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Analysis of volatile constituents in normal flower and insect induced flower gall of *Crataeva religiosa*

Surabhi Sharma, Preeti Mishra and Vidya Patni

Abstract
The “insect” galls are unique examples of complex interactions and mutual adaptation between the host and the pathogen, characterized by cellular hypertrophy and hyperplasia. *Crataeva religiosa* Hook and Frost, (Capparidaceae) is one of the herbal drug in urolithiasis. The present investigation was undertaken to evaluate the major and minor phytochemical compounds present in normal flowers and the changes that occur in flower galls of *Crataeva religiosa*. The methanolic extracts of both the tissues were analysed by Gas Chromatography-Mass Spectrometry technique for the study of major constituents. The GC-MS analysis of normal and galled flower of *Crataeva religiosa* showed the presence of eighty four and sixty four phytochemical compounds respectively. This analysis leads to the finding that under stressed conditions large no. of fatty acids and secondary metabolites were produced and the flower gall extract had a much higher unsaturated fatty acid content than normal flower extract. The fatty acids (oleic acid and palmitic acid) were found in higher amount in flower gall extract than normal flower extract. Whereas myristic acid could be detected only in gall extract. These compounds possess a wide range of proven industrial and medicinal values. This study will help in further investigation to predict the formula and structure of compounds which can be used as drugs and in industrial uses.

Keywords: *Crataeva religiosa*, galls, phytochemical, methanolic extract, GC-MS

Introduction
Crataeva religiosa Forst. (Family Capparidaceae) is a large tree distributed in the tropical zone and is common throughout India, Myanmar and Sri Lanka, either wild or cultivated. It is cultivated in the gardens for its ornamental as well as medicinal value [1]. The flowering tree *Crateva religiosa (syn Crataeva religiosa, Crateva adansonii*) is called the sacred garlic pear and temple plant, and many other names in a variety of dialects, including Balai Lamok, abiyuch, barna, varuna, and bidasi. The tree is sometimes called the spider tree because the showy flowers bear long, spidery stamens. It is native to Japan, Australia, much of Southeast Asia and several South Pacific islands. It is grown elsewhere for fruit, especially in parts of the African continent. The fruit of the tree is edible. The nectar-filled flowers are attractive to a multitude of insects and birds. The tree is sacred to Lord Shiva and the leaves are used to worship Him, especially on Mahashivaratri. The tree is also sacred to Rahu (Planet Neptune), one of the nine planets or the navagrahas. This tree is well known for its various pharmacological properties like diuretic, anti-inflammatory, laxative, antioxidant, antioxaluric, hepatoprotectant, lithonotriptic, antirehumatic, antiperiodic, antimycotic, contraceptive, antipyretic, antilithitic, antihelminthic, rubifacient and vasicant properties. The bark of the *Crataeva religiosa* is useful in the urinary disorders and kidney stone remover [2].
The flowers and fruits are resentfully afflicted by the insect, *Aschistonyx crataevae* Mani, order diptera [3]. Galls results from interactions between two individuals (and their genomes) and occur when a gall-inducing species attacks a suitable host plant. Most often, galling reflects a parasitic relationship, in which the gall inducer alters resources of the host plant to be more easily accessed or consumed [4]. They arise due to the growth reactions of plants to the attack of insects [5]. A special feature of higher plants is their capacity to produce a large number of secondary metabolites [6]. The most important bioactive constituents of the plants are alkaloids, tannins, flavonoids and phenolic compounds. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties. Abiotic stress is caused by insect to the plant. Stresses trigger a wide range of plant responses. Stress can have a devastating impact on plant growth and yield [7] or can result into enhancement of production of secondary metabolites [8]. These secondary metabolites are capable of triggering changes into plants cell which helps to overcome the stress [9].
In the present study the comparative analysis of volatile constituents in methanolic extracts of the normal flower and flower gall of *Crataeva religiosa* was done by GC-MS technique.

