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Estimation of Phytoconstituents from *Citrullus colocynthis* (L.) Schrad Roots Extract by GC-MS Spectroscopy

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Abstract: ***Citrullus colocynthis (L.) Schrad (family Cucurbitaceae) is wide spread annual uncultivated plant grows fast in sandy soil. The present study was undertaken on the roots of this plant to investigate the possible bioactive phytoconstituents using gas chromatography-mass spectroscopy (GC-MS) analysis. GC-MS analysis of hydro alcoholic extract from roots has led to the identification of 57 compounds by comparison of their retention indices and mass spectra fragmentation patterns with those stored in the GC-MS computer library. The main constituents identified as glycerol, 2-methoxy-4-vinlphenol, 1-pentadecanol, pluchidiol, myristic acid, oleic acid, codein, beta-codein, morphine, thebaol, thebaine, dimethylmorphine, 1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline, protopine, kryptopine, narceine, alpha-narcotine, methyl-3-hydroxycholest-5-en-26-oate, sebacic acid, 2,7-dimethylocta-7-en-5-yn-4-yl heptyl ester, verticiol etc. In spite of its high medicinal value, the GC/MS analysis of its root part is not reported earlier.***

Keywords: Gas Chromatography-Mass Spectroscopy (GC-MS), *Citrullus colocynthis* (L.) Schrad, Cucurbitaceae, Phytoconstituents

1. Introduction

Citrullus colocynthis (L.) Schrad (Family: Cucurbitaceae) is perennial herbs usually trailing[1]. It is commonly known as the colocynth[2], bitter apple[1,3], bitter cucumber, english colocynth, bitter gourd, wild gourd[4] etc. It resembles a common watermelon vine, but bears, small, hard fruit with a bitter pulp. *Citrullus colocynthis* (L.) Schrad is commonly found wild in the sandy lands of North West, the Punjab, Sind, and Central and southern India, and coromandal coast[1,5]. Also found indigenous in Arabia, West Asia, and Tropical Africa and in the Mediterranean region[3,4]. *Citrullus colocynthis* (L.) Schrad has the traditional use in remedy for cancer, carcinoma, endothelioma, leukemia, tumors of the liver and spleen, even the eye. A decoction of the whole plant, made with juice of fennel is said to help indurations of the liver. Roots may also be used as a purgative against as cites for jaundice, urinary diseases, rheumatism and for snake poison[3,4,6]. *Citrullus colocynthis* (L.) Schrad is widely used in folk medicine for centuries and as an energy source also. e.g. oilseed and biofuel[6,7]. In spite of its high medicinal value, the GC/MS analysis of its root part is not reported earlier. Gas chromatography-mass spectrometry (GC-MS) is a technique that combines the features of gas-liquid chromatography and mass spectrometry to recognize different substances within a test sample [8,9].

2. Materials and Methods

Citrullus colocynthis (L.) Schrad whole plants were collected from widely grown region of Southern Haryana in the month of June 2015. The plant was taxonomically identified and authenticated by Dr. Anjula Pandey, Principal Scientist Raw Materials, Herbarium and Museum Division, NISCAR, New Delhi, vide reference number NHCP/NBPGR/2016-14 as (*Citrullus colocynthis* (L.) Schrad) family Cucurbitaceae dated on 17 March, 2016. A voucher specimen of the same has been retained in the department for the future reference. *Citrullus colocynthis* (L.) Schrad roots were used to carry out the experimental work.

Preparation of Extracts

The shade dried roots of *Citrullus colocynthis* (L.) Schrad were crushed and coarsely grind[10]. Then sample was kept in a four necked round bottom flask with solvent ethanol (50%) and extracted in U-Wave 1000 Microwave synthesis reactor (SINEO Microwave Chemistry Technology, China) at Power-time mode[11]. The instrument operates at an input power of 2000W with operating frequency of 2450MHz and works at atmospheric pressure. The real time temperature was monitored by high precision platinum resistance temperature sensor. The flask was connected to outside condenser through a glass connecting tube (19mm U) and a X shaped tube. The pulverized drug was extracted at different operating conditions (Microwave power, ethanol concentration (%) and different volume of solvent/g of drug) as suggested by experimental design. The extracts obtained by different techniques were cooled for 5 min before filtration. Further the extracts was filtered and concentrated under reduced pressure by a rotary evaporator at 60^0C. The experiment was conducted in triplicate and percentage yield (w/w) was determined. The extracts were kept in a desiccator before further analysis[12].

