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PHYTOCHEMICAL EVALUATION AND GC-MS ANALYSIS OF ETHANOLIC LEAVES EXTRACT OF *VITEX LEUCOXYLON*.

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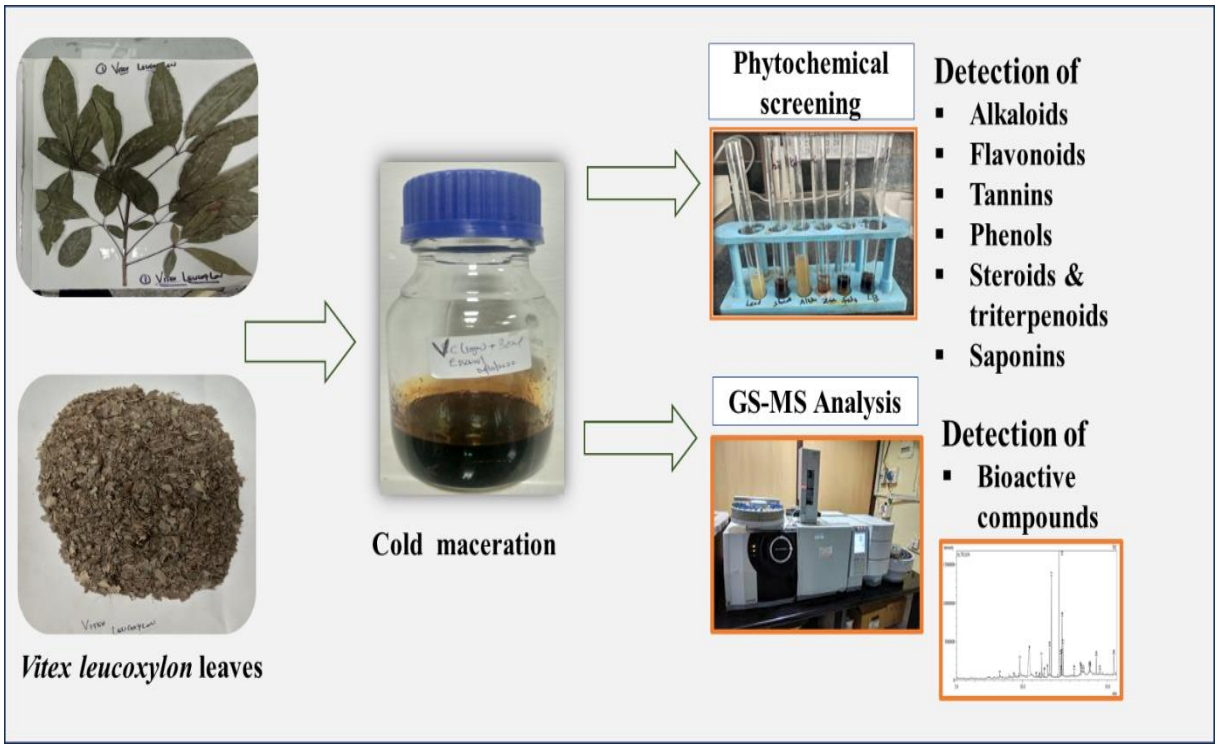
Abstract:

Medicinal plants show a foremost protagonist in the primitive healthcare systems of countries. The therapeutic properties of the plant are determined by the biological substances present in the plant components that are used to produce medicinal remedies. *Vitex leucoxylon* belongs to the Lamiaceae family. It is extensively distributed in the Eastern and Western Ghats of India, as well as in Sri Lanka. Traditionally, the leaves of this plant have been extensively used to treat leprosy, cancer, as an emetic, and for headaches. Objective: The current research aimed to explore the phytochemical constituents of the leaves of *Vitex leucoxylon* through phytochemical evaluation, and gas chromatography-mass spectrometry (GC-MS) analysis. Methods: The shade-dried leaves were powdered and extracted with ethanol using the cold maceration method; we conducted a phytochemical examination to evaluate the characteristics of secondary metabolites and used gas chromatography-mass spectrometry (GC-MS) to find the individual phytocompounds in the ethanolic leaf extract. Phytochemicals were determined using molecular weights (m/z) obtained from GC-MS chromatograms. Phytocompounds were identified by comparing data with the National Institute of Standards and Technology (NIST) library and interpreting spectral peaks.

Results: Phytochemical screening identified alkaloids, flavonoids, tannins, phenols, steroids, glycosides, and anthraquinones. GC-MS analysis identified twenty-six phytochemical compounds in Vitex leucoxylon leaf extract. The major bioactive compounds in the ethanol extract were beta-D-Glucopyranoside, methyl (22.11%), phytol (15.77%), hexadecanoic acid, ethyl ester (10.75%), and squalene (11.31%). Minor compounds included caryophyllene oxide (2.31%), guaiol (1.75%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (2.87%), decanoic acid, ethyl ester (2.10%), 9,12-octadecadienoic acid (9.38%), and octadecanoic acid (4.41%). The identification of bioactive compounds is based on retention time, peak area, molecular formula, and probability. Conclusion: The results suggest that *Vitex leucoxylon* may have antioxidant, antimicrobial, anticancer, antidiabetic, hypocholesterolemic, and hepatoprotective activities due to the secondary metabolites in the ethanol extract.

Keywords: *Vitex leucoxylon*, phytochemical, GC-MS analysis, ethanol extract.

Graphical abstract:



1. INTRODUCTION:

Plants are a rich source of herbal medicines, traditionally used in folklore for various diseases. Plant-based remedies have proven useful in disease treatment and management and are extensively used in ethnomedicine [1-3]. The high cost and limited availability of conventional medicines, especially in rural and developing areas, have sustained reliance on traditional therapeutics. About 75-90% of the world's population still depends on plants and plant extracts for primary health care. This widespread use has sparked global interest in screening medicinal plants for their bioactive phytochemicals and pharmacological efficacy. Most medicinal plant parts are used as raw drugs and are reported to possess various medicinal properties [4, 5].

In traditional medicine, medicinal plants are considered an essential component of drugs for treatment. The kingdom of plants is an ideal spot for finding new potential medications, and the importance of medicinal plants has come to prominence in recent years. Medicinal plants generate a diversity of bioactive molecules and are significant providers of several medicinal compounds. Herbal plant extracts are immensely helpful as well as one of the main forms of medicine. These serve as vital to fostering production and preventing an array of illnesses. These are the lower-cost sources for effective therapies and treatments for multiple infections. Because they hold an extensive number of secondary metabolites, like flavonoids, alkaloids, phenolics, and tannins, which enhance innate immune response, growth, and resistance to disease toward pathogenic microorganisms in humans and other organisms, extracts from medicinal plants have recently gained attention as a viable substitute. Approximately 80% of people in affluent nations utilize a variety of medicinal plants as traditional medicines, such

as antifungals, anticancer medications, and antibacterial pharmaceuticals, in a variety of ways. Secondary metabolites, which are incredibly varied chemically and taxonomically with unknown roles, are abundant in medicinal plants. Numerous phytochemicals are employed extensively in scientific study, veterinary medicine, agriculture, and human therapy [6-9].

