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GC-MS/MS PROFILE OF ETHANOL EXTRACT OF LEAVES & FLOWERS OF PHYTOTHERAPEUTIC PLANT – *CLEMATIS ZEYLANICA* (L.) PORI. FROM SANAMAVU FOREST, KRISHNAGIRI, TAMIL NADU, INDIA.

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ABSTRACT

Phytotherapeutic plants have been the main source of raw ingredients for the treatment of a wide range of ailments, since ancient times. In order to maintain a healthy lifestyle and manage illnesses, many people rely on medicinal plants. Ethnomedicinal plant *Clematis zeylanica* (L.) Pori. also known as *Naravelia zeylanica* (Linn) DC., belonging to the family ranunculaceae is used in the Indian ayurvedic medicinal system. Helminthiasis, dermatopathy, leprosy, rheumatalgia, odontalgia, colic inflammation, wounds, and ulcers are just a few of the conditions it helps treat. The purpose of the current study was to assess the photochemical and biological characteristics of *Clematis zeylanica*. The photochemical composition of the ethanol extract of leaves and flowers was examined using Gas chromatogram-Tandem Mass Spectrometry (GC-MS/MS). The ethanol extract of *Clematis zeylanica* leaves and flowers was subjected to GC-MS/MS analysis and identified 31 and 29 bioactive chemical compounds, respectively. n-Hexadecanoic acid (22.98%), Linoleic acid ethyl ester (9.69%), 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (6.94%), and 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (6.76%), Geranyl palmitoleate (4.30%) make up the majority of the bioactive components in leaves. 3,7,11,15-tetramethylhexadec-2en-1-yl acetate (8.52%), (Tanshinone IIA) Phenanthro[1,2-b]furan-10,11-dione, 6,7,8,9-tetrahydro-1,6,6-trimethyl- (5.84% +3.48% +5.36%), and beta-Tocopherol, O-methyl- (2.58%) are the principal bioactive chemicals found in flowers. This study reports compounds 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-, 9,12,15-Octadecatrienoic acid, (Z,Z,Z), Linoleic acid ethyl ester, Phenanthro[1,2-b]furan-10,11-dione, Geranyl palmioleate, Squalene as important new lead compounds for the first time from this species. Additional research is required to identify the extract's active ingredient and to clarify the precise mechanism of action in treating various illnesses and develop novel drugs.

Key Word: *Clematis zeylanica*, Ethanol extract, Tanshinone IIA, Squalene, Ethnomedicine, GC-MS/MS.

I. INTRODUCTION:

Plants have the ability to synthesize a variety of chemicals in order to better react to environmental stimuli. Humans have learned to recognize the significance of these chemicals in the treatment of human illnesses and the enhancement of quality of life as a result of the accumulation of practical knowledge. People are progressively finding the active components of conventional herbs through the application of technology in compound extraction, separation, and structural identification. Modern medicine has emerged as a result, using either a single substance or a combination of substances. New active compounds are continually discovered and employed in medicine, health care, and agriculture as a result of expanding study into plant natural products (Lv et al., 2022). Any plant whose substances can be used therapeutically or as raw materials for the creation of potent pharmaceuticals is referred to be a medicinal plant (Sofowora et al., 2013). In order to preserve good health and treat ailments, a large number of people in both developing and developed countries rely on medicinal plants and the products and chemicals generated from them (Umarani et al., 2022). It is estimated that there are between 750,000 and 1,000,000 different plant species in the world. Among them 500,000 have been identified and named. The number of plants that have been utilized as medicines since ancient times is steadily rising.

The plant *Naravelia zeylanica* (Linn) DC, which is a member of the Ranunculaceae family, is used in the Indian ayurveda system of medicine, to cure helminthiasis, dermatopathy, leprosy, rheumatalgia, odontalgia, colic inflammation, wounds, and ulcers. It is found all over India, but is most prevalent in the warm Eastern Himalayas, Assam, Bengal, Bihar, and larger portions of the Deccan Peninsula. *Naravelia zeylanica* leaves have traditionally been used to treat vitiated vata, pitta, inflammation, and skin conditions. The crushed leaves of *Naravelia zeylanica* release a strong aroma that is inhaled to treat migraines and all other types of headaches, including the common cold. The external application of root and stem paste treats psoriasis, itchiness, and skin allergies (Varghese, 2017). Back pain and headaches are treated externally using root paste (Udayan et al., 2006; Baby et al.,2018) *Clematis zeylanica* is utilized in Kerala as a source of medication for skin conditions, leprosy, toothaches, and intestinal worms. The leaf and stem juices are used by Karnataka-based practitioners of traditional medicine to treat dermatitis and psoriasis. The root and stem are used to relieve headaches and malarial fever because they have a potent, pervasive odour. Whole plant paste is administered topically to the affected area for two to three days to cure wounds and worm infections (Mohan et al., 2008). Bathing of boiled leaves in the water used to treat rheumatism (Kuru et al., 2011).

