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Phytochemical Analysis of *Vitex altissima* L. using UV-VIS, FTIR and GC-MS

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ABSTRACT
The present study was carried out to characterize the bioactive constituents present in different leaf extracts of *Vitex altissima* L. using UV-VIS, FTIR and GC-MS. The crude extracts were scanned in the wavelength ranging from 200-1100 nm by using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. For GC-MS analysis, 10 g sample is extracted with 30 ml ethanol, filtered in ash less filter paper with 2 g sodium sulphate and the extract is concentrated to 1 ml by bubbling nitrogen into the solution. The compound detection employed the NIST Ver. 2.0 - Year 2005 library. The biological activities are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA. The UV-VIS profile showed different peaks ranging from 400-700 nm with different absorption respectively. The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in different extracts. The results of the GC-MS analysis provide different peaks determining the presence of 21 phytochemical compounds with different therapeutic activities. The major phytoconstituents were n-Hexadecanoic acid (23.74%), 9, 12-Octadecadienoic acid [Z, Z] (23.41%) and Squalene (14.74%). Hence, this study offers a base of using *V. altissima* as herbal alternative for the synthesis of antimicrobial agents.

Keywords: Phytochemical, *Vitex altissima*, GC-MS, antimicrobial, ethnobotanical.

INTRODUCTION

The increase in prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs and has necessitated the search for new antimicrobials from alternative sources. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents. The only way to prevent antibiotic resistance is by using new compounds which are not based on the existing synthetic antimicrobial agents. [1] Plants are rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties. [2] Natural products from microbial sources have been the primary source of antibiotics. But with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant. [3-4]

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. [5] In order to promote the use of medicinal plants as potential sources of antimicrobial compounds, it is important to thoroughly investigate their composition and activity and thus validate their use. [6] Some phytochemicals produced by plants have antimicrobial activity and used for the development of new antimicrobial drugs. [7] It has been shown that *in-vitro* screening methods could provide the needed preliminary observations to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations. [8] The determination of phytoconstituents is largely performed by relatively expensive and often laborious techniques such as gas (GC) and liquid (LC) chromatography combined with specific detection schemes. [9-10] Analysis of small amounts of chemicals has become easier and more cost-effective owing to the development of hyphenated chromatographic techniques such as GC or LC-MS. GC-MS analysis can identify pure compounds present at less than 1 ng. [11] However, simple, cost-effective and rapid tests for detecting

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phytocomponents are necessary. Spectroscopic (UV-Vis, FT-IR) methods together or separate can be used in this sense as well as conventional methods. [12-14]

The genus *Vitex* consists of over 270 species, predominantly trees and shrubs and is restricted to tropical and subtropical regions, although a few species are found in temperate zones. *Vitex altissima* L. (Verbenaceae) commonly known as 'Mayilai notchi' is widely distributed in South East Asia. It is used in stomatitis, cardiac diseases, anorexia, blindness, leprosy, worm infestation, rheumatic swellings and chest pains. [15] It has also anti-inflammatory [16] and antioxidant [17] activities. In the last few years, spectroscopic methods have become firmly established as a key technological platform for secondary metabolite profiling in both plant and non plant species. [18-19] Therefore, the present research was conducted to investigate the phytochemical constituents of *V. altissima* using UV-VIS, FTIR and GC-MS.

MATERIALS AND METHODS

Collection and processing of plant material

The leaves of the plant *Vitex altissima* L. were collected from the natural habitats of Tiruchirappalli district, Tamil Nadu, India. The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The leaves were cut, shade dried, ground into fine powder and stored in air tight polythene bags until use.

Plant sample extraction

2 g of air dried powder of leaf sample was extracted with 50 ml of solvents such as ethanol, acetone, petroleum ether and chloroform with gentle stirring for 72 h. The sample was kept in dark for 72 h with intermittent shaking. After incubation, the solution was filtered through Whatmann No. 1 filter paper and the filtrate was collected (crude extracts). It was then transferred to glass vials and kept at 4°C before use.