GC-MS analysis

The extract was directly used for the analysis. GC-MS analysis was carried out on a GCMS-QP2010 Plus (Shimadzu, Kyoto, Japan) system with head space sampler (AOC-20s) and

auto injector (AOC-20i), equipped with mass selective detector, having ion source temperature of 230°C, interface temperature of 270°C, a solvent cut time of 3.50 min, detector gain mode relative, threshold of 1,000 and mass range of 40 to 650 m/z. Compounds were separated using a Rtx 5 MS capillary column (Restek Company, Bellefonte, USA: crossbond 5% diphenyl/ 95% dimethyl polysiloxane) having dimensions 30 m (length) × 0.25 mm (diameter) × 0.25 µm (film thickness). The split mode was used at a ratio of 10:1. The temperature of the injector was initialized to 260°C, having a split injection mode, pressure 69.0 kPa. The temperature was programmed from 50°C (3 min), then further increased to 280°C at a rate of 10°C/min (24 min hold). Helium (>99.999%) was used as the carrier gas at a linear flow velocity of 39.9 cm/s with constant flow of 1.21 mL/min and an injection volume of 1.0 µL was employed. The chemical constituents were identified by comparison of their retention indices (RI) relative to homologous alkane series (purchased from Sigma, St. Louis, USA) and by comparison of their mass spectral fragmentation patterns with those data provided in WILEY8.LIB, NIST08.LIB, NIST08s.LIB and NIST.LIB. Identification was assumed when a good match of mass spectrum and RI was achieved [13,14].

3. Results

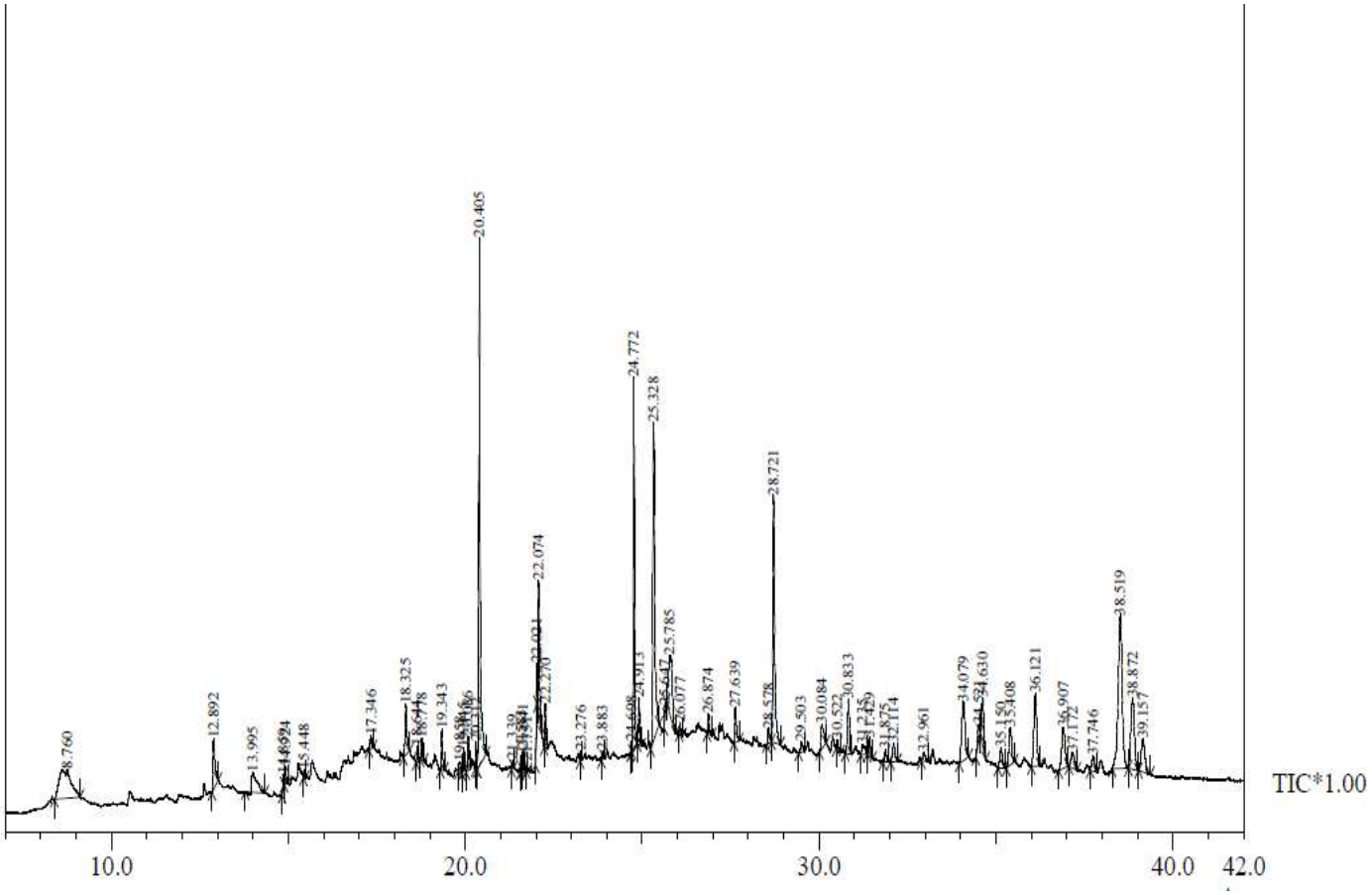
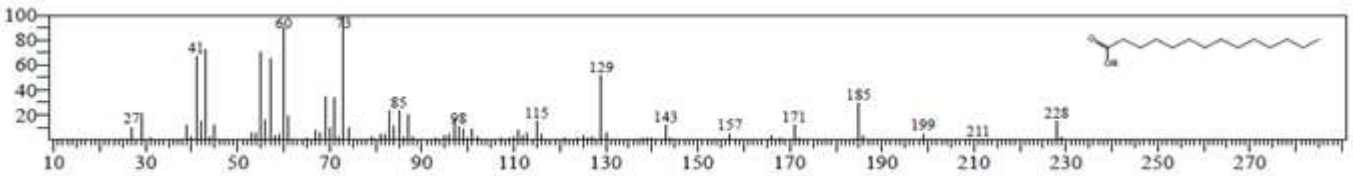


