International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 58-69
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Phytochemical Screening and GC-MS Analysis of Bio-Active Compounds in Ethanol Extract of *Crescentia cujete* Leaves

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Received: 12 Mar 2019 / Accepted: 29 Apr 2019 / Published online: 1 Jul 2019
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Abstract
Leaves of *Crescentia cujete* were collected, washed, shade dried and powdered. The ethanol extract was prepared using soxhlet apparatus and the phytochemicals were screened from this crude ethanol leaves extract of *Crescentia cujete*. The results showed the presence of secondary metabolites such as glycosides, flavonoids, phenols, alkaloids, phytosterols and tannins. Further the extracts were subjected to GC-MS for the identification of bioactive components present in the *Crescentia cujete* leaves. GC-MS analysis in the ethanol extract of *Crescentia cujete* leaves was done by using National Institute Standard and Technology (NIST) database 2005 to identify the compounds present. Thirty chemical constituents were identified. The results showed that the leaves containing a wide range of phyto constituents which could be exploited for the development of plant based novel drugs which may help to give treatment and protection from different kind of diseases.

Keywords
Crescentia cujete, Secondary metabolites, GC-MS, Bioactive phytocomponents

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INTRODUCTION
Medicinal plants play a very important role in protection of human health from ancient times and in modern culture it is reported that two-thirds of the world's plant species contain medicinal property [1]. Biochemical studies are important to explore more medicinal properties of the plants [2]. Many plants are potential sources of phytoconstituents such as volatile oils, steroids, alkaloids, flavonoids and other phenolic compounds, which have an integrated part of defence system against various diseases and stress conditions, so it can be utilized in the pharmaceutical industry [3]. Herbal medicine plays a central role among rural communities of developing countries for the provision of wellbeing in the absence of an efficient primary health care system [4]
The primary benefits of using plants derived medicines are that they are rich in secondary metabolites and relatively safer than synthetic alternatives to combat and cure various ailments [5]. Phytomedicines are derived from barks, leaves, flowers, roots, fruits, seeds and the knowledge of

chemical constituents of plants is important to provide value for synthesis of complex chemical substances [6]. Hence, Gas chromatography Mass spectroscopy (GC-MS) is a powerful detection technique with very high sensitivity and specificity for analysis of various volatile and semi-volatile compounds [7].

Crescentia cujete or calabash tree is a small tree belongs to the family *Binoniacea*. It grows about 6–10m tall with a wide crown and long branches covered with clusters of tripinnate leaves and gourd-like fruit and these branches are arranged as simple elliptical leaves clustered at the anode [8]. The leaves of the calabash tree are used to lower blood pressure, the tree bark is used to clean wounds and also to cure haematomas and tumours. Fruit decoction is used to treat diarrhoea, stomach-aches, cold, bronchitis, cough, asthma and urethritis [9].

A detailed investigation of other parts of *Crescentia cujete* tree have been reported to have medicinal uses but that of chemical components of leaves has not been well-documented [10]. and hence there is need for analysing the ethanolic *Crescentia cujete* leaf extract for separation and identification of the bioactive chemical compounds by using GC-MS analysis technique.

MATERIALS AND METHODS

Plant collection

The leaves of *Crescentia cujete* were collected from different localities of Coimbatore District and authenticated by Botanical Survey of India (BSI), Southern Regional Centre, Tamil Nadu Agricultural University campus, Coimbatore. A voucher specimen (No: BSI/SRC/5/23/2017/Tech 2021) has been deposited at the Herbarium of the Botany department.

Preparation of plant extracts

The leaves were cleaned, and shade dried for 6 days, then ground well to fine powder. About 500 g of dry powder was extracted with ethanol (80%) at 70°C by continuous hot percolation using soxhlet apparatus. The extraction was continued for 48 hours. The ethanolic extract was then filtered and kept in hot air oven at 40°C for 24 hours to evaporate the ethanol from it. A greenish brown residue was obtained. The residue was collected in airtight containers and stored in a deep freezer for further use.

Preliminary phytochemical screening

Preliminary phytochemical tests of ethanolic leaf extract of *Crescentia cujete* was carried out as described [11, 12, 13].

Alkaloids

Dragendroff's test (Kraut reagent Potassium bismuth iodide)

8 g of Bi $(NO)_3$ 5 H_2O was dissolved in 20 ml of HNO_3 and 2.72 g of potassium iodide in 50 ml of distilled water separately. They were mixed and allowed to stand till KNO_3 got crystallized. The supernatant was decanted and made up to 100 ml with distilled water. By treating the precipitate with Na_2CO_3 the alkaloids were regenerated followed by extraction of the liberated base with ether. To 0.5 ml of ethanol extract and 2 ml of HCl were added. Then 1ml of reagent was added to this acidic medium. An orange red precipitate was produced immediately, which indicated the presence of alkaloids.

Wagner's reagent (Iodine-Potassium iodide solution)

1.2 g of iodine and 2.0 g of potassium iodide were dissolved in 5 ml of H_2SO_4 and the solution was diluted to 100 ml. 10 ml of plant extract was acidified by adding 1.5% HCl and a few drops of Wagner's reagent. The formation of a yellowish-brown precipitate confirmed the presence of alkaloids.

Meyer's reagent (Potassium mercuric iodide)

1.36 g of mercuric chloride was dissolved in 60 ml of distilled water and 5 g of potassium iodide in 10 ml of water separately. Both solutions were mixed and diluted to 100 ml with distilled water. A few drops of the reagent were added to 1 ml of the leaf extract. The formation of a pale precipitate showed the presence of alkaloids.

Flavonoids

In a test tube containing 0.5 ml of plant extract, 5-10 drops of diluted HCl and a small pinch of zinc or magnesium were added, and the solution was boiled for a few minutes. In the presence of flavonoids, a reddish pink or dirty brown colour was produced.