UV-VIS and FTIR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis. For UV-VIS and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the UV-VIS and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

GC-MS analysis

10 g of powdered leaf sample is soaked with 30 ml ethanol overnight and filtered through ash less filter paper with sodium sulphate (2 g). The extract is concentrated to 1 ml by bubbling nitrogen into the solution. The extract contained both polar and non-polar phytocomponents. 2µl of the ethanolic extract of *V. altissima* was employed for GC-MS analysis. [20] The Clarus 500 GC used in the analysis employed a fused silica column packed with Elite-1 [100% dimethyl poly siloxane, 30 nm × 0.25 nm ID × 1µm df] and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The 2µl sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th minute GC extraction process, the oven was maintained at a temperature of 110°C with 2 minutes holding. The injector temperature was set at 250°C (mass analyser). The different parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet line temperature: 200°C; Source temperature: 200°C). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The MS detection was completed in 36 minutes.

Identification of components

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The detection employed the NIST (National Institute of Standards and Technology) Ver.2.0-Year 2005 library. The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA. Interpretation of GC-MS was conducted using the database of NIST having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

The qualitative UV-VIS spectrum profile of *Vitex altissima* L. ethanolic extract was selected at wavelength from 400 to 700 nm due to sharpness of the peaks and proper baseline. The profile showed the peaks at 422 and 664 nm with the absorption of 2.485 and 1.862 respectively (Fig. 1A; Table 1). The UV-VIS profile of acetone extract of *V. altissima* was chosen at a wavelength of 400 to 700 nm and the profile showed the peaks at 455, 533 and 664 nm with the absorption 2.571, 0.659 and 2.590 respectively (Fig. 1B; Table 1). The UV-VIS spectrum profile of *V. altissima* petroleum ether extract was taken at the wavelength of 400 to 700 nm. The profile showed the peaks at 410 and 669 nm with the absorption of 1.659 and 0.972 respectively (Fig. 1C; Table 1). The UV-VIS profile of *V. altissima* chloroform extract was selected at the wavelength of 500 to 700 nm and the spectrum profile showed the peaks at 537, 609 and 668 nm with the absorption 1.180, 0.912, and 3.147 respectively (Fig. 1D; Table 1).

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in Table 2. The FTIR spectrum profile was illustrated in the Figure 1E-1H. The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in different extracts. Hence, the crude extracts subjected to UV-VIS and FTIR analysis is used for the identification of chemical constituents present in *V. altissima*. In addition, UV-VIS and FTIR spectroscopy is proved to be a reliable and sensitive method

Table 1: UV-VIS peak values of different extracts of *Vitex altissima* L.

Ethanol		Acetone		Petroleum ether		Chloroform	
nm	**Abs**	**nm**	**Abs**	**nm**	**Abs**	**nm**	**Abs**
422	2.485	455	2.571	410	1.659	537	1.180
664	1.862	533	0.659	669	0.972	609	0.912
-	-	664	2.590	-	-	668	3.147