Vitex leucoxylon L.f. is an endemic tree in peninsular India and Sri Lanka, reaching 12 meters in height. It has 3-7 foliolate leaves with a 10 cm elongated petiole. The cream and purplish flowers are in corymbose panicles, and fruits are present year-round. These trees are mostly found along riverbanks and hills. *Vitex leucoxylon* is a valuable medicinal tree in India. Its pharmaceutically active extracts have demonstrated hypoglycemic and anti-inflammatory properties. Various parts of the tree are used for medicinal purposes, such as stem decoction for whooping cough, dried leaf powder smoke for asthma, leaf paste for pox scars, root bark for fever reduction, fresh twigs as insecticides, and dry leaf powder for food grain preservation. Native healers suggest leaf extract to inhibit blood sugar increase. It is traditionally used to relieve headaches and catarrh. Pharmacological studies have shown that aqueous and ethanolic extracts of *V. leucoxylon* leaves possess anti-psychotic, anti-depressant, analgesic, anti-inflammatory, anti-parkinsonian, and anti-microbial activities. Additionally, the crude alcoholic extract of the leaves has demonstrated anti-inflammatory and wound healing properties in an acute inflammation model. The roots and bark are astringent, and the roots are used as a febrifuge. In Tamil Nādu, the wood is primarily used for construction, general purposes, and cartwheels. Due to its attractive color, small pores, and conspicuous medullary rays with silvery flecks, the wood is recommended for decorative cabinets and similar work (The Wealth of India, Raw Material). This research analyzes bioactive compounds in the ethanolic leaf extract of *Vitex leucoxylon*. [10-17].

2. MATERIALS AND METHODS

2.1. Plant material.

Collection and identification of plant material

Vitex leucoxylon exploited for the research was found from Tirupati, Andhra Pradesh, India. The plant was authenticated by Dr. Sankararao Mudadla, Botanical Survey of India, Hyderabad. The voucher number is BSI/DRC/2021-22/Tech./Identification/522. The *Vitex leucoxylon* plant material was washed with tap water and air-dried.

Fig: 1. *Vitex leucoxylon* plant leaves

2.2. Preparation of extract

The plant material was dried out and ground to powder in a motorized grinder. The leaf powder of Vitex leucoxylon was weighed, placed in a reagent bottle, immersed in ethanol for 72 hours, and filtered through Whatman No. 41 paper. The extracts were collected and

concentrated using a rotary evaporator under reduced pressure. Before the phytochemical and GC/MS analysis, the concentrated extracts were kept in an air-tight container at a temperature of 4°C [18-20].

2.3. Phytochemical evaluation:

Standard procedures were used for qualitatively identifying numerous secondary metabolites using the ethanolic leaf extract of *Vitex leucoxylon* [21-25].

2.4. GC/MS Experimental System and Measurements

The GC/MS analysis was carried out with a Shimadzu TQ8040 NX GS-MS instrument connected to a silica capillary column TG-5-MS with dimensions of 30.0 m×0.25 mm and a film thickness of 0.25µm. The GC/MS detection utilised an electron impact ionisation system with an ionising energy of 70 eV. The scanning mass range was set at 29–400 (m/z) and a helium carrier gas with a flow rate corresponding to a linear velocity of 41.4 cm/s was used. When initiating the phytochemical analysis technique, the oven temperature was set at 60°C for a duration of 1 minute. During the completion of the initial stage, the temperature will be gradually raised to 300°C at a rate of 3°C per minute, and then retained at a constant temperature for 15 minutes. The temperature specifications for the injector port, ion source, and detector were 280°C, 220°C, and 280°C, respectively. The overall duration of the GC process was 20 minutes. The NIST Library database provided the components' names, molecular weights, and structures [26-28].

2.5. Statistical Analysis:

All qualitative tests/analyses were done in triplicate.

3. RESULTS AND DISCUSSION

3.1. Qualitative phytochemical screening

The qualitative analysis of *Vitex leucoxylon* leaf extracts, detailed in Table 3, reveals the phytochemical constituents present in the ethanolic extract.

Table 1: Qualitative phytochemical analysis of *Vitex leucoxylon* leaf extract

S. No	Phytochemicals	Tests/Reagents	Results
1	Alkaloids	Mayer's test, Wagner's test, Hager's test, Dragendorff's test, Tannic acid test	+
2	Flavonoids	Lead acetate test, Shinoda test, Alkaline test, Zinc Hcl test	+
3	Tannins & Phenols	$Fecl_3$ test, Lead acetate test	+
4	Steroids	Libermann-Buchard test, Salkowski test	+
5	Anthocyanins	Anthocyanins test	-
6	Glycosides	Glycoside test	+
7	Saponins	Honeycomb test, Foam test	-
8	Anthraquinone	Bontrager's test	+

Note: + indicates the presence of constituents and – indicates the absence of constituents.

It is evident from the table.1 that the ethanol extract detailed the maximum number of chemical constituents including alkaloids, flavonoids, tannins, phenols, glycosides and

anthraquinone. Presence of alkaloids compounds is of importance in pharmaceutical application as these compounds are responsible for numerous biological functions like antiasthma, anticancer, antimalarial, vasodilatory, analgesic, cholinomimetic, antiarrhythmic, and antibacterial in the human body. The existence of flavonoids, known to be effective free radical scavengers, indicates that this plant may have antioxidant qualities. Tannins and phenols are linked to antimicrobial, virucides, anticancer, antioxidant, and anti-inflammatory, antdiabetic, antidiarrhoics, cardiovascular protection and wound healing. Glycosides have been shown to be linked to the reduction of blood pressure and anthraquinones act as chemotherapeutic agent utilized for the medicine of progressive relapsing, secondary progressive, or worsening relapsing-remitting multiple sclerosis.