Figure 1: GC-MS Chromatogram of *Citrullus colocynthis* L. Schrd. root extract

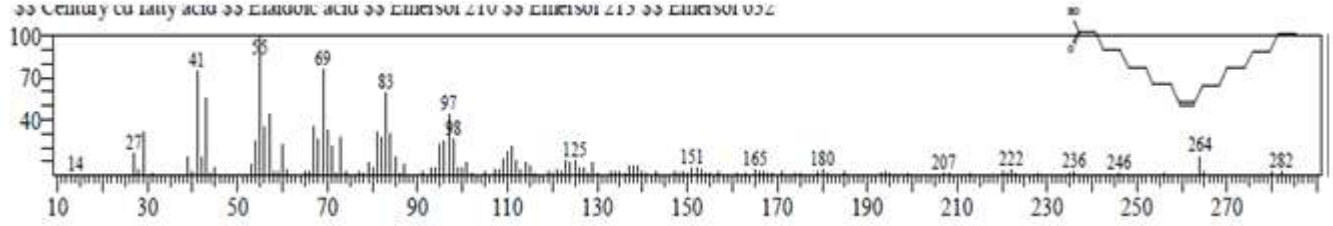
Table 1: Different phytoconstituents identified in the GCMS study of Citrullus colocynthis L. Schrd. root extract

Peak	*Retention Time*	*Area*	*Area %*	*Name*	*Molecular Weight*	*Molecular Formula*
1	8.760	4769572	5.54	Glycerol; 1,2,3-Propanetriol	92	$C_3H_8O_3$
2	12.892	1421572	1.65	2-Methoxy-4-vinylphenol	150	$C_9H_{10}O_2$
3	13.995	1427758	1.66	DL-Proline, 5-oxo-methyl ester	143	$C_6H_9NO_3$
4	14.859	166312	0.19	Dodecane, 1-chloro-	204	$C_{12}H_{25}Cl$
5	14.924	274928	0.32	1-Dodecanol	186	$C_{12}H_{26}O$
6	15.448	93934	0.11	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-	226	$C_{13}H_{22}O_3$
7	17.346	281388	0.33	1-Pentadecanol	228	$C_{15}H_{32}O$
8	18.325	1268482	1.47	Tetradecanoic acid; Myristic acid	228	$C_{14}H_{28}O_2$
9	18.64	229405	0.27	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a tetrahydrobenzofuran-2(4H)-one	196	$C_{11}H_{16}O_3$
10	18.778	415778	0.48	Pluchidiol	208	$C_{13}H_{20}O_2$

