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(RESEARCH ARTICLE)

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Phytochemical profiling of methanolic extract of *Periploca aphylla* Decne. using GC-MS analysis

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Abstract

Introduction: *Periploca aphylla* Decne. (Asclepiadoideae) locally named as "Bata", is a medicinally important plant. The milky juice of *P. aphylla* is externally applied to tumors and swellings, for cerebral fever treatment and as stomachic. The flowers are used as vegetables, emetic purposes, expectorant, laxative, diuretic and for wart removal.

Objectives: In this study, crude methanolic extract of *P. aphylla* (whole plant) was investigated for its phytochemical composition using GC-MS analysis.

Methodology: THERMO GC-TRACE ULTRA VER: 5.0, THERMO MS DSQ II and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a ZB 5-MS CAPILLARY STANDARD NON-POLAR COLUMN (30 m x 0.25 mm ID x 0.25 µm FILM) was used to carry out analysis of extract. The interpretation of mass spectrums was performed utilizing National Institute of Standard and Technology (NIST) database.

Results: Thirty compounds were recognized in *P. aphylla* methanol extract during GC-MS analysis in which hexadecanoic acid, methyl ester (13.65%) was a major component followed by 1,1,6-trimethyl-3-methylene-2-(3,6,9,13 tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane (9.41%), 9-Octadecenal, (Z)-(CAS) (8.00%), Pyrrolidine, 1-(6-phenyl-1-cyclohexen-1-yl)-(CAS) (7.50%) and 5 (Dimethylamino)-3-ethoxy-5-isopropyl-1-(4'-trifluoromethylphenyl)-2-[2"-(4-"'trifluoromethyl phenyl) ethynyl]-1,3-cyclopentadiene (5.28%).

Conclusion: *Periploca aphylla* is a rich source of chemical compounds having therapeutic potentials which validates its usage for the treatment of different diseases.

Keywords: Asclepiadoideae; GC-MS; *Periploca aphylla; Tumor*

1. Introduction

The kingdom of plants is considered as a treasure house of potential drugs. In the last few years, awareness about the importance of plants has been increased among the masses. A wide variety of natural health care practices including Siddha, Ayurveda, Unani and Amchi are referred as traditional medicine. The origin of these medical practices is time immemorial. They mostly get developed by relying on practical experiences without having any significant reference to the scientific principles [1].

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Organic compounds including alkaloids, tannins, terpenoids, carbohydrates, flavonoids and steroids having well-defined physiological effect on the human body are richly supplied by medicinal plants. Secondary metabolism of living organisms synthesized these compounds. Chemically and taxonomically, secondary metabolites are varied compounds with ambiguous functions. They have a wide use in agriculture, veterinary, human therapy and research. It is very important to gain the information of chemical components of plants for the synthesis of complex substances [2].

The pharmacological and chemical information can be obtained by screening the medicinal plants using different techniques such as chromatography and spectrometry, which is helpful for the selection of biologically active plants [3]. Recently, gas chromatography-mass spectrometry (GC-MS) as well as fourier-transform infrared (FTIR) have been employed to find various bioactive compounds in the medicinal plants along with the detection of functional groups [4,5].

Ideally, volatile and semi-volatile compounds are analyzed by GC–MS which combines the best separation (GC) and identification (MS) techniques. It is very effective in identifying the bioactive components of ester, long and branched chain hydrocarbons, alcohols and acids etc. [6]. A small volume of extract is required in GC-MS. Hence, it is employed in the present study to detect and identify phytochemical compounds present in *Periploca aphylla*.

Periploca aphylla Decne. belongs to the family Asclepiadoideae having 348 genera and 2,900 species. They are found mainly in the tropics to subtropics, particularly in South America and Africa. In Pakistan, it is mostly found in the northern regions [7]. Its local name is “Bata”. Traditionally, it is utilized as stomachic and for treating cerebral fever. Its milky juice is applied externally on tumors and swellings [8]. Its flowers are utilized as vegetables. Latex from its stem is used as chewing gum. They are also used as diuretic, laxative and expectorant. Many known triterpenes and steroids have been isolated from it [9].

Considering the medicinal importance of *P. aphylla*, its methanolic extract was analyzed using GC-MS for the first-time. This research will be useful for the identification of compounds having therapeutic value.

2. Material and methods

2.1. Plant material

The collection of *Periploca aphylla* was done in April-May 2010 from Margalla Hills Islamabad, Pakistan. Dr. Saleem Ahmad identified the plant sample and voucher specimen was deposited at Herbarium of Pakistan Museum of Natural History, Islamabad (Voucher No. 069721). Whole plant was collected and shade dried at temperature range of 21-30 °C. The dried plant material was powdered in the blender and stored in polythene bags (at room temperature). Extraction was performed according to the following procedure.

2.2. Extract Preparation

2.2.1. Methanol extract

Dried powdered sample of *P. aphylla* (1.5 kg) was soaked in 4 liters of methanol (95%) for one week (3 times) at room temperature with random shaking and stirring. The filtration of extract was carried out by using Whattman filter paper No. 42 (125 mm). After combining the resulting filtrates, they were concentrated at 40 °C in a rotary vacuum evaporator to get a solid, gummy mass (PAME). The extract was kept at -4 °C in airtight vials till further study.

2.3. GC-MS analysis

GC-MS analysis of methanol extract was carried out employing a THERMO GC - TRACE ULTRA VER: 5.0, THERMO MS DSQ II and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a ZB 5 - MS CAPILLARY STANDARD NON - POLAR COLUMN (30 m x 0.25 mm ID x 0.25 µm FILM). The components of the sample were ionized in electron impact mode (EI, 70 eV). Helium gas (99.999%) was employed as the carrier gas at constant flow rate of 1 ml/min and 1 µl of injection volume; split ratio of 10:1 while 250 °C and 280 °C were the injector and ion-source temperatures respectively. The oven temperature was programmed from 70 °C (isothermal for two minutes) with an increase of 6 °C / min to 260 °C. The sample was injected in split mode as 10:1. Scan range of mass spectra was set at 45-450 (m/z). The total running time of GC was 38.50 minutes.

2.4. Identification of components

The interpretation of mass spectrum (GC-MS) was made employing National Institute of Standard and Technology (NIST) database which has more than 62,000 patterns. The spectrums of the unknown components were compared with those of the known compounds stored in the NIST library. The structure, molecular weight and name of the components of the extract were established.

3. Results

3.1. GC-MS analysis

GC-MS analysis of *P. aphylla* (methanolic extract) was done for the detection of compounds (Fig. 1-15). The molecular formula, retention time (RT), molecular weight (MW) and concentration (%) of the active principles in the *P. aphylla* methanolic extract are presented in the Table 1. The chromatogram shows 30 prominent peaks in the 7.07 - 40.41 retention time range. The peak area of a compound at 21.65 retention time is 13.65. This largest peak identified is of Hexadecanoic acid, methyl ester (CAS). The peak area of second less prominent peak at 31.73 retention time is 9.41. It is due to the presence of 1, 1, 6- trimethyl-3-methylene-2-(3, 6, 9,13 tetramethyl-6-ethenye-10,14-dimethylenepentadec-4-enyl)cyclohexane. 9-Octadecenal, (Z)- (CAS) characterizes the third less significant peak at 35.13 retention time with the peak area of 8.00. Pyrrolidine, 1-(6-phenyl-1-cyclohexen-1-yl)- (CAS) shows the peak area of 7.50 at 22.31 retention time and is the fourth less prominent peak. The fifth less prominent peak at 34.66 retention time with the peak area 5.28 is characteristic of 5 (Dimethylamino)-3-ethoxy-5-isopropyl-1-(4'-trifluoromethylphenyl)-2-[2"-(4-"' trifluoromethylphenyl)ethynyl]-1,3-cyclopentadiene. All the other less prominent peaks at different retention times are given in Table 1. The probability, common name, structure, nature of the compounds and their pharmacological activities are given in Table 2 and 3.