3.2. Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

GC-MS analysis of Vitex leucoxylon leaf ethanolic extract identified twenty-six compounds. Table 1 lists the active compounds with their retention time (RT), concentration (peak area%), molecular formula, similarity, and biological activity. Figure 1 shows the GC-MS chromatogram of the twenty-six detected compounds. The compounds identified by mass spectroscopy are presented. The GC-MS revealed a total number of components in the ethanol extract. The results revealed that .beta.-D-Glucopyranoside, methyl (22.11%), Phytol (15.77%), Hexadecanoic acid, ethyl ester (10.75%), Ethyl linolenate (8.84%), Benzoic acid, 4- hydroxy (5.66%) and 1-Hexacosanol (5.11%) was found as the 6 major components in the ethanol extract, the eight minor compounds such as Neophytadiene (2.54%), Bicyclo [3.2.0] hept-2-ene, 4-ethoxy-, exo- (2.16%), n-Hexadecanoic acid (3.83%), Linolenic acid (4.37%), Linoleic acid ethyl ester (2.93%), Octadecanoic acid, ethyl ester (3.32%), squalene (2.32%), and 2-Hexadecanoyl glycerol (1.27%).

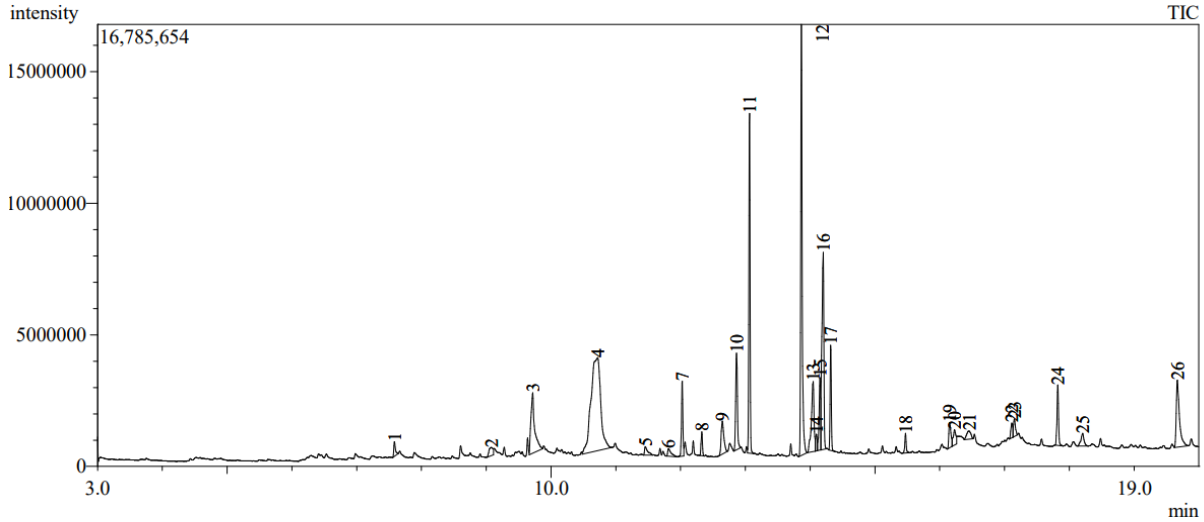


Fig:2 GC-MS spectral analysis of leaf ethanolic extract of *Vitex leucoxylon*

Table:2 Bioactive compounds identified in the ethanolic extract of *Vitex leucoxylon*

S. No	Bioactive compound name	Retention time (min)	Peak area %	Molecular formula	Similarity	Biological activity reported	Reference
1	Bicyclo[3.3.0]oc	7.583	0.61	$C_{10}H_{14}O$	84	Not reported	

	tan-2-one, 7-ethylidene-						
2	Methane, trimethylolnitro-	9.079	0.80	$C_4H_9NO_5$	82	Not reported	
3	Benzoic acid, 4-hydroxy-	9.718	5.66	$C_7H_6O_3$	96	Antimicrobial, Antimutagenic, Hypoglycemic Antiestrogenic, Anti-inflammatory, Antialgal, Anti-platelet aggregating, Nematicidal, Antioxidant, Antiviral.	29
4	beta.-D-Glucopyranoside, methyl	10.721	22.11	$C_7H_{14}O_6$	88	Antimicrobial	30
5	Tetradecanoic acid	11.456	0.62	$C_{14}H_{28}O_2$	91	Anti-Virulence	31
6	2,5-Furandione, 3-(2-decenyl) dihydro-	11.812	0.63	$C_{14}H_{22}O_3$	74	Antifungal	32
7	Neophytadiene	12.023	2.54	$C_{20}H_{38}$	95	Anti-inflammatory agent, antimicrobial agent	33
8	Neophytadiene	12.326	0.73	$C_{20}H_{38}$	93	Anti-inflammatory agent, antimicrobial agent	34
9	Bicyclo [3.2.0] hept-2-ene, 4-ethoxy-, exo-	12.642	2.16	$C_9H_{14}O$	73	Not reported	
10	n-Hexadecanoic acid	12.864	3.83	$C_{16}H_{32}O_2$	94	Antioxidants, hypocholesterolemic, nematicide, and pesticide	35
11	Hexadecanoic acid, ethyl ester	13.063	10.75	$C_{18}H_{36}O_2$	94	Antioxidants, hypocholesterolemic, nematicide, and pesticide	36
12	Phytol	13.865	15.77	$C_{20}H_{40}O$	97	Cytotoxic, metabolism-modulating, autophagy, apoptosis-inducing, Anxiolytic, immune-modulating,	37

						antioxidant, antimicrobial, antinociceptive, and anti-inflammatory	
13	Linolenic acid	14.044	4.37	$C_{18}H_{30}O_2$	96	Antibacterial	38
14	3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate	14.097	0.63	$C_{22}H_{42}O_2$	92	Cell viability	39
15	Linoleic acid ethyl ester	14.150	2.93	$C_{20}H_{36}O_2$	89	Antibacterial	40
16	Ethyl linolenate	14.199	8.84	$C_{20}H_{34}O_2$	95	Anti-inflammatory	41
17	Octadecanoic acid, ethyl ester	14.317	3.32	$C_{20}H_{40}O_2$	94	Anti-inflammatory	42
18	Eicosanoic acid, ethyl ester	15.470	0.68	$C_{22}H_{44}O_2$	92	Antioxidant	43
19	2-Hexadecanoyl glycerol	16.152	1.27	$C_{19}H_{38}O_4$	93	Analgesic	44
20	Octacosanal	16.230	1.00	$C_{28}H_{56}O$	79	Antibacterial	45
21	Arbutin	16.455	0.84	$C_{12}H_{16}O_7$	91	Skin lighting	46
22	1-cis-Vaccenoylglycerol	17.111	0.68	$C_{21}H_{40}O_4$	85	Antibacterial	47
23	Methyl linolenate	17.152	0.84	$C_{19}H_{32}O_2$	90	Anti-inflammatory	48
24	Squalene	17.821	2.32	$C_{30}H_{50}$	96	Anti-inflammatory	49
25	1-Pentacosanol	18.203	0.95	$C_{25}H_{52}O$	90	Not reported	
26	1-Hexacosanol	19.668	5.11	$C_{26}H_{54}O$	96	antibacterial	50

