International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 03-01-2022, Accepted: 18-01-2022, Published: 03-02-2022
Volume 7, Issue 2, 2022, Page No. 133-141

Spectroscopic analysis (GC-MS and FT-IR) of Melia azedarach leaf extract

Sownthariya C, Shanthi P*
PG and Research, Department of Botany, Holy Cross College, Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Abstract
The current study was designed to examine the phytochemical constituents in the acetone extract of *Melia azedarach*, L. leaves through Gas Chromatography – Mass Spectrometry (GC-MS) and Fourier Transform – Infrared (FT-IR) spectroscopic analysis. Totally, about 28 phytocompounds were identified in GC - MS and the chromatogram graph showed relative peaks with individual compounds. The major phytocompounds with peak area percentage identified in the acetone extract were 2-methyl-Z,Z-3,13 (44.43%); n-hexadeconoic acid (21.83%); 2-pentanone,4-hydroxy-4-methyl- (10.05%); 3,7,11,15-tetramethyl-2-hexadecen-1-ol (4.08%); 2-furancarboxaldehyde,5-hydroxymethyl- (3.42%); octadecanoic acid (2.69%); 9-nonadecene (2.19%); dodecanoic acid (1.61%); phytol (1.29%); hydroquinone (1.25%). The presence of alcohol, alkane, aldehyde, conjugated alkene group, alkyl aryl, ether, primary alcohol and halo compound with stretching and bending functional groups were confirmed in the FT-IR analysis. The results obtained in the present study is an evident that, the phytocompounds present in acetone extract of *M. azedarach* leaves, might be a potential herbal alterative for various diseases and offers a base to take further investigation to find out its bioactivity and pharmaceutical importance.

Keywords: phytocompounds, acetone extract, GC-MS, FT-IR, *Melia azedarach*

Introduction
Medicinal plants are known to possess enormous healing properties and are considered to be major source of herbal medicine against various human ailments. *Melia azedarach* L. is one such medicinal plant, predominantly found in Indian sub-continent, belonging to the mahogany family Meliaceae. In India, the plant is well known for its therapeutic importance used in the treatment of traditional system of medicine against certain common ailments, diseases and disorders, which include skin infections, fever, stomach disorders, inflammation, menstural cramps and diabetes. Studies revealed that leaves of the plant were used in the treatment of skin diseases, heal arthritis, rheumatism and possess bioactive properties such as, antiseptic, arbortifacient, purgative, diuretic, insect-repellent, antihelmintic, antimalarial, antifungal, antibacterial, cathartic, emetic and emmenagogic properties [1, 2]. Since, *M. azedarach*, was known to possess invariable therapeutic importance the plant was selected for further study. Plants serve as an effective substitute with few side effects in combating serious diseases, due to the presence of certain bioactive phytoconstituents (alkyl phenols, tannins, alkaloids, glyalkaloids, flavonoids, lactones, saponins, terpenoids, sesquiterpenes, phenols) that help in certain physiological function on the human body. Researches have reported that the most important bioactive constituents were regarded to be flavonoids, tannins, alkaloids and phenolic compounds [3, 4]. These phytoconstituents may be considered as secondary metabolites with different structural arrangements, properties and biological activities. A number of experimental studies on medicinal plants has proved several therapeutic effect of bioactive compounds in the maintenance of human health have been reported [5, 6, 7]. Owing to an increasing demand in screening of varied biochemical diversity for the development of new therapeutic drugs from plant origin through analytical methodologies has elevated in recent research works.
The primary step to utilize the biologically active compound from plant resources includes extraction, pharmacological screening, isolation and characterization of bioactive compound, toxicological evaluation and clinical evaluation [8]. Inspite of advances on number of analytical techniques within in an era, Gas Chromatography – Mass Spectroscopy (GC-MS) and Fourier Transform Infrared spectroscopy are widely used techniques in the identification and determination of phytochemical compounds. Spectroscopy has developed drastically and provides a significant progress as an important tool in the field of clinical evaluation and biomedical applications. GC-MS is very compatible and most commonly used technique in the identification and quantification of compounds. Whereas, FT-IR is known to identify the functional groups present in the compounds [9, 10]. In recent years, GC-MS and FT-IR has a significant role in pharmaceutical analysis [11, 12]. Several research works have been carried out using spectroscopic techniques in the analysis and structure elucidation of phytoconstituents [13]. Therefore, the present research work was conducted to investigate the presence of various phytochemical (bioactive) compounds in the acetone extract of *Melia azedarach* L. leaves, using GC-MS and FT-IR Spectroscopic methods.