Materials and Methods

Collection of plant material

The normal flower and flower galls were collected from the Central park, Jaipur, India. They were identified and authenticated by the herbarium of Department of Botany, University of Rajasthan, Jaipur.

Preparation of powder and extract

Flowers and flower galls were shade dried, powdered separately and extracted with methanol for 6-8 hours using soxhlet apparatus. The extract was then filtered through whatmann filter paper, evaporated under reduced pressure and vacuum dried to get the viscous residue. The methanolic extracts of the plant was used for preliminary phytochemical and GC-MS analysis. 1 µl of both the extract of *C. religiosa* were employed for GC-MS analysis.

GC-MS analysis

The GC-Ms analysis of methanolic extract of normal and galled leaves of *Crataeva religiosa* was carried out on Shimadzu QP-2010 plus with thermal desorption system TD 20. It includes auto sampler and a gas chromatograph which interfaced to a mass spectrophotometer. The column size of this system is 30m × 0.25mm id × 0.26µm with a film thickness of 0.26mm, composed of 5MS (5% diphenyl/ 95% dimethyl poly siloxane). Helium gas (99.999%) was used as carrier gas at constant flow rate of 1ml/min. The 2µl injection volume of sample was utilized with split ratio of 10:1. The injector temperature was programmed initially at 280 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (for 4 min), with an increase of 10 °C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectra were analysed using electron impact ionization at 70 eV. The total running time for each sample was 45 min.

Component Identification

Chemical constituent components of the extracts were identified by matching the peaks with Computer NIST MS libraries and confirmed by comparing mass spectra of the peaks and those from Literature. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials was ascertained.

Results

Phytochemical characterization

The plants are sources of several bio-active compounds that also produce large amounts of secondary metabolites and substances of medicinal and industrial importance. Several studies have proved that the phytoconstituents are widely responsible for the therapeutic and industrial potential of the plant. Phytochemical investigation of methanolic extract of normal flower showed the presence of carbohydrates, steroids, proteins and amino acid while galled flower showed the presence of carbohydrates, steroids, tannins, glycosides, proteins and amino acids, flavonoids and alkaloids. Results are summarised in Table 1.

Table 1: Shows Phytoconstituents.

S. No.	Phytoconstituents	Methanolic extract of normal flower	Methanolic extract of flower gall
1.	Carbohydrates	+	+
2.	Steroids	+	+
3.	Tannins	-	+
4.	Glycosides	-	+
5.	Proteins and Amino acids	+	+
6.	Saponins	-	-
7.	Flavonoids	-	+
8.	Alkaloids	-	+

GC-MS: Phytocomponents in methanolic extract of *Crataeva religiosa*

GC-MS is a combined technique of Gas Chromatography with Mass Spectrometry. MS is wide ranging analytical technique, which identify the charged species according to their mass to charge ratio (M/Z). GC-MS is one of the best techniques to identifies the constituents of volatile compounds. The GC-MS analysis of normal and galled flower of *Crataeva religiosa* showed the presence of eighty four (figure 1) and sixty four (Figure 2) phytochemical compounds respectively. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their retention time (RT), area %, compound name, of normal and leaf galls are presented in Table 2 and 3 respectively.