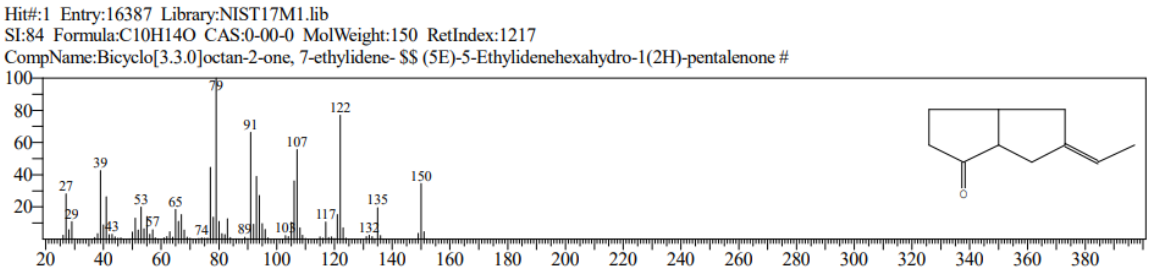


Fig: 2 GC-MS spectra of 7-ethylidene-bicyclo[3.3.0]octan-2-one

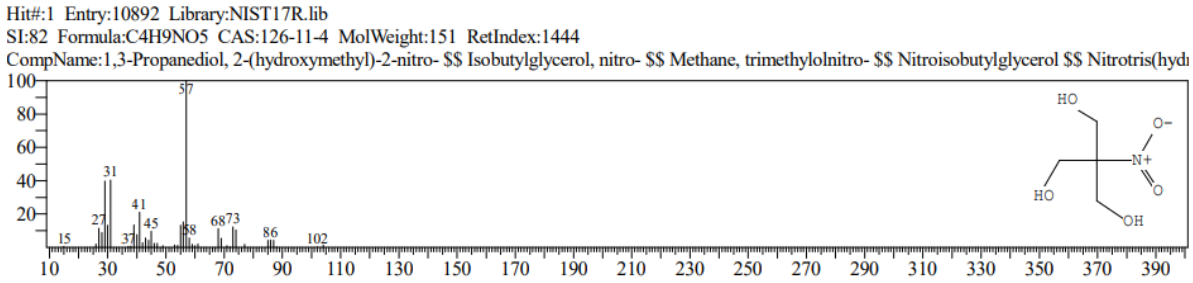


Fig: 3 GC-MS spectra of trimethylolnitro methane

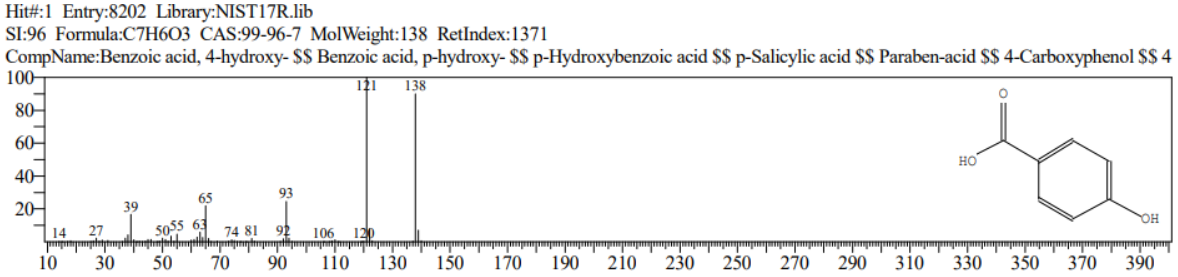


Fig: 4 GC-MS spectra of 4-hydroxy benzoic acid

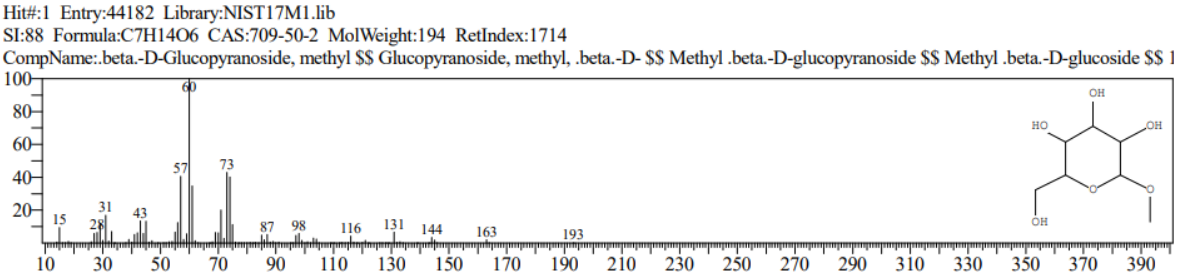


Fig: 5 GC-MS spectra of methyl beta.-D-Glucopyranoside

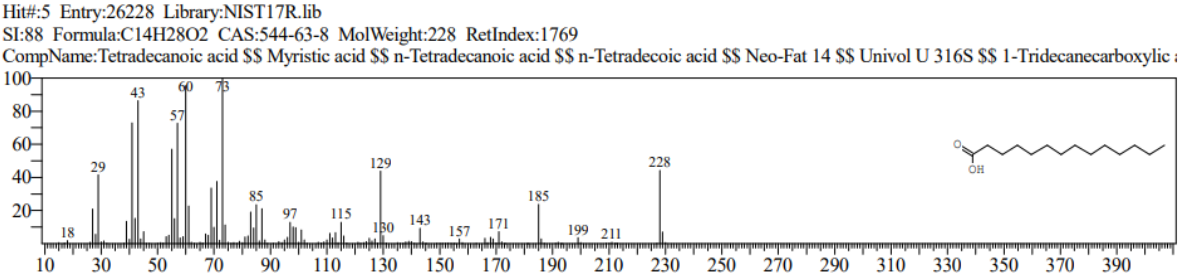


Fig: 6 GC-MS spectra of Tetradecanoic acid

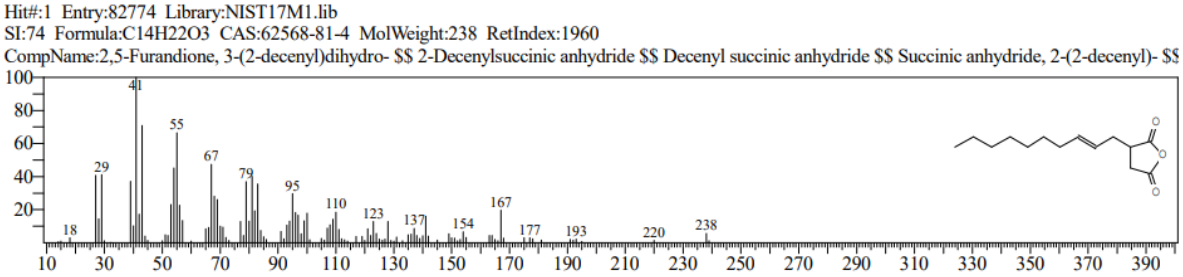


Fig: 7 GC-MS spectra of 3-(2-decenyl)dihydro-2,5-Furandione