Materials and Methods

Collection and Preparation of Plant Materials

Traditional antidermatophytic plant *Melia azedarach* L. (leaves) was collected from Tiruchirappalli district, Tamil Nadu, South India. Fresh leaves were dried under shade at room temperature for several days. Then dried leaves were powdered using an electric grinder. 15g of fine powder was then mixed with 100ml of acetone, in closed dark container and soaked for 3 days at room temperature (25-30ºC) and filtered using standard Whatmann No.1 filter paper. The acetone extract was then used for GC-MS and FT-IR Spectroscopic analysis.

GC-MS Analysis

The plant sample was investigated through GC-MS analyzer (Perkin Elmer Clarus 500). The GC-MS was modelled with capillary column elite-5 (crossbond 5%phenyl and 95% dimethylpolysiloxane), length 30m, internal diameter 0.25mm. The carrier gas helium (99.999%) was used at a flow rate of 1ml per minute in split ratio (1:10). 2µl acetone extract of the sample was injected into the column at 280°C injector temperature. The oven temperature starts from 60°C without holding and increased at the rate of 6.0 reached 150°C with 2 minutes holding. Finally at the rate of 280°C holding for 5 minutes at the rate of 4.0 was programmed. The injector temperature was set at 280°C and detector temperature at 160°C. The mass spectrum of compounds present in the sample was obtained by operating the mass spectrometer in the positive Electron Ionization (EI) energy at 70eV. The detector was operated in scan mode at an interval of 0-5 seconds and fragments from 40-600 Da atomic units were maintained. The total running time was 40 minutes.

Identification of Components

The chemical components of the sample was identified based on molecular mass, molecular structure and calculated fragments. The data interpretation of mass spectrum GC-MS was done using National Institute Standard and Technique (NIST library) database having more than 62,000 patterns. The name, molecular weight and structure of the components of the sample was ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. The spectrum of unknown components were compared with the version, 2005, Software, Turbo mass 5.2. This is done inorder to determine whether the selected plant specimen contain any individual compound or group of compounds, which may validate its current use as herbal medicine.

FTIR Spectroscopic Analysis

The type of chemical bonds (functional groups) present in compounds are identified using Fourier transform infrared spectrophotometer (FTIR). The dried powdered plant sample (10mg) was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc. The powdered sample of plant specimen was loaded in FTIR Spectroscope (Shimadzu, IR Affinity 1, Japan) with a scan range of 400-4000 cm^{-1} with resolution of 4 cm^{-1}.