Discussion

The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in *C. religiosa*. The GC-MS analysis showed that the methanolic extract of normal flower of *C. religiosa* had more compounds than the flower gall extract of the plant. The enhancement of polyunsaturated fatty acids due to insect attack could be detected. This analysis indicates that the transformation from larva to adult during metamorphosis increased the unsaturated fatty acid content. Normal flower extract showed the major compound present as 1, 3-Propanediol with 20.19 % peak area which retention time is 14.072. 1, 3-Propanediol has numerous applications in polymers, cosmetics, foods, lubricants, and medicines. Industrial 1, 3-PD production has attracted attention as an important monomer to synthesize a new type of polyester, polytrimethylene terephthalate [09] The next highest found compound was Silane, which retention time is 29.074 with 9.99% peak area. Silanes are used as coupling agents to adhere fibers such as glass fibers and carbon fibers to certain polymer matrices, stabilizing the composite material. In the galled flower extract the highest found compound was Glycerin which retention time is 7.942 with 36.64% peak area and can be exploited commercially, However Glycerin was not detected in the normal flower. This could be related with the insect feeding requirements. Glycerin is a simple polyol compound. It is a colorless, odorless, viscous liquid that is sweet-tasting and non-toxic. The glycerol backbone is found in all lipids known as triglycerides. It is widely used in the food industry as a sweetener and humectant and in pharmaceutical formulations. The another maximum found compound in gall was n-haxadecanoic acid, (Palmitic acid) with peak area 14.22%. Acexamic acid and Tetradecanoic acid (Myristic acid) were found in galled flower with peak area 3.13% and 3.86% respectively. Myristic acid is used in soaps and cosmetic production. Oleic acid is monounsaturated omega -9- fatty acid which decreases LDL cholesterol and blood pressure [10]. This fatty acid have much economic and commercial significance and are used widely in food industry and pharmaceuticals. A compound which is used to make plastics more flexible i.e, Diethyl phthalate is found more in galled tissues with 2.14 % peak area. From the another result of our study we could find that in normal flower extract the amount of free amino acids was high in comparison of galled extract thus the free amino acids were probably used up by insect during their metamorphosis process The L-Proline, an important compound responsible for tissue repair, collagen formation, arteriosclerosis prevention and blood pressure maintenance was found less in galled flower with peak area 1.88% as compared to the normal flower (peak area 3.13%). Acexamic acid is also found in galled flower, A drug used to treat gastrointestinal ulcers, protecting the mucosa of the stomach and intestine. However, isolation of individual phytochemical constituents may proceed to find a novel drug. The effect of hexadecanoic acid have been the focus of several dietary guidelines targeting the reduction of cardiovascular disease (CVD) [12, 13], obesity related diseases and recently, cancer prevention [14]. Other various compounds such Divinyl sulphide, Agrospirol, Steric acid, Octadecanoic acid, and D-ribose etc. were found in galled flower. whereas in normal flower many other compounds are found such as Guanazine, Glutamine, alpha-D-Galactopyranoside, succinic acid, oxalic acid and colensanone etc.