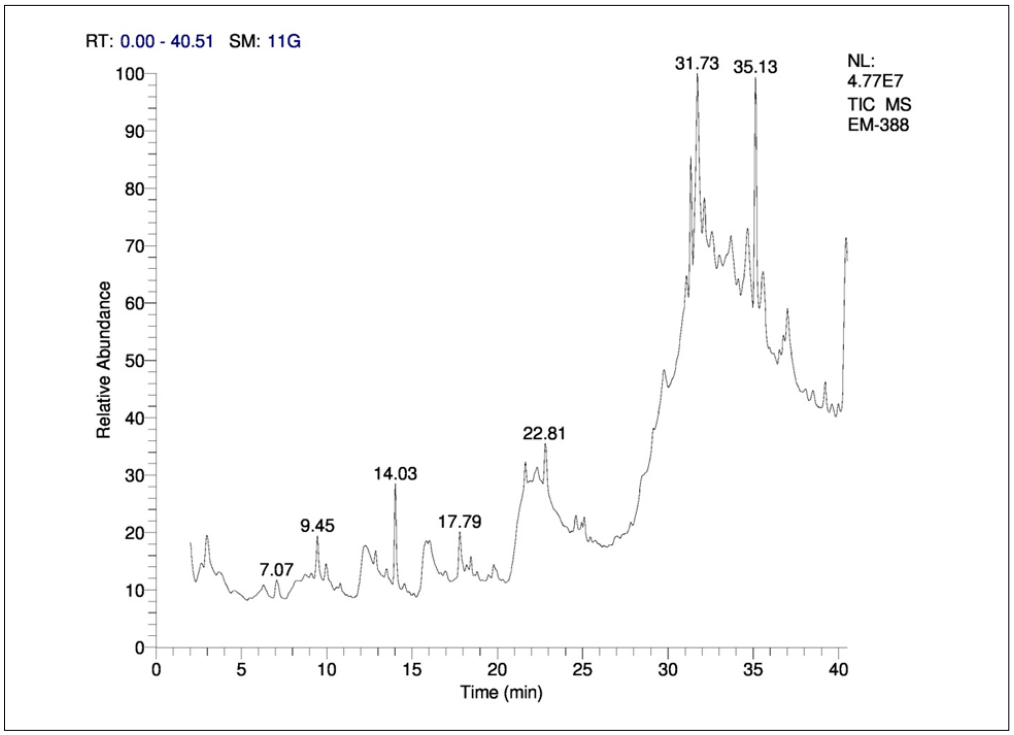


Figure 1 GC-MS chromatogram of *P. aphylla* methanol extract

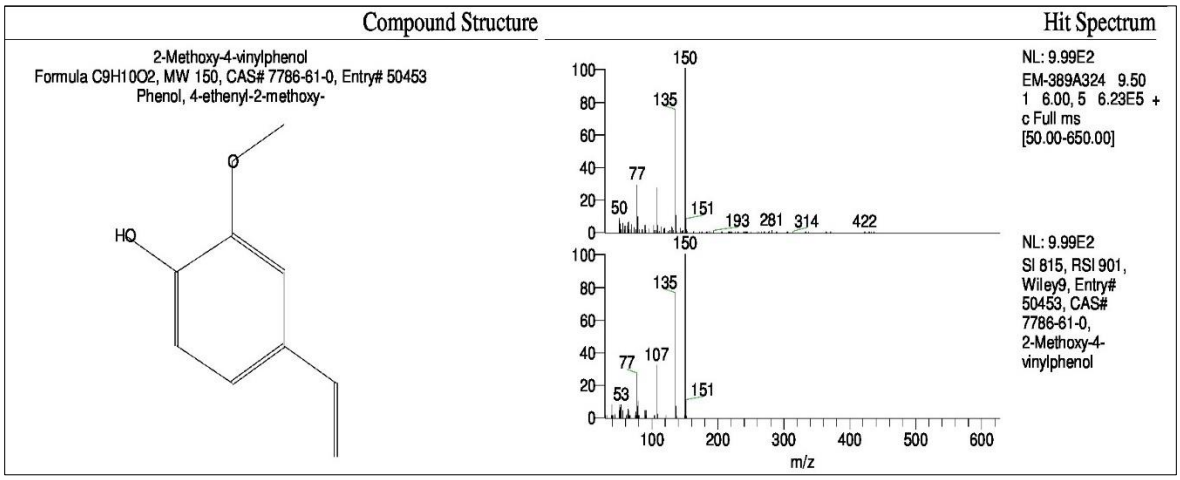


Figure 2 2-Methoxy-4-vinylphenol with RT: 9.45 present in *P. aphylla*

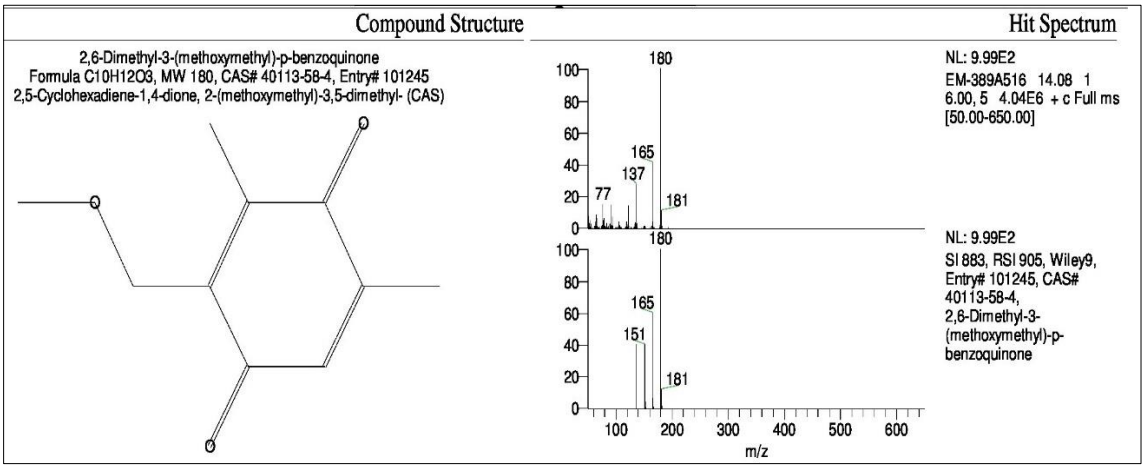


Figure 3 2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone with RT: 14.03 present in *P. aphylla*

