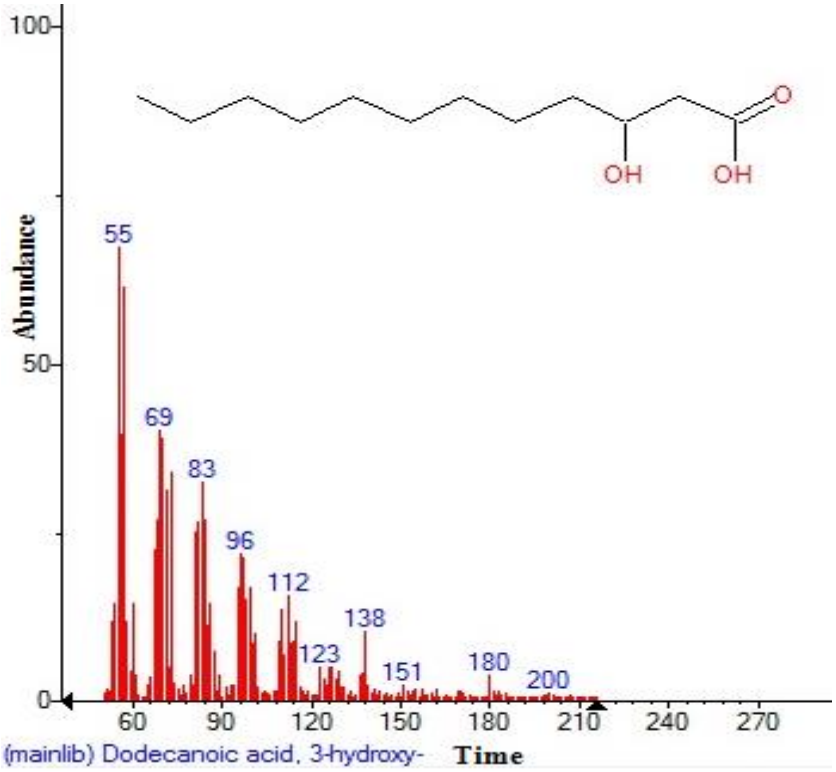


Figure 4. Structure of dodecanoic acid, 3-hydroxy present in the *Citrullus colocynthis* by using GC-MS analysis.

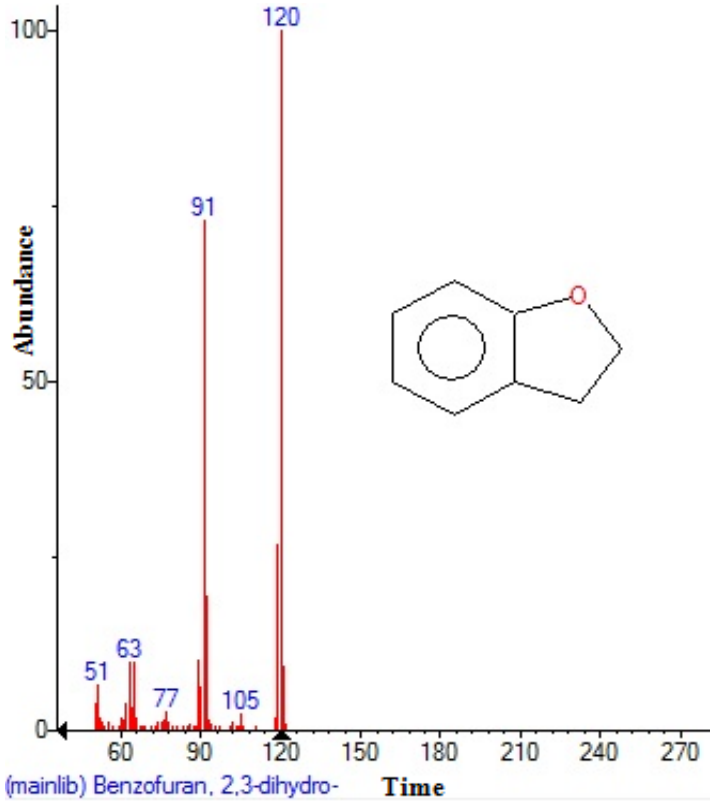


Figure 5. Structure of benzofuran,2,3-dihydro present in the *Citrullus colocynthis* by using GC-MS analysis.

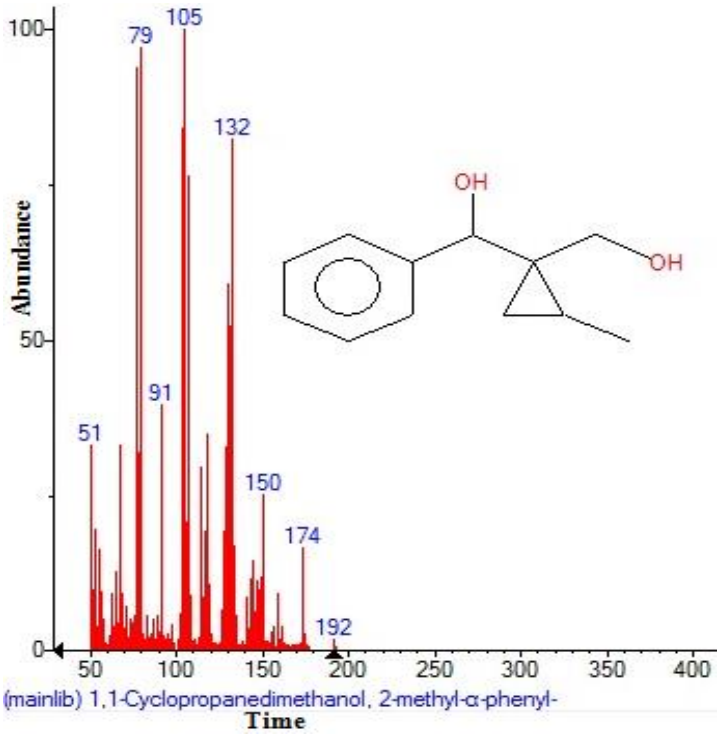


Figure 6. Structure of 1,1-cyclopropanedimethanol, 2-methyl-α-phenyl present in the *Citrullus colocynthis* by using GC-MS analysis.

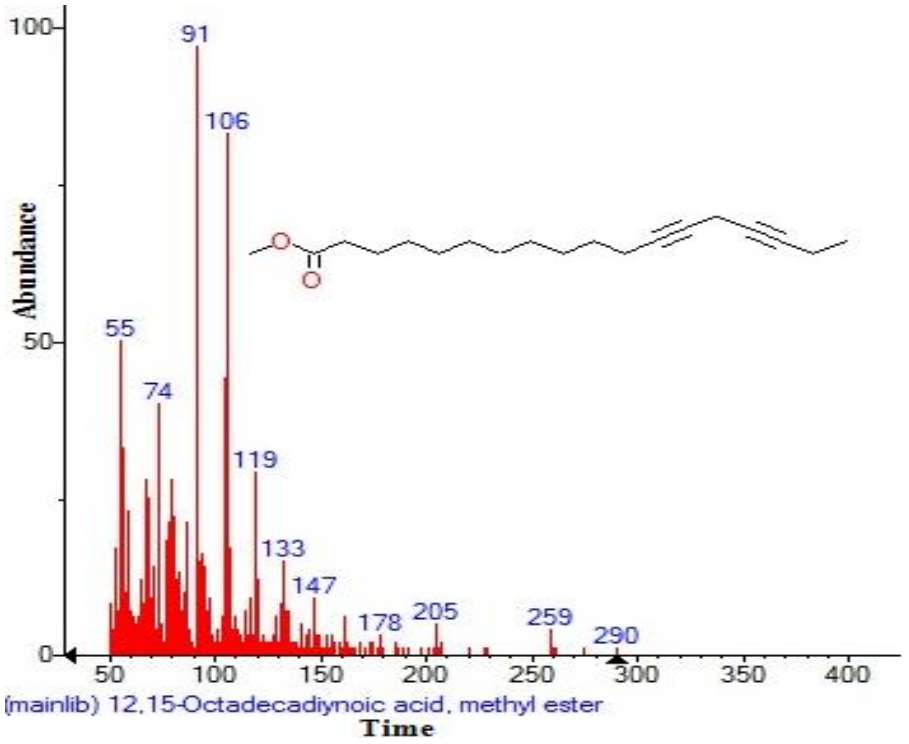


Figure 7. Structure of 12,15-octadecadiynoic acid, methyl ester present in the *Citrullus colocynthis* by using GC-MS analysis.