Results and Discussion

In the present study, the GC-MS analysis of acetone leaf extract of *Melia azaderach*, Linn., revealed the presence of about 28 compounds (Table 1) and the chromatogram graph (Fig. 1) showed peaks with individual compounds. The major phytocompounds with peak area percentage identified in the acetone extract were 2-methyl-Z,Z-3,13 (44.43%); n-hexadeconoic acid (21.83%); 2-pentanone,4-hydroxy-4-methyl- (10.05%); 3,7,11,15-tetramethyl-2-hexadecen-1-ol (4.08%); 2-furancarboxaldehyde,5-hydroxymethyl- (3.42%); octadecanoic acid (2.69%); 9-nonadecene (2.19%); dodecanoic acid (1.61%); phytol (1.29%); hydroquinone (1.25%). The result of present investigation finds a supportive evidence with other species of meliaceae (mahogany family) like acetone leaf extract of *Melia dubia* [14]; root, stem, leaves and seeds of *Swietenia macrophylla* [15]; methanolic leaf extract of *M. azedarach* [16], sap of *Azadirachta indica* [17]. The identified compounds are known to exhibit various biological activities. The acetone leaf extract of *Melia dubia* revealed the presence of 42 phytocompounds rich in unsaturated fatty acids, phenolic derivatives, terpenoids (diterpenes and sesquiterpenes), lipophilic organic compounds. The compounds included linolenic acid, phthalic acid, palmitic acid, humulene, aromadendrene, caryophyllene, probucol, germacrene-D, butylated hydroxy toluene. Also the plant was reported to possess antibacterial, antifungal, insecticidal, antifeedant, antitumor, anti-inflammatory and antioxidant properties [14]. The predominant phytocompounds reported in root, stem, leaves and seeds of *Swietenia macrophylla* (methanol extract) were n-hexadeconoic acid; benzene,1,2,3,trimethy-; phytol; linoleic acid and the crude extracts were proved to have a promising antimicrobial activity [15]. About 13 bioactive compounds identified from methanolic leaf extract of *M. azedarach* were reported to possess distinctive antifungal activity and compounds such as hexadeconoic acid, octadecane with antibacterial, antifungal, antioxidant, anti-inflammatory and antihelmintic activities [16]. The study on *Azadirachta indica* (neem sap) revealed the presence of 30 volatile compounds belonging to esters, steroids, alcohols, n-hydroxyimine deravatives, isothiocyanate, aromatics, halocompounds, alkalamide, cyanides, unsaturated alkenamides, alkyne and indol groups, but predominantly rich in fatty acids like hexadeconoic acid and pentadeconoic acid [17].

The functional groups of the bioactive components in the plant sample is identified based on the peak value in the region of infrared radiation with absorbance or transmittance percentage (%T) using FTIR spectrum . The

acetone leaf extract of *M. azedarach* showed a characteristic absorption peaks (Fig-2 and Table – 2) and confirmed the presence of alcohol, alkane, aldehyde, conjugated alkene group, alkyl aryl, ether, primary alcohol and halo compound with strong and medium appearance in stretching and bending functional groups. The absorption spectra exhibited a peak at 3419.74 representing the presence of alcohol (O-H stretching). The peak at 2924.52 and 2852.09 represented the presence of alkane (C-H stretching) and 1382.82 with alkane (C-H bending). The peak at 1736.26 represented aldehyde group (C=O stretching) and 1625.64 with conjugated alkene group with (C=C stretching). The peak at 1249.14 represented alkyl aryl ether and 1052.71 as primary alcohol with C-O stretching. Peak at 602.54 represented halo compound (C-I stretching). In general, it is known that, FT-IR analysis aids to identify the functional group of active compounds based on the peak values in the region of infrared radiation [18]. Alkanes are normally found in the cuticle and epicuticular wax of many plants [19]. FTIR is known to be a valuable tool for characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extract [20, 21]. A few reports have documented that the GC-MS spectrum shows the presence of long chain hydrocarbons with complex chemical compositions. When the number of carbon atoms increases in the molecule, hydrophilicity is reduced and lipophilicity is increased. Similarly, the lipophilicity of a drug is higher due to its distribution. So, when a drug is in systemic circulation, it is distributed to all the tissues at a particular rate depending upon its physiochemical characteristics such as lipophilicity and charges [22, 23]. Similarly, a classical fragment pattern of long chain saturated hydrocarbons ranging from 16-36 C atoms belonging to alkane group have been reported in *Ficus benghalensis* sprouted arial root extract through GC-MS and FTIR analysis [24].