Carbohydrates

Fehling's test

Solution A: 34.65 g of copper sulphate was dissolved and made up to 500 ml with distilled water.

Solution B: 125 g of potassium hydroxide and 173 g of Rochelle's salt (sodium potassium tasrtarate) and made upto 500 ml of distilled water.

The solutions 'A' and 'B' were added into the test samples. The contents were boiled for a few minutes. The formation of a red or brick red precipitate indicated the presence of carbohydrates.

Benedict's test

173 g of sodium citrate and 100 g of sodium carbonate were dissolved in 500 ml of distilled water. 17.3 g of copper sulphate dissolved in 100 ml of distilled water was added to the above solution. To 0.5 ml of plant extract, 5 ml of Benedict's reagent

was added and boiled for 5 min. The formation of a bluish green colour showed the presence of carbohydrates.

Proteins

Millon's test

One part of mercury was digested with 2 parts of concentrated HNO_3 and the resulting solution was diluted with 2 volumes of water. To a small quantity of plant extract, 5-6 drops of Millon's reagent was added. A white precipitate which turned red on heat indicated the presence of proteins.

Phenols

One ml of plant extract, 2 ml of distilled water followed by a few drops of 10 % aqueous $FeCl_3$ solution was added. Formation of a blue or green precipitate indicated the presence of phenols.

Lead acetate test

One ml of ethanol leaf extract was diluted to 5 ml with distilled water and then a few drops of 1% aqueous solution of lead acetate was added. Appearance of yellow precipitate indicated the presence of phenols.

Liebermann's test

A small amount of plant extract was dissolved in 0.5 ml of 20 % sulphuric acid solution followed by the addition of a few drops of aqueous sodium nitrate solution. A red colour was obtained on dilution and it turned blue when made alkaline with aqueous sodium hydroxide solution, which indicated the presence of phenol.

Saponins

In a test tube containing about 5 ml of plant extract, a drop of sodium bicarbonate solution was added. The mixture was shaken vigorously and kept for 3 min. Honeycomb like stable froth formation showed the presence of saponins.

Tannins

Ferric chloride test

Two ml of plant extract, a few drops of 5 % aqueous FeCl3 solution was added. A bluish black colour was formed, which then disappeared and addition of few ml of dilute H_2SO_4 formed yellowish brown precipitate.

Lead acetate test

In a test tube containing about 5 ml of plant extract, a few drops of 1 % solution of lead acetate was added. The presence of tannins was indicated by the formation of yellow or red precipitate.

Phytosterols

About 0.5 ml of test solution was mixed with minimum quantity of chloroform and the 3-4 drops of acetic acid and one drop of concentrated H_2SO_4 were added. Formation of a deep blue or green colour showed the presence of steroids.

Terpenoids

5 ml of plant extract was mixed in 2 ml of chloroform; 3 ml of concentrated H_2SO_4 was added to form a layer. A reddish-brown precipitate was formed which indicates the presence of terpenoids.

Glycosides

Salkowski's Test. We added 2 ml H_2SO_4 concentrated to the whole aqueous plant crude extract. A reddish brown color formed which indicated the presence of steroidal aglycone part of the glycoside.

Liebermann's Test. We added 2.0 ml of acetic acid and 2 ml of chloroform with whole ethanol plant crude extract. The mixture was then cooled, and we added H_2SO_4 concentrated. Green color showed the entity of aglycone, steroidal part of glycosides.

Resins

To 2.0ml of sample extract, 5-10ml of acetic anhydride was added, dissolved by gently heating, cooled and then 0.5ml of H_2SO_4 was added. A bright purple colour rapidly changing into violet indicating the presence of resins.

Tannins

5 ml of bromine water was added to the 1.0 ml of ethanol extract. Decoloration of bromine water showed the presence of tannins.

Thiols

To about 0.5ml of sample extract, enough ammonium sulphate was added. To saturate the solution, 2-4 drops of 5% sodium nitroprusside was then added followed by one or more drops concentrated nitric acid. A transient magenta colour develops in the presence of thiols.

GC-MS ANALYSIS

30 g powdered sample of *Crescentia cujete* were soaked and dissolved in 75 ml of ethanol for 24 hrs. Then the filtrates were collected by evaporated under liquid nitrogen. GC- MS analysis of the *Crescentia cujete* leaves extract was performed by using the equipment Thermo GC Trace Ultra Version: 5.0, Thermo MS DSQII. The equipment has a DB 35 - MS Capillary Standard non-polar column with dimensions of 30 mm ×0.25 mm ID ×0.25 µm film. The carrier gas used is Helium with at low of 1.0 ml/min. The injector was operated at 250°C and the oven temperature was programmed as follows: 60ºC for 15 min, then gradually increased to 280°C at 3 min. The identification of components was based on Willey and NIST libraries as well as comparison of their retention indices.

RESULTS AND DISCUSSSION

Phytochemical Screening

Preliminary qualitative phytochemical analysis of ethanolic extract of *Crescentia cujete* leaves are

ISSN: 2230-7605 (Online); ISSN: 2321-3272 (Print)
Int J Pharm Biol Sci.

shown in (Table: 1) and it revealed the presence of alkaloids, carbohydrates, glycosides, phytosterols, tannins, phenols, proteins, flavonoids and absence of saponins, fixed oils and fats. These secondary metabolites protect the plants to overcome temporary or continuous threats integral to their surroundings, which is useful to the human for medical purposes [14]. The medicinal plants contributing towards the ethno medicine have been broadly screened for their phytochemicals including alkaloids, tannins, saponins, steroid, terpenoid, flavonoids, phenols and cardic glycoside [15]. They are related with protection and treatment of chronic diseases such as heart disease, cancers, diabetes, neurodegenerative disease and hypertension and also other medical conditions [16].