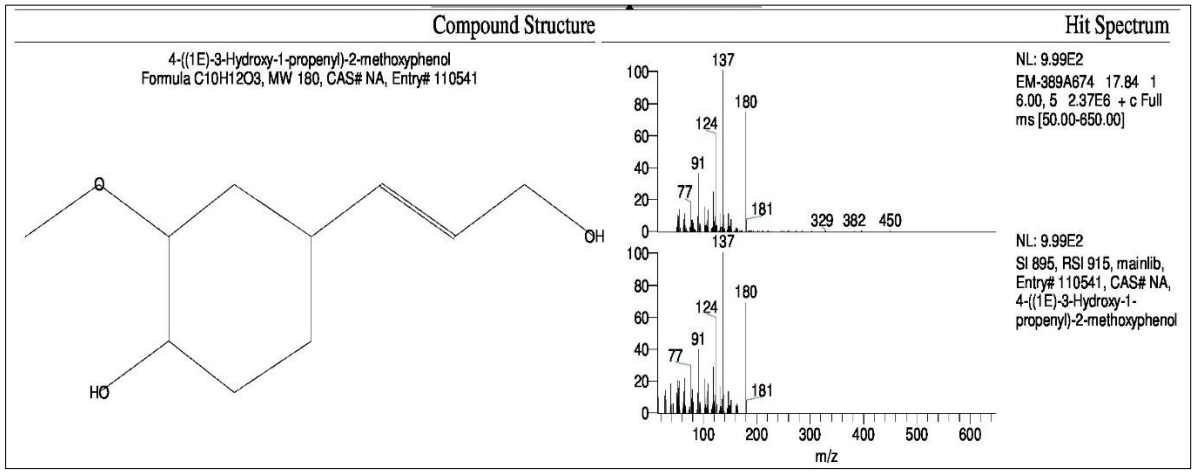


Figure 4 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol with RT: 17.79 present in *P. aphylla*

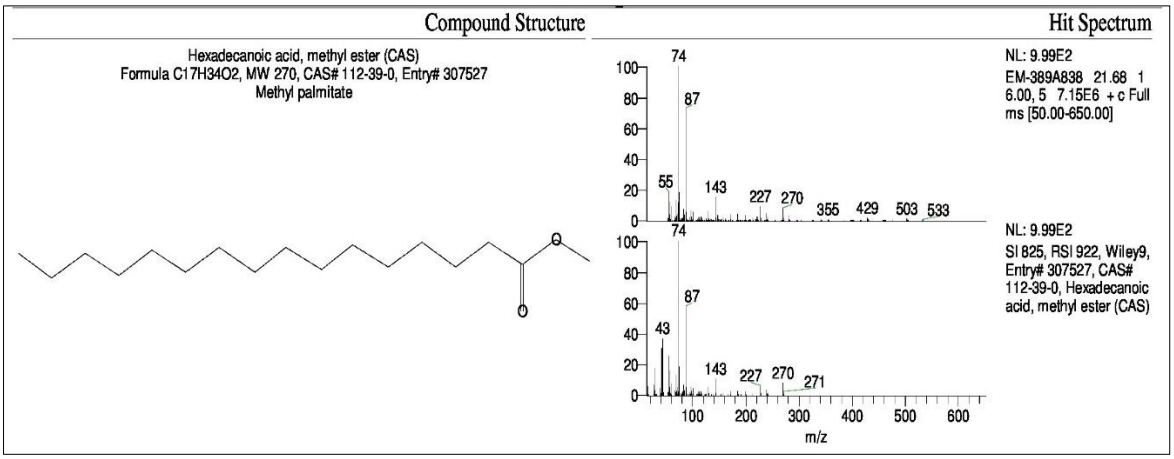


Figure 5 Hexadecanoic acid, methyl ester (CAS) with RT: 21.68 present in *P. aphylla.*

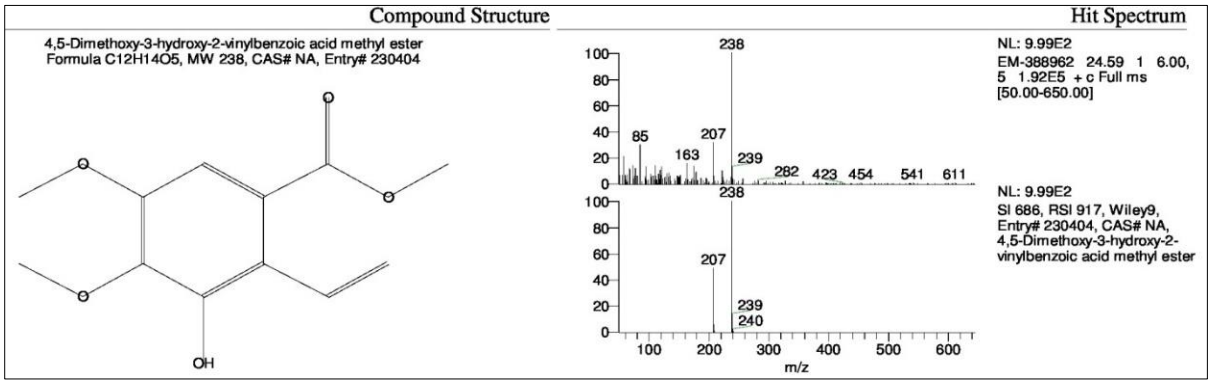


Figure 6 4, 5-Dimethoxy-3-hydroxy-2-vinylbenzoic acid methylester with RT: 24.59 present in *P. aphylla*

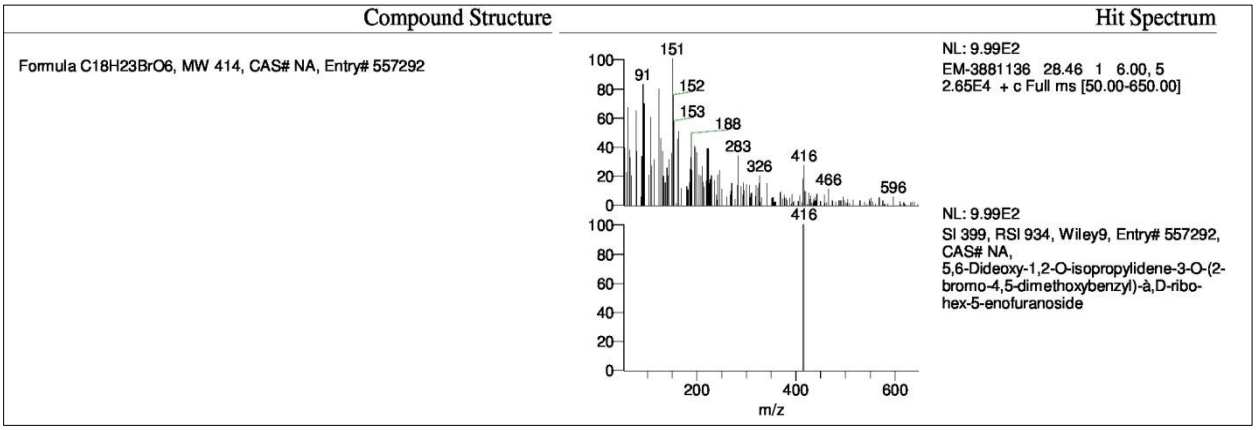


Figure 7 5,6-Dideoxy-1,2-O-isopropylidene-3-O-(2-bromo-4,5-dimethoxybenzyl)-à,D-ribo-hex-5-enofuranoside with RT: 28.46 present in *P. aphylla*

