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Phytochemical Studies on *Ipomoea sepiaria* Roxb

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Abstract: *Ipomoea sepiaria* Roxb., also known as Purple heart glory is a slender vine belonging to family Convolvulaceae. In Sanskrit it is known as lakshmana, in hindi as Bankalmi and in Malayalam as Tirutali. It is one among the ten sacred plants known collectively as "Dasapushpam" in Kerala. These plants are used for rejuvenating the body in the form of 'karkidakakanji' in the monsoon season in Kerala which is also prescribed in text books of ayurveda. Juice of the plant is used as deobstruent, diuretic, hypotensive, uterine tonic and antidote to arsenic poisoning. The present study aims at the preliminary phytochemical screening of the successive solvent extracts of *Ipomoea sepiaria* Roxb. and identification of compounds in the Hexane extract by Gas Chromatography- Mass Spectrometry technique. The preliminary phytochemical screening revealed the presence flavanoids, tannins, flavanoids, terpenoids, steroids, sterols, cardiac glycosides and carbohydrates. The GC-MS analysis revealed the presence of the compounds Caryophyllene, α –Curcumene, β –Cadinene, ϒ –Elemene, Caryophyllene oxide, Asarone, Germacrone, Methyl isopimarate, Abietic acid, Lupeol, β –Amyrin and α –Amyrin which have not been so far reported in *Ipomoea sepiaria.* The higher percentages of compounds α-amyrin(33.341) β –Amyrin(16.28) which form the basic skeleton of triterpenoid saponins are promising.

Keywords: Ipomoea sepiaria, Dasapushpam, Tirutali, Phytochemical , GC-MS, β –Amyrin and α –Amyrin.

INTRODUCTION

Nature is a master craftsman of molecules and have created an inexhaustible array of molecular entities. Plants serve as a rich source for drug development, novel chemotypes and pharmacophores, and scaffolds for amplification into potent drugs for an array of disease indications and a variety of bioactive agents which are valuable. Since ancient times, plant based products have been the backbone of traditional system of healing throughout the world and have also been an integral part of history and culture[1].

One of the ancient treatment systems, Ayurveda, whose history can be traced back to 5000 b.c.. The Ayurvedic treatment methodology was developed through day today life experiences and with the mutual relationship between human beings and nature. The ancient text books of Ayurveda like Charaka Samhita reports that more than 2000 plant species are used for their therapeutic potentials. Other traditional and folklore systems of healing and health care were practiced and developed in the different times in Indian subcontinent, where more than 7500 plants were used. The plants referred in the ancient texts of Ayurveda like Charaka Samhita and other systems may be explored with the developed scientific approaches for better heights in the health care system[2]. India is having a rich vegetation with a wide variety of plants, because of the extreme variations in geographical and climatic conditions prevailing in the country. The efficacy and safety of all the reported ethnomedical plants needs to be evaluated for phytochemical and pharmacological studies, especially the plants with high informant consensus factor, fedility level and use value should be given priority to carryout bioassay and toxicity studies[3].

Ipomoea sepiraia Roxb. also known as Purple heart glory is a slender vine belonging to family Convolvulaceae. The plant is found on sea costs and on saline soil. In Sanskrit it is known as lakshmana, in hindi as Bankalmi and in Malayalam as Tirutali. It is one among the ten sacred plants known collectively as "Dasapushpam" in Kerala. These plants are used for rejuvenating the body in the form of karkidakakanji in the monsoon season in kerala which is also prescribed

in text books of ayurveda. This plant preparation is useful in preventing the aggravation of vata dosha which usually occurs in the monsoon. Most of the plants are scientifically validated. Juice of the plant is used as deobstruent, diuretic, hypotensive, uterine tonic, antidote to arsenic poisoning. The plant is reported to show aphidicidal activity and appeared to be useful as pesticides. Seeds are used as cardiac depressant, hypotensive, spasmolytic. Plant is also used in the treatment of sterility in women, urinary retention, constipation and gynaecological disorders. Ipomoea resin in the seeds contain non-ergoline type indole alkaloids, ipobscurine A & B, and a serotonin alkaloid ipobscurines C[4].