In recent years, research on medicinal plants and the active ingredients obtained from them has raised interest in researchers. Patients with chronic medical illnesses, such as malignancies (2%), liver diseases (21%), HIV (22%), asthma (24%) and rheumatologic disorders (26%), typically prefer and utilize plant-based products. Many people think that using natural treatments is safe (Keskin, 2018) Years of research have shown that plants can be used as herbal remedies to treat and prevent disease. This is because they are inexpensive and easily accessible (Rouf et al., 2021).

Medicinal plants might be a source of phytochemical substances with significant biological and pharmacological effects. Identification of bioactive compounds from medicinal plants opens the door to future biological and pharmacological investigations. This study aims to identify bioactive compounds from leaves and flowers of *Clematis zeylanica* (L.) Pori.

II. MATERIALS AND METHODS:

2.1. Plant Collection:

The Seeds and flowers of *Clematis zeylanica* (L.) Pori. were harvested and collected (Latitude $12^0$66’61”N and Longitude $77^0$91’54”E.) in the Sanamavu Forest of Tamil Nadu, Krishnagiri district, India. It is located 8 kilometres southeast of Samanapalli village, 9 kilometres southwest of Kelamangalam village. The nearest town station is Hosur.

a. Habitat b. Young twig c. Flower d. Fruit (Achenes)

Fig. 1.Different parts of *Clematis zeylanica* (L.)Pori.

2.2. Leaves and Flower Extraction:

Collected leaves and flowers were washed using clean running tap water and distilled water, dried in the room temperature under the shade, powdered and stored in the closed vessel. The solvent ethanol (10mg/50ml) is added to the dried powdered material. The mixture was kept for 72 hours with frequent shaking at room temperature. Afterward, the extract was filtered from marc (the damp solid substance) through Whatman No. 41 filter paper. The extraction procedure was repeated thrice. The extract thus obtained was preserved in the refrigerator for further GC-MS/MS analysis.

2.3. Gas Chromatogram– Tandem Mass Spectroscopy (GC–MS/MS) analysis:

The extracts from leaves and flowers of *Clematis zeylanica* L. (Pori.) were analyzed using an Agilent 7890B Gas Chromatography system fitted with 7000C Mass Spectrometer Triple Quad (Agilent Technologies Inc. Sant Clara, CA, USA). Helium (99.999% purity) was employed as the carrier gas, with a 3 ml/min front inlet purge flow rate and a 1.2 ml/min constant gas flow rate through the column. An Agilent HP-5ms UI (30m x 250 µm x 0.25 µm – Agilent Technologies Inc., Santa Clara, CA, USA) column was used for Volatile Organic Compounds (VOCs) separation. The oven temperature program began at 130^0C for 1 minute, then ramped up to 270^0C at a rate of 10^0C/min and was hold for 12 minutes and run time set to 27 min. Temperature for the Mass Selective Detector (MSD) transfer line, Ion source and Quadrupole mass detectors were set to 290^0C, 300^0C and 150^0C respectively. At ionization energy of 70eV, Mass Spectra in Electron Impact Ionizations (ME_EI) mode were observed. Data was collected using the mass spectrometer's scanning mode from m/z 40 to 500. The solvent delay time was 3.2 minutes.

2.4. Identification of compounds:

The components were correlated with National Institute Standard and Technology (NIST) library. NIST has more than 62,000 patterns. Mass spectrum of unknown component was compared with the spectrum of the known components stored in the NIST library.

III. RESULT AND DISCUSSION:

3.1. GC–MS/MS Analysis

The medicinal plants are exhibiting foundation of various phytochemical constitutes which are revealed by GC–MS/MS analysis. Current work examined the presence of phytochemical constitutes in ethanol extracts of Leaves and flowers of *Clematis zeylanica* L. (Pori.) through GC–MS/MS analysis. Different kinds of phytochemicals were identified along with retention time, Confirmation of acceptance for studies (CAS), molecular formal and peak of this extracts. **Tables 1 & 2** give some of the high peak

compounds including its molecular formula and Conformation of Acceptance for Studies (CAS#) of each extract. Based on the GC–MS/MS report, *Clematis zeylanica* ethanol extract (CZEE) of leaves and flowers, reported a number of higher peak compounds.

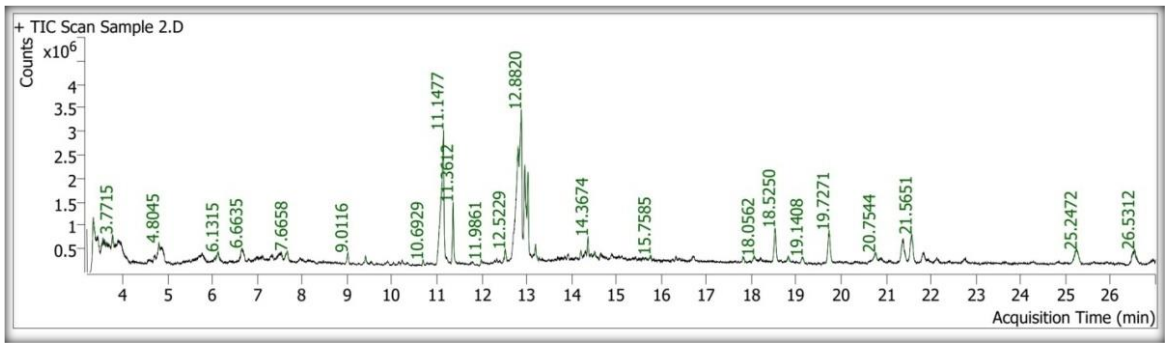


Fig. 2: GC-MS/MS chromatogram of ethanol fraction of *Clematis zeylanica* (L.)Pori. leaves.