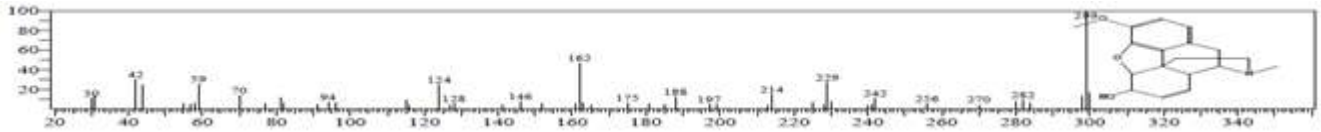
11	19.343	629154	0.73	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	278	$C_{16}H_{22}O_4$
12	19.858	239716	0.28	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276	$C_{17}H_{27}NO_2$
13	19.945	274217	0.32	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$
14	20.086	705938	0.82	7-oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-	226	$C_{13}H_{22}O_3$
15	20.312	272871	0.32	Dibutyl phthalate	278	$C_{16}H_{22}O_4$
16	20.405	10633174	12.36	N-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
17	21.339	104359	0.12	Heptadecanoic acid	270	$C_{17}H_{34}O_2$
18	21.583	167479	0.19	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	294	$C_{19}H_{34}O_2$
19	21.641	228982	0.27	6-Octadecenoic acid, methyl ester, (Z)-	296	$C_{19}H_{36}O_2$
20	21.751	87006	0.10	3,7,11,15-tetramethylhexadec-2-en-1-ol	296	$C_{20}H_{40}O$
21	22.021	829407	0.96	9,12-Octadecadienoic acid (Z,Z)-	280	$C_{18}H_{32}O_2$
22	22.074	2270034	2.64	Oleic acid	282	$C_{18}H_{34}O_2$
23	22.270	665180	0.77	Stearic acid; Octadecanoic acid	284	$C_{18}H_{36}O_2$
24	23.276	89765	0.10	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	213	$C_{12}H_{23}NO_2$
25	23.883	104651	0.12	4,8,12,16-Tetramethylheptadecan-4-olide	324	$C_{21}H_{40}O_2$
26	24.698	114013	0.13	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	213	$C_{12}H_{23}NO_2$
27	24.772	5686687	6.61	Codein	299	$C_{18}H_{21}NO_3$
28	24.913	800697	0.93	Beta Codein; Morphinan-6-ol,8,14-didehydro-4,5-epoxy-3-methoxy-17-methyl-	299	$C_{18}H_{21}NO_3$
29	25.328	7816128	9.08	Morphine	285	$C_{17}H_{19}NO_3$
30	25.647	162181	0.19	Thebaol; 3,6-dimethoxy-4-phenanthrenol	253	$C_{16}H_{14}O_3$
31	25.785	3181844	3.70	Thebaine	311	$C_{19}H_{21}NO_3$
32	26.077	176806	0.21	Dimethylmorphine; Morphinan, 7,8-didehydro-4,5-epoxy-3,6-dimethoxy-17-methyl-, (5.alpha.,6.	313	$C_{19}H_{23}NO_3$
33	26.874	336517	0.39	1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline	357	$C_{21}H_{27}NO_4$
34	27.639	789621	0.92	1-Carbomethoxy-1,2,5,5-tetramethyl-cis-decalin(1R,2S,4as,8as)	252	$C_{16}H_{28}O_2$