Ipomoea sepiraia Roxb. is a glabrous or occasionally pubescent,slender twinning with a slightly thickened or tuberous perennial root and a very short stem producing annually or seasonally a number of villous, grayish purple branches bearing simple, cordate or ovate, variable mediam sized leaves, very often blotches with dull purplish patches in the centre and pink to purplish flowers in clusters on fairy long thickened clavate peduncles . Leaves are simple alternate, entire, blotched with brownish patches towards the middle . Flowers are delicate purple or white with a purple eye, along with short to long peduncles and short pedicels. The root system consists of a fairly long, somewhat thickened taproot and several slightly thinner or slender branches, arising from its base with very few wiry rootlets[5].

The phytochemical evaluation of roots of *Ipomoea sepiaria* for the presence of various functional groups revealed the presence of alkaloids, saponins, phenols and resin[6]. The methanolic extract of roots found to possess significant antifungal activity against *Candida albicans* substantiating the use of root in the treatment of leucorrhoea[7]. Gas Chromatography and Mass Spectrometry of ethanolic extract of the leaves of *Ipomoea sepiaria* confirmed the presence of 20 compounds and the main ones are: Stigmasterol, Sitosterol and Hexatriacontane[8]. Molecular docking studies were carried out to identify the best bioactive compound among 32 compounds separated by GC-MS analysis against type 2 diabetes. Out of 32 compounds 1-Monolinoleoyglycerol trimethyl silylether was identified as the best based on energy values by targeting transcription factor7-like 2(TCFL2) gene which is responsible for Type 2 diabetes[9]. The flavanoid Quercetin was estimated from *Ipomoea sepiaria* by RP-HPLC method[10]. An antioxidant principle Dodecyl-p-coumarate was isolated from methanolic extract of *Ipomoea sepiaria*[11]. The powdered leaves and extracts of Bankalmi as a 3% mixture provided good protection for black gram seeds by reducing insect oviposition, adult emergence and grain infestation rates[12].

The present study aims at conducting a preliminary phytochemical screening of *Ipomoea sepairia* Roxb. and combined Gas Chromatography-Mass Spectometry to identify the presence of compounds in the hexane extract.

MATERIALS AND METHODS

The plants were collected from Thodupuzha part of Idukki District, Kerala. The plant was identified at Department of Botany, Nirmala College of Arts and Sciences, Muvattupuzha, Kerala and a voucher specimen (NCH 577) was kept there. The careful observations of collected plant are made according to the standard procedure[13]. The plants were washed properly and was dried in shade.

Successive solvent extraction

100 g of the dried powdered whole plant of *Ipomoea sepiaria* Roxb. was subjected to extraction using hot continuous percolation using Soxhlet apparatus. The extraction was carried out in the increasing order of polarity ie, Hexane, Chloroform, Ethyl acetate and Methanol.

Preliminary Phytochemical screening [14,15]

1.Test for flavonoids

0.1 g of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution and observed a yellow coloration.

2.Test for Tannins

0.1g of the extract was stirred with 10 ml of distilled water. This was filtered and ferric chloride reagent was added to the filtrate, a blue-black precipitate was taken as evidence for the presence of tannin.

3.Test for terpenoids

0.1g of the extract was mixed with 2ml of chloroform and concentrated H2SO4 (3ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive results of the presence of terpenoids.

4. Test for steroids

0.1 g of the extract was dissolved in 5 ml of methanol. 1 ml of the solution was treated with 0.5 ml of acetic acid anhydride and cooled in ice. This was mixed with 0.5 ml of chloroform and 1 ml of concentrated sulfuric acid was then added carefully by means of a pipette. At the separations level of the two liquids, a reddish-brown ring was formed, as an indication of the presence of steroids.

Test for Cardiac Glycosides: **Keller –killiani test**

0.5g of the extract was mixed with 5 ml of methanol. This solution was mixed with 2 ml of glacial

acetic acid containing one drop of ferric chloride (FeCl3) solution,followed by the addition of 1 ml concentrated sulfuric acid. Brown ring was formed at the interface which indicated the presence of deoxysugar of cardenolides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer.

6.Test for alkaloids

0.5 g of the extract was dissolved in methanol. Methanolic extract was warmed with 2% H2SO4 for two minutes. It is filtered and a few drops of Dragendorff's reagents were added and the red precipitate indicates the presence of alkaloids.

7. Test for Phenols

0.5g of the extract was dissolved in 5ml ofalcohol. To 1ml of the above solution added one drop of neutral ferric chloride (5%) solution. Formation of an intense blue color indicates the presence of phenols.