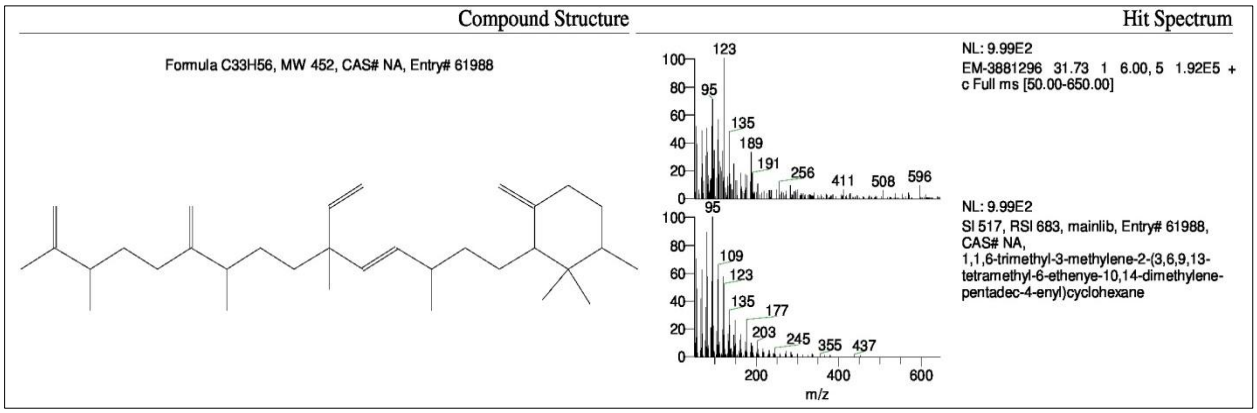


Figure 8 1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane with RT: 31.73 present in *P. aphylla*

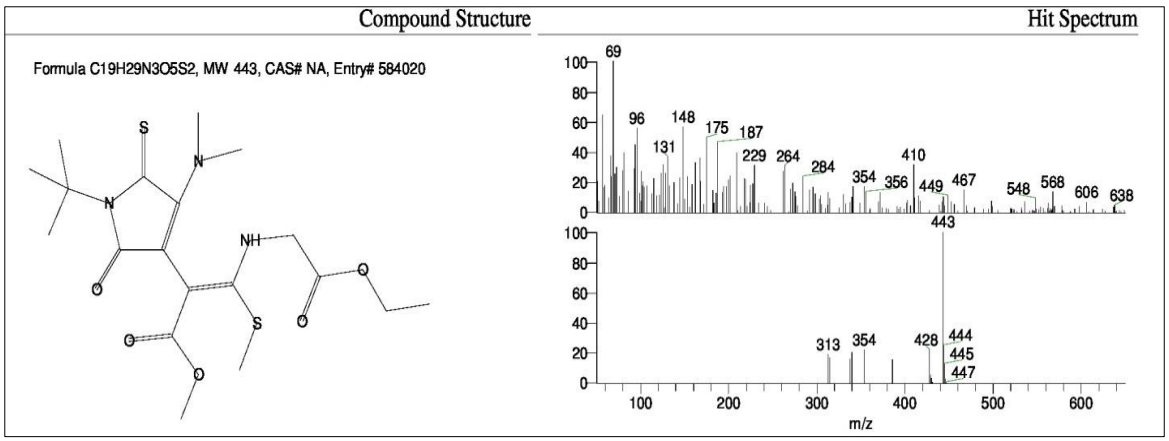


Figure 9 1-tert-Butyl-3-(dimethylamino)-4-[á.-[[[(ethoxycarbonyl)methyl]amino]-à-(methoxycarbonyl)-á-(methylthio)vinyl]-2-thiomaleimide with RT: 32.60 present in *P. aphylla*

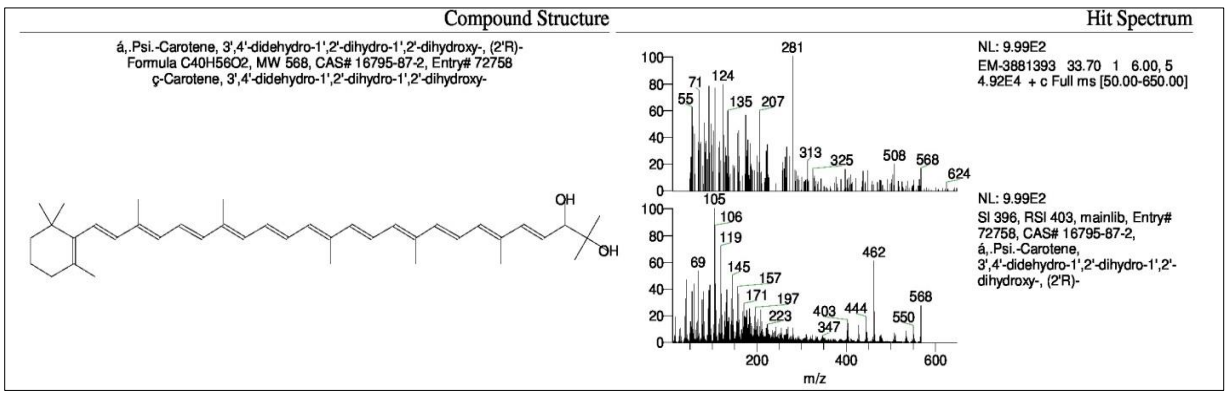


Figure 10 á,.Psi.-Carotene,3',4'-didehydro-1',2'-dihydro-1',2'-dihydroxy-, (2'R)- with RT: 33.70 present in *P. aphylla*

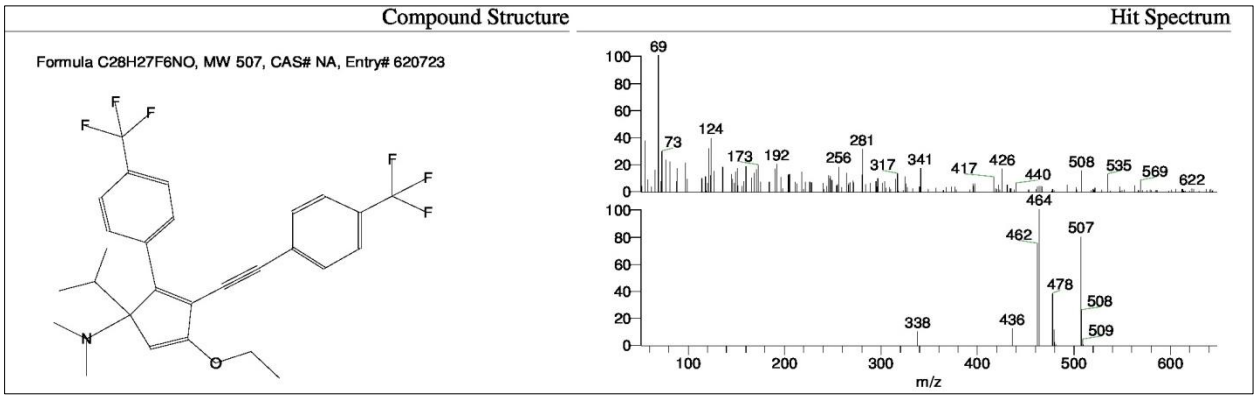


Figure 11 5-(Dimethylamino)-3-ethoxy-5-isopropyl-1-(4'-trifluoromethylphenyl)-2-[2"-(4-"'-trifluoromethylphenyl)ethynyl]-1,3-cyclopentadiene with RT: 34.66 present in *P. aphylla*