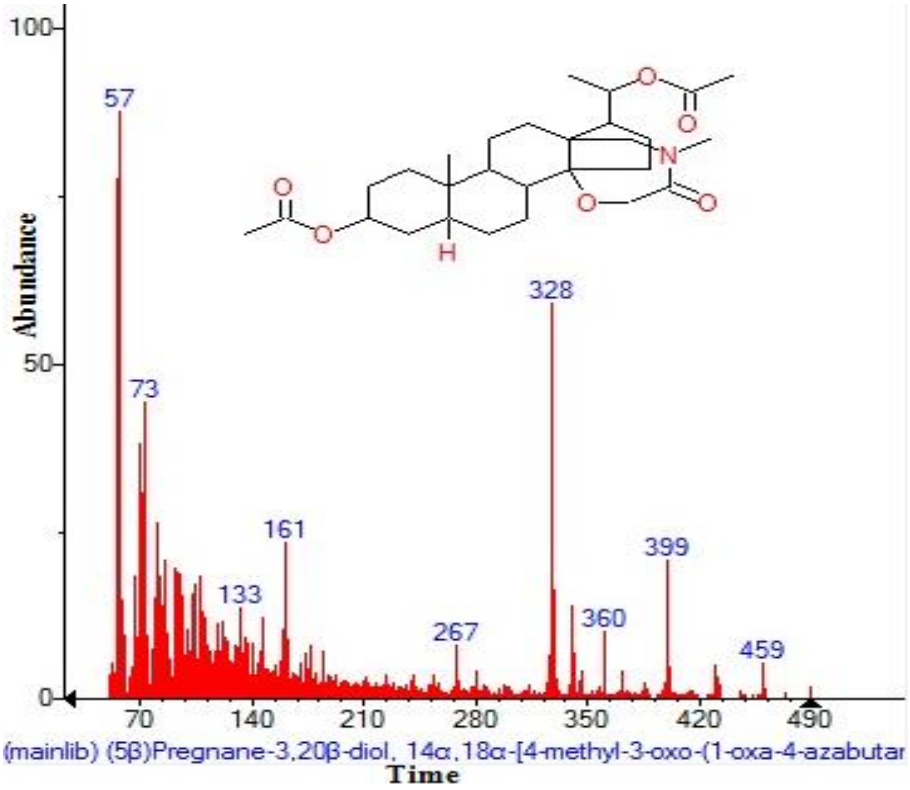


Figure 8. Structure of (5ß)pregnane-3,20ß-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutan present in the *Citrullus colocynthis* by using GC-MS analysis.

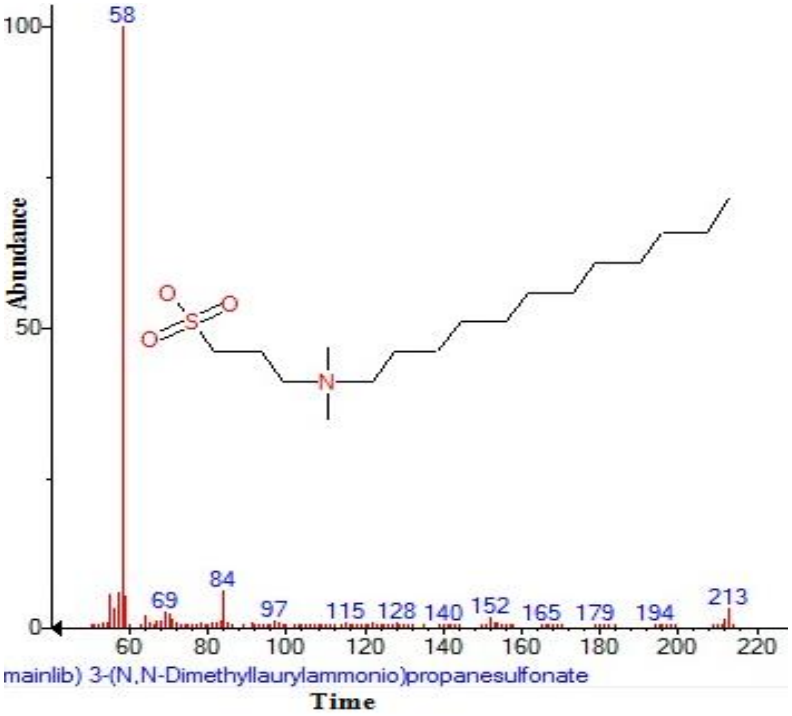


Figure 9. Structure of 3-(N,N-dimethyllaurylammonio)propanesulfonate present in the *Citrullus colocynthis* by using GC-MS analysis.

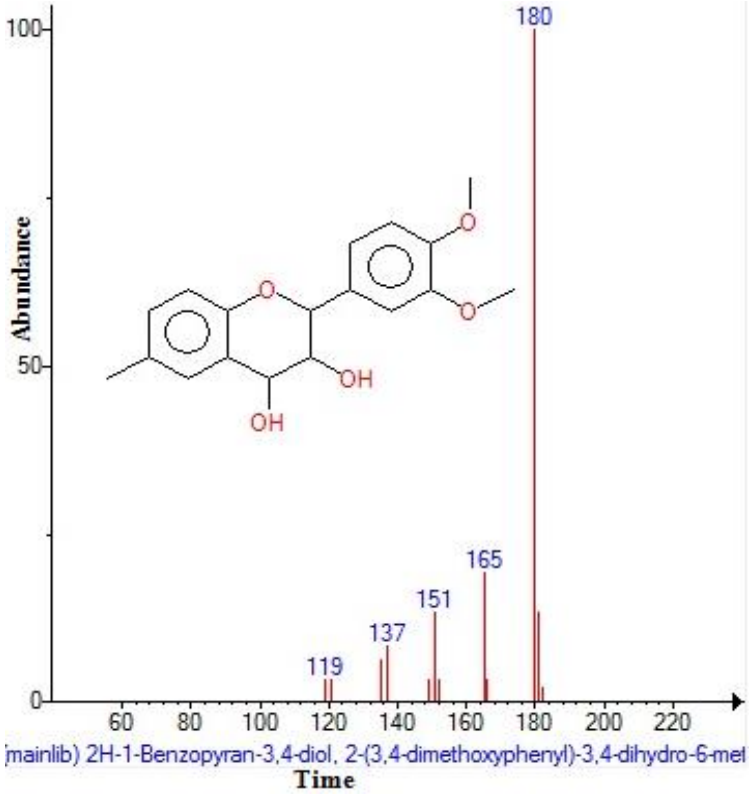


Figure 10. Structure of 2H-1-benzopyran-3,4-diol,2-(3,4-dimethoxyphenyl)-3,4dihydro-6-met present in the *Citrullus colocynthis* by using GC-MS analysis.

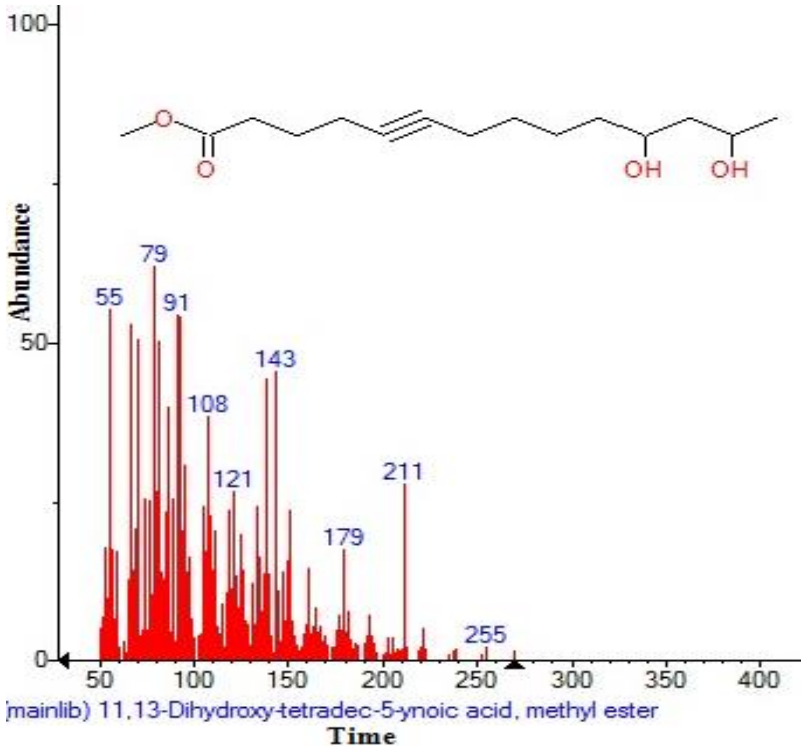


Figure 11. Structure of 11,13-dihydroxy-tetradec-5-ynoic acid, methyl ester present in the *Citrullus colocynthis* by using GC-MS analysis.