8.Test for sterols

0.5g of the extract was dissolved in minimum quantity of chloroform are added with 3-4 drops of acetic anhydride and one drop of concentrated Sulphuric acid. Formation of purple color changes into green color that indicates the presence of steroids.

9.Test for carbohydrates

To 0.5g of the etract few drops of Molisch reagent was added and mixed well. Followed by the addition of 1ml concentrated Sulphuric acid to form a layer below the aqueous solution. A brown ring is formed at the interface indicating a positive result.

10. Determination of amino acid

Ninhydrin test- To the extract add 0.25% ninhydrin reagent and boil for a few minutes. Formation of blue colour indicate presence of aminoacid.

GC-MS Analysis of the Hexane Extract

GC-MS analysis of the hexane extract was carried out by splitless injection of 0.2 µl of the sample on a Hewlett Packard 6890 gas chromatograph fitted with an HP-5 MS cross-linked 5% PH ME siloxane, 30 m x 0.32mm x 0.25µm capillary column, coupled with a model 5973 mass detector. GC-MS operation conditions: injector temeperature – 220^0C; transfer line -240^0C; oven temperature programme -60^0C – 243^0C (3^0C min^{-1}); carrier gas – He at 1.4 ml min^{-1}. Mass spectra: Electron Impact (EI^+) mode 70eV, ion source temperature 240^0C. Individual components were identified by Relative percentages of individual components in the extract were obtained from the peak-area percent report of volatiles from GC-MS data.

RESULTS

The results of the chemical tests of the hexane,methanol, ethyl acetate and chloroform extracts of *Ipomoea sepiaria* extracts are as follows.

Table-1: Phytochemical screening of *Ipomoea sepiaria* Roxb. extracts

SL.NO		HEXANE	METHANOL	ETHYL ACETATE	CHLOROFORM
1	Flavanoids	-ve	+ve	+ve	-ve
2	Tannin	-ve	+ve	-ve	+ve
3	Terpenoids	+ve	+ve	+ve	+ve
4	Steroids	+ve	+ve	+ve	+ve
5	Cardiac glycosides	-ve	+ve	+ve	+ve
6	Alkaloids	-ve	+ ve	+ ve	+ ve
7	Phenols	-ve	+ve	+ve	+ve
8	Sterols	+ve	-ve	-ve	-ve
9	Carbohydrate	-ve	+ve	+ve	+ve
10	Aminoacid	-ve	-ve	-ve	-ve