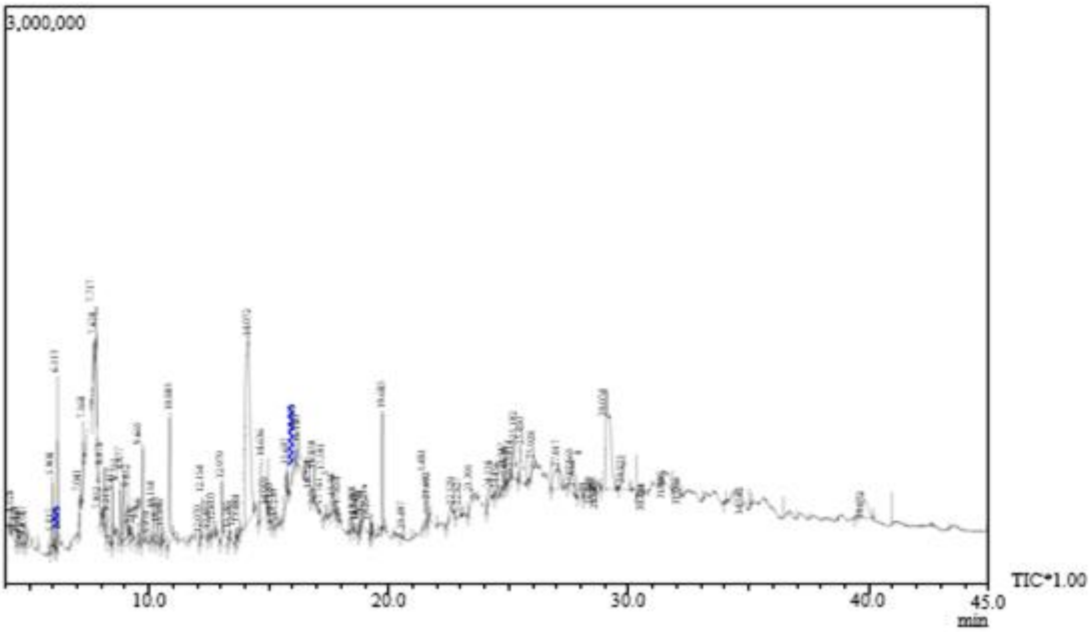

Fig 1: Shows GC-MS Chromatogram of Normal flower of *Crataeva religiosa*

Table 2: Compounds identified from methanolic extract of Normal flower Part of *Crataeva religiosa*using GC-MS analysis.

Peak#	R. Time	Area	Area%	Name
1	4.128	127854	0.29	METHYL2-OXOPROPANOATE
2	4.434	75250	0.17	PYRAZINE, METHYL-
3	4.526	38196	0.09	DODECANALDIMETHYLACETAL
4	4.673	66091	0.15	PROPANOIC ACID, 3-HYDROXY-, METHYLESTER
5	4.787	96800	0.22	METHANE, SULFINYLBIS-
6	5.817	107176	0.25	2-PROPENOICACID, 2-METHYL-, 1, 2-ETHANEDIYLE
7	5.908	489380	1.12	2(3H)-FURANONE, DIHYDRO-
8	6.032	109607	0.25	HEXANAL, 3-METHYL-
9	6.113	932415	2.14	2-Cyclopenten-1-one, 2-hydroxy-
10	7.041	212316	0.49	2,4-Dihydroxy-2, 5-dimethyl-3(2H)-furan-3-one
11	7.168	362186	0.83	2-Hydroxy-gamma-butyrolactone
12	7.620	295633	0.68	PYRAZINECARBOXAMIDE
13	7.717	1053778	2.41	1, 2, 3-PROPANETRIOL
14	7.952	70657	0.16	Guanazine
15	8.078	279625	0.64	7-TRIDECANONE
16	8.213	316889	0.73	2,5-ANHYDRO-1, 6-DIDEOXYHEXO-3, 4-DIULOSE
17	8.417	436958	1.00	2-Pyrrolidinone
18	8.732	373289	0.86	2,5-ANHYDRO-1, 6-DIDEOXYHEXO-3, 4-DIULOSE
19	8.877	438560	1.01	BUTANE, 2-METHYL-
20	9.052	243390	0.56	1H-Pyrrole, 2, 5-dihydro-
21	9.210	62202	0.14	Ethanol, 1-(1-cyclohexenyl)-