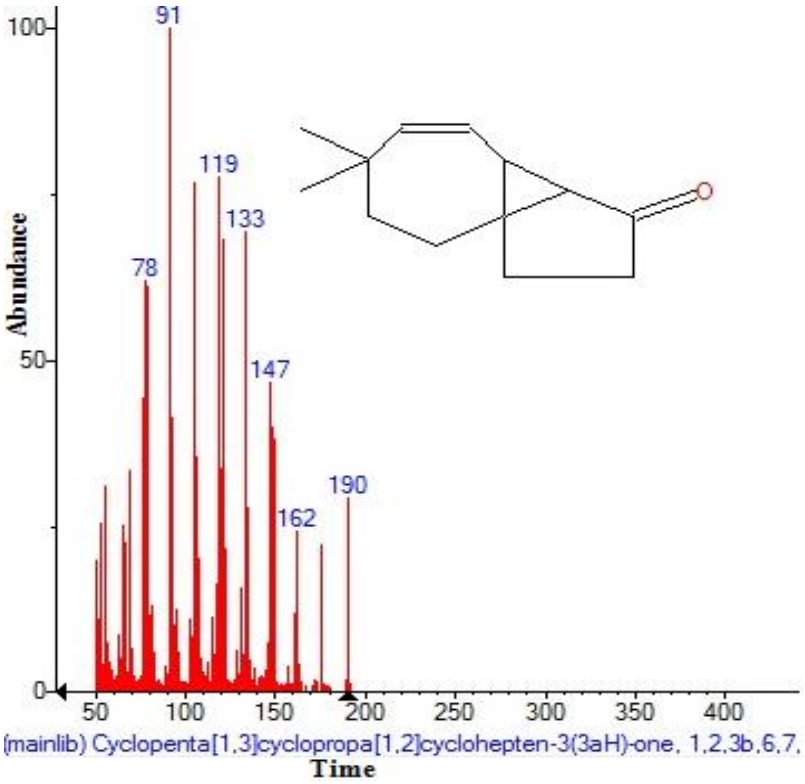


Figure 12. Structure of cyclopenta[1,3]cyclopropa[1,2]cycloheptan-3(3aH)-one,1,2,3b,6,7 present in the *Citrullus colocynthis* by using GC-MS analysis.

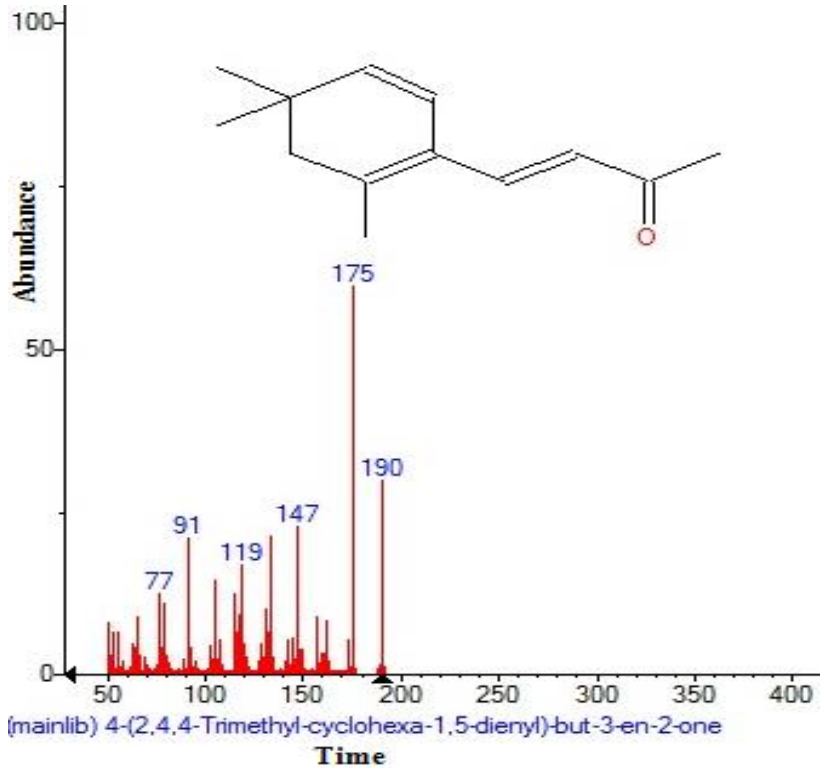


Figure 13. Structure of 4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one present in the *Citrullus colocynthis* by using GC-MS analysis.

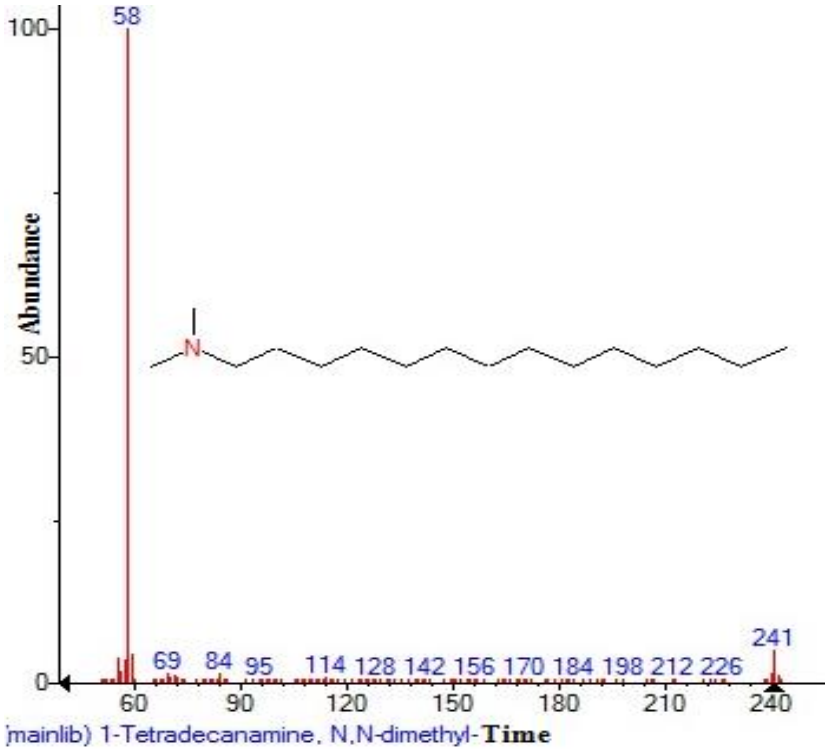


Figure 14. Structure of 1-tetradecanamine, N,N-dimethyl present in the *Citrullus colocynthis* by using GC-MS analysis.

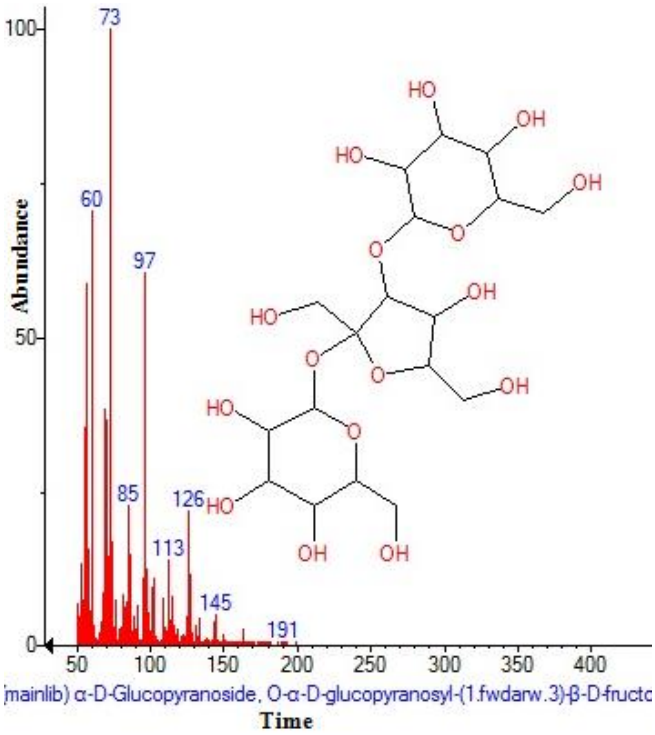


Figure 15. Structure of α-D-glucopyranoside, O-α-D-glucopyranosyl-(1,fwdarw.3)-ß-D-fructo present in the *Citrullus colocynthis* by using GC-MS analysis.