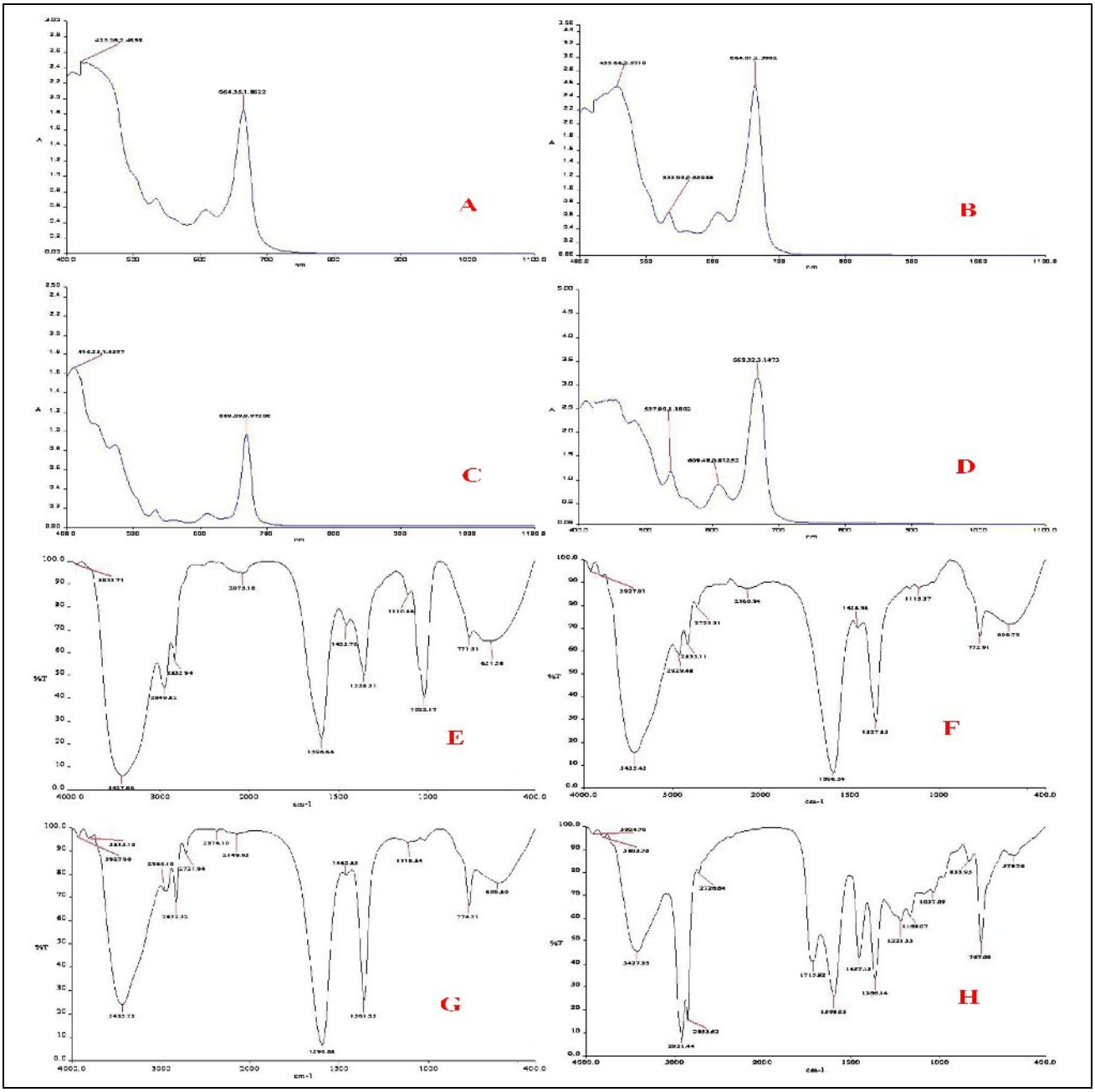

Fig. 1: UV-VIS and FTIR Spectrum of *V. altissima* A. UV-VIS Spectrum of Ethanolic Extract of *V. altissima* B. UV-VIS Spectrum of Acetone Extract of *V. altissima,* C. UV-VIS Spectrum of Petroleum Ether Extract of *V. altissima*, D. UV-VIS Spectrum of Chloroform Extract of *V. altissima,* E. FTIR Spectrum of Ethanolic Extract of *V. altissima,* F. FTIR Spectrum of Acetone Extract of *V. altissima,* G. FTIR Spectrum of Petroleum Ether Extract of *V. altissima* and H: FTIR Spectrum of Chloroform Extract of *V. altissima.*

for detection of biomolecular composition. [21]

The results pertaining to GC-MS analysis leads to the identification of number of compounds from the GC fractions of the ethanolic extract of *V. altissima*. These compounds were identified through mass spectrometry attached with GC. These observations may be due to the nature of biological active components and the stronger extraction capacity of ethanol could have been produced number of active constituents responsible for antibacterial activity.