35	28.578	385499	0.45	1-((4-hydroxy-3-methoxyphenyl)methyl)-6,7-dimethoxy isoquinoline	325	$C_{19}H_{19}NO_4$
36	28.721	6446190	7.49	Isoquinoline, 1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxy-	339	$C_{20}H_{21}NO_4$
37	29.503	342116	0.40	1-((4-hydroxy-3-methoxyphenyl)methyl)-6,7-dimethoxy isoquinoline	325	$C_{19}H_{19}NO_4$
38	30.084	992888	1.15	1-((4-hydroxy-3-methoxyphenyl)methyl)-6,7-dimethoxy isoquinoline	325	$C_{19}H_{19}NO_4$
39	30.522	207511	0.24	Protopine	353	$C_{20}H_{19}NO_5$
40	30.833	1431755	1.66	Kryptopine;	369	$C_{21}H_{23}NO_5$
41	31.235	428360	0.50	7-(2,6-dimethyl-hepta-1,5-dienyl)-3,8,8-trimethyl-bicyclo[4.2.0] oct-2-ene	272	$C_{20}H_{32}$
42	31.429	511541	0.59	Tetratriacontyl pentafluoropropionate	640	$C_{37}H_{69}F_5O_2$
43	31.875	375842	0.44	Methyl 3-hydroxycholest-5-en-26-oate	430	$C_{28}H_{46}O_3$
44	32.114	642882	0.75	Sebacic acid, 2,7-dimethylocta-7-en-5-yn-4-yl heptyl ester	434	$C_{27}H_{46}O_4$
45	32.961	309446	0.36	alpha.-(N,N-Dimethylamino)-3'-hydroxy-4'-methoxyacetophenone	209	$C_{11}H_{15}NO_3$
46	34.079	2817852	3.27	Alpha- Narcotine	413	$C_{22}H_{23}NO_7$
47	34.521	437567	0.51	Delta-7- strongyloster	424	$C_{30}H_{48}O$
48	34.630	1448067	1.68	Narceine	445	$C_{23}H_{27}NO_8$
49	35.150	768671	0.89	cis-3,14-Clerodadien-13-ol	290	$C_{20}H_{34}O$
50	35.408	1453499	1.69	1-acetyl-11a-[(acetyloxy)methyl]-3a-hydroxy-2-(hydroxymethyl)-	466	$C_{25}H_{38}O_8$
51	36.121	2954324	3.43	9,19-Cyclolanost-24-ene-3,26-diol, diacetate	526	$C_{34}H_{54}O_4$
52	36.907	1952843	2.27	9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	440	$C_{31}H_{52}O$
53	37.172	755372	0.88	Alpha-Narcotine; 3-Chloro-5-cholestene	413	$C_{22}H_{23}NO_7$
54	37.746	560776	0.65	Verticol	290	$C_{20}H_{34}O$
55	38.519	8902210	10.34	Narceine	445	$C_{23}H_{27}NO_8$
56	38.872	3584160	4.16	1,4,5,8,9,10,11,12,13,14,17,18,21,22,23,24,25,26-octadecahydrodicyclodeca[	404	$C_{30}H_{44}$
57	39.1	1636168	1.90	7-(2,6-dimethyl-hepta-1,5-dienyl)-3,8,8-trimethyl-bicyclo[4.2.0]	272	$C_{20}H_{32}$