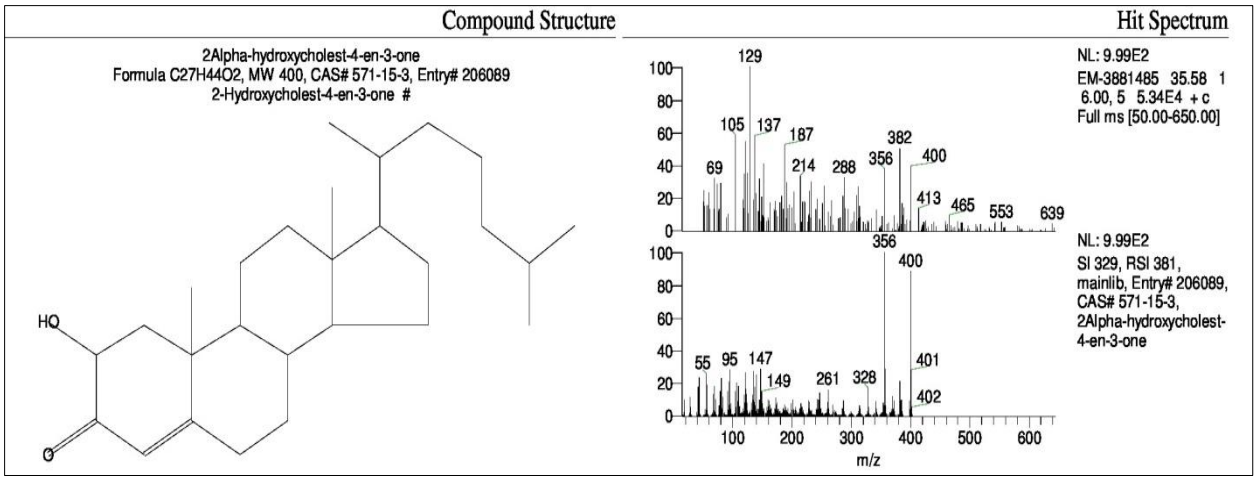


Figure 12 2Alpha-hydroxycholest-4-en-3-one with RT: 35.58 present in *P. aphylla*

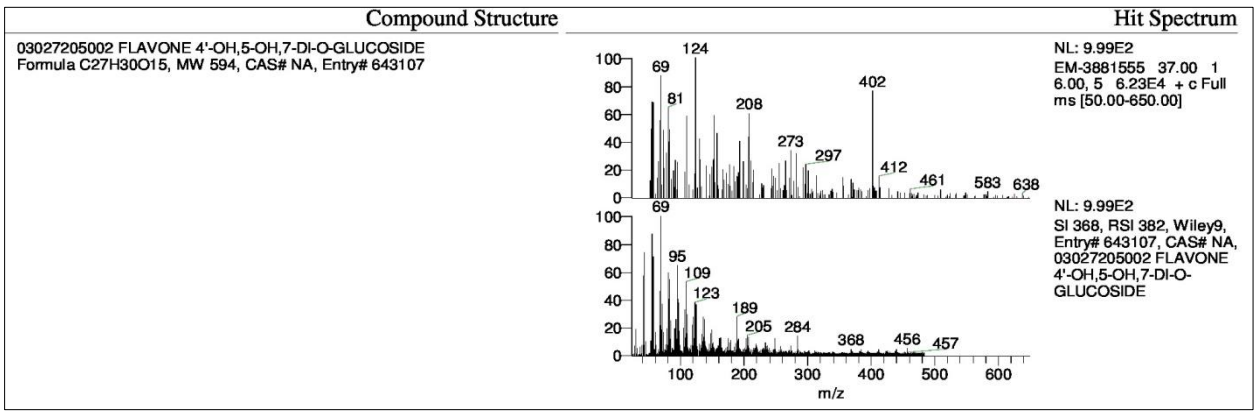


Figure 13 FLAVONE 4'-OH,5-OH,7-DI-O-GLUCOSIDE with RT: 37.00 present in *P. aphylla*

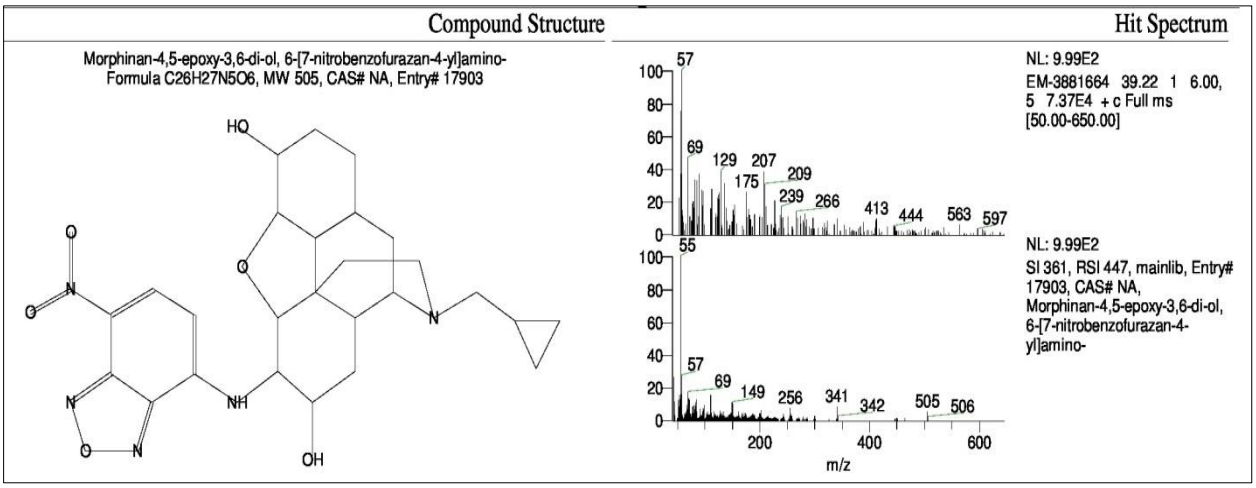


Figure 14 Morphinan-4,5-epoxy-3,6-di-ol,6-[7-nitrobenzofurazan-4-yl]amino- with RT: 39.22 present in *P. aphylla*