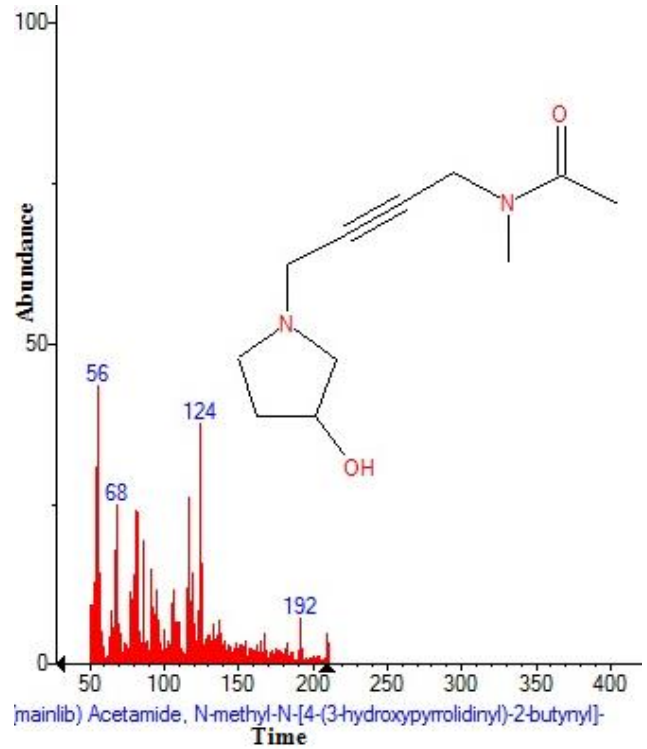


Figure 16. Structure of acetamide,N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl] present in the *Citrullus colocynthis* by using GC-MS analysis.

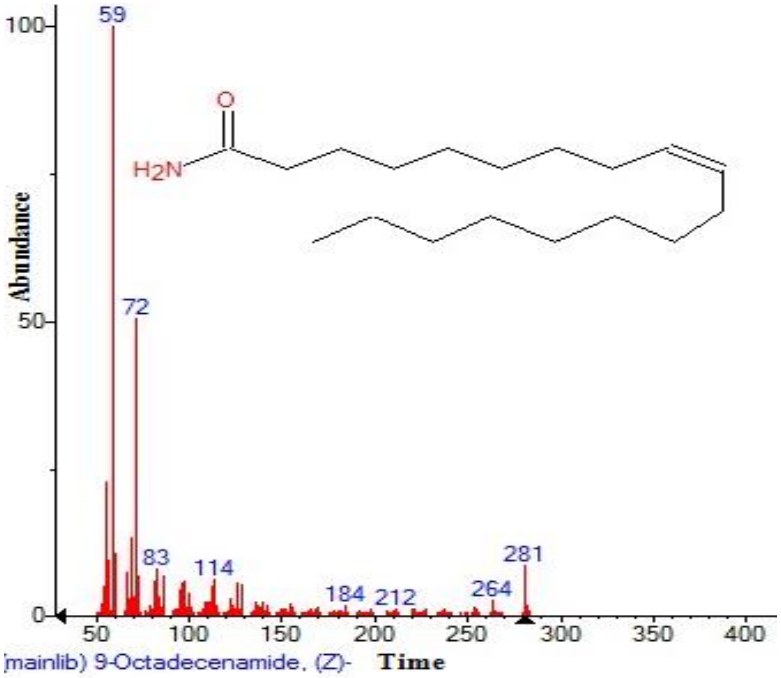


Figure 17. Structure of 9-octadecenamide,(z) present in the *Citrullus colocynthis* by using GC-MS analysis.

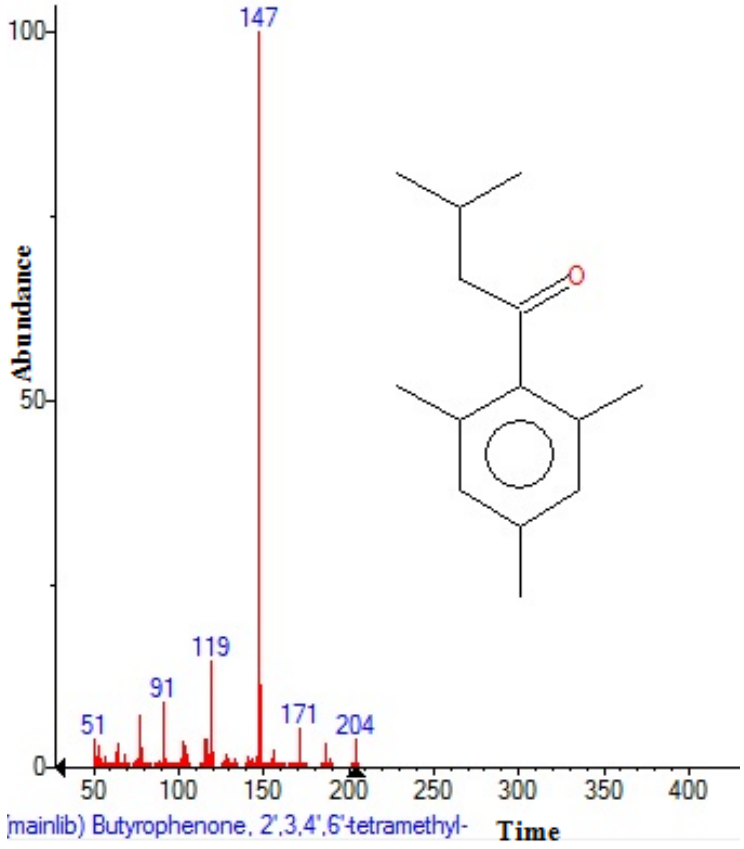


Figure 18. Structure of butyrophenone,2´,3,4´,6´-tetramethyl present in the *Citrullus colocynthis* by using GC-MS analysis

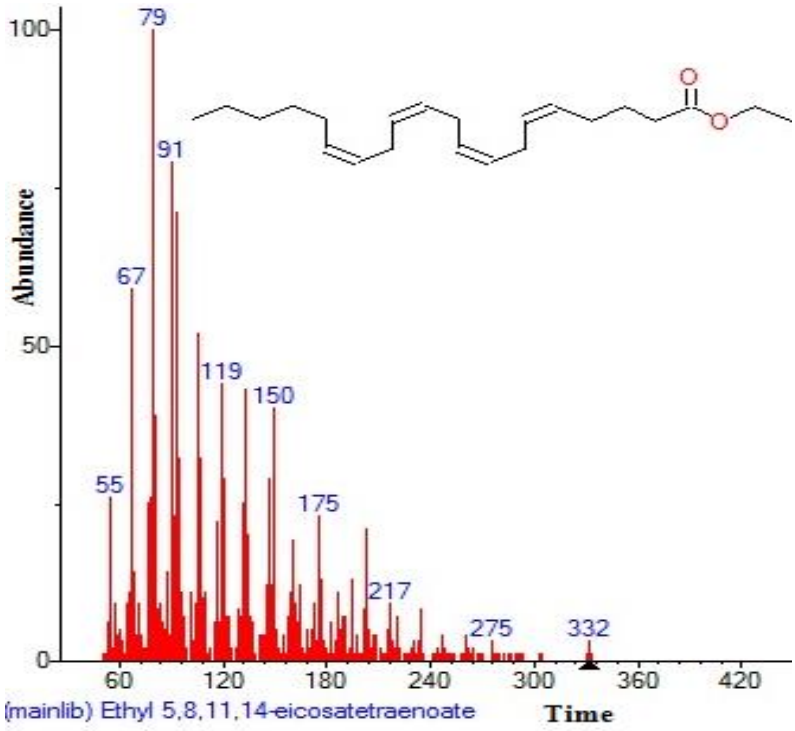


Figure 19. Structure of ethyl 5,8,11,14,-eicosatetraenoate present in the *Citrullus colocynthis* by using GC-MS analysis.