The GC-MS/MS chromatogram of ethanol leaves extract depicted different peaks resulting from the presence of 31 compounds. Based on abundance, the top compound identified was n-hexadecanoic acid (22.98%), followed by Linoleic acid ethyl ester (9.69%), 9,12,15-octadecatrienoic acid, ethyl ester, (z,z,z)- (6.94%), 9,12,15-octadecatrienoic acid (6.76%), Hexadecanoic acid, ethyl ester (5.24%), Squalene (5.19%) Geranyl oleate (4.94%), Geranyl palmitoleate (4.30%), 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (3.88%), Heptacosane (3.75%), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (1.28%) and trace percentage of other bioactive compounds.

Table 1: Compounds from the ethanol leaves extract of *C. zeylanica* (L.) Pori. by GC-MS/MS.

RT	Compound Name	Area %	Component	CAS#	Match Factor	Formula
3.3462	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	3.88	1833017.1	28564-83-2	71.7	$C_6H_8O_4$
3.5303	2(3H)-Furanone, 5-heptyldihydro-	0.12	58432.3	104-67-6	54.3	$C_{11}H_{20}O_2$
3.5581	3,3-Dimethylbutan-2-yl 2-methylbutanoate	0.37	172623.9	1000372-72-8	54.4	$C_{11}H_{22}O_2$
3.6354	1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol	0.18	87069.3	1000127-68-4	52.6	$C_8H_{16}N_2O_7$
3.7715	5-Hydroxymethylfurfural	0.91	431648.6	67-47-0	64.7	$C_6H_6O_3$
4.8045	Benzene, 1-(4-methyl-4-pentenyl)-4-(trifluoromethyl)-	0.69	323733.0	74672-15-4	57.8	$C_{13}H_{15}F_3$
6.1315	1-(4-Ethoxyphenyl)propan-1-ol	0.91	428314.2	1031927-88-4	57.3	$C_{11}H_{16}O_2$
6.6635	Benzoic acid, 4-ethoxy-, ethyl ester	0.43	204201.9	23676-09-7	71.3	$C_{11}H_{14}O_3$
7.6658	11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester	1.26	597470.9	1010193-81-6	57.6	$C_{15}H_{26}O_4$
9.0116	Tetradecanoic acid	1.22	575653.7	544-63-8	62.2	$C_{14}H_{28}O_2$
9.4106	Hexadecane, 1,1-bis(dodecyloxy)-	0.50	234419.0	56554-64-4	53.6	$C_{40}H_{82}O_2$
10.6929	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-	0.34	160528.9	38520-31-9	54.8	$C_{19}H_{36}O_3$
11.1477	n-Hexadecanoic acid	22.9	10859464.5	57-10-3	72.5	$C_{16}H_{32}O_2$

11.3612	Hexadecanoic acid, ethyl ester	5.24	2474092.6	628-97-7	81.2	$C_{18}H_{36}O_2$
11.9861	7-Methyl-Z-tetradecen-1-ol acetate	0.36	170389.2	1000130-99-6	52.4	$C_{17}H_{32}O_2$
12.5229	Phytol	1.14	538730.9	150-86-7	63.2	$C_{20}H_{40}O$
12.8138	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	2.44	1151488.3	463-40-1	75.0	$C_{18}H_{30}O_2$
12.8820	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	6.76	3193412.8	463-40-1	71.8	$C_{18}H_{30}O_2$
12.9563	Linoleic acid ethyl ester	9.69	4578482.4	544-35-4	81.5	$C_{20}H_{36}O_2$
13.0306	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	6.94	3277672.3	1191-41-9	78.8	$C_{20}H_{34}O_2$
13.1992	Ethyl 14-methyl-hexadecanoate	1.03	487793.8	1000336-64-7	57.0	$C_{19}H_{38}O_2$
14.2111	cis-5,8,11,14,17-Eicosapentaenoic acid	0.62	290776.7	10417-94-4	61.3	$C_{20}H_{30}O_2$
14.3674	Ethyl 5,8,11,14,17-icosapentaenoate	1.29	610242.2	84494-70-2	73.0	$C_{22}H_{34}O_2$
14.5113	Sterebin A	0.38	177926.3	107647-14-3	56.2	$C_{18}H_{30}O_4$
15.7585	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	0.55	261996.6	55282-12-7	51.4	$C_{26}H_{54}$
17.8195	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	0.60	285460.7	55282-12-7	55.1	$C_{26}H_{54}$
18.0562	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-	0.67	314857.8	22393-98-2	53.7	$C_{34}H_{64}O_2$
18.5250	Geranyl palmitoleate	4.30	2031252.6	1000414-43-3	73.9	$C_{26}H_{46}O_2$
18.8205	5,6,7,8,9,10-Hexahydro-9-benzyl-spiro [2H-1,3-benzoxazine-4,1'-cyclohexane]-2-thione	0.67	316454.1	1000216-50-2	51.5	$C_{20}H_{27}NOS$
19.1408	Dodecyl cis-9,10-epoxyoctadecanoate	0.60	281984.4	92332-53-1	53.9	$C_{30}H_{58}O_3$
19.7271	Squalene	5.19	2452267.4	111-02-4	74.1	$C_{30}H_{50}$
20.7544	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	1.28	604197.2	55282-12-7	56.7	$C_{26}H_{54}$
21.3671	Geranyl oleate	1.56	737149.6	81601-03-8	70.8	$C_{28}H_{50}O_2$
21.5651	Geranyl oleate	4.94	2332080.9	81601-03-8	68.8	$C_{28}H_{50}O_2$
25.2472	Heptacosane	3.75	1771241.8	593-49-7	65.4	$C_{27}H_{56}$
26.5312	beta.-Tocopherol, O-methyl-	0.62	291238.5	1000374-73-6	58.7	$C_{29}H_{50}O_2$