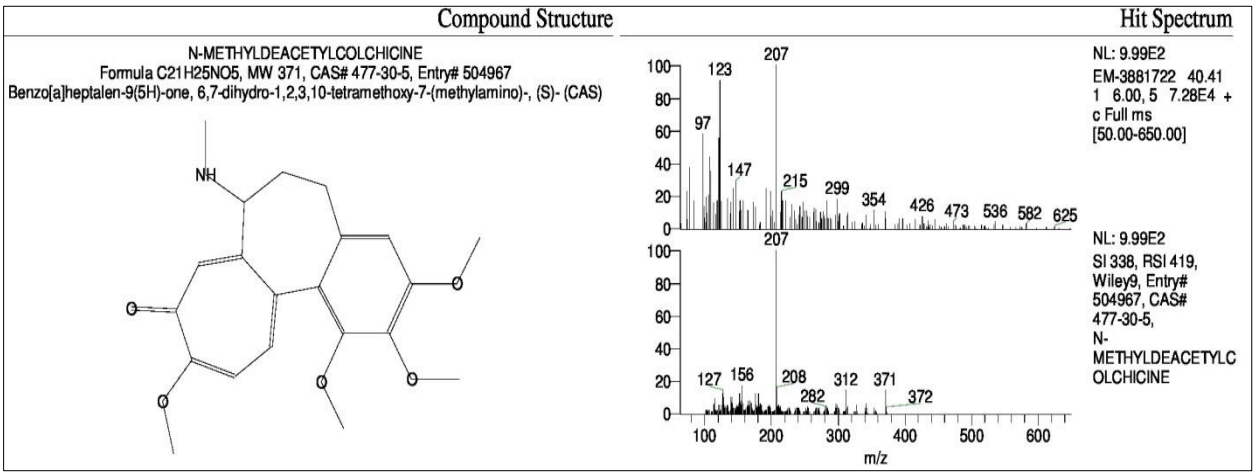


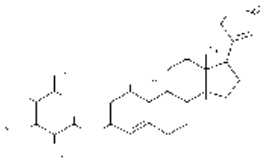
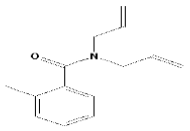
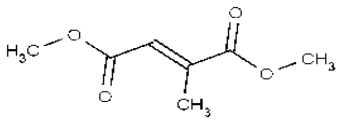
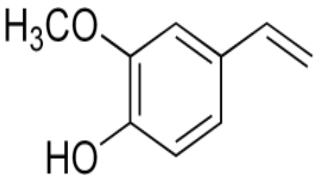
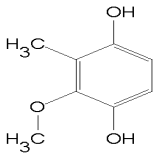
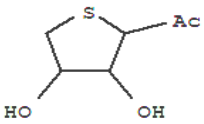
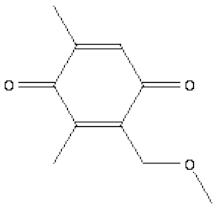
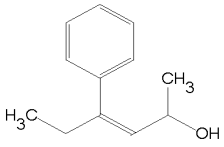
Figure 15 N-methyldeacetylcolchicine with RT: 40.41 present in *P. aphylla*

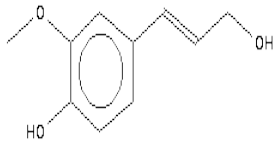
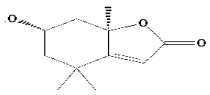
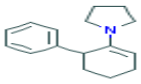
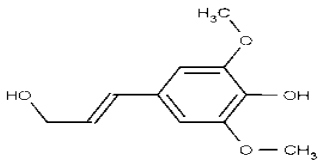
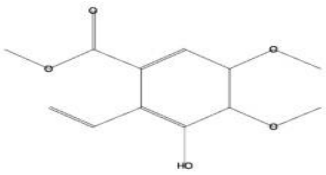
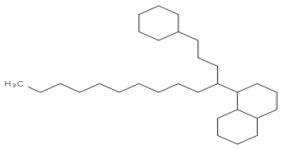
Table 1 Total ionic chromatogram (GC-MS) of *P. aphylla*

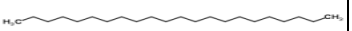
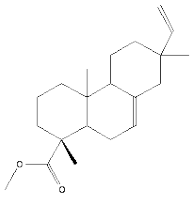
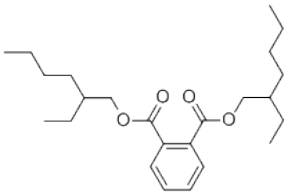
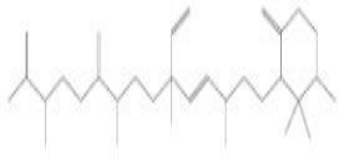
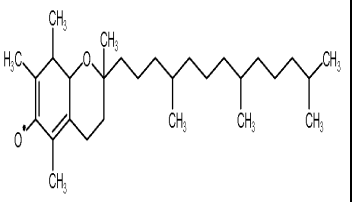
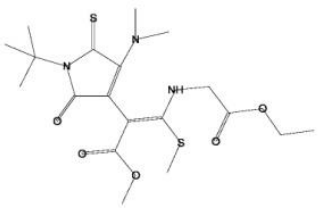
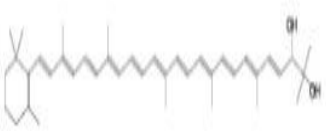
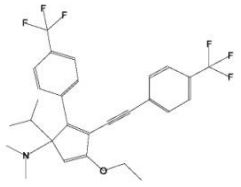
No	RT	Name of the compound	Molecular Formula	MW	Peak area %
1	7.07	Oppovenoside Carda-4,20(22)-dienolide,3-[(6-deoxy-3-O-methyl-à-D-allopyranosyl)oxy]-1,14-dihydroxy-, (1á,3á)-	C30H44O9	548	0.96
2	8.16	Benzamide, 2-methyl-N,N-diallyl-	C14H17NO	215	1.55
3	8.74	1-trideuteromethyl 4-methyl citraconate	C7H7D3O4	158	1.32
4	9.45	2-Methoxy-4-vinylphenol	C9H10O2	150	2.07
5	9.97	2-Methoxy-3-methylhydroquinone	C8H10O3	154	0.99
6	12.19	D-Fructose, 1,3,6-trideoxy-3,6-epithio-	C6H10O3S	162	4.04
7	14.03	2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone	C10H12O3	180	3.41

8	15.81	3-Methyl-4-phenylhex-5-en-3-ol	C13H18O	190	5.11
9	17.79	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C10H12O3	180	2.22
10	18.46	(-)-Loliolide	C11H16O3	196	1.16
11	19.80	2-(3,4-Dimethoxyphenyl)tetrahydropyran	C12H16O3	208	1.19
12	21.65	Hexadecanoic acid, methyl ester (CAS)	C17H34O2	270	13.65
13	22.31	Pyrrolidine, 1-(6-phenyl-1-cyclohexen-1-yl)- (CAS)	C16H21N	227	7.50
14	22.81	3,5-Dimethoxy-p-coumaric alcohol	C11H14O4	210	4.39
15	24.59	4,5-Dimethoxy-3-hydroxy-2-vinylbenzoic acid methylester	C12H14O5	238	0.96
16	25.09	Naphthalene,1-[1-(3-cyclohexylpropyl)undecyl]decahydro-(CAS)	C30H56	416	1.35
17	28.46	5,6-Dideoxy-1,2-O-isopropylidene-3-O-(2-bromo-4,5-dimethoxybenzyl)-à,D-ribo-hex-5-enofuranoside	C18H23BrO6	414	1.01
18	29.77	Docosane (CAS)	C22H46	310	2.09
19	31.07	1-Phenanthrenecarboxylic acid,1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, methyl ester, [1R-(1à,4aá,10aà)]- (CAS)	C21H30O2	314	1.84
20	31.34	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester(CAS)	C24H38O4	390	3.24
21	31.73	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane	C33H56	452	9.41
22	32.13	Vitamin E	C29H50O2	430	1.41
23	32.60	1-tert-Butyl-3-(dimethylamino)-4-[á.-[[[(ethoxycarbonyl)methyl]amino]-à-(methoxycarbonyl)-á-(methylthio)vinyl]-2-thiomaleimide	C19H29N3O5S2	443	1.44
24	33.70	á,.Psi.-Carotene,3',4'-didehydro-1',2'-dihydro-1',2'-dihydroxy-, (2'R)-	C40H56O2	568	3.22
25	34.66	5-(Dimethylamino)-3-ethoxy-5-isopropyl-1-(4'-trifluoromethylphenyl)-2-[2"-(4-"'-trifluoromethylphenyl)ethynyl]-1,3-cyclopentadiene	C28H27F6NO	507	5.28
26	35.13	9-Octadecenal, (Z)- (CAS)	C18H34O	266	8.00
27	35.58	2Alpha-hydroxycholest-4-en-3-one	C27H44O2	400	4.06
28	37.00	03027205002 Flavone 4'-OH,5-OH,7-DI-O-Glucoside	C27H30O15	594	3.83
29	39.22	Morphinan-4,5-epoxy-3,6-di-ol,6-[7-nitrobenzofurazan-4-yl]amino-	C26H27N5O6	505	1.06
30	40.41	N-methyldeacetylcolchicine	C21H25NO5	371	2.24