The biological activities based on Dr. Duke's Phytochemical and Ethnobotanical Databases were tabulated in Table 3. The results revealed the presence of 21 different compounds namely Benzene, 1, 4-dichloro (2.92%), 4, 6-Octadienoic acid (0.80%), Eugenol (2.86%), Germacrene D (0.67%), Caryophyllene (0.53%), Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl,[S-(R*, S*)] (3.07%), 1, 3-Cyclohexadiene, 5-(1, 5-dimethyl-4-hexenyl)-2-methyl (3.23%), à-Caryophyllene (0.70%), 1,6,10-Dodecatriene, 7, 11-dimethyl-3-methylene-[Z] (0.35%), Dodecanoic acid (0.73%), 3-Pyridine carboxylic acid,6-amino (1.76%), d-Mannose (2.43%), Tetradecanoic acid (1.47%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (3.71%), 1, 2-Benzene dicarboxylic acid, butyl octyl ester (1.26%), n-Hexadecanoic acid (23.74%), Hexadecanoic acid ethyl ester (1.69%), Phytol (3.85%), 9, 12-Octadecadienoic acid [Z, Z] (23.41%), Octadecanoic acid (6.08%) and Squalene (14.74%).

Table 2: FTIR peak values and functional groups of different extracts of *Vitex altissima* L.

Ethanol		Acetone		Petroleum ether		Chloroform	
Peak values	**Functional groups**	**Peak values**	**Functional groups**	**Peak values**	**Functional groups**	**Peak values**	**Functional groups**
3933.71	Unknown	3927.91	Unknown	3927.90	Unknown	3924.70	Unknown
3427.66	Alcohols, Phenols	3435.43	Alcohols, Phenols	3815.15	Unknown	3803.76	Unknown
2949.82	Alkanes	2929.48	Alkanes	3435.73	Alcohols, Phenols	3427.35	Alcohols, Phenols
2835.94	Carboxylic acids	2833.11	Carboxylic acids	2960.10	Alkanes	2921.44	Alkanes
2075.16	Unknown	2725.31	Aldehydes	2832.32	Carboxylic acids	2853.62	
1596.64	Primary amines	2160.94	Alkynes	2721.94	Aldehydes	2726.64	Aldehydes
1455.76	Aromatics	1596.54	Aromatics	2374.10	Unknown	1715.92	Esters
1358.31	Nitro compounds	1458.98	Alkanes	2149.95	Alkynes	1598.03	Aromatics
1110.86	Aliphatic amines	1357.85	Nitro compounds	1596.68	Aromatics	1457.13	Alkanes
1022.17	Aliphatic amines	1115.37	Aliphatic amines	1462.83	Alkanes	1366.14	
771.51	Alkyl halides	772.91	Amines	1361.53		1221.33	Alcohols
651.58	Alkynes	606.75	Alkyl halides	1116.84	Aliphatic amines	1168.07	Alkyl halides
-	-	-	-	774.31	Alkyl halides	1037.09	Aliphatic amines
-	-	-	-	609.80		833.95	
-	-	-	-	-	-	767.09	Alkyl halides
-	-	-	-	-	-	578.76	