Generally, medicinal plants are used in traditional system of medicine with ethnopharmacological activities in the treatment of various diseases [25, 26, 27]. Scientific evidences have been reported that *M.azedarach* and other plant species belonging to meliaceae are store house of limonoids like azadirachtin, fatty acids, terpenoids and other related compounds, which has a wide range of pharmacological uses with certain bioactive properties such as analgesic, anticancer, antimalarial, antibacterial, antifungal, antifeedant, and antifertility [28, 29, 30]. Therefore, the chemical nature, concentration and its presence in the plant system attributes to the bioactive principles which are responsible for therapeutic nature of medicinal plants.

Table 1: GC-MS analysis of acetone extract of *Melia azedarach*, L. Leaves

S. No	Peak Name	Retention time	Peak area	%Peak area
1.	2-Pentanone, 4-hydroxy-4- methyl-	3.61	232841200	10.0508
2.	1,2-Cyclopentanedione	5.22	16919182	0.7303
3.	2,4-Dihydroxy-2,5-dimethyl-3(2H)- furan-3-one	5.96	2837145	0.1225
4.	2,4-Heptadienal, (E,E)-	6.29	1693548	0.0731
5.	4-Acetylbutyric acid	6.79	12108571	0.5227
6.	a - [5-Methyl-2-tetrahydrofuranyl] alanine	8.46	3809386	0.1644
7.	Maltol	8.94	7941136	0.3428
8.	4H-Pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6-methyl-	10.18	9552189	0.4123
9.	2-Furancarboxaldehyde, 5- (hydroxymethyl)-	12.56	79341912	3.4249
10.	(E,Z,Z)-2,4,7-Tridecatrienal	13.66	2117105	0.0914
11.	2-Methoxy-4-vinylphenol	13.96	17506746	0.7557
12.	1,6;3,4-Dianhydro-2-O-acetyl-á- d-allopyranose	14.27	8114083	0.3503
13.	Hydroquinone	14.99	28926764	1.2487
14.	1,2-Octadecanediol	17.35	1287329	0.0556
15.	2-Propenoic acid, 3-phenyl-	17.71	10303892	0.4448
16.	2(4H)-Benzofuranone, 5,6,7,7a- tetrahydro-4,4,7a-trimethyl-	19.41	4317448	0.1864
17.	D-Allose	19.92	17855844	0.7708
18.	Dodecanoic acid	20.62	37377576	1.6134
19.	2-Cyclohexen-1-one, 4-(3- hydroxybutyl)-3,5,5-trimethyl-	24.48	3083361	0.1331
20.	Tetradecanoic acid	26.03	28817833	1.2440
21.	3,7,11,15-Tetramethyl-2- hexadecen-1-ol	27.14	94628176	4.0847
22.	1,21-Docosadiene	27.77	13691443	0.5910
23.	1-Hexadecen-3-ol, 3,5,11,15- tetramethyl-	30.10	3119297	0.1346
24.	n-Hexadecanoic acid	31.35	505784896	21.8327
25.	Phytol	34.16	30071296	1.2981
26.	2-Methyl-Z,Z-3,13- octadecadienol	35.66	1029267328	44.4294
27.	Octadecanoic acid	35.98	62537884	2.6995
28.	9-Nonadecene	44.17	50783348	2.1921

Table 2: FT – IR peak values of acetone extract of *Melia azedarach*, L. leaves

Transmittance percentage (%T)	Peak Value (cm^{-1})	Frequency Ranges (cm^{-1})	Functional group	Compound class	Intensity
58.50	3419.74	3200-3550	O-H stretching	Alcohol	strong, broad

57.28	2924.52	2840-3000	C-H stretching	Alkane	medium
60.11	2852.09	2840-3000	C-H stretching	Alkane	medium
59.45	1736.26	1720-1740	C=O stretching	Aldehyde	strong
58.99	1625.64	1600-1650	C=C stretching	conjugated alkene	medium
60.64	1382.82	1380-1385	C-H bending	Alkane	medium
59.78	1249.14	1200-1275	C-O stretching	alkyl aryl ether	strong
57.78	1052.71	1050-1085	C-O stretching	primary alcohol	strong
64.54	602.54	500-600	C-I	halo compound	strong