Table 1: **Preliminary phytochemical screening of *Crescentia cujete* leaves.**

Phytochemical Constituents	Indication
Carbohydrates	+
Proteins	+
Oils and fats	_
Phytosterols	+
Thiols	+
Alkaloids	+
Flavonoid	+
Phenols	+
Saponins	_
Glycosides	+
Tannins	+

"+" Present "–" Absent

GC-MS analysis

GC-MS analysis of plant material play an important role in the development, modernization, quality control of herbal formulations and understanding the nature of medicinal properties the results pertaining to GC-MS analysis of the ethanolic extract of *Crescentia cujete* lead to the identification of a number of bioactive components as shown in Figure 1. GC-MS analysis of the ethanolic extract of *Crescentia cujete* showed the presence of thirty compounds. The active principles with their retention time, molecular formula, molecular weight and peak area as a percentage are presented in Table 2 and Table 3 shows the mass spectrum and structure of the compounds

Identification of compounds

Interpretation of mass spectrum of GC – MS was done using the database of National Institute Standard and Technology (NIST4) and WILEY9. The spectrum of the unknown component was compared with the spectrum of the known components stored in the inbuilt library.

Figure 1: GC-MS chromatogram of ethanolic extract of *Crescentia cujete leaves*

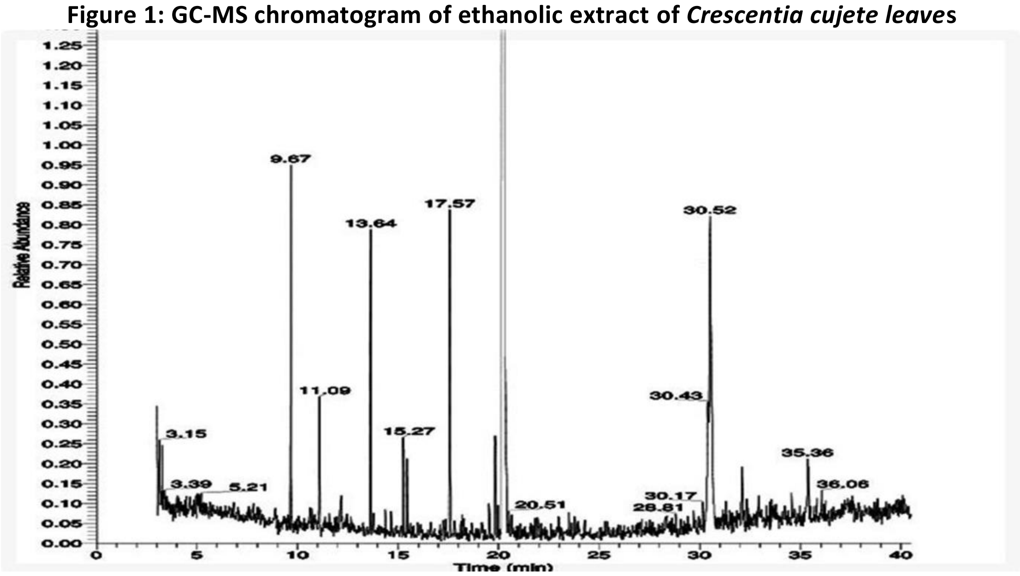
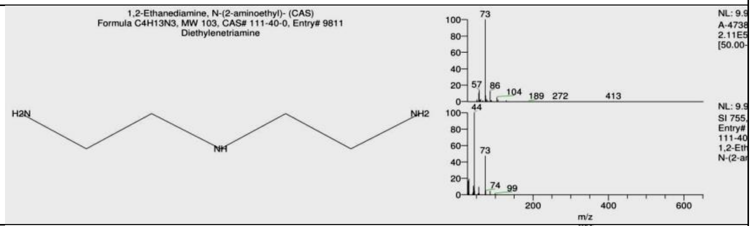
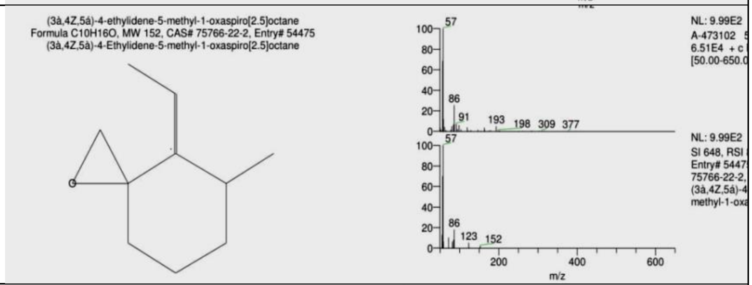
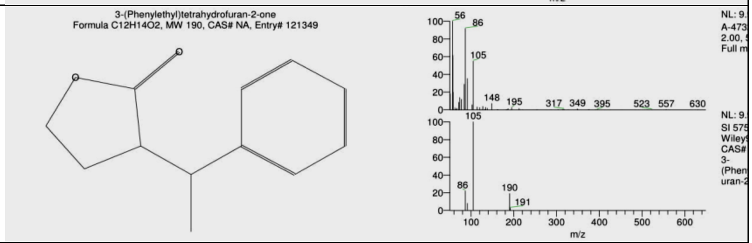
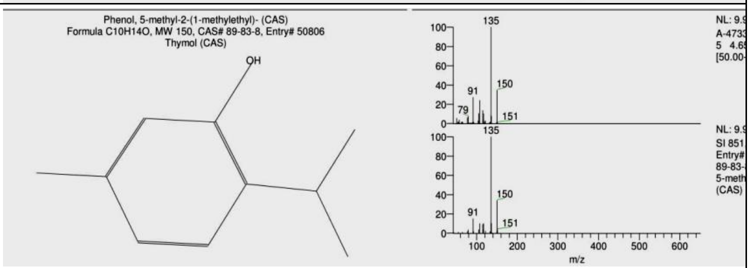
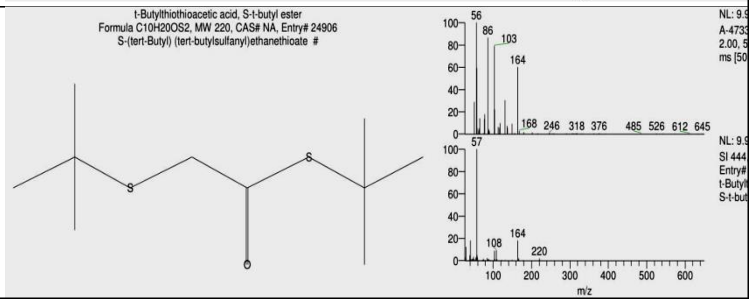
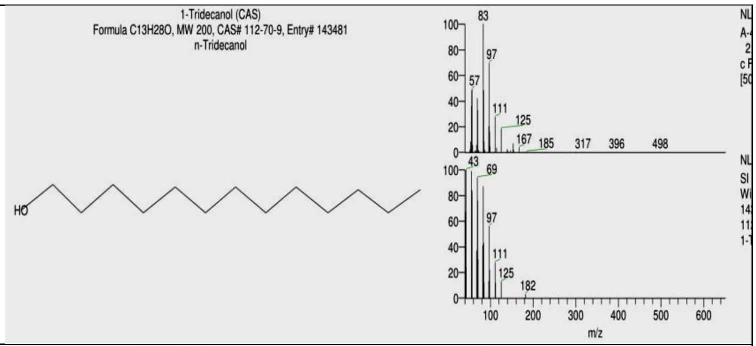
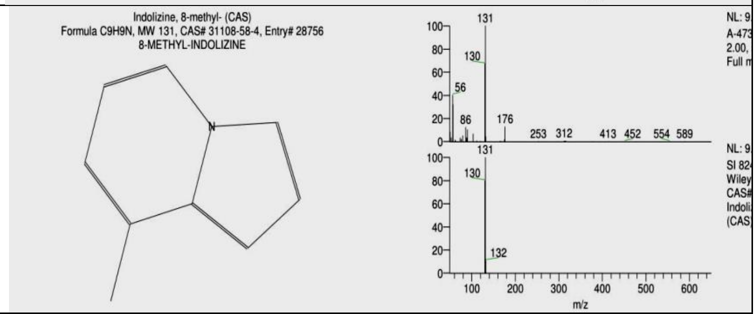
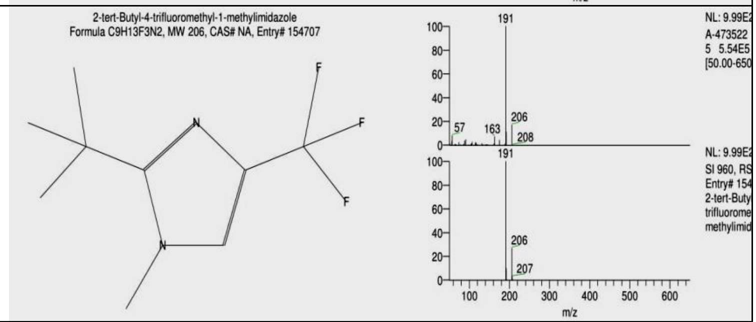
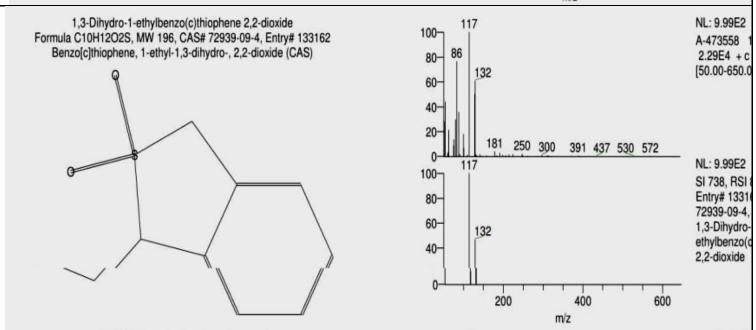
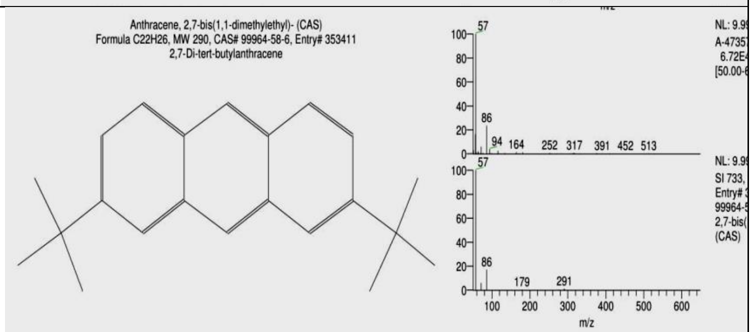