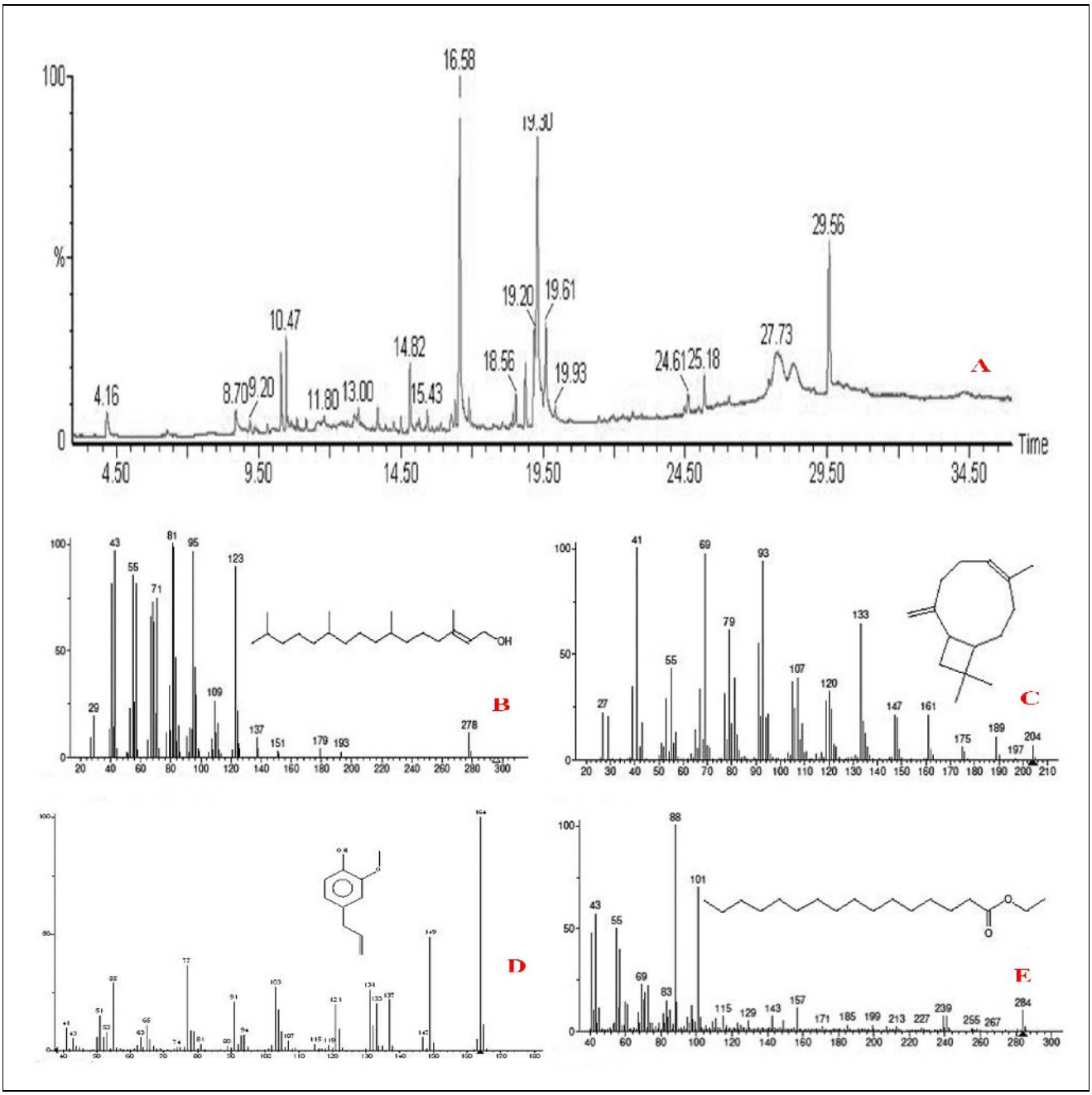

Fig. 2: GC-MS chromatogram of *V. altissima,* A. GC-MS Chromatogram of ethanolic leaf extract of *V. altissima*, B. Mass spectra of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, C. Mass spectra of Caryophyllene, D. Mass spectra of Eugenol and E. Mass spectra of Hexadecanoic acid, ethyl ester