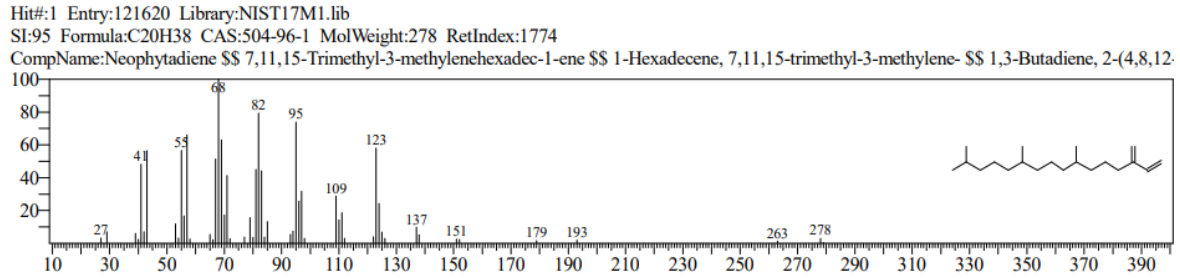


Fig: 8 GC-MS spectra of Neophytadiene

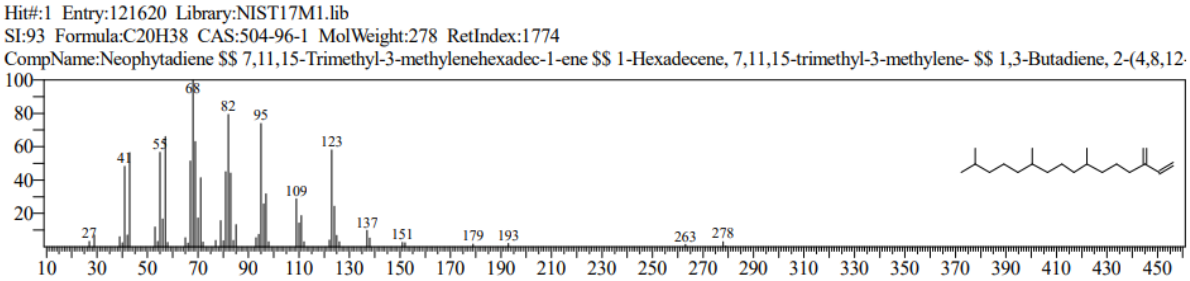


Fig: 9 GC-MS spectra of Neophytadiene

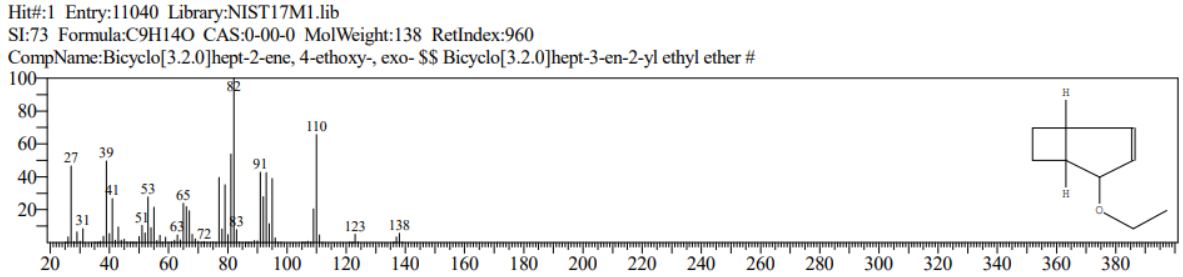


Fig: 10 GC-MS spectra of 4-ethoxy-, exo-bicyclo [3.2.0] hept-2-ene

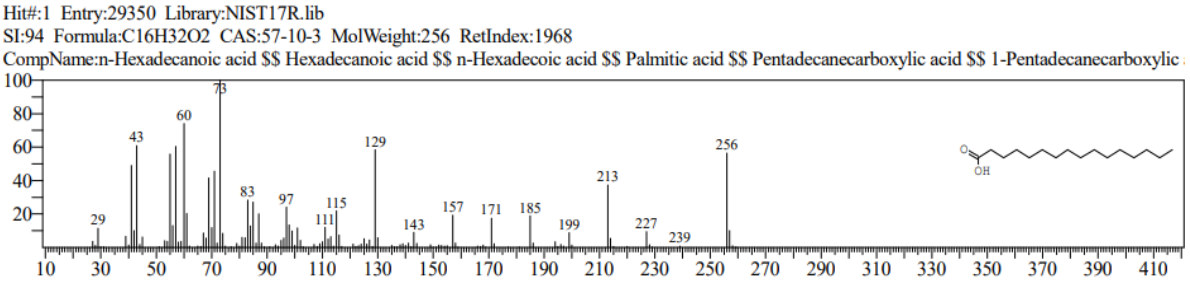


Fig: 11 GC-MS spectra of n-Hexadecanoic acid

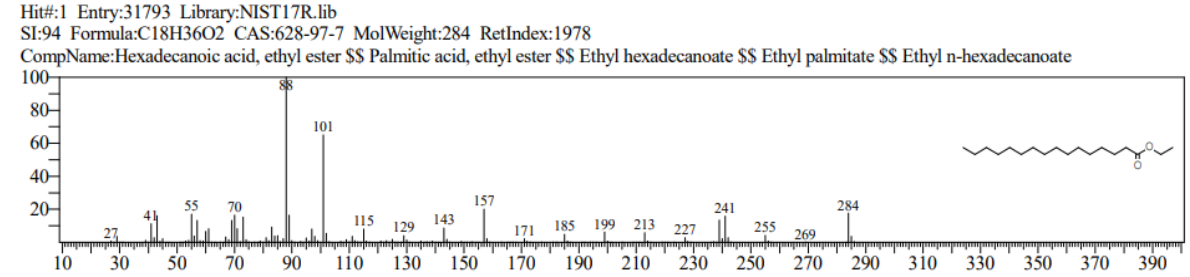


Fig: 12 GC-MS spectra of Hexadecanoic acid, ethyl ester

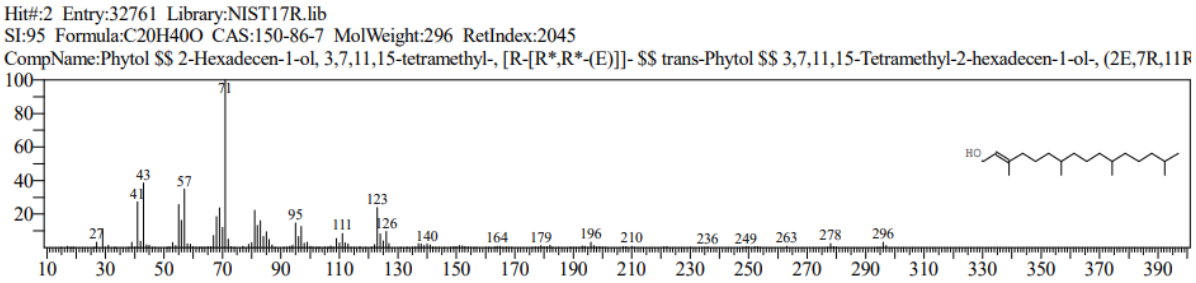


Fig: 13 GC-MS spectra of phytol

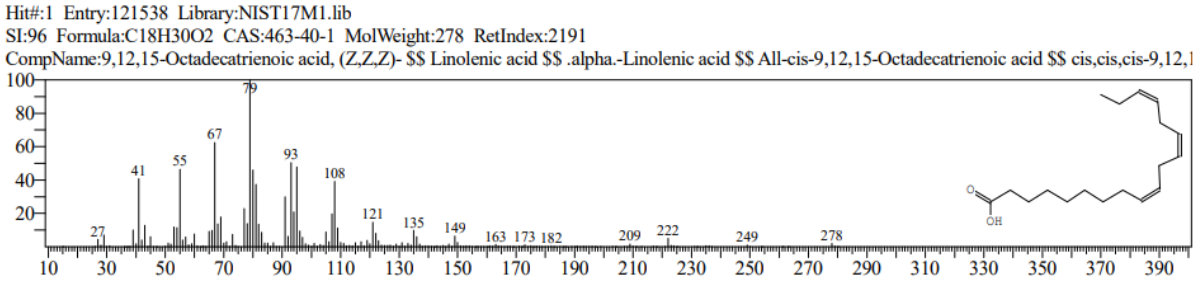