Peak#	R. Time	Area	Area%	Name
22	9.466	221920	0.51	Butanoicacid, 2-methyl-3-oxo-, ethylester
23	9.665	660459	1.51	4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-
24	9.970	82257	0.19	2-ACETYLTHIO-3-METHYL-NORTICYCLENE
25	10.114	331351	0.76	Glutamine
26	10.311	369487	0.85	2(3H)-FURANONE, 5-BUTYLDIHYDRO-
27	10.496	97650	0.22	METHYL2-OXOPROPANOATE
28	10.803	1364314	3.13	**L-Proline**,1-acetyl-
29	12.070	59543	0.14	N-(3-Hydroxypropyl)-2-pyrrolidone
30	12.154	759133	1.74	3,4,4-D3-TRANS-3, 5-DIHYDROXY-CYCLOPENTENE
31	12.417	121458	0.28	Triacetin
32	12.610	275958	0.63	3, 4, 4-D3-TRANS-3, 5-DIHYDROXY-CYCLOPENTENE
33	12.979	498448	1.14	5-OXO-PYRROLIDINE-2-CARBOXYLICACIDMETHY
34	13.285	169692	0.39	2-(2, 3-DIMETHYL-2-OXIRANYL) PYRIDINE
35	13.555	95258	0.22	Pyrrolidine, 1-(1-cyclohexen-1-yl)-
36	13.684	85066	0.19	PROPANE, 1, 3-DIMETHOXY-2, 2-BIS(METHOXYMETH
37	14.072	8810977	20.19	1, 3-Propanediol, 2-(hydroxymethyl)-2-nitro-
38	14.636	2350014	5.39	.beta.-D-Glucopyranose, 1, 6-anhydro-
39	14.959	154600	0.35	(3R, 3aR, 7R, 8aS)-3, 8, 8-Trimethyl-6-methyleneoctahydro-1H
40	15.120	174269	0.40	Spiro [4.5] decane-2, 6-dione
41	15.230	202052	0.46	Acetoxyaceticacid, nonylester
42	15.697	697347	1.60	Diethyl Phthalate
43	16.002	522022	1.20	5-Hydroxy-2-isopropyl-[1, 3, 4] thiadiazolo[3, 2-a]pyrimidin-7-
44	16.180	761505	1.75	.alpha.-D-Galactopyranoside,methyl
45	16.724	152559	0.35	Dodecylpropylether
46	16.850	264851	0.61	1,1-DIMETHYL-1, 2, 3, 5, 7, 8, 9, 9A-OCTAHYDRO-6H-BEN
47	17.281	126549	0.29	Benzeneaceticacid,.alpha.-hydroxy-, 2-methylpropylester
48	17.591	437757	1.00	9-OCTADECENOICACID(Z)-
49	17.735	89979	0.21	2, 3-Diazatetracyclo[3.3.0.0(4, 7).0(6, 8)]octane-3-carboxylic
50	17.854	94463	0.22	6-Hydroxy-4, 4, 7a-trimethyl-5, 6, 7, 7a-tetrahydrobenzofuran-2
51	18.380	82398	0.19	Succinicacid, 3-methylbut-2-en-1-yl3-methoxyphenylester
52	18.513	55950	0.13	Butanoicacid, 3-hydroxy-4-(phenylthio)-,methylester
53	18.715	60772	0.14	1,2-BENZENEDICARBOXYLICACID, BIS(2-METHYLP
54	18.794	67493	0.15	Octyl-.beta.-D-glucopyranoside
55	19.214	96067	0.22	7, 9-Di-tert-butyl-1-oxaspiro(4, 5)deca-6, 9-diene-2, 8-dione
56	19.257	40192	0.09	3-SEC-BUTYL-4-(2, 2, 3, 3-TETRAMETHYLCYCLOPROP
57	19.685	678839	1.56	n-Hexadecanoicacid
58	20.497	47659	0.11	Palmitic Acid, TMS derivative
59	21.481	302352	0.69	MONOMETHYLMONOBUTYL "CAPPED" TETRAETH
60	21.602	112908	0.26	Octadecanoicacid
61	22.529	400642	0.92	Oxalicacid, 3, 5-difluorophenylnonylester
62	22.827	251804	0.58	Hexacontane
63	23.391	253458	0.58	13-[(1-Phenylethylimino) methyl] tricycle [8.2.2.24,7] hexade
64	24.138	273722	0.63	Tetrapentacontane
65	24.456	132630	0.30	Hexacontane
66	24.747	49978	0.11	Bis (2-ethylhexyl) phthalate
67	24.830	79039	0.18	3, 6-Undecandione
68	25.014	191304	0.44	OCTADECANE
69	25.192	821526	1.88	Octatriacontylpentafluoropropionate
70	25.450	1074554	2.46	Hexacontane
71	25.928	231946	0.53	HEXACONTANE
72	27.017	1049068	2.40	Tetrapentacontane
73	27.565	470585	1.08	Tetrapentacontane
74	27.968	212253	0.49	1-Bromodocosane
75	28.230	366387	0.84	OCTADECANE
76	28.463	222796	0.51	TETRAPENTACONTANE
77	28.697	154926	0.36	Fumaricacid, 2-ethylhexyltridec-2-yn-1-ylester
78	29.074	4360094	9.99	**Silane**, (1, 1-dimethylethyl) (dotriacontyloxy) dimethyl-
79	29.823	1334172	3.06	5-(7A-ISOPROPENYL-4, 5-DIMETHYLOCTAHYDRO-1H
80	30.491	351431	0.81	Heptadecane, 3-methyl-
81	31.498	636250	1.46	DOCOSANEDIOICACID, DIMETHYLESTER
82	31.975	459489	1.05	Hexacontane
83	34.592	812464	1.86	TETRAPENTACONTANE
84	39.672	1683419	3.86	COLENSANONE
		43637757	100.00	