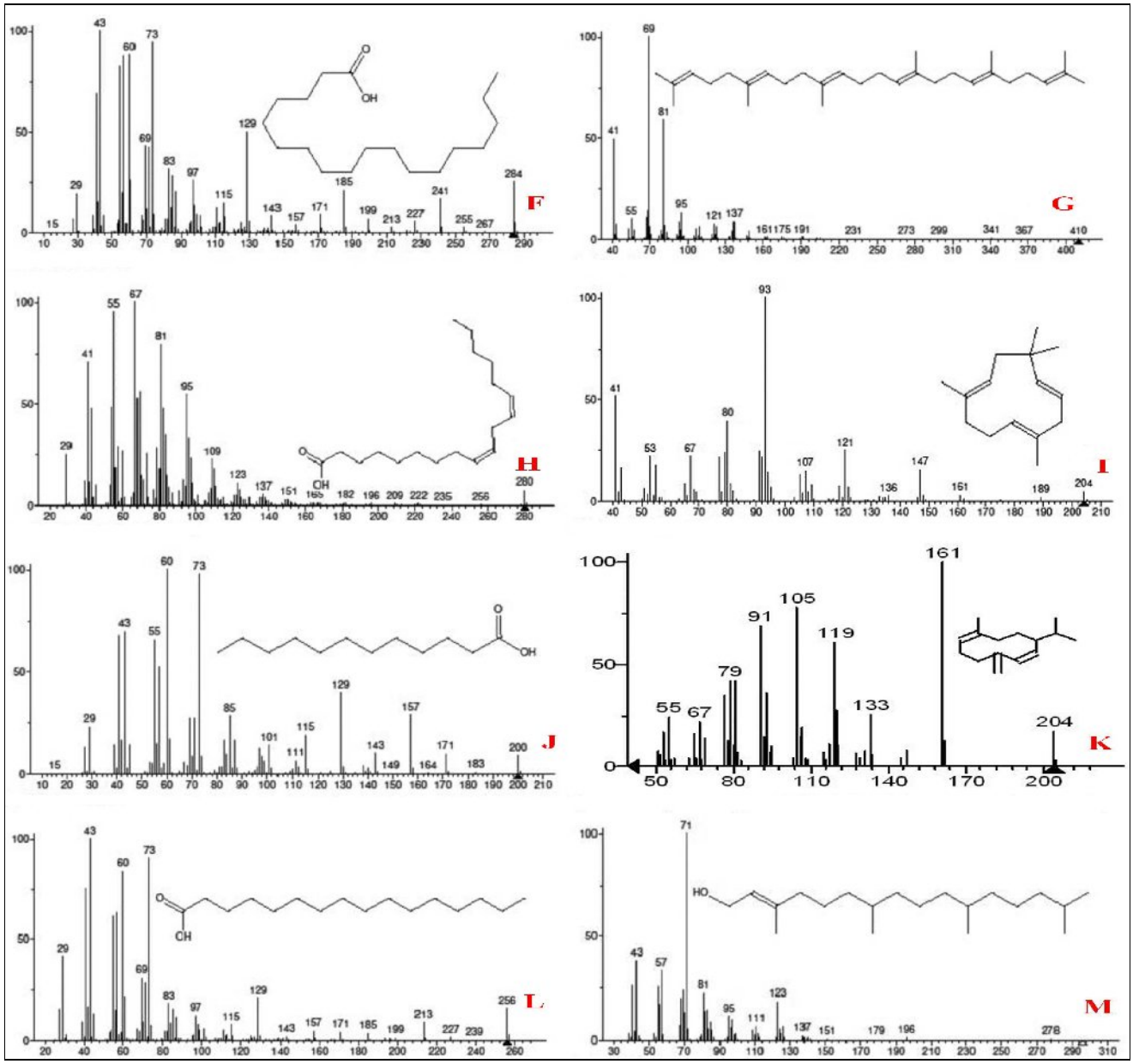

Fig. 2F: Mass spectra of Octadecanoic acid, G. Mass spectra of Squalene, H. Mass spectra of 9,12-Octadecadienoic acid (Z,Z), I. Mass spectra of à-Caryophyllene, J. Mass spectra of Dodecanoic acid, K. Mass spectra of Germacrene D, L. Mass spectra of n-Hexadecanoic acid and M. Mass spectra of Phytol