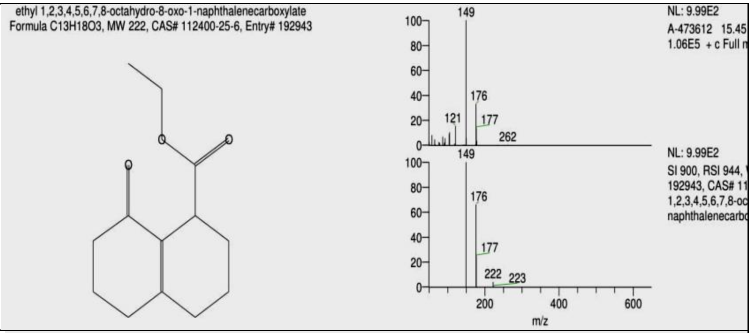
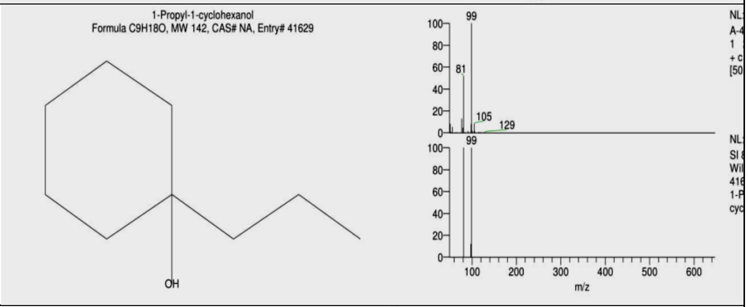
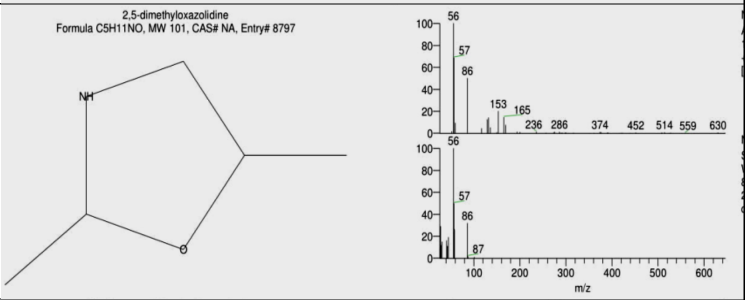
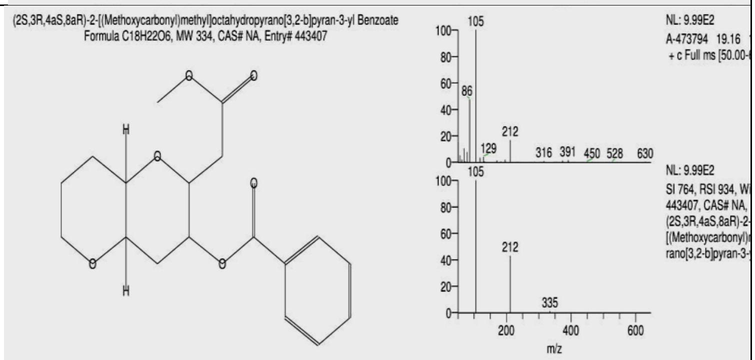
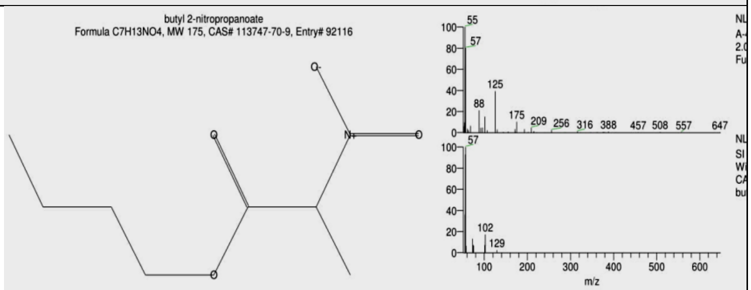
Table 2*: *Bioactive compounds identified in ethanolic extract of *Crescentia cujete* leaves

S.No	RT (min)	Name of compound	Molecular formula	MW	Peak area%
1.	3.15	1,2-Ethanediamine	$C_4H_{13}N_3$	103	0.02
2.	5.06	(3à,4Z,5á)-4-ethylidene-5-methyl-1-oxaspiro [2.5]octane	$C_{10}H_{16}O$	152	0.07
3.	8.89	3-(Phenylethyl) tetrahydrofuran-2-one	C_{12} $H_{14}O_2$	190	3.05
4.	9.67	Phenol, 5-methyl-2-(1-methylethyl)-	$C_{10}H_{14}O$	150	0.34
5.	10.62	t-Butylthiothioacetic acid, S-t-butyl ester	$C_{10}H_{20}OS_2$	220	0.03
6.	11.09	1-Tridecanol	$C_{13}H_{28}O$	200	1.15
7.	12.15	Indolizine, 8-methy	C_9H_9N	131	0.07
8.	13.62	2-tert-Butyl-4-trifluoromethyl-1-methylimidazole	$C_9H_{13}F_3N_2$	206	0.44
9.	14.35	1,3-Dihydro-1-ethylbenzo(c)thiophene,2,2-dioxide	$C_{10}H_{12}O_2S$	196	0.02
10.	14.66	Anthracene,2,7-bis(1,1-dimethylethyl)-	$C_{22}H_{26}$	290	0.03
11.	15.45	ethyl1,2,3,4,5,6,7,8-octahydro-8-oxo-1-naphthalenecarboxylate	$C_{13}H_{18}O_3$	222	0.15
12.	17.57	1-Propyl-1-cyclohexanol	$C_9H_{18}O$	142	0.34
13.	18.22	2,5- dimethyloxazolidine	$C_5H_{11}NO$	101	3.07
14.	19.16	Methoxycarbonyl) methyl]octahydropyrano[3,2-b]pyran-3-yl Benzoate	$C_{18}H_{22}O_6$	334	0.03
15.	19.53	butyl 2-nitropropanoate	$C_7H_{13}NO_4$	175	1.56
16.	19.83	Tridec-2-en-11-ynedial	$C_{13}H_{18}O_2$	206	0.19
17.	20.26	Isopropyl myristate	$C_{17}H_{34}O_2$	270	95.97
18.	22.98	Di(endo-3-camphoryl) Ditelluride	$C_{20}H_{30}O_2Te_2$	562	0.04
19.	23.50	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	2.05
20.	23.95	Xycaine	$C_{14}H_{22}N_2O$	234	0.05
21.	29.70	3-Pentanone	$C_5H_{10}O$	85	0.04
22.	30.52	5,8-Dibromo-7-methoxy-3-methoxycarbonylpyrimido [1,6- a] indole	$C_{14}H_{10}Br_2N_2O_3$	412	1.23
23.	32.10	4-n-Butylbenzopyran-4-ol	$C_{13}H_{18}O_2$	206	0.09
24.	32.94	Hexanoic acid, 4-methyl	$C_7H_{14}O_2$	130	0.06
25.	33.71	2-(-N, N-Di-isopropylaminomethyl)-1-methylpyrrole	$C_{12}H_{22}N_2$	194	0.07
26.	34.59	1-(4-(2-methoxyethyl) phenoxy)-3-(N-methyl-N-isopropylamino) propan-2-ol	$C_{16}H_{27}NO_3$	281	0.04
27.	35.38	Azidophenylacetoamide	$C_8H_8N_4O$	176	0.15
28.	35.79	5a,8-Dimethyl-9-phenyl-5a,6-dihydronaphtho[3,2,1-kl] xanthen-6-ol	$C_{28}H_{22}O_2$	390	0.03
29.	36.06	(3S)-(3-2H1)-2,2-Dimethylcyclobutyl acetate	$C_8H_{13}DO_2$	142	0.08
30.	40.17	Eicosane, 2-cyclohexyl	$C_{26}H_{52}$	364	0.07