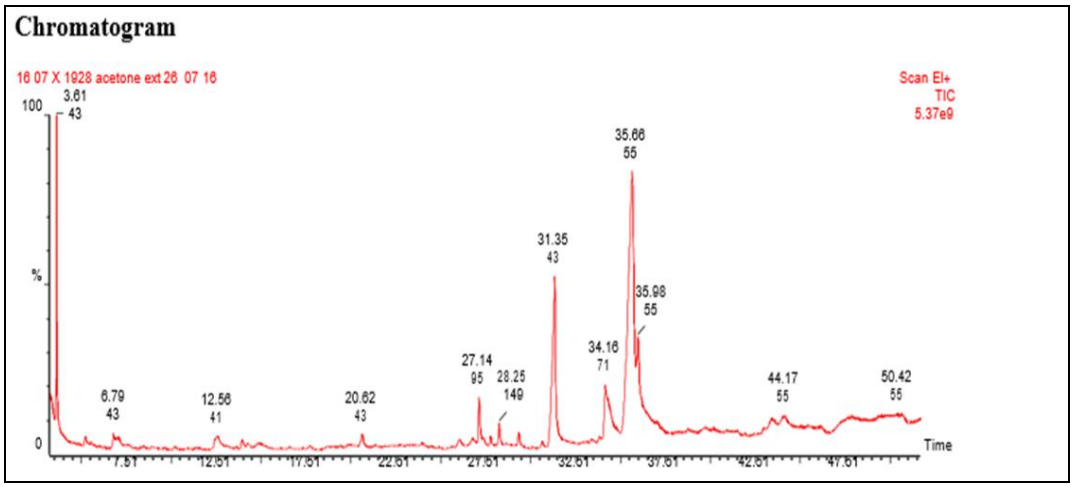