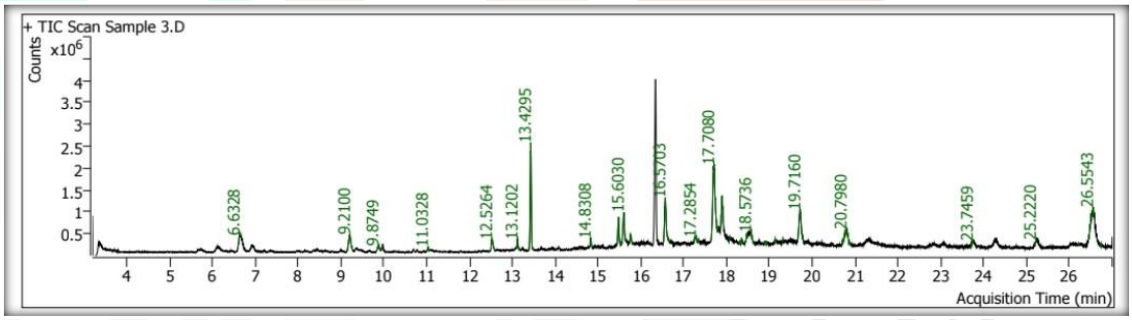


Fig. 3: GC-MS/MS chromatogram of ethanol fraction of *Clematis zeylanica* (L.) Pori. flowers

The GC-MS/MS chromatogram of ethanol flower extract depicted different peaks resulting from the presence of 29 compounds. Based on abundance, the top compound identified was 3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate (8.52%), followed by Phenanthro[1,2-b]furan-10,11-dione, 6,7,8,9-tetrahydro-1,6,6-trimethyl- (5.84%), Phenanthro[1,2-b]furan-10,11-dione, 6,7,8,9-tetrahydro-1,6,6-trimethyl- (5.36%) , Phenanthro[1,2-b]furan-10,11-dione, 6,7,8,9-tetrahydro-1,6,6-trimethyl- (3.48%), 1H-2,8a-Methanocyclopenta[a] cyclopropa[e] cyclodecen 11-one,1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6- trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1S- 1.alpha.1a.alpha., 2.alpha., 5.beta., 5a.beta., 6.beta., 8a.alpha., 9.alpha.,10a.alpha.)]-(3.16%), Acetic acid, 6-hydroxy-2,2-dimethyltetrahydro- [1,3] dioxolo [4,5-c] pyran-7-yl ester (2.80%), .beta.-Tocopherol, O-methyl- (2.58%), Pyrimidine, 4-(4-methylphenyl)-6-phenyl-2-(1-piperazinyl)- (2.55%), Pregn-5-ene-3,11,12,14,20-pentol, 11-acetate 12-(3-methylbutanoate), 3.beta.,11.alpha.,12.beta.,14beta.)- (2.28%), Phytol (1.52%), dl-.alpha.-Tocopherol (1.35%), 4a,7b-Dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-9a-((2-methylpropanoyl)oxy)-5-oxo- 1a,1b,4,4a,5,7a,7b,8,9,9a-decahydro-1H-cyclopropa[3,4]benzo[1,2-e]azulen-9-yl 2-methylbutanoate, TMS derivative(1.29%), Benzene, 1-[(4-butylphenyl)ethynyl]-4-ethoxy-2-methyl- (1.01%), and trace percentage of other bioactive compounds.

Table 2: Compounds identified from the ethanol flower extract of *C. zeylanica* by GC MS/MS.