Fig: 14 GC-MS spectra of Linolenic acid

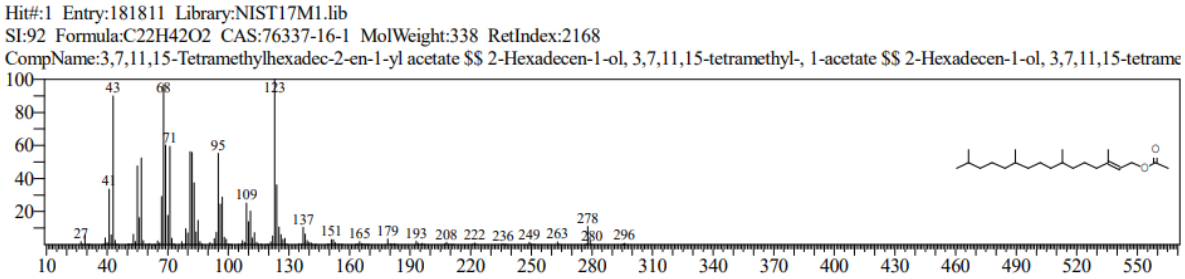


Fig: 15 GC-MS spectra of 3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate

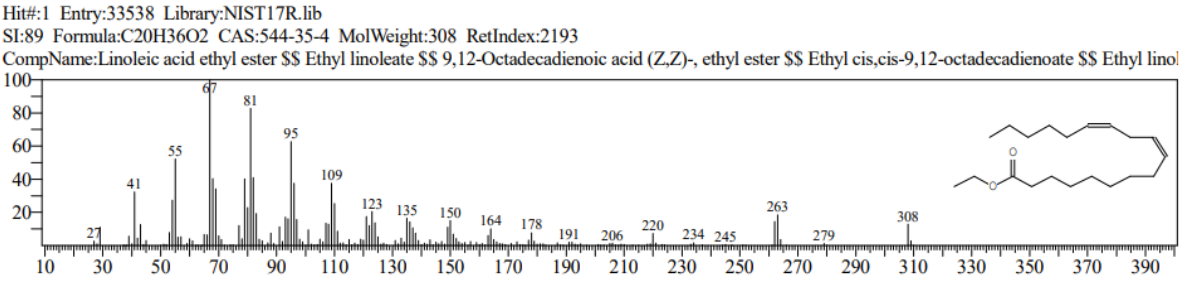


Fig: 16 GC-MS spectra of Linoleic acid ethyl ester

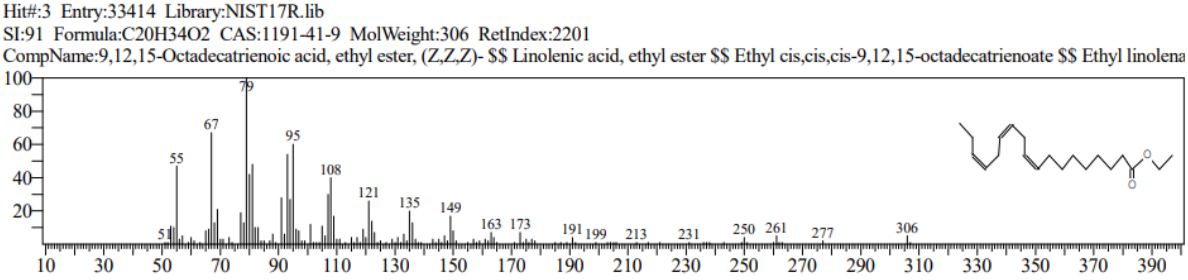


Fig: 17 GC-MS spectra of Ethyl linolenate

Hit#:2 Entry:33852 Library:NIST17R.lib
SI:94 Formula:C20H40O2 CAS:111-61-5 MolWeight:312 RetIndex:2177
CompName:Octadecanoic acid, ethyl ester $$ Stearic acid, ethyl ester $$ Ethyl n-octadecanoate $$ Ethyl octadecanoate $$ Ethyl stearate $$ Radia 7185