These phytochemicals have been shown to possess analgesic, anesthetic, allergenic, antibacterial, anti-inflammatory, antioxidant, antipyretic, antiseptic sedative, anticancer, hypocholesterolemic, nematicide, anticoronary, antiarthritic and hepatoprotective activities [22]. The therapeutic benefits of secondary metabolites of plant origin have been researched in several recent studies [23]. Most of identified compounds have antimicrobial and antioxidant activities. Phenols, aromatic compounds, lauric acid, myristic acid, palmitic acid, fatty acid and terpenes are reported to have antioxidant properties. The compounds possessing antimicrobial activity are phenols, terpenes, lauric acid, alkaloids and plasticizer. The spectrum profile of GC-MS confirmed the presence of 21 major components with the retention time 4.16, 6.28, 8.70, 9.20, 9.82, 10.29, 10.47, 10.53, 10.85, 11.17, 11.80, 12.87, 13.69, 14.82, 16.41, 16.58, 16.90, 18.89, 19.30, 19.61 and 29.56 respectively (Fig. 2A). This gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in *V. altissima.* The individual fragmentation patterns of the components were illustrated in Fig. 2B-M.

The mass spectrum of the compound with retention time 14.82 (3.71%) gave 10 major peaks (m/z) at 81.999, 82.986, 43.965, 95.962, 123.892, 55.852, 41.811, 57.811, 71.748 and 68.728 (Fig. 2B). The mass spectrum of the compound with retention time 9.82 (0.53%) gave 10 major peaks (m/z) at 41.999, 69.976, 93.937, 133.646, 79.614, 91.551, 55.432, 81.389, 107.389 and 105.372 (Fig. 2C). The mass spectrum of the compound with retention time 8.70 (2.86%) gave 10 major peaks (m/z) at 164.999, 149.485, 77.362, 55.287, 103.269, 131. 259, 137. 216, 91. 205, 133.199 and 121.195 (Fig. 2D). The mass spectrum of the compound with

retention time 16.90 (1.69%) gave 10 major peaks (m/z) at 88.999, 101.700, 43.570, 55.500, 41.480, 57.400, 69.226, 73.224, 71.190 and 70.152 (Fig. 2E). The mass spectrum of the compound with retention time 19.61 (6.08%) gave 10 major peaks (m/z) at 43.999, 73.942, 60.881, 57.875, 55.827, 41.688, 129.500, 69.432, 71.429 and 83.317 (Fig. 2F). The mass spectrum of the compound with retention time 29.56 (14.74%) gave 10 major peaks (m/z) at 69.999, 81.590, 41.496, 68.143, 95.132, 67.108, 55.103, 93.88, 137.87 and 136.84 (Fig. 2G). The mass spectrum of the compound with retention time 19.30 (23.41%) gave 10 major peaks (m/z) at 67.999, 55.954, 81.793, 41.708, 69.560, 95.545, 68.530, 54.486, 43.480 and 82.479 (Fig. 2H). The mass spectrum of the compound with retention time 10.53 (0.70%) gave 10 major peaks (m/z) at 93.999, 41.517, 80.394, 121.249, 91.247, 79.238, 67.222, 53.221, 77.219 and 92.217 (Fig. 2I). The mass spectrum of the compound with retention time 11.17 (0.73%) gave 10 major peaks (m/z) at 60.999, 73.978, 43.697, 41.675, 55.657, 57.526, 129.398, 157.290, 85.284 and 71.276 (Fig. 2J). The mass spectrum of the compound with retention time 9.20 (0.67%) gave 10 major peaks (m/z) at 161.999, 105.775, 91.685, 41.619, 119.604, 79.415, 81.414, 93.359, 77.346 and 27.315 (Fig. 2K). The mass spectrum of the compound with retention time 16.58 (23.74%) gave 10 major peaks (m/z) at 43.999, 73.905, 60.838, 41.749, 57.634, 55.616, 29.414, 69.310, 71.285 and 61.218 (Fig. 2L). The mass spectrum of the compound with retention time 18.89 (3.85%) gave 10 major peaks (m/z) at 71.999, 43.381, 57.334, 41.260, 55.259, 69.239, 81.223, 68.199, 123.184 and 56.169 (Fig. 2M). These mass spectra are fingerprint of the compound which can be identified from the NIST data library. Hence, the identified phytocomponents using GC-MS can be used as a pharmacognostical tool for the identification of adulterants.