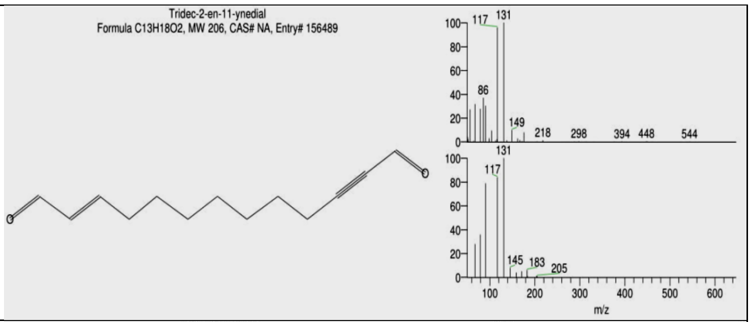
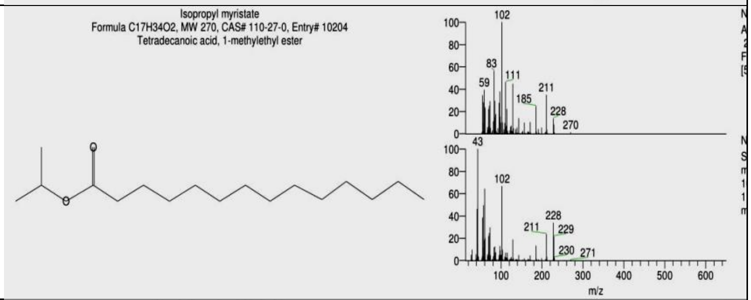
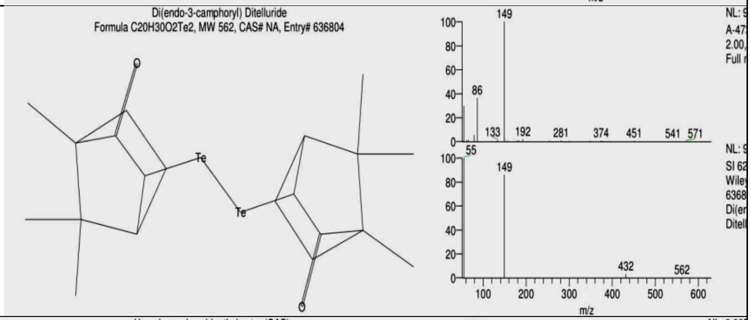
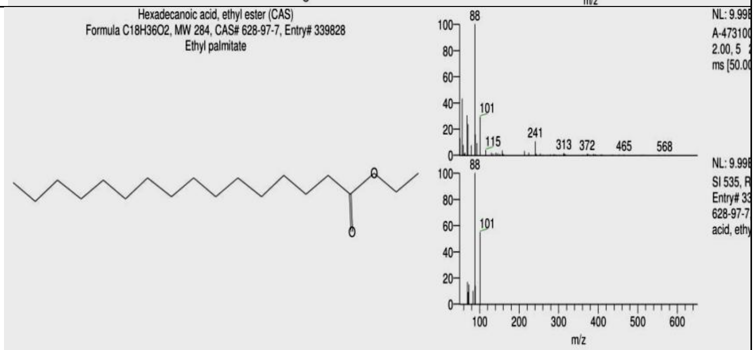
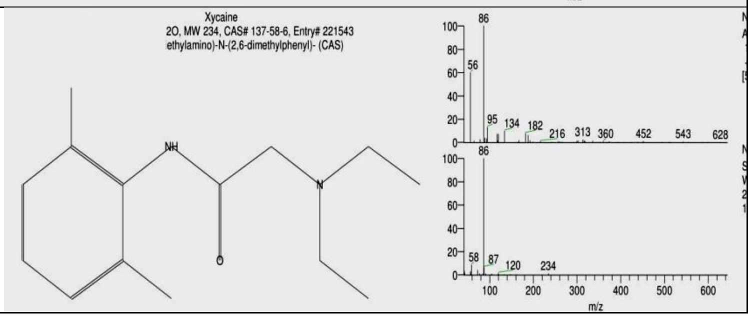
Table 3: Mass spectrum and structure of the bioactive compounds in ethanolic extract of *Crescentia cujete* leaves