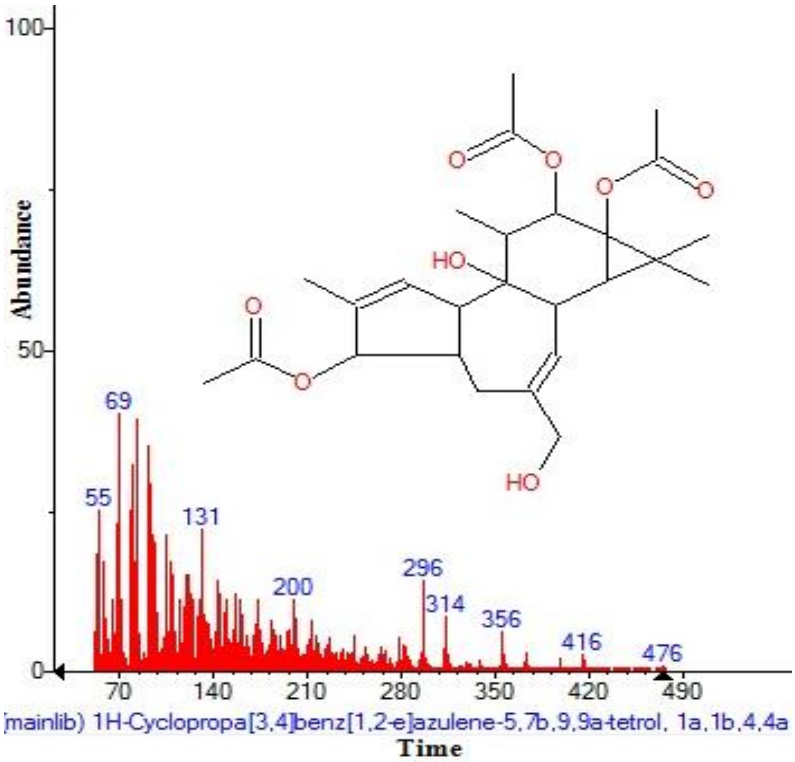


Figure 20. Structure of 1H-cyclopropa[3,4]benz[1,2-e]ezulene -5,7b,9,9a tetrol,1a,1b,4,4a present in the *Citrullus colocynthis* by using GC-MS analysis.

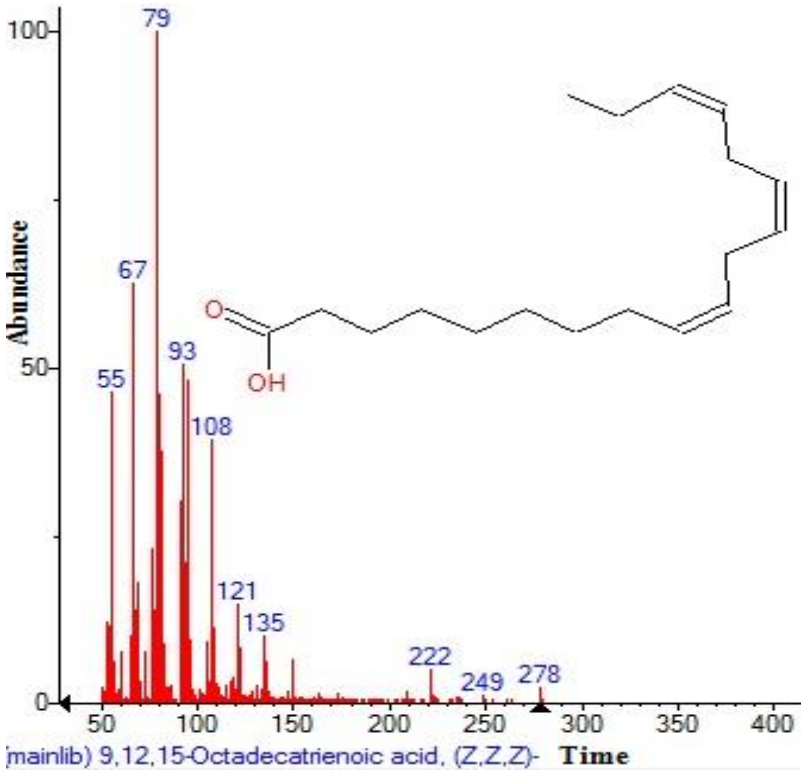


Figure 21. Structure of 9,12,15-octadecatrienoic acid present in the *Citrullus colocynthis* by using GC-MS analysis.

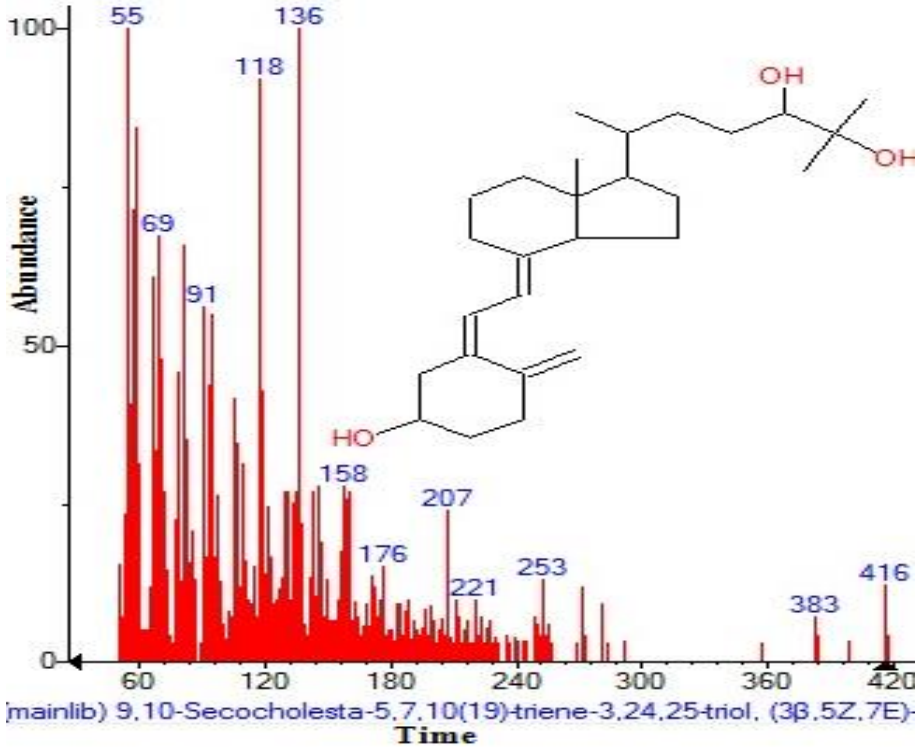


Figure 22. Structure of 9,10-secocholesta -5,7,10(19)-triene-3,24,25,-triol,(3ß,5Z,7E) present in the *Citrullus colocynthis* by using GC-MS analysis.

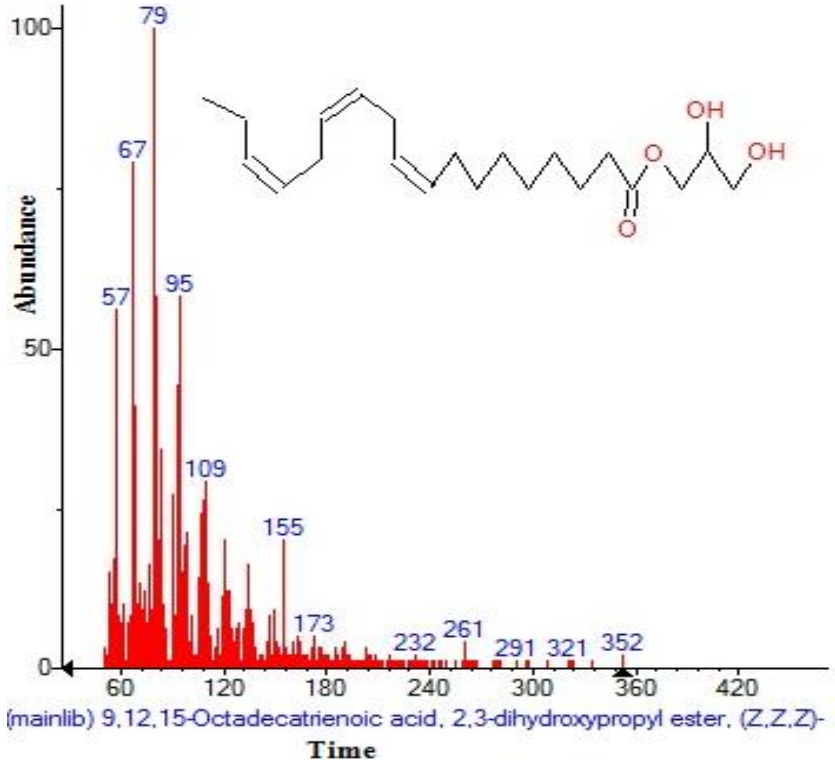


Figure 23. Structure of 9,12,15-octadecatrienoic acid ,2,3-dihydroxypropyl ester, (Z,Z,Z) present in the *Citrullus colocynthis* by using GC-MS analysis.