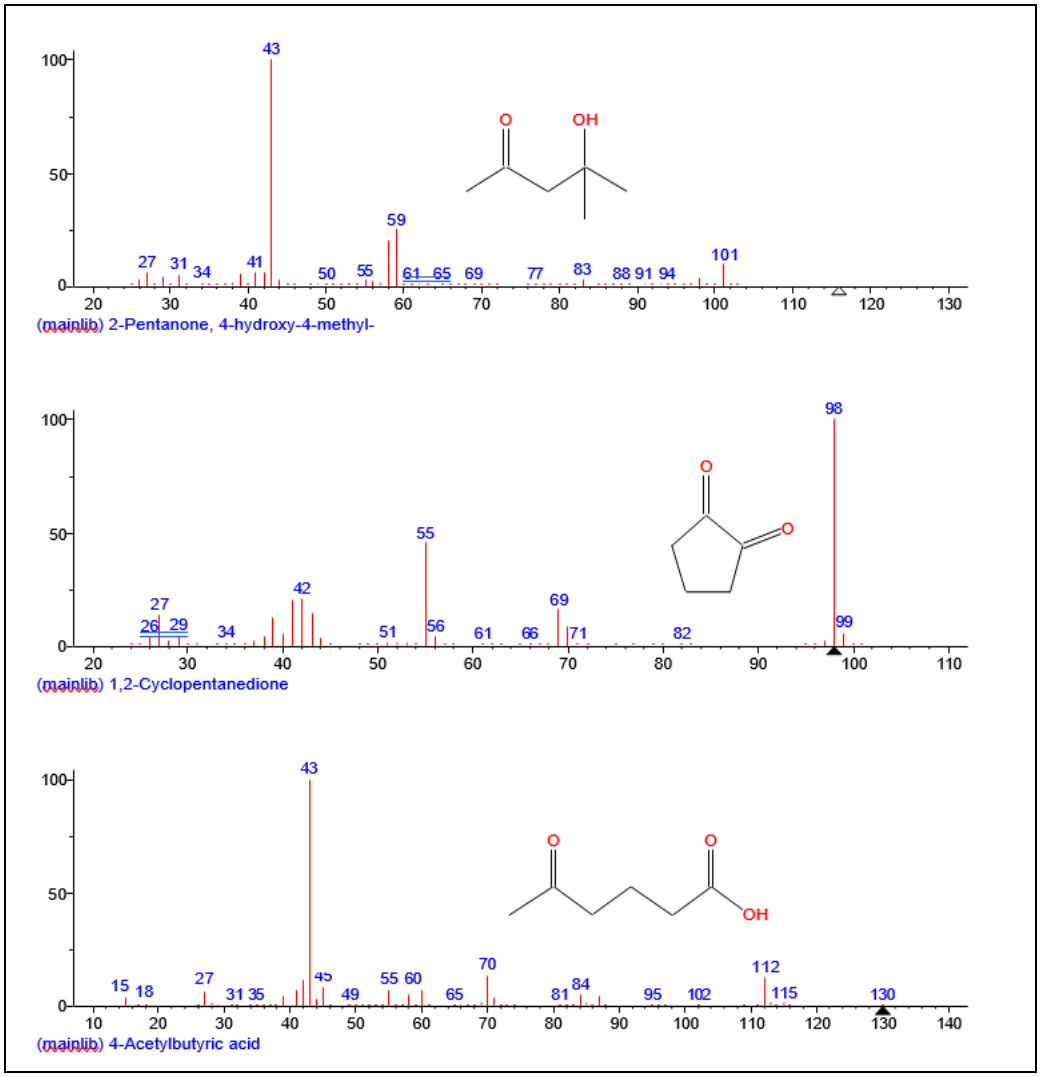
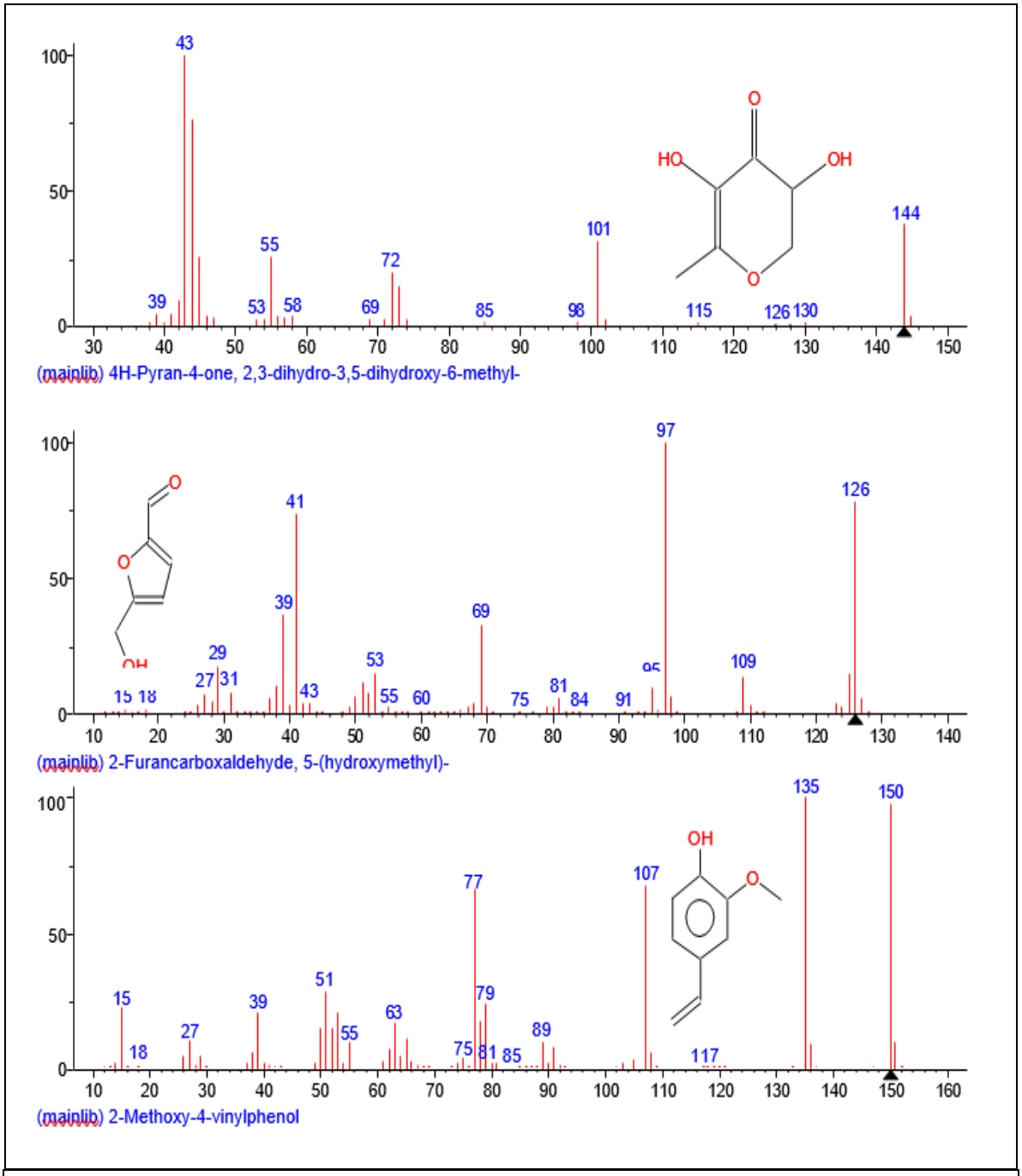