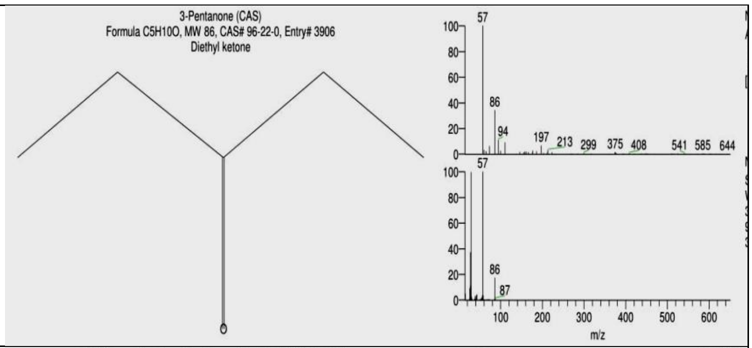
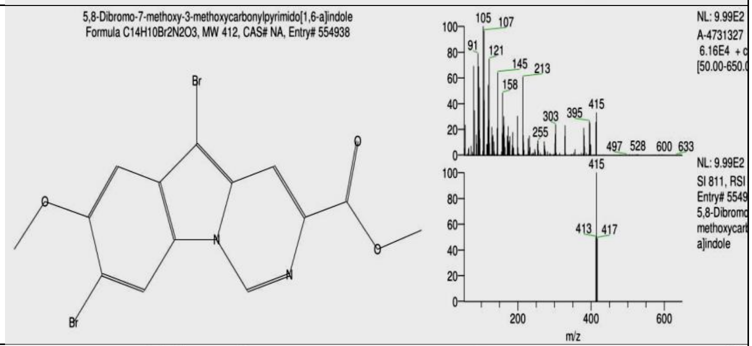
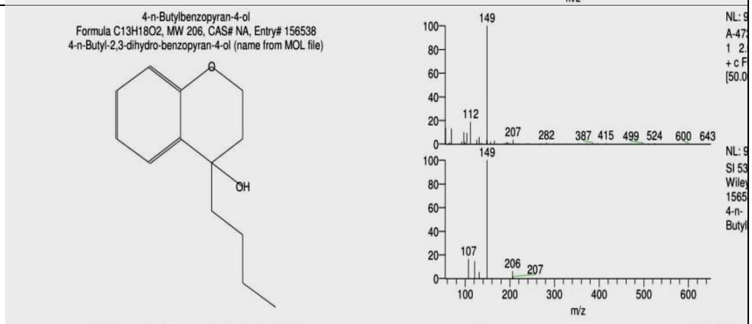
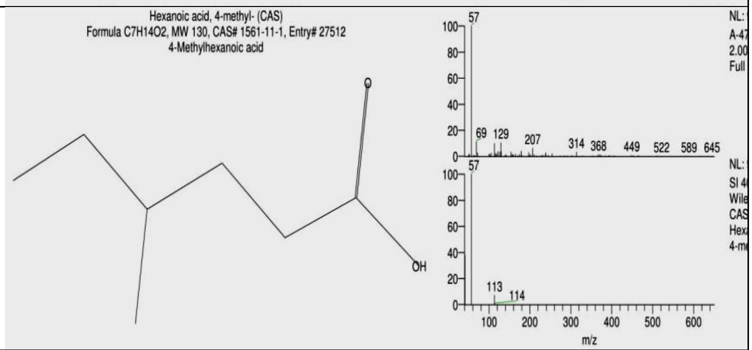
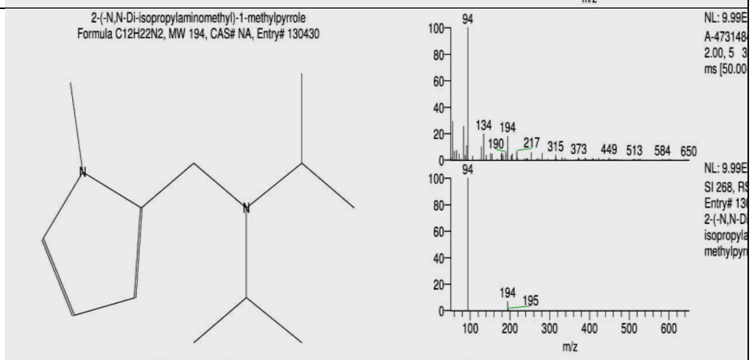
S.No	Name of the compound	Spectrum and structure of the compound
1	1,2-Ethanediamine, N-(2-aminoethyl)	
2	(3à,4Z,5á)-4-ethylidene-5-methyl-1-oxaspiro[2.5]octane	
3	3-(Phenylethyl)tetrahydrofuran-2-one	
4	Phenol, 5-methyl-2-(1-methylethyl)-	
5	t-Butylthiothioacetic acid, S-t-butyl ester	