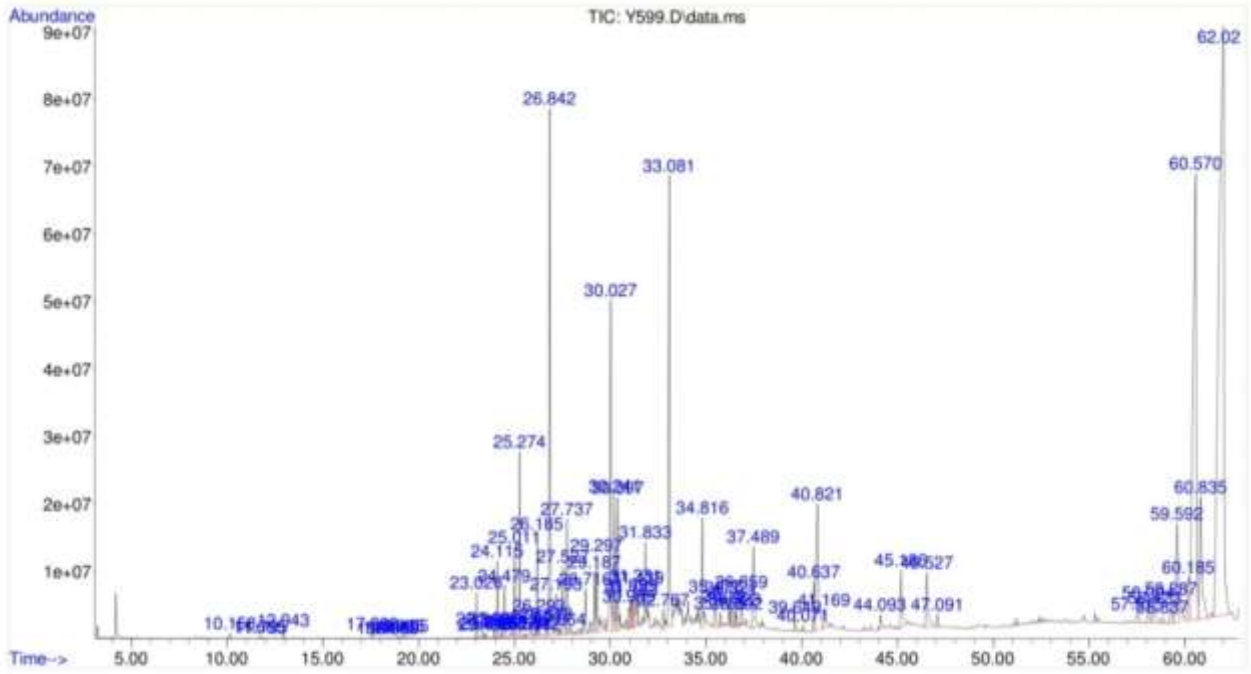


Fig-1: GC-MS Chromatogram of Hexane Extract of *Ipomoea sepiaria* Roxb.

The compounds in the Hexane extract are separated on the basis of Retention time and 50 compounds are identified by comparison of the Mass Spectra with the spectra in NIST Library. The major compound identified were Caryophyllene, α –Curcumene, β –Cadinene, ϒ –Elemene, Caryophyllene oxide, Asarone, Germacrone, Palmitic acid, 9,12-Octadecenoic acid, Methyl isopimarate, Abietic acid, Dioctyl phthalate Lupeol, β –Amyrin and α –Amyrin. The peaks and mass spectra indicating fragmentation pattern of compounds with highest percentages, α-amyrin(33.341) β –Amyrin(16.28) and α –Curcumene(5.103) are given.

Table-2: Percentage of components present in Hexane extract of *Ipomoea sepiaria* Roxb

Sl.No.	Retention time In min	Name of the compound	Percentage Content
1	10.16	α-pinene	0.040
2	11.79	β -pinene	0.015
3	12.94	3-Carene	0.071
4	18.150	Borneol	0.009
5	18.96	α-terpinol	0.028
6	23.02	α- elemene	0.364
7	23.34	α -cubebene	0.094
8	23.42	α- longipinene	0.032
9	23.54	α-Guaiene	0.038
10	23.94	Ylangene	0.080
11	24.11	Copaene	0.629
12	24.32	β -Bourbonene	0.047
13	24.48	β - Elemene	0.620
14	24.82	α -Cedrene	0.047
15	25.01	D- Longifolene	0.723
16	25.27	Caryophyllene	1.533
17	25.52	ϒ -Cadinene	0.024
18	25.59	α -Bergamotene	0.027
19	26.01	Seychellene	0.016
20	26.09	β -Farnesene	0.028
21	26.18	α- Caryophyllene	0.854
22	26.29	Allo-aromadendrene	0.181
23	26.68	ϒ -Muurolene	0.158
24	26.84	α -Curcumene	5.103
25	27.19	Elixene	0.475
26	27.74	β -Cadinene	1.036
27	27.52	β -Himachalene	0.892
28	28.72	ϒ -Elemene	0.572
29	29.19	Spathalenol	0.651
30	29.29	Caryophyllene oxide	0.839
31	30.03	Asarone	3.672
32	31.09	Ledene alcohol	0.431
33	31.16	Ar-Turmerone	0.264
34	31.83	Germacrone	0.783
35	35.47	Platambin	0.456
36	35.75	Corymbolone	0.138
37	37.48	Palmitic acid	1.353
38	39.65	n-Heptadecanol-1	0.169
39	40.01	Phytol	0.065
40	40.82	9,12-Octadecenoic acid	3.355
41	41.17	Stearic acid	0.472
42	44.09	Pimaric acid	0.263
43	45.18	Methyl isopimarate	0.983
44	46.52	Abietic acid	1.159
45	47.09	Dioctyl phthalate	1.168
46	57.51	Campesterol	0.277
47	58.09	Stigmasterol	0.430
48	60.18	Lupeol	1.114
49	60.57	β -Amyrin	16.28
50	62.02	α -Amyrin	33.341