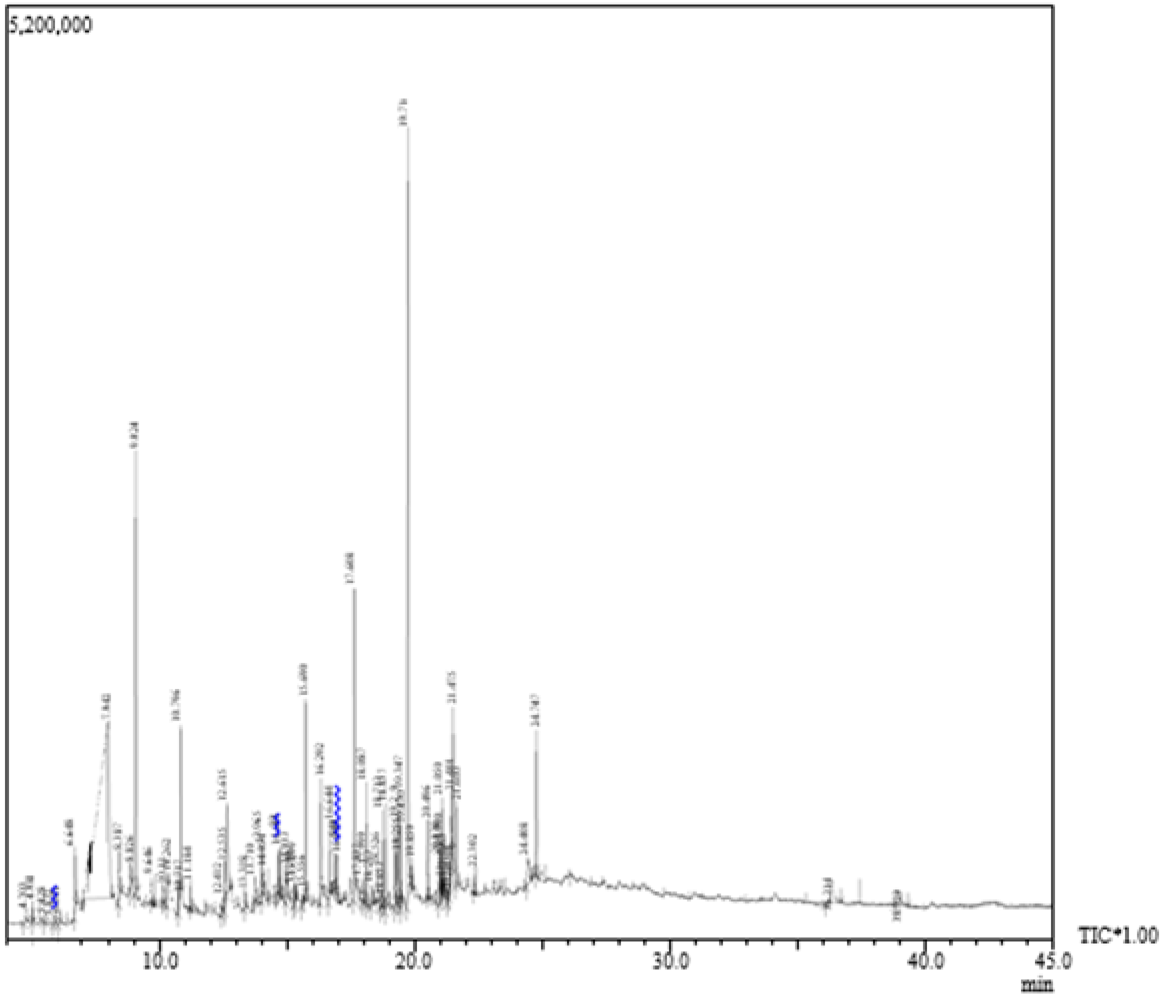

Fig 2: Shows GC-MS Chromatogram of methanolic galled flowers of *Crataeva religiosa*

Table 3: Compounds identified from methanolic extract of flower gall of *Crataeva religiosa* using GC-MS analysis.

Peak#	R. Time	Area	Area%	Name
1	4.703	114820	0.18	BUTANOIC ACID, 3-METHYL-
2	4.981	133721	0.21	Butanoicacid, 3-hydroxy-, methylester,(S)-
3	5.428	61312	0.10	1, 3-Dioxan-5-ol
4	5.832	92939	0.15	2(3H)-FURANONE, DIHYDRO-
5	6.041	90446	0.14	2-Cyclopenten-1-one, 2-hydroxy-
6	6.648	544813	0.85	ETHANOL, 2, 2'-OXYBIS-
7	7.942	23401416	36.64	**Glycerin**
8	8.387	493513	0.77	2-Pyrrolidinone
9	8.826	280358	0.44	1, 2, 3-Propanetriol, 1-acetate
10	9.024	3218504	5.04	2, 5-DIHYDRO-1H-PYRROLE
11	9.646	286423	0.45	4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-
12	10.112	124971	0.20	Pyrrolidine-5-one, 2-[3-hydroxypropyl]-
13	10.262	149425	0.23	ETHANOL, 2-(2-BUTOXYETHOXY)-