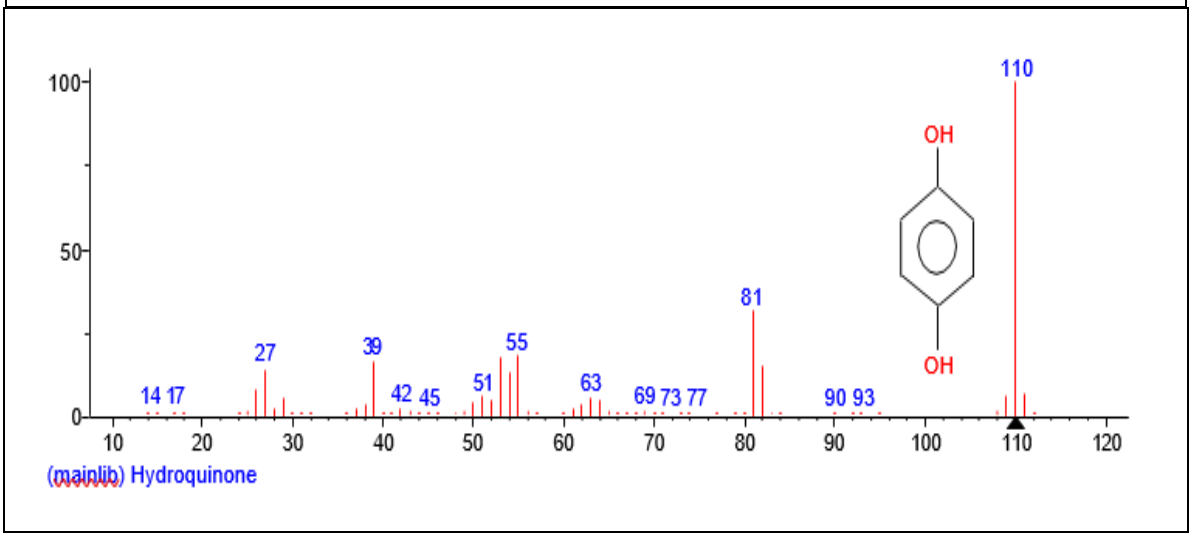