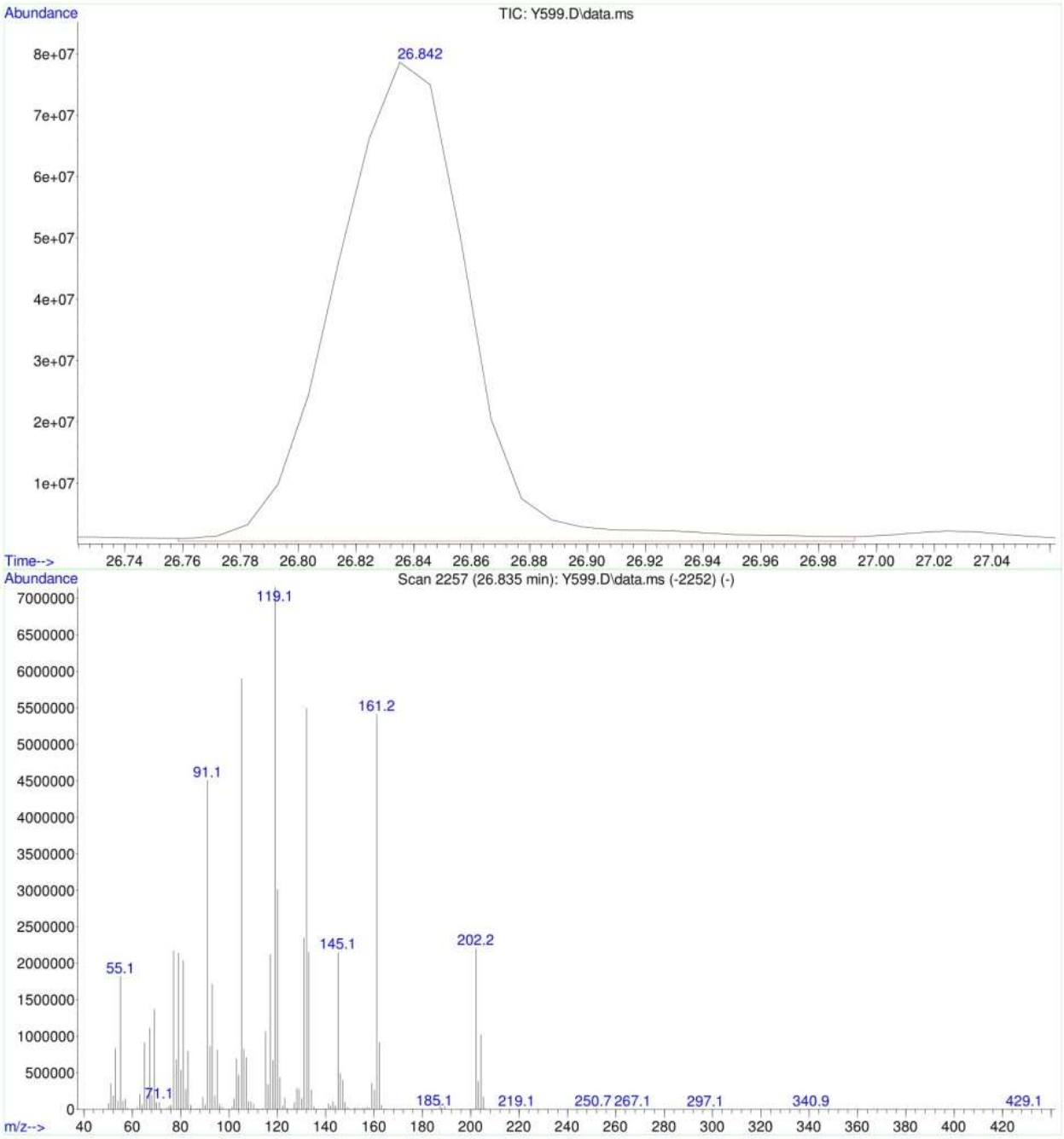


Fig-2 : GC-MS Pattern of α –Curcumene

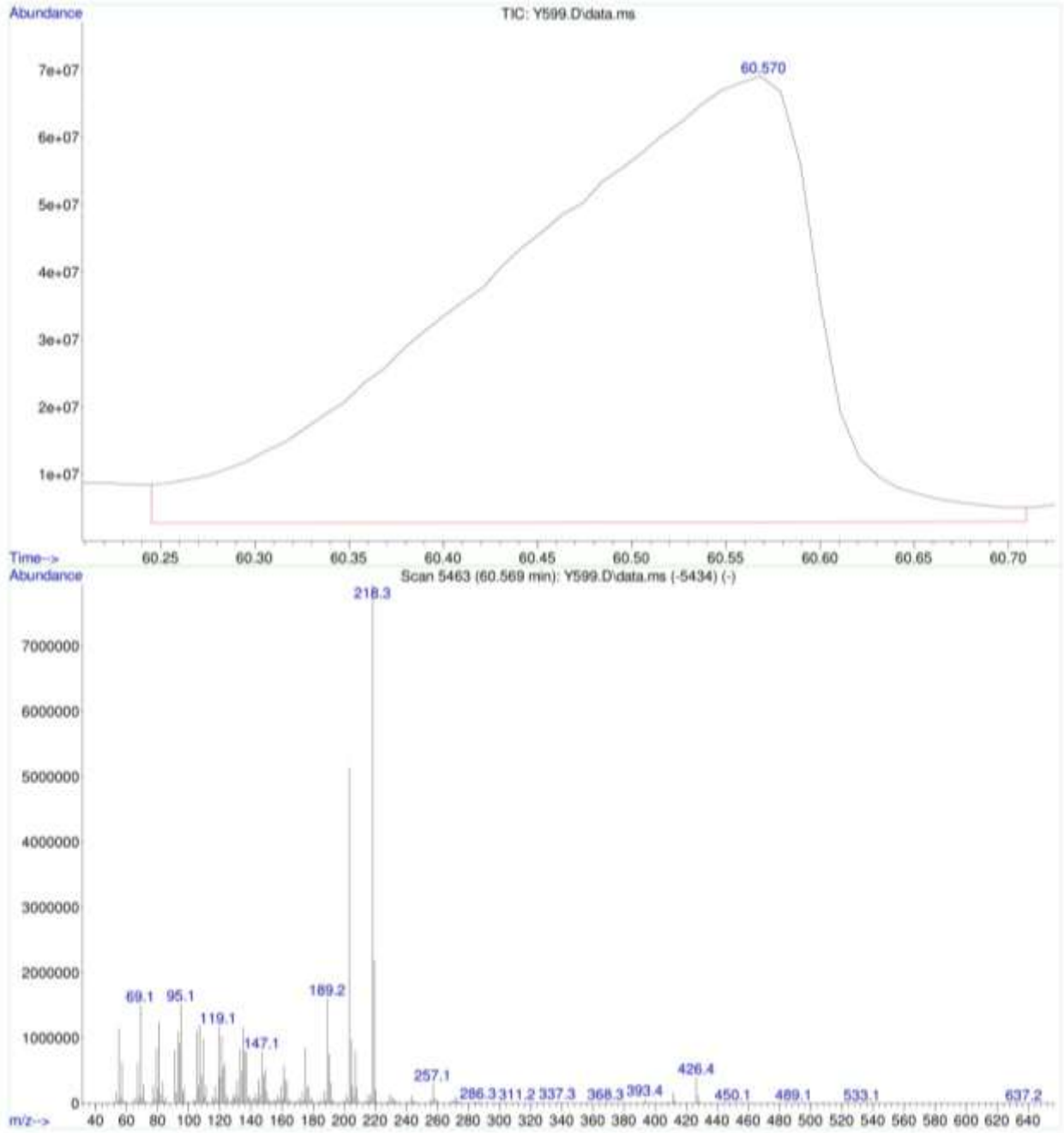


Fig-3 : GC-MS Pattern of β –amyrin

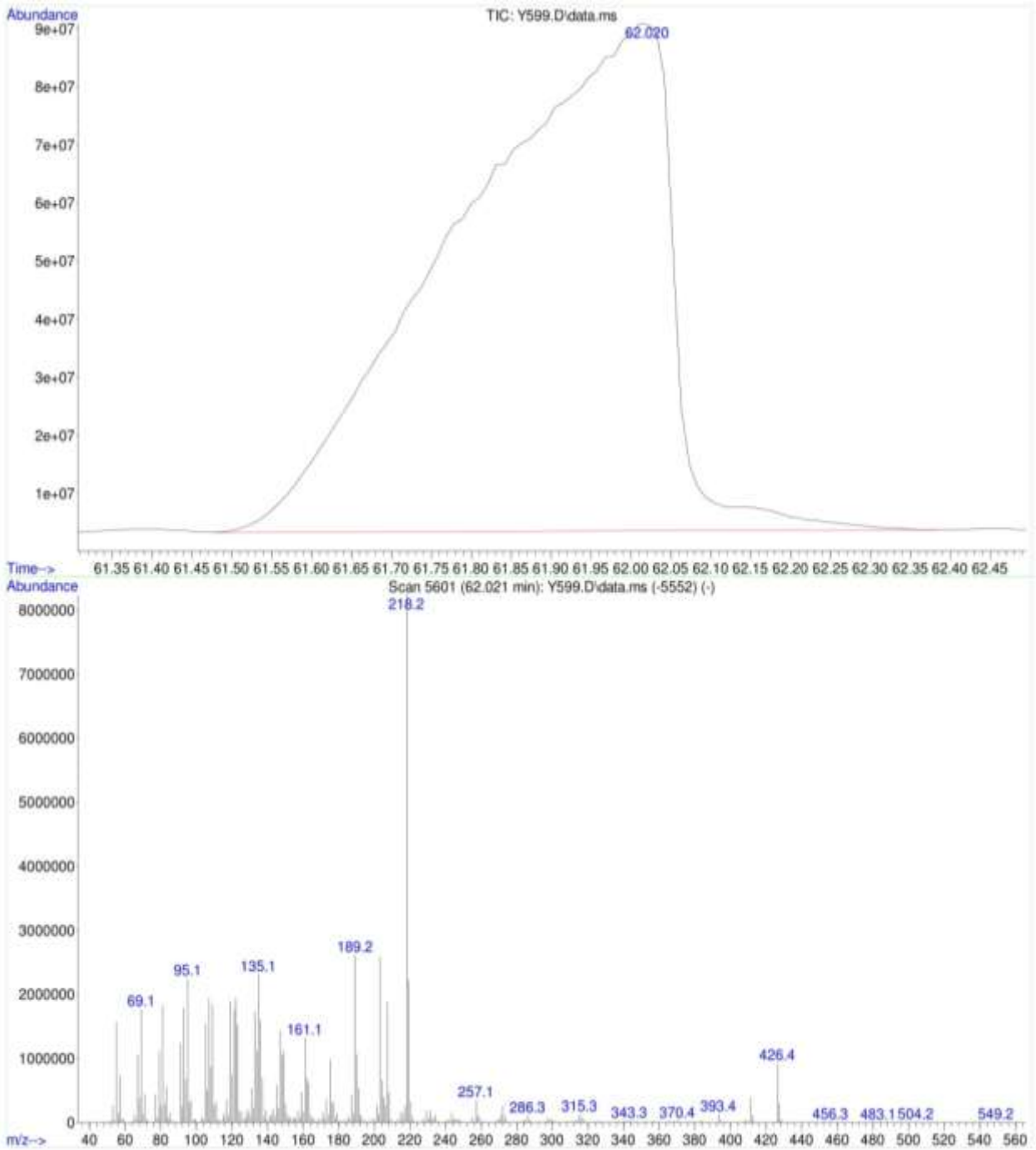