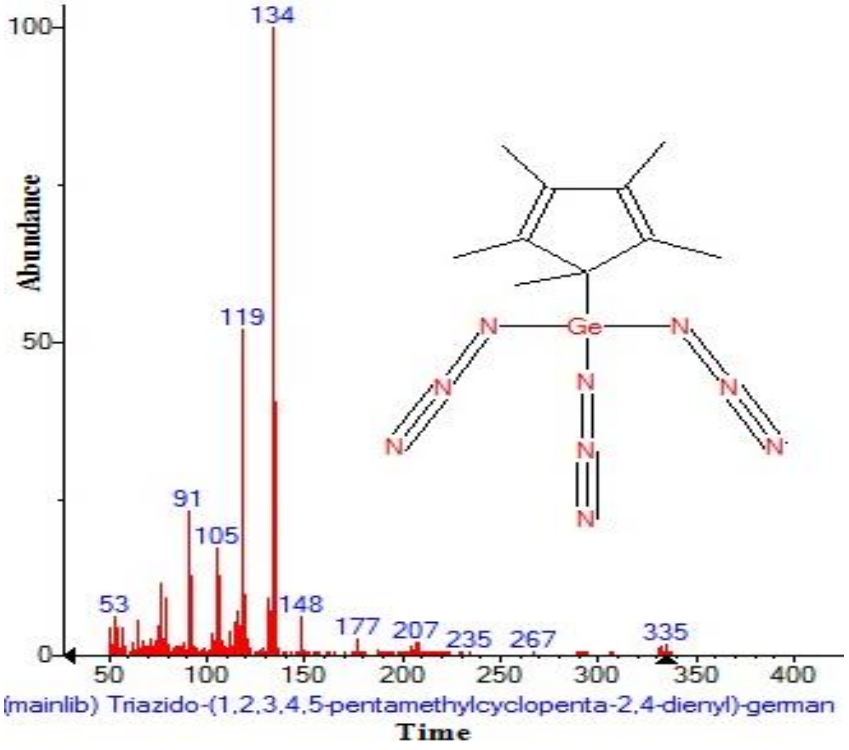


Figure 24. Structure of triazido-(1,2,3,4,5-pentamethylcyclopenta-2,4-dienyl)-german present in the *Citrullus colocynthis* by using GC-MS analysis.

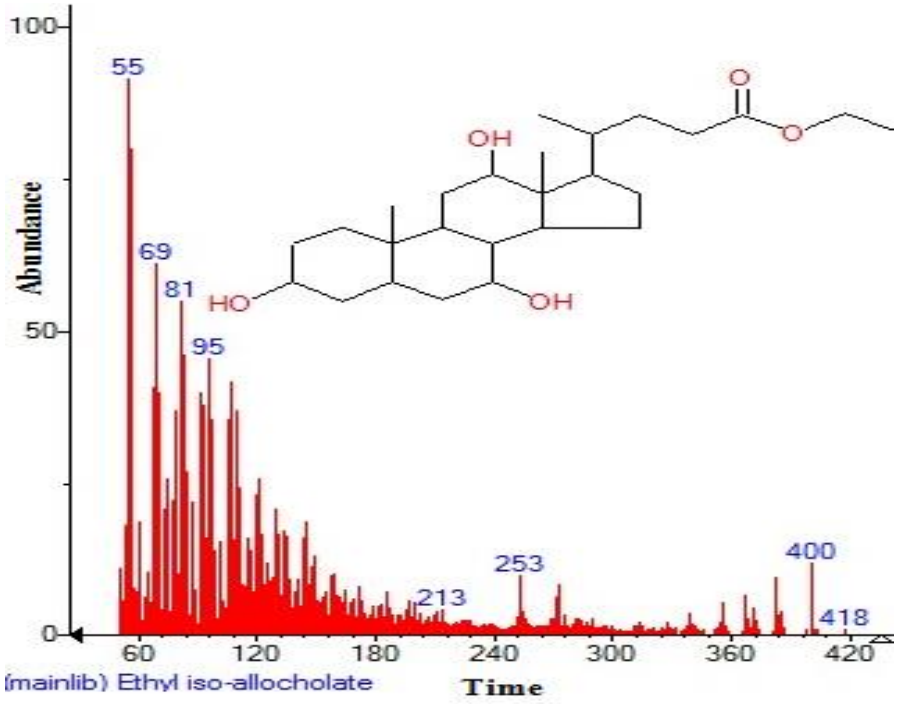


Figure 25. Structure of ethyl iso-allocholate present in the *Citrullus colocynthis* by using GC-MS analysis.

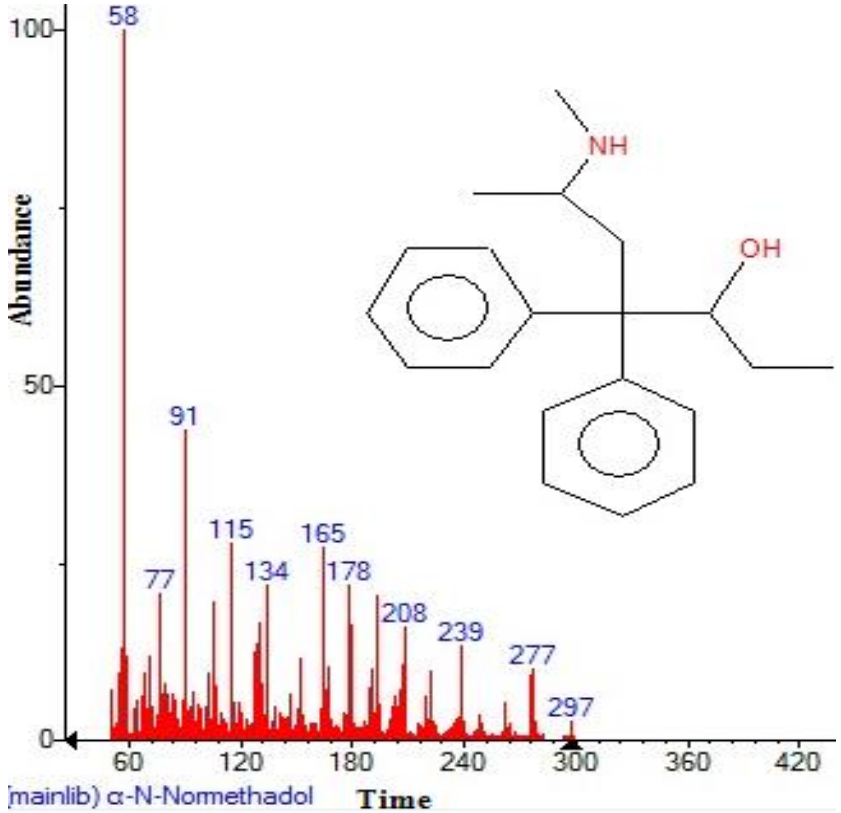


Figure 26. Structure of α-N-normethadol present in the *Citrullus colocynthis* by using GC-MS analysis.

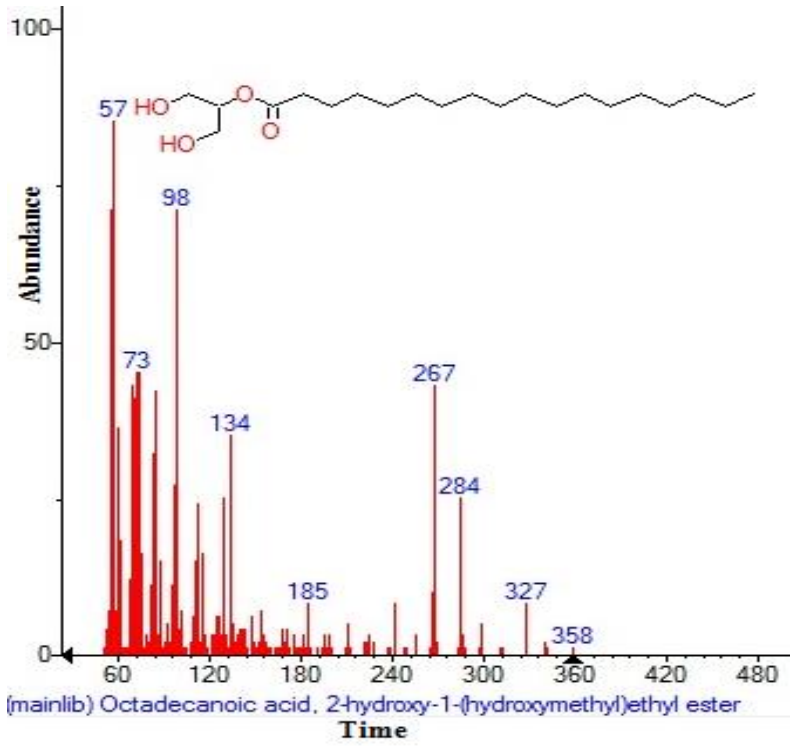


Figure 27. Structure of octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester present in the *Citrullus colocynthis* by using GC-MS analysis.