The current pioneering study suggests that ethanolic extract is a potent therapeutic agent. It paves the way for the development of several treatment regimens based on this extract. In addition, further research is necessary to identify and purify the active compounds responsible for therapeutic activity.

Table 3: Phytocomponents identified in ethanolic leaf extract of *Vitex altissima* L. using GC-MS

RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature	Activity
4.16	Benzene, 1,4-dichloro-	$C_6H_4Cl_2$	146	2.92	Aromatic compound	No activity
6.28	4,6-Octadienoic acid	$C_8H_{12}O_2$	140	0.80	Fatty acid	No activity
8.70	Eugenol	$C_{10}H_{12}O_2$	164	2.86	Phenolic compound	Analgesic, Anesthetic, Allergenic, Antibacterial, Anticonvulsant, Anti-inflammatory, Antioxidant, Antipyretic, Antisalmonella, Antistaphylococcus, Antiseptic
9.20	Germacrene D	$C_{15}H_{24}$	204	0.67	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory, Sedative, Fungicide
9.82	Caryophyllene	$C_{15}H_{24}$	204	0.53	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory, Sedative, Fungicide
10.29	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- [Ar-Curcumene]	$C_{15}H_{22}$	202	3.07	Aromatic compound	Antioxidant, Anti-inflammatory, Anticancer
10.47	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl, [S-(R*,S*)]-	$C_{15}H_{24}$	204	3.23	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory, Sedative, Fungicide
10.53	à-Caryophyllene	$C_{15}H_{24}$	204	0.70	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory, Sedative, Fungicide
10.85	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-	$C_{15}H_{24}$	204	0.35	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory, Sedative, Fungicide
11.17	Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.73	Lauric acid	Antioxidant, Antibacterial, COX-1 & COX-2 inhibitor, Antiviral, Hypocholesterolemic, Candidicide.
11.80	3-Pyridinecarboxylic acid, 6-amino-	$C_6H_6N_2O_2$	138	1.76	Alkaloid	Antimicrobial, Anti-inflammatory
12.87	d-Mannose	$C_6H_{12}O_6$	180	2.43	Sugar compound	Preservative
13.69	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.47	Myristic acid	Antioxidant, Cancer preventive Nematicide, Hypocholesterolemic, Lubricant
14.82	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	3.71	Terpene alcohol	Antimicrobial, Anti-inflammatory
16.41	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	334	1.26	Plasticizer compound	Antimicrobial, Antifouling
16.58	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	23.74	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
16.90	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.69	Fatty acid ester	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
18.89	Phytol	$C_{20}H_{40}O$	296	3.85	Diterpene	Antimicrobial, Anti cancer, Anti-inflammatory
19.30	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	23.41	Linoleic acid	Hypocholesterolemic, Nematicide, Anticoronary, Antiarthritic, Hepatoprotective, Anti -androgenic, Hypocholesterolemic, 5-Alpha reductase inhibitor, Antihistaminic, Insectifuge, Antieczemic, Antiacne
19.61	Octadecanoic acid	$C_{18}H_{36}O_2$	284	6.08	Stearic acid	No activity
29.56	Squalene	$C_{30}H_{50}$	410	14.74	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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