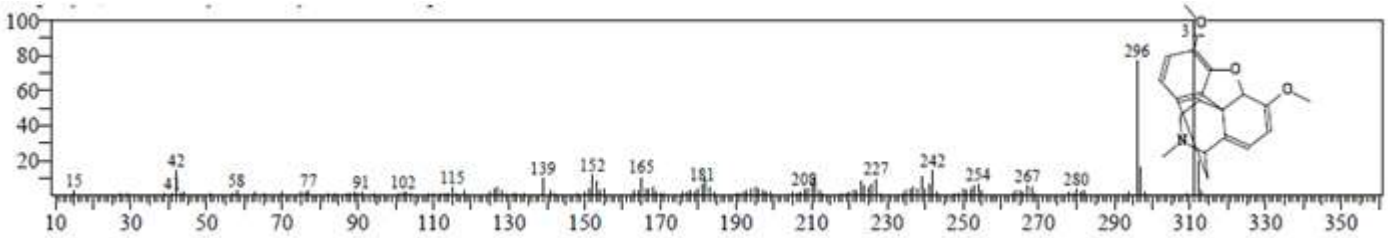
Myristic acid



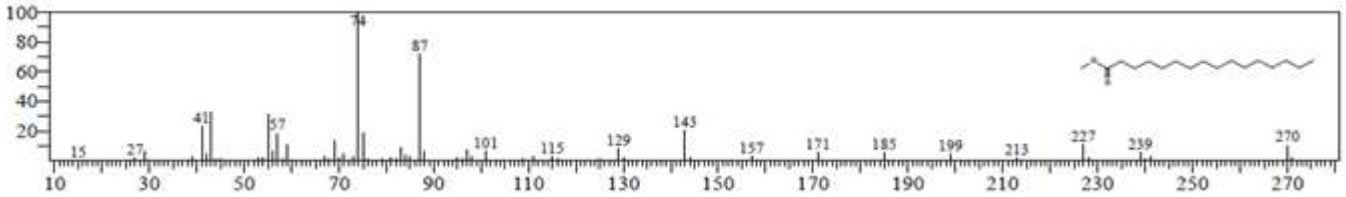
Oleic acid



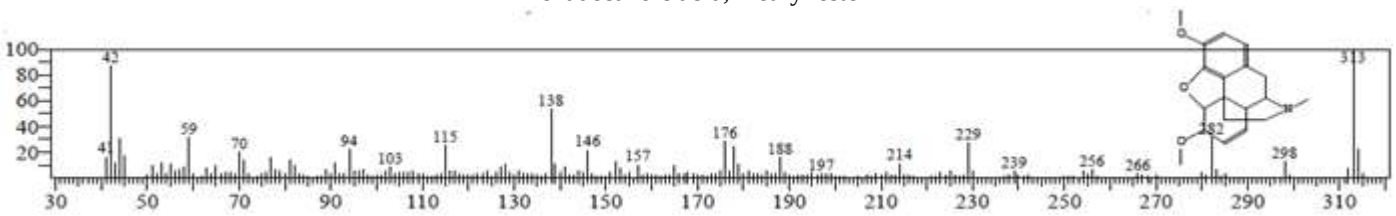
Codein



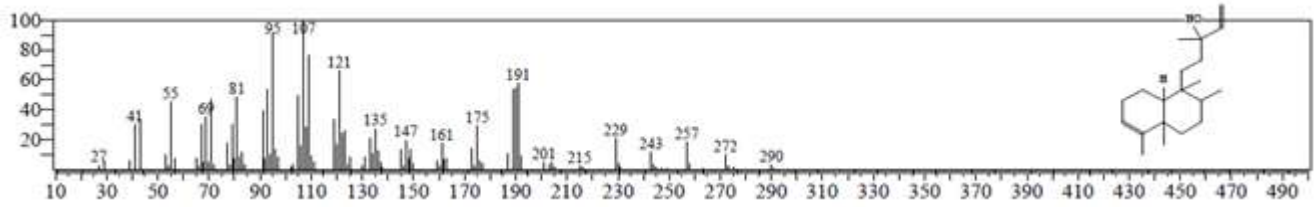
Thebaine



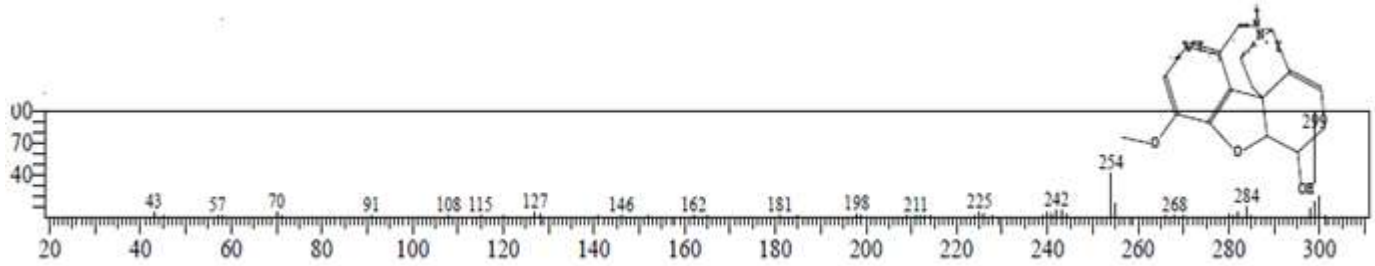
Hexadecanoic acid, methyl ester



Dimethylmorphine



cis-3,14-Clerodadien-13-ol



Beta Codein

4. Conclusions

The GC/MS investigation led to the identification of 57constituents components in the roots of plant *Citrullus colocynthis* L. Schrd. Table1. revealed the important bioactive constitutents presumed to be responsible for eliciting the traditional activity of this plant. The results revealed the major compounds are fatty acid esters and alkaloids which showed antioxidant, antimicrobial, anticancer, antineuropathic, anti-inflammatory activities of *Citrullus colocynthis* L. Schrd roots.

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