14	10.717	105790	0.17	Divinylsulfide
15	10.796	1199059	1.88	L-Proline,1-acetyl-
16	11.184	211853	0.33	Benzaldehyde, 4-(1-methylethyl)-
17	12.412	106282	0.17	Glycerol1, 2-diacetate
18	12.535	253177	0.40	DIMEROF.BETA.-HYDROXYBUTYRICACID,METH
19	12.615	1997981	3.13	**Acexamicacid**
20	13.318	167776	0.26	3-Methoxy-4-[3-oxo-3-(pyrrolidin-1-yl)propoxy]benzaldehy
21	13.710	216235	0.34	N-Propionylglycine, TMS derivative
22	13.965	311499	0.49	Dimethylphthalate
23	14.054	223573	0.35	METHYL4-HYDROXYBENZOATE
24	14.623	296710	0.46	1,4-Benzenedicarboxylicacid, dimethylester
25	14.695	301717	0.47	Phenol, 3, 5-bis(1, 1-dimethylethyl)-
26	14.933	185466	0.29	(3S, 3aR, 3bR, 4S, 7R, 7aR)-4-Isopropyl-3, 7-dimethyloctahydr
27	15.240	163508	0.26	Fumaricacid,di(3-(2-methoxyethyl)heptyl) ester
28	15.299	175638	0.27	Dodecanoicacid
29	15.556	131440	0.21	2H-3, 9a-Methano-1-benzoxepin, octahydro-2, 2, 5a, 9-tetrame