Table 2 Structure of phytocomponents identified in the methanol extract of *P. aphylla*

No	Name of the compound	Probability	Common name	Structure
1	OPPOVENOSIDE Carda-4,20(22)-dienolide, 3-[(6-deoxy-3-O-methyl-à-D-allopyranosyl)oxy]-1,14-dihy droxy-, (1á,3á)-	6.80	-	
2	Benzamide, 2-methyl-N,N-diallyl-	7.51	N,N-Diallyl-2-methylbenzamide	
3	1-trideuteromethyl 4-methyl citraconate	26.69	Citraconate, 4-methyl-, 1-trideuteromethyl	
4	2-Methoxy-4-vinylphenol	30.62	Phenol, 4-ethenyl-2-methoxy-	
5	2-Methoxy-3-methylhydroquinone	21.15	2,4-Dimethoxyphenol	
6	D-Fructose, 1,3,6-trideoxy-3,6-epithio-	6.95	3,6-Anhydro-1-deoxy-3-thiohex-2-ulose	
7	2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone	36.90	2,5-Cyclohexadiene-1,4-dione, 2-(methoxymethyl)-3,5-dimethyl- (CAS)	
8	3-Methyl-4-phenylhex-5-en-3-ol	18.95	-	

9	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	64.57	(E)-Conipheryl alcohol	
10	(-)-Loliolide	84.65	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-, (6S-cis)-(CAS)	
11	2-(3,4-Dimethoxyphenyl)tetrahydropyran	19.97	-	-
12	Hexadecanoic acid, methyl ester (CAS)	35.59	Methyl palmitate	
13	Pyrrolidine, 1-(6-phenyl-1-cyclohexen-1-yl)- (CAS)	10.90	3-phenyl-2-(1-pyrrolidinyl)-1-cyclohexene	
14	3,5-Dimethoxy-p-coumaric alcohol	45.24	-	
15	4,5-Dimethoxy-3-hydroxy-2-vinylbenzoic acid methyl ester	77.03	-	
16	Naphthalene,1-[1-(3-cyclohexylpropyl)undecyl]decahydro- (CAS)	11.58	1-cyclohexyl-4-(1-decahydronaphthyl)tetradecane	
17	5,6-Dideoxy-1,2-O-isopropylidene-3-O-(2-bromo-4,5-dimethoxybenzyl)-à,D-ribo-hex-5-enofuranoside	29.73	-	-

18	Docosane (CAS)	9.29	n-Docosane	
19	1-Phenanthrenecarboxylic acid,1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methyl ethyl)-, methyl ester, [1R-(1à,4aá,10aà)]- (CAS)	25.01	Methyl dehydroabietate; Cannabidiol	
20	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS)	21.28	Bis(2-ethylhexyl) phthalate	
21	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane	5.87	-	
22	Vitamin E	25.27	Alpha-tocopherol	
23	1-tert-Butyl-3-(dimethylamino)-4-[á.-[[[(ethoxycarbonyl) methyl]amino]-à-(methoxycarbonyl)-á-(methylthio)vinyl]-2-thiomaleimide	13.26	-	
24	á,.Psi.-Carotene, 3',4'-didehydro-1',2'-dihydro-1',2'-dihydroxy-, (2'R)-	16.05	ç-Carotene, 3',4'-didehydro-1',2'-dihydro-1',2'-dihydroxy-	
25	5-(Dimethylamino)-3-ethoxy-5-isopropyl-1-(4'-trifluoromethylphenyl)-2-[2"-(4-"'-trifluoromethylphenyl)ethynyl]-1,3-cyclopentadiene	9.58	-	

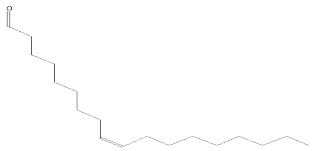
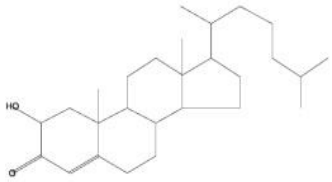
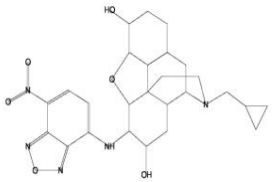
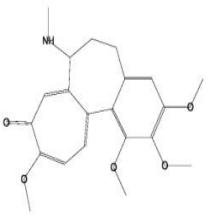
26	9-Octadecenal, (Z)- (CAS)	12.82	Olealdehyde	
27	2Alpha-hydroxycholest-4-en-3-one	7.08	2-Hydroxycholest-4-en-3-one	
28	03027205002 FLAVONE 4'-OH,5-OH,7-DI-O-GLUCOSIDE	17.78	-	-
29	Morphinan-4,5-epoxy-3,6-di-ol,6-[7-nitrobenzofurazan-4-yl]amino-	7.92	-	
30	N-methyldeacetylcolchicine	9.49	Demecolcine	

Table 3 Bioactivities of phytocomponents identified in the methanol extract of *P. aphylla*

No	Name of the compound	Compound Nature	Bioactivity/Pharmacological activity
1	OPPOVENOSIDE Carda-4,20(22)-dienolide, 3-[(6-deoxy-3-O-methyl-à-D-allopyranosyl)oxy]-1,14-dihy droxy-, (1á,3á)-	-	-
2	Benzamide, 2-methyl-N,N-diallyl-	Aromatic amide	-
3	1-trideuteromethyl 4-methyl citraconate	-	-
4	2-Methoxy-4-vinylphenol	Phenol	Antimicrobial, antioxidant, anti-inflammatory, analgesic, anti-germination activities, inhibiting platelet aggregation and reducing the risk of coronary heart disease, flavoring agent
5	2-Methoxy-3-methylhydroquinone	Phenol/Quinone	-
6	D-Fructose, 1,3,6-trideoxy-3,6-epithio-	Sugar	-
7	2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone	Quinone	-
8	3-Methyl-4-phenylhex-5-en-3-ol	-	-