RT	Compound Name	Area %	Component	CAS#	Match Factor	Formula

6.6328	Benzoic acid, 4-ethoxy-, ethyl ester	0.95	490879.3	23676-09-7	74.2	$C_{11}H_{14}O_3$
9.2100	Acetic acid, 6-hydroxy-2,2-dimethyltetrahydro- [1,3] dioxolo [4,5-c] pyran-7-yl ester	2.80	1445489.8	1000186-40-6	56.7	$C_{10}H_{16}O_6$
9.8749	d-Mannitol, 1-O-ethyl-, pentaacetate	0.82	422834.7	59921-39-0	51.7	$C_{18}H_{28}O_{11}$
11.0328	l-(+)-Ascorbic acid 2,6-dihexadecanoate	0.36	185372.0	28474-90-0	57.5	$C_{38}H_{68}O_8$
12.5264	Phytol	1.52	782241.3	150-86-7	64.4	$C_{20}H_{40}O$
13.1202	Hexadecanoic acid, butyl ester	0.91	470564.1	111-06-8	64.6	$C_{20}H_{40}O_2$
13.4295	3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate	8.52	4391732.7	76337-16-1	88.3	$C_{22}H_{42}O_2$
14.8308	Octadecanoic acid, eicosyl ester	0.71	366555.3	22413-02-1	54.1	$C_{38}H_{76}O_2$
15.4745	Pregn-5-ene-3,11,12,14,20-pentol, 11-acetate 12-(3-methylbutanoate), 3.beta.,11.alpha.,12.beta.,14beta.)-	2.28	1177134.5	20230-38-0	66.8	$C_{28}H_{44}O_7$
15.6030	1H-2,8a-Methanocyclopenta[a] cyclopropa[e] cyclodecen 11-one,1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1S- 1.alpha.1a.alpha., 2.alpha., 5.beta., 5a.beta., 6.beta., 8a.alpha., 9.alpha.,10a.alpha.)]-	3.16	1628056.9	52557-29-6	64.8	$C_{20}H_{28}O_6$
15.7655	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	0.76	394239.9	55282-12-7	59.0	$C_{26}H_{54}$
16.5703	Phenanthro[1,2-b]furan-10,11-dione, 6,7,8,9-tetrahydro-1,6,6-trimethyl-	5.84	3011012.1	568-72-9	74.2	$C_{19}H_{18}O_3$
17.2854	3,9-Epoxypregnane-11,14,18-triol-20-one, 16-cyano-3-methoxy-,11-acetate	0.74	379489.6	1000194-66-5	56.2	$C_{25}H_{35}NO_7$
17.3458	2,4,6-Decatrienoic acid, 1a,2,5,5a,6,9,10,10a-octahydro- 5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-teteramethyl-11-oxo-1H-2,8a-methanocylopenta[a] cyclopropa[e]cyclodecen-6-yl ester, [1aR- (1a.alpha.,2.alpha.,5.beta.,5a.beta., 6.beta.,8a. alpha.,9.alpha.,10a.alpha.)]-	0.06	29062.7	52557-28-5	58.1	$C_{30}H_{40}O_6$
17.8072	9-(Acetyloxy)-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-ox0-1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-9aH-cyclopropa[3,4]benzo[1,2-e]azulen-9a-yl 2-methylbutanoate,TMS	0.08	43032.6	1000503-50-5	56.4	$C_{30}H_{46}O_8Si$
17.8954	Phenanthro[1,2-b]furan-10,11-dione, 6,7,8,9-tetrahydro-1,6,6-trimethyl-	3.48	1791679.8	568-72-9	74.4	$C_{19}H_{18}O_3$
18.0704	4-[(2R,3R)-2,3-Dihydro-3-methyl-5-(1E)-1-propen-1-yl-2-benzofuranyl]-2-methoxyphenol	0.12	63969.7	109225-40-3	51.1	$C_{19}H_{20}O_3$
18.3150	3-Desoxo-3,16-dihydroxy-12-desoxyphorbol 3,13,16,20-tetraacetate	0.11	57694.2	1000203-00-5	55.0	$C_{28}H_{38}O_{10}$
18.3584	Demecolcine	0.26	133156.8	477-30-5	50.2	$C_{21}H_{25}NO_5$
18.4776	Hydrocortisone Acetate	0.68	350053.9	50-03-3	62.4	$C_{23}H_{32}O_6$
18.5736	Benzene, 1-[(4-butylphenyl)ethynyl]-4-ethoxy-2-methyl-	1.01	518384.8	107949-29-1	51.1	$C_{21}H_{24}O$
18.9110	2,3,14-Trihydroxypregn-7-ene-6,20-dione, 2TMS	0.21	106902.8	1000497-45-1	52.5	$C_{27}H_{46}O_5Si_2$
19.1370	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9a-tris(acetyloxy)-3-[(acetyloxy) methyl]-2-chloro-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-,[1aR (1a.alpha.,1b.beta.,2.alpha.,3.beta.,4a.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]-	0.09	44421.1	77573-36-5	55.7	$C_{28}H_{37}C_lO_{11}$
19.3166	Androst-11-ene-14-carboxylic acid, 3-(acetyloxy) -4,4,8,12,16-pentamethyl-15,17-dioxo-16-hydroxy,methyl ester, Ac	0.07	37958.4	1000494-30-0	52.9	$C_{30}H_{42}O_8$
19.7160	Phenanthro[1,2-b]furan-10,11-dione, 6,7,8,9-tetrahydro-1,6,6-trimethyl-	5.36	2764129.8	568-72-9	64.6	$C_{19}H_{18}O_3$
20.7732	4a,7b-Dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-9a-((2-methylpropanoyl)oxy)-5-oxo-1a,1b,4,4a,5,7a,7b,8,9,9a-decahydro-1H-cyclopropa[3,4]benzo[1,2-e]azulen-9-yl 2-methylbutanoate, TMS derivative	1.29	663621.6	1000488-81-0	62.1	$C_{32}H_{50}O_8Si$
20.7980	Pyrimidine, 4-(4-methylphenyl)-6-phenyl-2-(1-piperazinyl)-	2.55	1313692.3	326481-01-0	50.0	$C_{21}H_{22}N_4$
23.7459	4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one,8,8a-bis(acetyloxy)-2a-[(acetyloxy)methyl]- 1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-3,3a,6b-trihydroxy-1,1,5,7-teteramethyl-	0.39	199825.1	77646-26-5	55.1	$C_{26}H_{34}O_{11}$