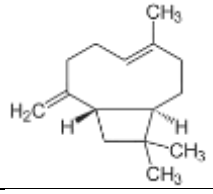
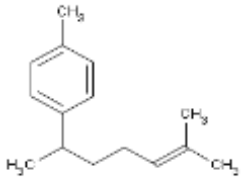
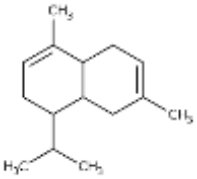
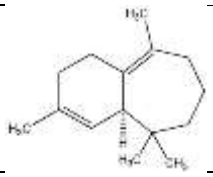
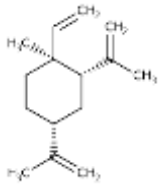
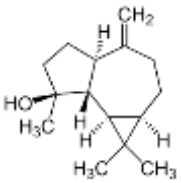
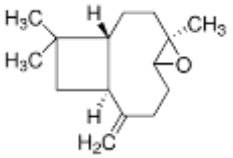
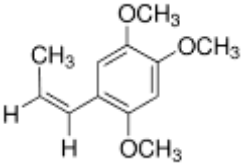
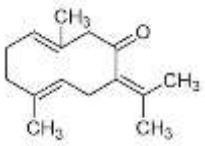
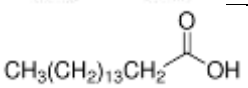