Peak#	R. Time	Area	Area%	Name
30	15.690	1368021	2.14	DiethylPhthalate
31	16.292	914039	1.43	METHANONE, DIPHENYL-
32	16.644	692729	1.08	8-Pentadecanone
33	16.844	257806	0.40	BICYCLO[3.1.0]HEX-3-EN-2-ONE, 4-METHYL-1-(1-ME
34	16.933	422871	0.66	Agarospirol
35	17.608	2467608	3.86	Tetradecanoicacid
36	17.837	148678	0.23	Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6, 6-dimethyl-
37	17.999	290926	0.46	Tetradecanoicacid, 12-methyl-, methylester, (S)-
38	18.087	995560	1.56	DIMEROF.BETA.-HYDROXYBUTYRICACID, METH
39	18.293	207978	0.33	1H-Benzocyclohepten-7-ol, 2, 3, 4, 4a, 5, 6, 7, 8-octahydro-1, 1, 4
40	18.526	389862	0.61	TETRADECANOICACID, TRIMETHYLSILYLESTER
41	18.653	79989	0.13	PENTADECANOICACID
42	18.713	641990	1.01	1, 2-Benzenedicarboxylicacid, bis(2-methylpropyl)ester
43	18.837	692855	1.08	8-Octadecanone
44	19.209	623954	0.98	7, 9-Di-tert-butyl-1-oxaspiro(4, 5)deca-6, 9-diene-2, 8-dione
45	19.255	264189	0.41	7, 9-Di-tert-butyl-1-oxaspiro(4, 5)deca-6, 9-diene-2, 8-dione
46	19.347	818269	1.28	HEXADECANOICACID, METHYLESTER
47	19.430	527750	0.83	Benzenepropanoicacid, 3, 5-bis(1, 1-dimethylethyl)-4-hydrox
48	19.716	9079430	14.22	n-Hexadecanoicacid
49	19.859	177619	0.28	Phthalicacid, 5-methylhex-2-ylbutylester
50	20.496	643616	1.01	Palmitic Acid, TMS derivative
51	20.930	340790	0.53	n-Nonadecanol-1
52	20.990	220413	0.35	9, 12-Octadecadienoicacid, methylester
53	21.050	651860	1.02	HEXADECADIENOICACID, METHYLESTER
54	21.103	107626	0.17	cis-10-Heptadecenoicacid, methylester
55	21.163	179313	0.28	2-HEXADECEN-1-OL, 3, 7, 11, 15-TETRAMETHYL-,[R-[R
56	21.283	65072	0.10	TETRADECANOICACID, METHYLESTER
57	21.340	101396	0.16	D7-DODECENE-1-OL
58	21.401	503995	0.79	cis-9-Hexadecenal
59	21.475	946906	1.48	MONOMETHYLMONOBUTYL "CAPPED" TETRAETH
60	21.610	798777	1.25	Octadecanoicacid
61	22.302	186551	0.29	Stearicacid, TMS derivative
62	24.408	515914	0.81	D-RIBOSE, 2-DEOXY-BIS(THIOHEPTYL)-
63	24.747	1009837	1.58	Bis(2-ethylhexyl)phthalate
64	36.314	856693	1.34	STIGMAST-5-EN-3-OL, (3.BETA.)-
65	38.929	637399	1.00	LUP-20(29)-ENE-3, 28-DIOL, (3.BETA.)-
		63868646	100.00	

Conclusion

The aim of the present study is to provide more information about the essential phytoconstituents of *C. religiosa*, the results from the present investigation are very encouraging and indicates that this plant should be studied more extensively to explore its potential to use as plant medicinal nutritive. Phytochemical investigation and GC-MS analysis of methanolic extract of *C. religiosa* showed the presence of carbohydrates, steroids, alkaloids, glycosides, flavonoids, tannins and saponins. The presence of various bioactive compounds confirms the application of *C. religiosa* for various ailments by traditional practitioners. Under stress condition glycerin is a compound which is not found in normal flower but in galled flower it is found in highest amount and can be exploited commercially. Acexamic acid is also found in galled flower, a drug used to treat gastrointestinal ulcers, protecting the mucosa of the stomach and intestine. However, isolation of individual phytochemical constituents may be useful in the pathway to find a novel drug.

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