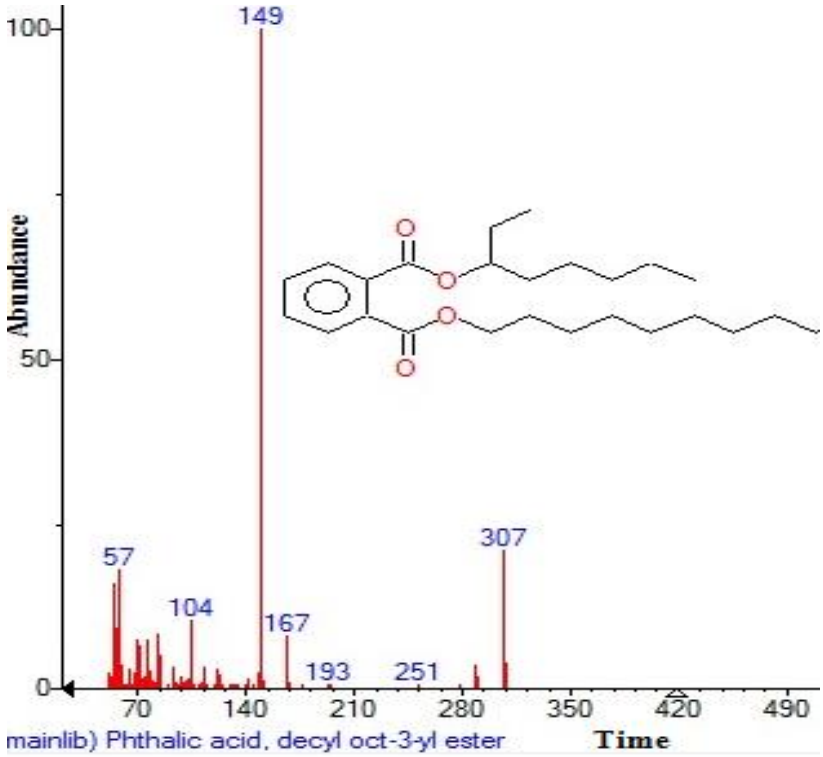


Figure 28. Structure of phthalic acid, decyl oct-3-yl ester present in the *Citrullus colocynthis* by using GC-MS analysis.

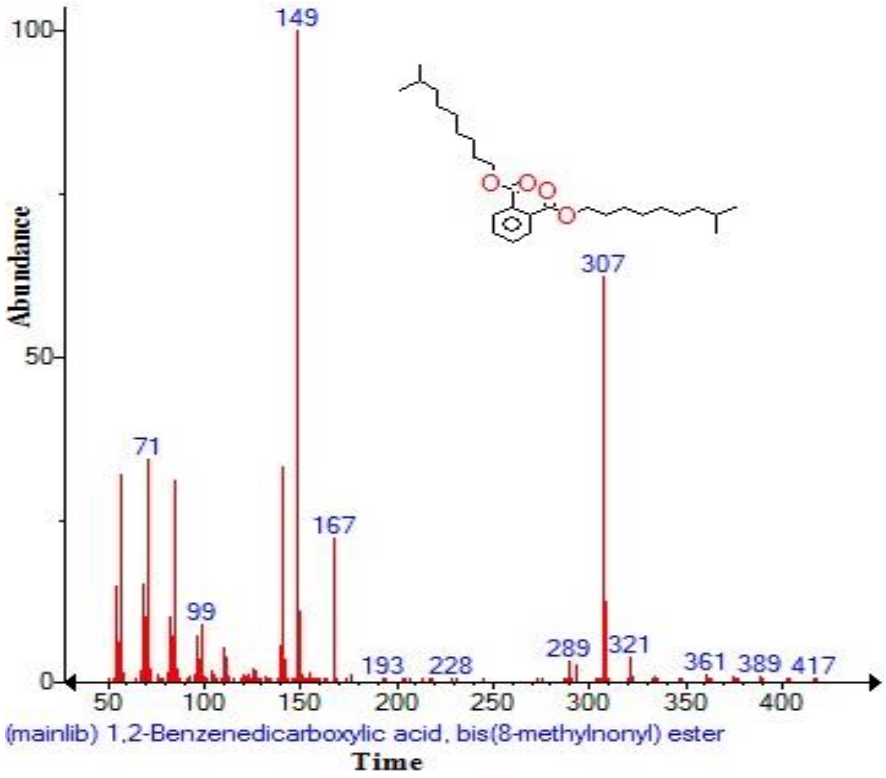


Figure 29. Structure of 1,2-benzenedicarboxylic acid, bis(8-methylnonyl)ester present in the *Citrullus colocynthis* by using GC-MS analysis.

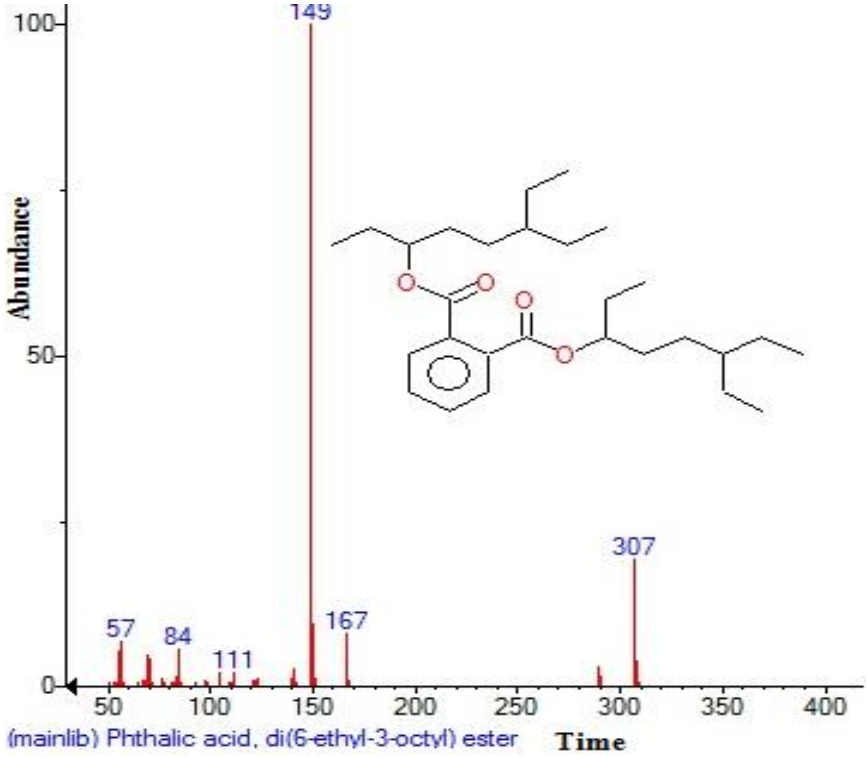


Figure 30. Structure of phthalic acid, di(6-ethyl-3-octyl)ester present in the *Citrullus colocynthis* by using GC-MS analysis.

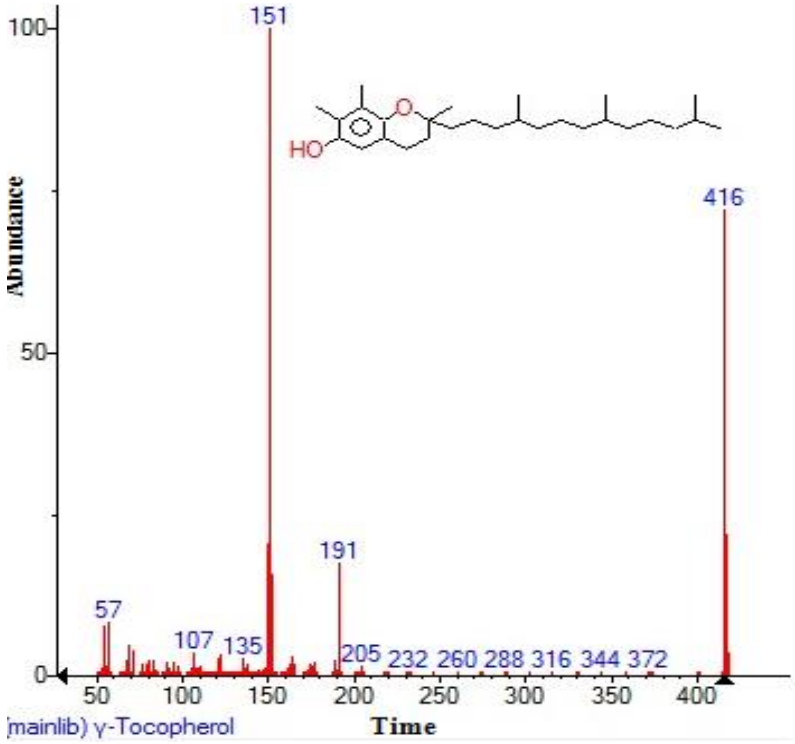


Figure 31. Structure of γ-tocopherol present in the *Citrullus colocynthis* by using GC-MS analysis.