9	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	Methoxyphenol	Antimicrobial, antioxidant, anti-inflammatory, analgesic activities
10	(-)-Loliolide	Carotenoid/Terpene	Antioxidant potential
11	2-(3,4-Dimethoxyphenyl)tetrahydropyran	Aromatic Compound	-
12	Hexadecanoic acid, methyl ester (CAS)	Fatty acid methyl ester/Palmitic acid ester	Antimicrobial, antioxidant, anti-inflammatory, hypocholesterolemic, hemolytic, cancer prevention, antifeedant agent, nematicide, pesticide, lubricant, antiandrogenic, flavouring agent
13	Pyrrolidine, 1-(6-phenyl-1-cyclohexen-1-yl)- (CAS)	Alkaloid	-
14	3,5-Dimethoxy-p-coumaric alcohol	Phenol	-
15	4,5-Dimethoxy-3-hydroxy-2-vinylbenzoic acid methyl ester	Polyphenol	-
16	Naphthalene,1-[1-(3-cyclohexylpropyl)undecyl]decahydro-(CAS)	Triterpenoid	-
17	5,6-Dideoxy-1,2-O-isopropylidene-3-O-(2-bromo-4,5-dimethoxybenzyl)-à,D-ribo-hex-5-enofuranoside	-	-
18	Docosane (CAS)	Alkane	Antimicrobial activity
19	1-Phenanthrenecarboxylic acid,1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methyl ethyl)-, methyl ester, [1R-(1à,4aá,10aà)]- (CAS)	Aromatic compound	-
20	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS)	Phthalate	Anti-cancer, immunomodulatory B-cell stimulant
21	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane	Hydrocarbon	-
22	Vitamin E	Alpha-tocopherol/Vitamin	Anti-inflammatory activities and their role in disease prevention and therapy, potent antioxidant and cytoprotective activities, plays a key role in neurological function, inhibits platelet aggregation and enhances vasodilation, inhibits angiogenesis and tumor dormancy, antiageing, antioxidant, hypoglycaemmic, analgesic, anti-tumor, anti-inflammatory, anticancer, antileukemic, vasodilator, antispasmodic, anticoronary, antibronchitic
23	1-tert-Butyl-3-(dimethylamino)-4-[á.-[[[(ethoxycarbonyl) methyl]amino]-à-(methoxycarbonyl)-á-(methylthio)vinyl]-2-thiomaleimide	-	-
24	á,.Psi.-Carotene, 3',4'-didehydro-1',2'-dihydro-1',2'-dihydroxy-, (2'R)-	Carotenoid	Anticancer, antioxidant

25	5-(Dimethylamino)-3-ethoxy-5-isopropyl-1-(4'-trifluoromethylphenyl)-2-[2"-(4-"'-trifluoromethylphenyl)ethynyl]-1,3-cyclopentadiene	-	-
26	9-Octadecenal, (Z)- (CAS)	Fatty aldehyde	-
27	2Alpha-hydroxycholest-4-en-3-one	-	-
28	Flavone 4'-OH,5-OH,7-DI-O-Glucoside	Isoflavonoid	A radical scavenger and a plant metabolite
29	Morphinan-4,5-epoxy-3,6-di-ol,6-[7-nitrobenzofurazan-4-yl]amino-	-	Anti-aging, analgesic and antidiabetic
30	N-methyldeacetylcolchicine	Alkaloid	Anti-leukemic agent, used in chemotherapy

4. Discussion

Since the dawn of medicine, natural products specifically those obtained from plants have been helping mankind to maintain human health. Traditional medicine exists since ancient times, and it is recognized and used by the people during the course of history. Medicinal plants are an excellent source of medicine since time immemorial. Medicinal products derived from plants have gained the attention of scientists all over the world due to their positive effects on health with minimum side effects. Several infections and diseases can be treated by a valuable source of substances provided by ethnomedicine. There are a wide variety of therapeutic potentials related with medicinal plants including antiviral, anti-inflammatory, antitumor, analgesic and antimalarial activities. Hence, medicinal plants can be considered a repository of various kinds of bioactive compounds having pharmacological potentials [10].

In order to defend themselves against harmful insects, pathogenic microbes and unfavorable environmental conditions, plants produce a variety of secondary metabolites and chemicals which are useful in defense mechanism but are non-nutritive [11]. These are called phytochemicals, and to some extent essential oils. They protect plants as well as animals and humans from various ailments caused by microorganisms. This is due to their antimicrobial potential [12]. Phytochemicals can be used as chemopreventive agents as well [13]. Many phytochemicals have been discovered till now and have been classified as major groups based on chemical and structural differences [14]. Phytochemicals can be classified into flavonoids, phytosterols, saponins, terpenoids, alkaloids, aromatic acid, protease inhibitors, organic acid and essential oils [15]. These metabolites possess some properties which enable them to protect directly or indirectly against pathogenic organisms and diseases such as antimicrobial, anthelmintic, anticarcinogenic, anti-inflammatory, antiproliferative, antigenotoxic, antioxidative and antimutagenic [16].

Several analytical and extraction techniques have been employed to study active compounds in the plants [17]. Among them, GC-MS is most accurate and fast method used for the detection of numerous compounds including alkaloids, alcohols, nitro compounds, organic compounds, long chain hydrocarbons, esters, amino acids and steroids [18]. In the past few years, it has developed as a major technological platform for the metabolite profiling of plants as well as other species [19-23]. GC-MS instrumentation was accessible to a few plant research laboratories until recently. However, it is increasingly becoming more commonplace. Different compounds have been eluted as a function of retention time and their relative concentrations are shown by gas chromatogram. Peak heights indicates the relative concentrations of the constituents in *P. aphylla*. The structure and nature of compounds which are eluted at different times, are identified by the mass spectrometer analysis. The larger compounds get fragmented into smaller compounds which appeared as peaks at different m/z ratios. These mass spectra are the fingerprints of those compounds which can be identified from the data library.

Thirty phytochemical compounds have been discovered in the GC-MS investigation of *P. aphylla* (methanolic extract), which could contribute to the therapeutic potentials of this specie. The compounds identified in *P. aphylla* possess various biological activities. For instance, hexadecanoic acid, methyl ester has anti-inflammatory, antioxidant, antimicrobial, pesticide, hypocholesterolemic, hemolytic, nematicide, lubricant, flavor, antiandrogenic and cancer prevention potentials [24-27]. It's presence may be used as potential antifeedant agent against insects [28]. Loliolide is a monoterpenoid and it has an antioxidant potential [29]. Vitamin E has hypocholesterolemic, antioxidant, cancer preventive and anti-coronary potentials. It prevents the clumping of blood platelets by acting as a blood thinner which

is another important health benefit. High levels of vitamin E reduces the risk of sunstroke [30]. Vitamin E has antioxidant, anti-ageing, hypoglycaemic, antitumor, analgesic, anti-inflammatory, antileukemic, anticancer, vasodilator, anticoronary, antibronchitic and antispasmodic properties [31].

2-Methoxy-4-vinylphenol has antimicrobial, antioxidant, anti-inflammatory, analgesic and anti-germination activities. It also acts as flavoring agent, inhibits platelet aggregation and reduces the risk of coronary heart diseases [31-33]. 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol possess antimicrobial, antioxidant, analgesic and anti-inflammatory activities [34]. Docosane possess antimicrobial activity [35]. Morphinan-4, 5-epoxy-3, 6-di-ol, 6-[7-nitrobenzofurazan-4-yl]amino- has anti-aging, analgesic and antidiabetic activities [36]. Flavone 4'-OH, 5-OH, 7-DI-O-Glucoside is a radical scavenger [37]. á,.Psi.-Carotene, 3',4'-didehydro-1',2'-dihydro-1',2'-dihydroxy-, (2'R)- has anticancer and antioxidant activities [26]. 1, 2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) is used as anti-cancer and immunomodulatory B-cell stimulant [38]. N-methyldeacetylcolchicine is a highly potent anti-leukemic agent and used in chemotherapy [39].

5. Conclusion

In this investigation, GC-MS analysis of *Periploca aphylla* Decne. (methanolic extract) was performed for the first time to identify various bioactive compounds. These compounds possess different therapeutic and pharmacological properties. Further investigations can be performed on the isolation of these bioactive compounds for drug formulation.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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