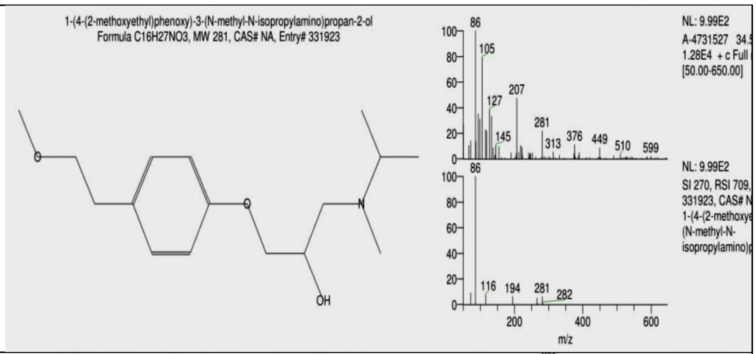
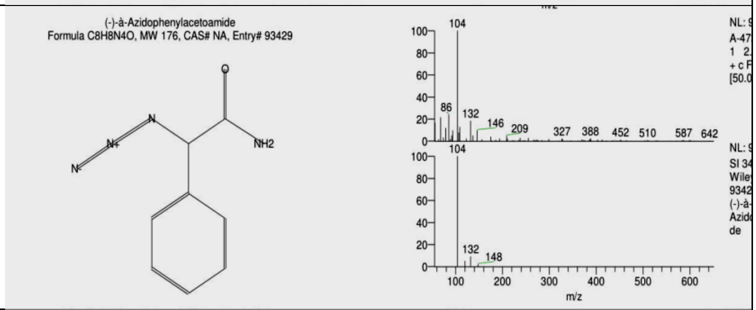
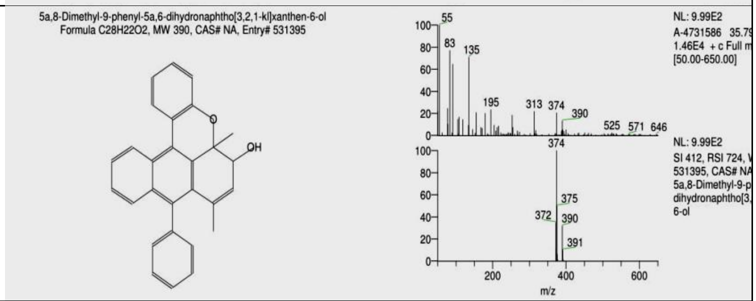
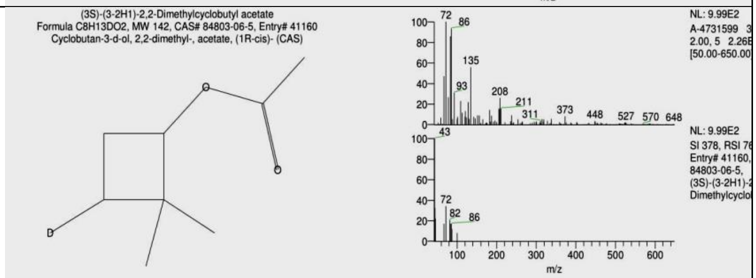
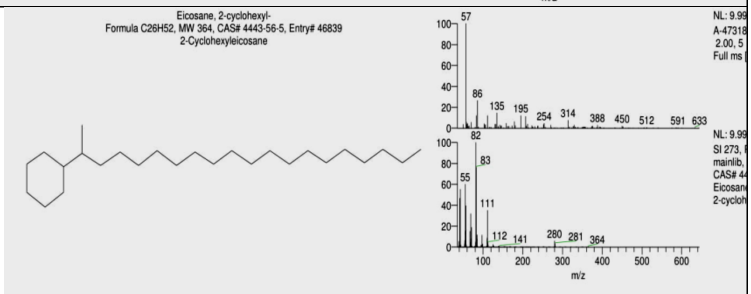
6	1-Tridecanol	
7	Indolizine, 8-methy	
8	2-tert-Butyl-4-trifluoromethyl-1-methylimidazole	
9	1,3-Dihydro-1-ethylbenzo(c)thiophene 2,2-dioxide	
10	Anthracene,2,7-bis(1,1-dimethylethyl)-	

11	ethyl1,2,3,4,5,6,7,8-octahydro-8-oxo-1-naphthalenecarboxylate	
12	1-Propyl-1-cyclohexanol	
13	2,5- dimethyloxazolidine	
14	(2S,3R,4aS,8aR)-2-[(Methoxycarbonyl)methyl]octahydrop yrano[3,2-b]pyran-3-yl Benzoate	
15	butyl 2-nitropropanoate	

ISSN: 2230-7605 (Online); ISSN: 2321-3272 (Print)
Int J Pharm Biol Sci.

16	Tridec-2-en-11-ynedial	
17	Isopropyl myristate	
18	Di(endo-3-camphoryl) Ditelluride	
19	Hexadecanoic acid, ethyl ester	
20	Xycaine	

21	3-Pentanone	
22	5,8-Dibromo-7-methoxy-3-methoxycarbonylpyrimido[1,6-a]indole	
23	4-n-Butylbenzopyran-4-ol	
24	Hexanoic acid, 4-methyl	
25	2-(-N,N-Di-isopropylaminomethyl)-1-methylpyrrole	

26	1-(4-(2-methoxyethyl)phenoxy)-3-(N-methyl-N-isopropylamino)prppan-2-ol	
27	Azidophenylacetoamide	
28	5a,8-Dimethyl-9-phenyl-5a,6-dihydronaphtho[3,2,1-kl]xanthen-6-ol	
29	(3S)-(3-2H1)-2,2-Dimethylcyclobutyl acetate	
30	Eicosane, 2-cyclohexyl	

The identified compounds occupy many biological properties. GC-MS analysis of phytoconstituents in plants gives a clear view of the pharmaceutical value of that plant. Thus, this type of GC-MS analysis is the first step towards understanding the nature of medicinal properties [17].

CONCLUSION

From the present research, the result confirms that the work is significant and preliminary qualitative phytochemical analysis revealed the presence of secondary metabolites which are reported to have many biological and therapeutic properties, so this plant is expected to have many medicinal uses. Thirty phytoconstituents have been identified from ethanolic extract by GC-MS analysis. Identification of these compounds in the plant serves as the basis in determining the possible medicinal benefits of the plant leading to further biological and phytopharmaceutical studies.

Acknowledgement

The authors are greatly thankful to the South Indian Textile and Research Association (SITRA), Coimbatore, Tamil Nadu for providing laboratory support.

Conflict of interest

There is no conflict of interest.

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