25.2220	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	0.38	194489.5	55282-12-7	55.2	$C_{26}H_{54}$
26.2231	Demecolcine	0.05	27183.2	477-30-5	50.9	$C_{21}H_{25}NO_5$
26.4800	dl-.alpha.-Tocopherol	0.76	392972.5	10191-41-0	69.1	$C_{29}H_{50}O_2$
26.5063	dl-.alpha.-Tocopherol	1.39	715248.8	10191-41-0	65.8	$C_{29}H_{50}O_2$
26.5218	dl-.alpha.-Tocopherol	1.10	567642.8	10191-41-0	51.5	$C_{29}H_{50}O_2$
26.5543	beta.-Tocopherol, O-methyl-	2.58	1329488.8	1000374-73-6	53.0	$C_{29}H_{50}O_2$
26.7245	Vitamin E	0.10	53727.1	59-02-9	51.5	$C_{29}H_{50}O_2$

3.2. Discussion:

The crude extract of this plant is frequently used by tribal and rural populations in India for medical and other purposes. Leaves have rich in n-hexadecanoic acid which is used for the treatment of rheumatism in the traditional medicinal system of India, Ayurveda (Aparna et al., 2012). The most abundant compound in ethanol extract was n-hexadecanoic acid, and it has anti-inflammatory, anticancer (Mazumder et al., 2020). antioxidant, and hypocholesterolemic properties(Tulika et al., 2017). Hexadecanoic acid ethyl ester has been reported to have antioxidant activities (Guerrero et al., 2017). Linoleic acid ethyl ester has been reported to be effective in the treatment of infantile neuroaxonal dystrophyl (Adams et al., 2020). 9, 12, and 15-Octadecatrienoic Acid exhibits anti-inflammatory, cancer-preventive, hepato protective, antioxidant, and hypocholesterolemic properties (Praveen Kumar et al., 2010). 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- is used as Antiinflammatory, Insectifuge Hypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary (Sermakkani et al., 2012). 9,12,15-Octadecatrienoic acid, ethyl ester (Z,Z,Z) is used for a variety of purposes, including anti-inflammatory, insecticide, hepatoprotective, nematicide, anti-histaminic, anti-eczema, anti-acne, and cancer prevention. Antiandrogenic, antiarthritic, and anticoronary 5-Alpha reductase inhibitor (Sermakkani et al., 2012). Squalene acts as a protective agent and has been shown to mitigate the side effects of chemotherapy. Furthermore, squalene has chemopreventive properties on its own. Although it is just a minor inhibitor of tumour cell proliferation, its potentiating activity contributes to cancer treatment either directly or indirectly (Reddy et al., 2009). Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor (Sermakkani et al., 2012). Octadecane, 3-ethyl-5-(2-ethylbutyl)- has antioxidant and anti-inflammatiory property(Kumar et al., 2021). 5- Hydroxymethylfurfural is used as antioxidant and in baking industry. Tetradecanic acid used as Antioxidant, Cancer preventive (Rajendran et al., 2017; Gangaprasad et al., 2017). 11,13-Dihydroxytetradec-5-ynoic acid, methyl ester used as Arachidonic acid inhibitor(Dahiya et al., 2019).

Table 3: Biological activity of specific phytochemicals identified in Leaves of *C. zeylanica* (L.)Pori.

S.No	Name of the compound	Compound nature	Biological activities
1.	n-hexadecanoic acid	Fatty acid	Anti-rheumatism, anti-inflammatory, anticancer, antioxidant, and hypocholesterolemic.
2.	Hexadecanoic acid ethyl ester	Fatty acid ester	Antioxidant, Flavor, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5- Alpha reductase inhibitor
3.	Linoleic acid ethyl ester	Fatty acid ester	Infantile neuroaxonal dystrophyl
4.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Linolenic acid	Antiinflammatory, Insectifuge Hypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary
5	9,12,15-Octadecatrienoic acid, ethyl ester (Z,Z,Z)	Fatty acid ester compound	anti-inflammatory, insecticide, hepatoprotective, nematicide, anti-histaminic, anti-eczema, anti-acne, and cancer prevention.
6.	Squalene	Triterpene	Antibacterial, Antioxidant,Pesticide, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor.
7.	Phytol	Diterpene	Antimicrobial, anti-inflammatory, antioxidant, diuretic properties.