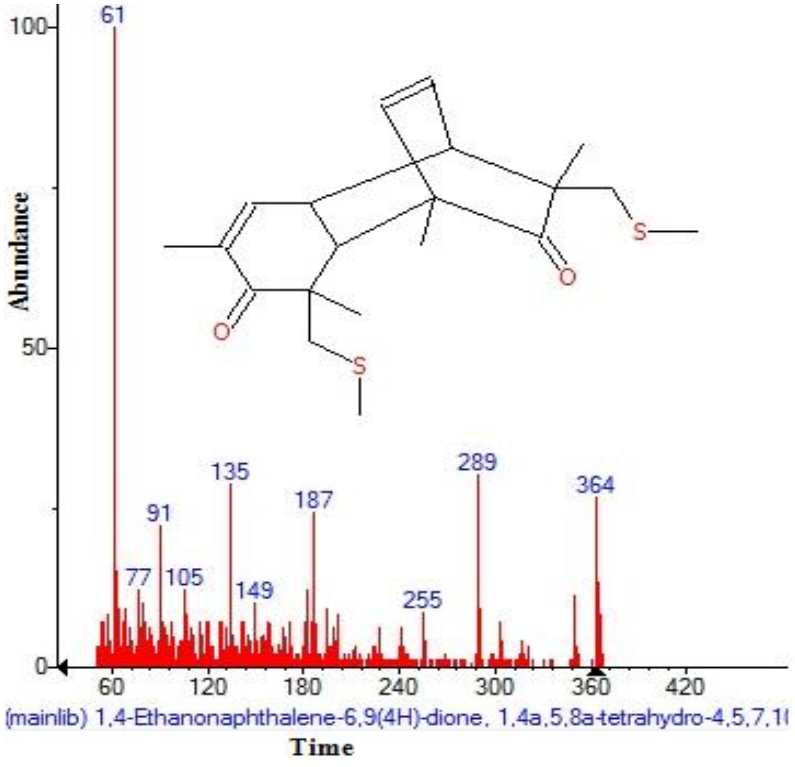


Figure 32. Structure of 1,4-ethanonaphthalene -6,9(4H)-dione,1,4a,5,8a-tetrahydro-4,5,7,10 present in the *Citrullus colocynthis* by using GC-MS analysis.

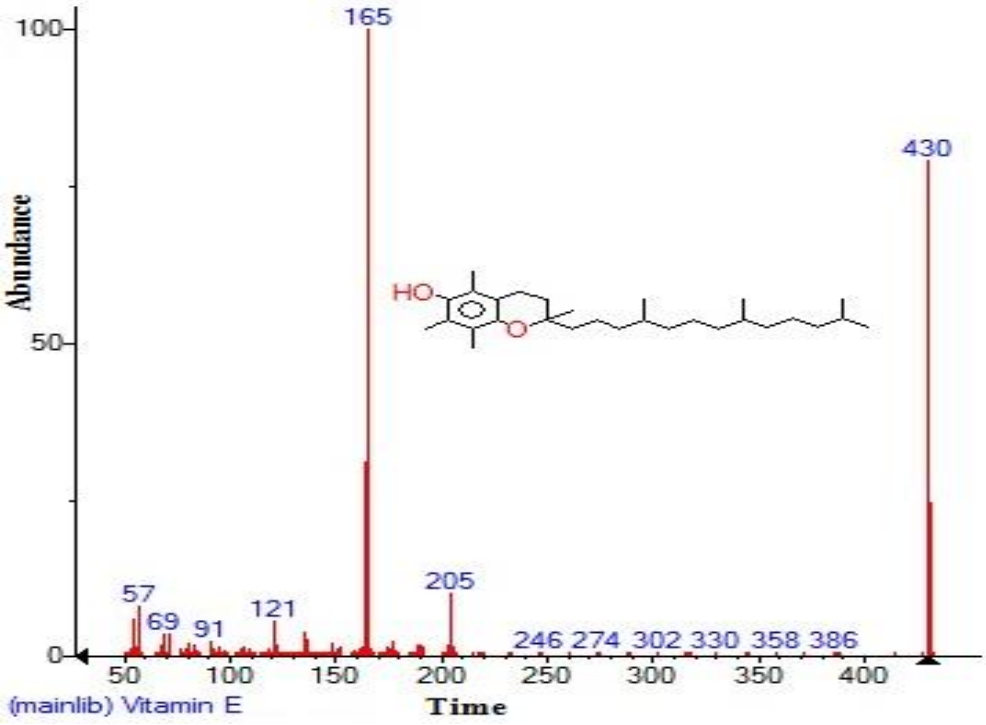


Figure 33. Structure of vitamin E present in the *Citrullus colocynthis* by using GC-MS analysis.

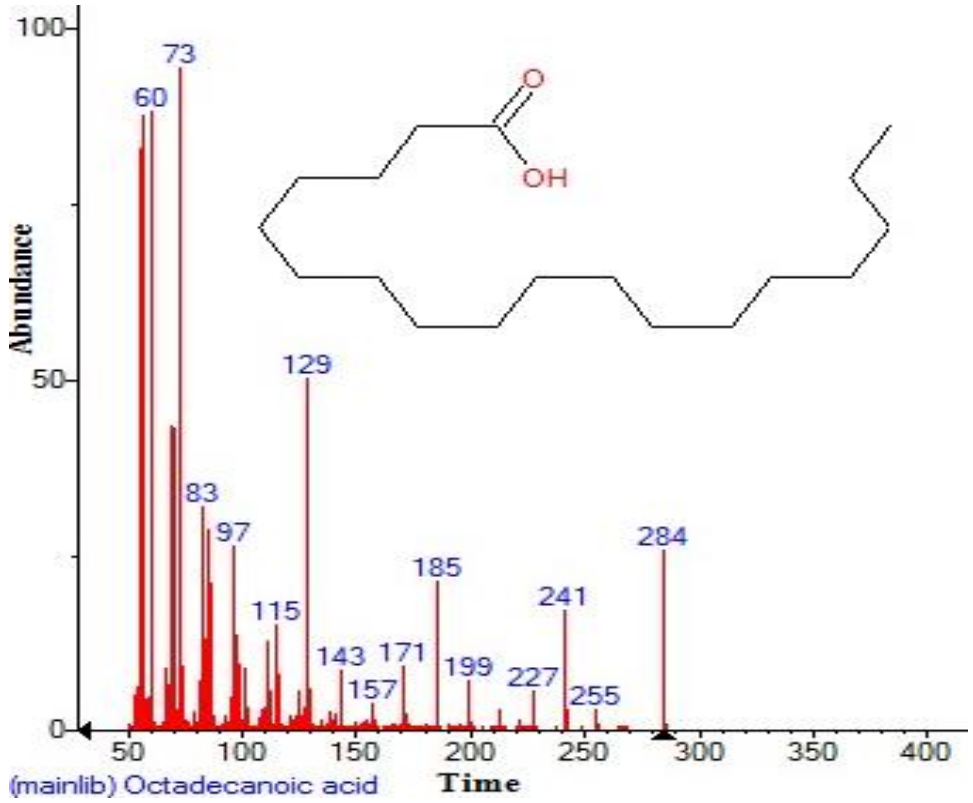


Figure 34. Structure of octadecanoic acid present in the *Citrullus colocynthis* by using GC-MS analysis.

Table 2. Zone of inhibition (mm) of test bacterial strains to *Citrullus colocynthis* bioactive compounds and standard antibiotics.

Bacteria	Plant (*Citrullus colocynthis*)/Antibiotics			
	Citrullus colocynthis	**Streptomycin**	**Rifambin**	**Cefotoxime**
Klebsiella pneumonia	3.95±0.330	1.98±0.251	2.04±0.100	1.03±0.200
Proteus mirabilis	2.06±0.140	1.90±0.120	1.69±0.160	2.00±0.350
Pseudomonas eurogenosa	4.91±0.260	1.08±0.110	2.06±0.140	2.187±0.130
Staphylococcus aureus	2.95±0.160	2.00±0.300	3.00±0.180	2.07±0.101
Escherichia coli	3.00±0.201	2.20±0.141	1.00±0.200	3.10±0.240

concluded that *C. colocynthis* possesses remarkable antimicrobial activity, which is mainly due to α-D-glucopyranoside, O-α-D-glucopyranosyl, phthalic acid and y-tocopherol. According to these findings, it could be said that the methanolic extract act as antibacterial agents.

Conflict of interests

The author(s) did not declare any conflict of interest.

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