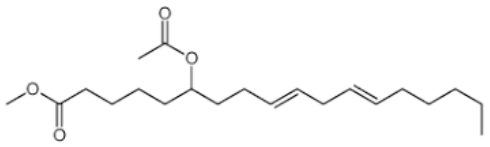
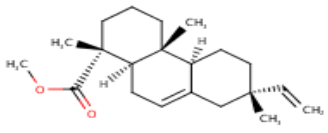
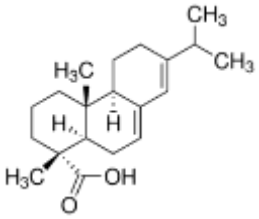
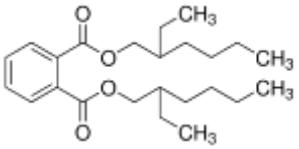
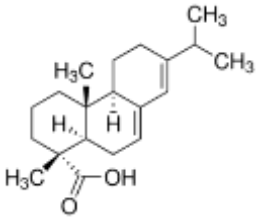
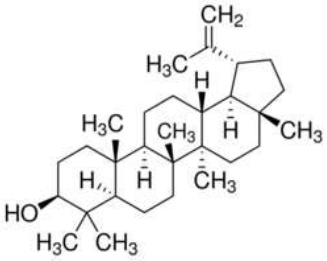
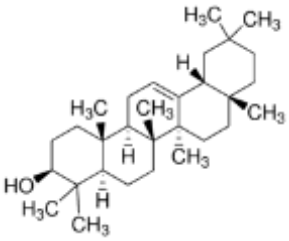
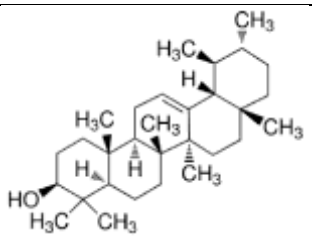
Fig-4: GC-MS Pattern of α –amyrin

Identification of α-amyrin and β –Amyrin by Mass Spectra

Most of the triterpenes belonging to the oleanene/ursene series are characterized by a base peak at m/z 218. Unequivocal differentiation between the compounds α-amyrin and β-amyrin could be seen by examination of the relative intensities of the peaks at m/z 189 and 203: β-amyrin (Fig 3) has a m/z 203 peak around twice the intensity of the m/z 189 peak, while α-amyrin (Fig 4) spectra shows both peaks with similar intensities [16].

Table-3: Chemical structure of some important compounds identified in the Hexane Extract

Sl.No	Compound	Chemical structure
1.	Caryophyllene	
2.	α -Curcumene	
3.	β -Cadinene	
4.	β-Himachalene	
5.	β - Elemene	
6.	Spathalenol	
7	Caryophyllene oxide	
8	Asarone	
9	Germacrone	
10	Palmitic acid	

11.	9,12-Octadecenoic acid	
12.	Methyl isopimarate	
13.	Abietic acid	
14.	Dioctyl phthalate	
15.	Abietic acid	
16.	Lupeol	
17.	β -Amyrin	
18.	α -Amyrin	

CONCLUSION

Phytochemical screening of *Ipomoea sepiaria* Roxb. revealed the presence of flavanoids in Ethyl acetate and Methanol extracts, tannins in Chloroform and Methanol extracts, terpenoids and steroids in all extracts, cardiac glycoside, phenols and alkaloids in Chloroform, Ethyl Acetate and Methanol extracts, Sterols in Hexane extract. Carbohydrates were present in Chloroform, Ethyl acetate and Methanol extracts.

The compounds Caryophyllene, α –Curcumene, β –Cadinene, ϒ –Elemene, Caryophyllene oxide, Asarone, Germacrone, Methyl isopimarate, Abietic acid, Lupeol, β –Amyrin and α –Amyrin have not been so far reported in *Ipomoea sepiaria*. The higher percentages of compounds β –Amyrin and α –Amyrin which form the basic skeleton of triterpenoid saponins are promising as β and α -amyrins have been shown to exhibit various pharmacological actitivies in vitro and invivo conditions against various health-related conditions, including conditions such as inflammation, microbial, fungal, and viral infections and cancer cells [17]. Further studies can be carried out in the direction of isolating these compounds from the hexane extract of *Ipomoea sepiaria* Roxb.

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