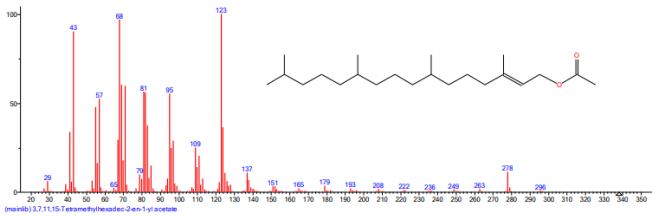
8.	Geranyl oleate	Terpene	Bioactivity in food, pharmaceutical, cosmetic.
9.	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	Flavonoid	Strong antioxidant.
10.	5- Hydroxymethylfurfural	Organic compound	Antioxidant
11.	Tetradecanic acid	Fatty acid	Antioxidant, Cancer preventive.
12.	Beta –tocopherol, o-methyl-	Vitamin E	Antioxidant
13.	Sterebin A	Sesquiterpenoids	Sweetener

Table 4: Biological activity of specific phytochemicals identified in Flowers of *C. zylanica* (L.)Pori.

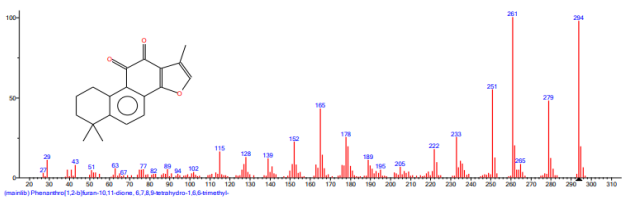
S.No	Name of the compound	Compound nature	Biological activities
1.	Phenanthro [1, 2-b] furan-10, 11-dione, 6, 7, 8, 9-tetrahydro-1, 6, 6-trimethyl (Tanshinone IIA).	Lipophilic components	Cancer, heart disease, inflammatory disease, and neurodegenerative diseases.
2.	Beta Tocopherol, O methyl	Vitamin-E	Anti-aging, analgesic, anti-diabetic, anti-inflammatory, antioxidant, anti-dermatitic, anti-leukemic, anti-tumor, anti-cancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, and anticoronary properties

One of the main components of flower extract is Phenanthro [1, 2-b] furan-10, 11-dione, 6, 7, 8, 9-tetrahydro-1, 6, 6-trimethyl (Tanshinone IIA). Since it has been demonstrated to have therapeutic potentials against a number of illnesses, such as cancer, heart disease, inflammatory disease, and neurodegenerative diseases, Traditional Chinese Medicine holds it in high respect (Ansari et al., 2021). Phytol biologically active compound possesses anti-obesity (Momoh et al., 2022) and Antimicrobial, anti-inflammatory, antioxidant, and diuretic properties. Vitamin E (Beta Tocopherol, O methyl-) has anti-aging, analgesic, anti-diabetic, anti-inflammatory, antioxidant, anti-dermatitic, anti-leukemic, anti-tumor, anti-cancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, and anticoronary properties(Waghmare et al., 2014). 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (or) 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) is a strong antioxidant (Xiangying et al., 2013).

Identified compound from ethanol extract of *Clematis zeylanica* flower

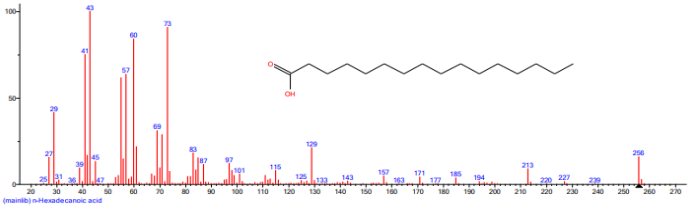


3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate

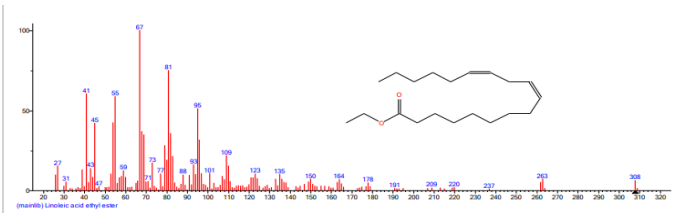


Phenanthro[1,2-b]furan-10,11-dione, 6,7,8,9-tetrahydro-1,6,6-trimethyl-

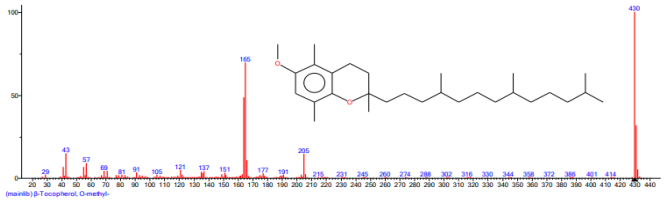
Identified compound from ethanol extract of *Clematis zeylanica* leaves