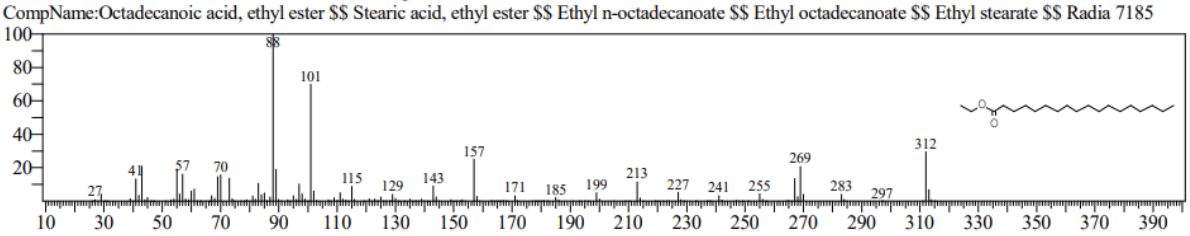


Fig: 18 GC-MS spectra of Octadecanoic acid, ethyl ester

Hit#:2 Entry:35507 Library:NIST17R.lib
SI:89 Formula:C22H44O2 CAS:18281-05-5 MolWeight:340 RetIndex:2375
CompName:Eicosanoic acid, ethyl ester $$ Ethyl icosanoate $$ Ethyl arachidate $$ Ethyl eicosanoate $$ Arachidic acid, ethyl ester

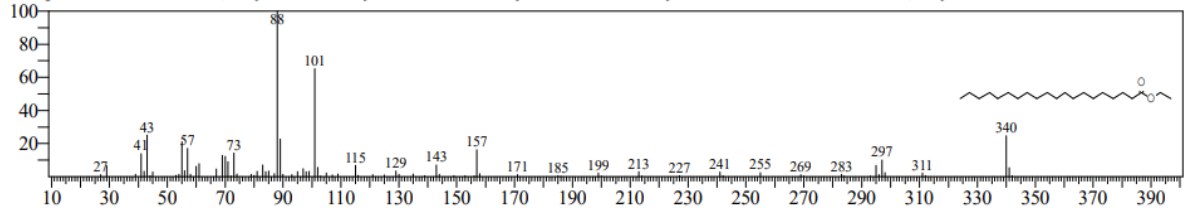


Fig: 19 GC-MS spectra of Eicosanoic acid, ethyl ester

Hit#:2 Entry:174082 Library:NIST17M1.lib
SI:89 Formula:C19H38O4 CAS:23470-00-0 MolWeight:330 RetIndex:2498
CompName:Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester $$ Palmitin, 2-mono- $$ Palmitic acid .beta.-monoglyceride $$ 2-Hexadecanoyl gl

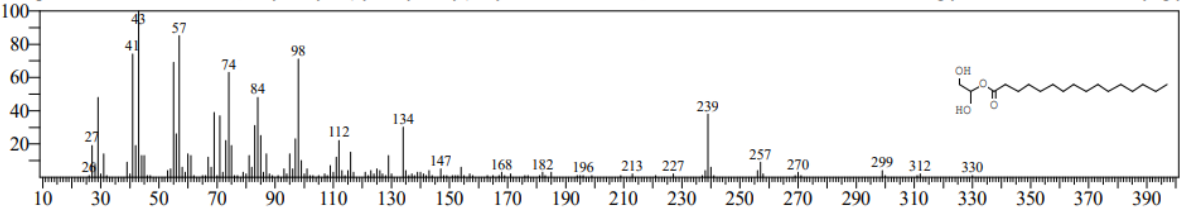


Fig: 20 GC-MS spectra of 2-Hexadecanoyl glycerol

Hit#:1 Entry:231562 Library:NIST17M1.lib
SI:79 Formula:C28H56O CAS:22725-64-0 MolWeight:408 RetIndex:2993
CompName:Octacosanal $$ n-Octacosanal

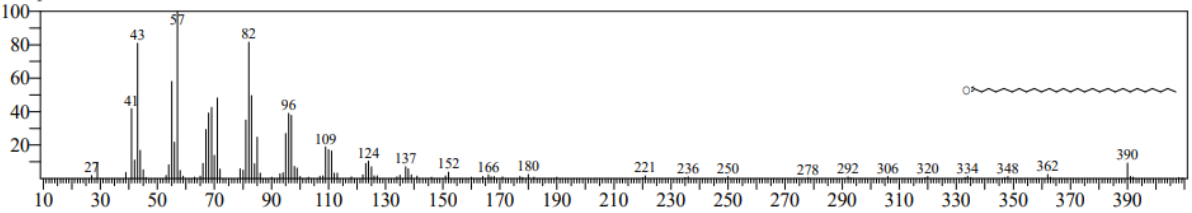


Fig: 21 GC-MS spectra of Octacosanal

Hit#:1 Entry:114874 Library:NIST17M1.lib
SI:91 Formula:C12H16O7 CAS:497-76-7 MolWeight:272 RetIndex:2508
CompName:Arbutin $$ .beta.-D-Glucopyranoside, 4-hydroxyphenyl $$ p-Hydroxyphenyl .beta.-D-glucopyranoside $$ p-Hydroxyphenyl .beta.-D-glucoside

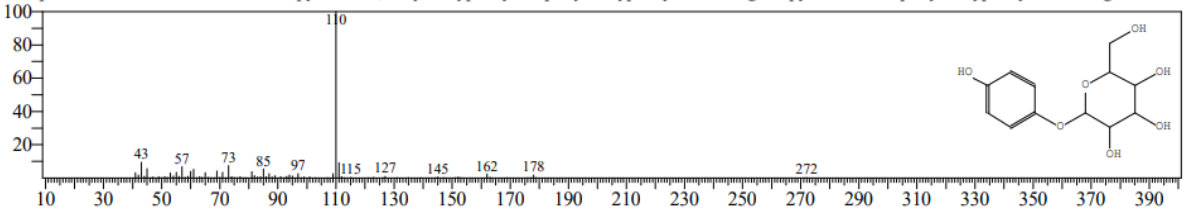


Fig: 22 GC-MS spectra of Arbutin

Hit#:1 Entry:197760 Library:NIST17M1.lib
SI:85 Formula:C21H40O4 CAS:20379-67-3 MolWeight:356 RetIndex:2689
CompName:1-cis-Vaccenoylglycerol $$ 11-Octadecenoic acid, 2,3-dihydroxypropyl ester, (11Z)- $$ 11-Octadecenoic acid, 2,3-dihydroxypropyl ester, (Z)- $