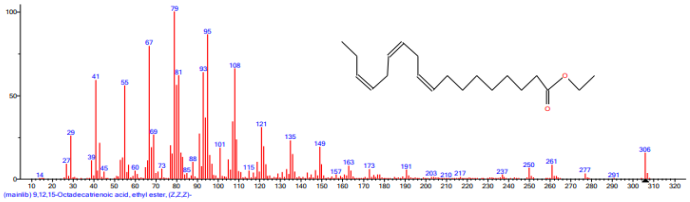
n-hexadecanoic acid



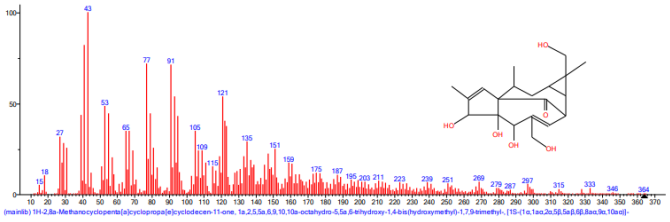
Linoleic acid ethyl ester



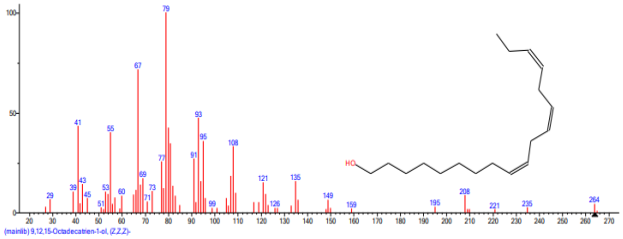
beta.-Tocopherol, O-methyl-



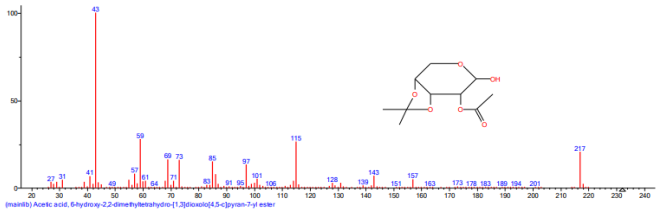
9,12,15-Octadecatrienoic acid, ethyl ester, (z,z,z)-



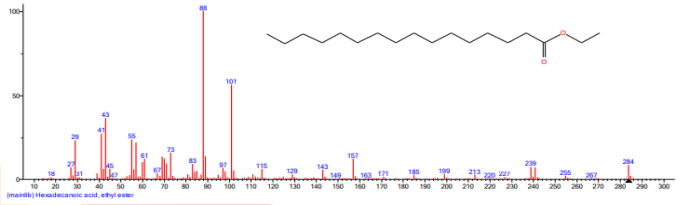
1H-2,8a-Methanocyclopenta[a] cyclopropa[e] cyclodecen 11-one,1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1S- 1.alpha.1a.alpha., 2.alpha., 5.beta., 5a.beta., 6.beta., 8a.alpha., 9.alpha.,10a.alpha.)]-



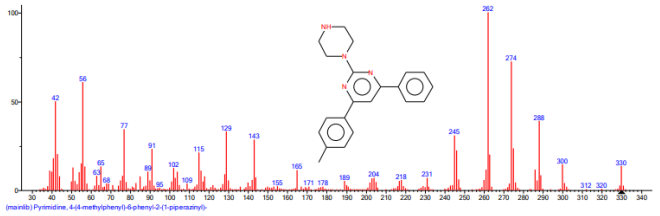
9, 12, 15- Octadecatrienoic acid (z,z,z)-



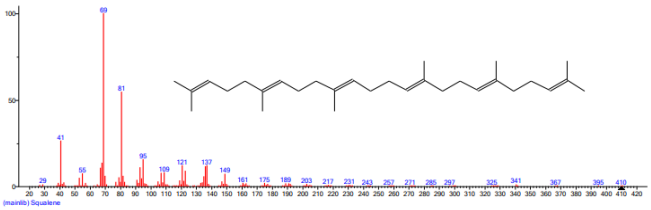
Acetic acid, 6-hydroxy-2,2-dimethyltetrahydro- [1,3] dioxolo [4,5-c] pyran-7-yl ester



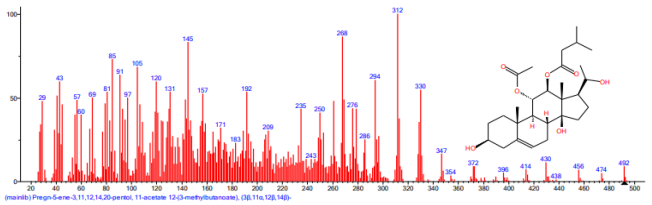
Hexadecanoic acid, ethyl ester



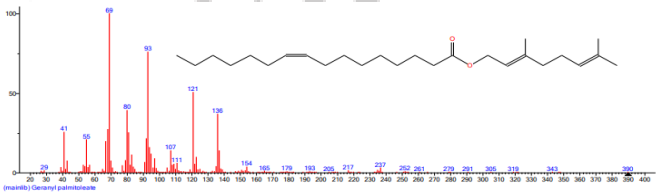
Pyrimidine, 4-(4-methylphenyl)-6-phenyl-2-(1-piperazinyl)-



Squalene



Pregn-5-ene-3,11,12,14,20-pentol, 11-acetate 12-(3-methylbutanoate), 3.beta.,11.alpha.,12.beta.,14beta.)-



Geranyl palmioleate

Fig. 5: Mass spectra of some of the identified compound from ethanol extract of *C. zeylanica* flower and Leaves

IV. CONCLUSION:

The GC-MS/MS study revealed that the ethanol extract of the leaves and flowers of *Clematis zeylanica* contained 31 and 29 bioactive compounds, respectively.

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