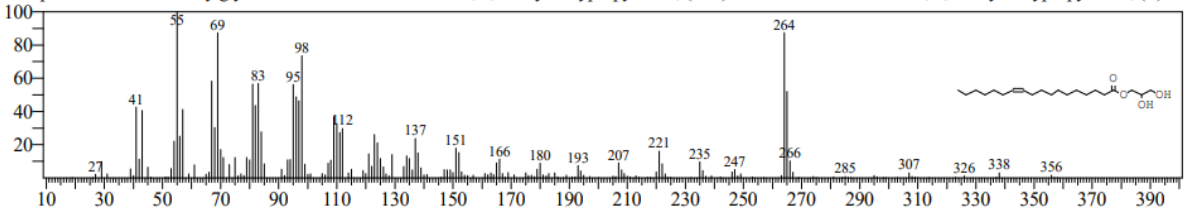


Fig: 23 GC-MS spectra of 1-cis-Vaccenoylglycerol

Hit#:1 Entry:135724 Library:NIST17M1.lib
SI:90 Formula:C19H32O2 CAS:301-00-8 MolWeight:292 RetIndex:2101
CompName:9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- $$ Linolenic acid, methyl ester $$ Methyl all-cis-9,12,15-octadecatrienoate $$ Methyl lino

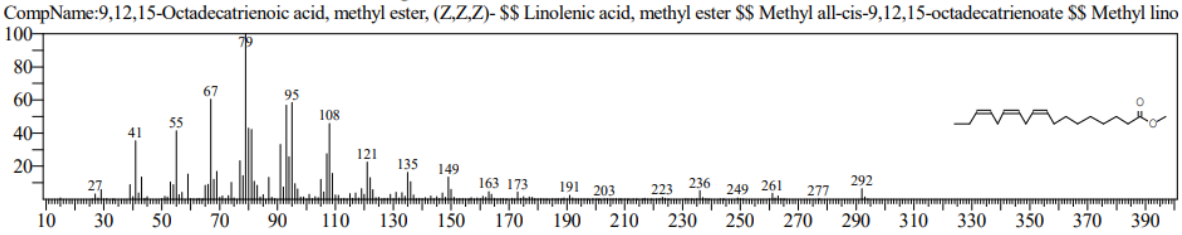


Fig: 24 GC-MS spectra of Methyl linolenate

Hit#:1 Entry:232590 Library:NIST17M1.lib
SI:96 Formula:C30H50 CAS:111-02-4 MolWeight:410 RetIndex:2914
CompName:Squalene $$ 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- $$ all-trans-Squalene $$ trans-Squalene $$ Spinacen $$ S

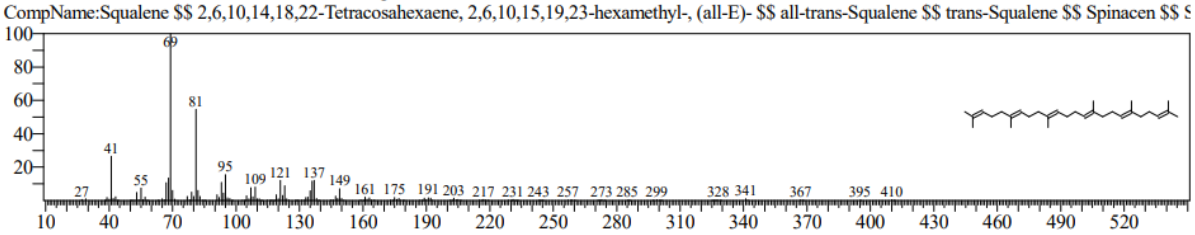


Fig: 25 GC-MS spectra of Squalene

Hit#:1 Entry:207358 Library:NIST17M1.lib
SI:90 Formula:C25H52O CAS:26040-98-2 MolWeight:368 RetIndex:2749
CompName:1-Pentacosanol

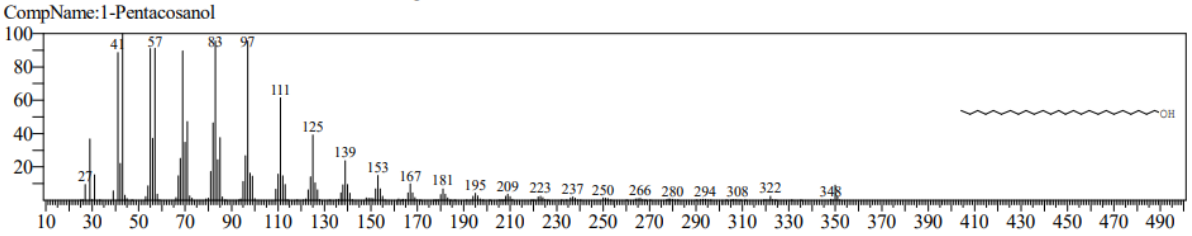


Fig: 26 GC-MS spectra of 1-Pentacosanol

Hit#:5 Entry:37114 Library:NIST17R.lib
SI:94 Formula:C26H54O CAS:506-52-5 MolWeight:382 RetIndex:2848
CompName:1-Hexacosanol $$ n-Hexacosanol $$ Ceryl alcohol $$ Cerylic alcohol $$ Hexacosyl alcohol

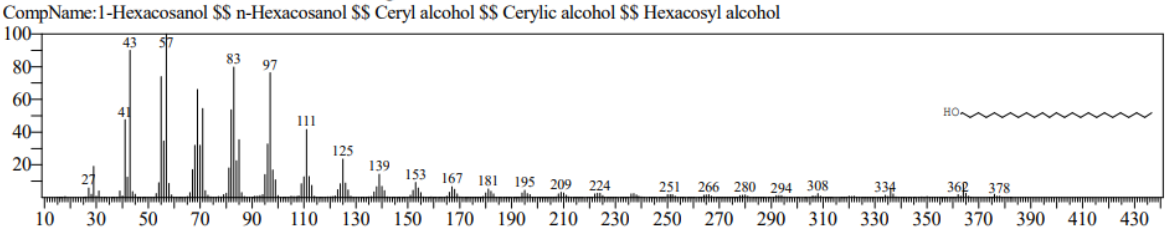


Fig: 27 GC-MS spectra of 1-Hexacosanol

4. CONCLUSION:

In this work, phytochemical screening indicated the existence of various phytochemicals like alkaloids, flavonoids, tannins, phenols, glycosides, and anthocyanins. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was used to find twenty-six chemical components from an ethanolic extract of *Vitex leucoxylon* leaves. Traditional healers utilize plant leaves for a variety of illnesses, which is justified by the existence of several bioactive chemicals.

Based on the results attained in this investigation, it can be concluded that the identified phytocomponents exhibit significant biological activities, including antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and anticancer effects. Consequently, *Vitex leucoxylon* is recommended for its phytopharmaceutical value.

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Conflict of interest:

Author has no conflict of interest.

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