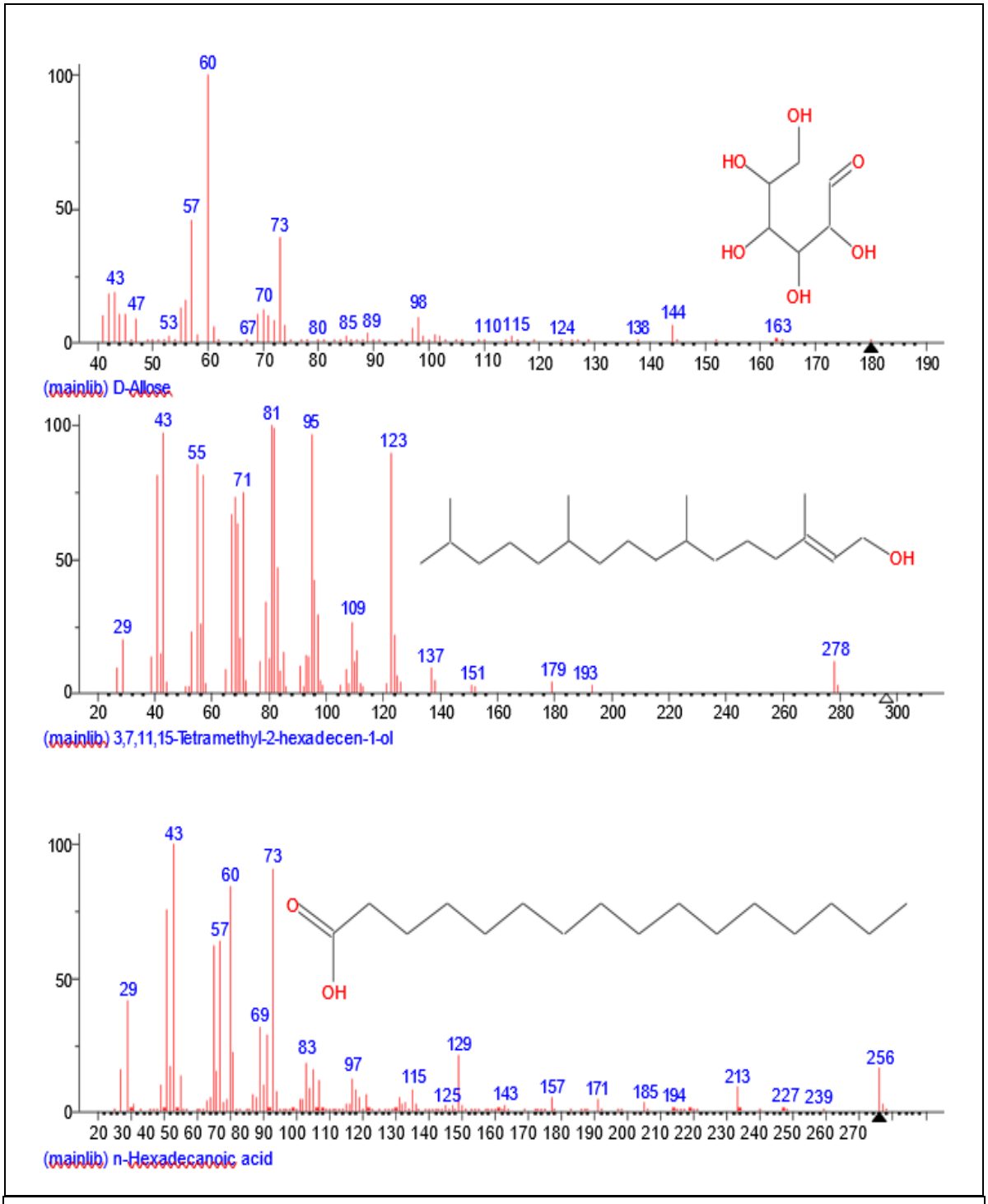


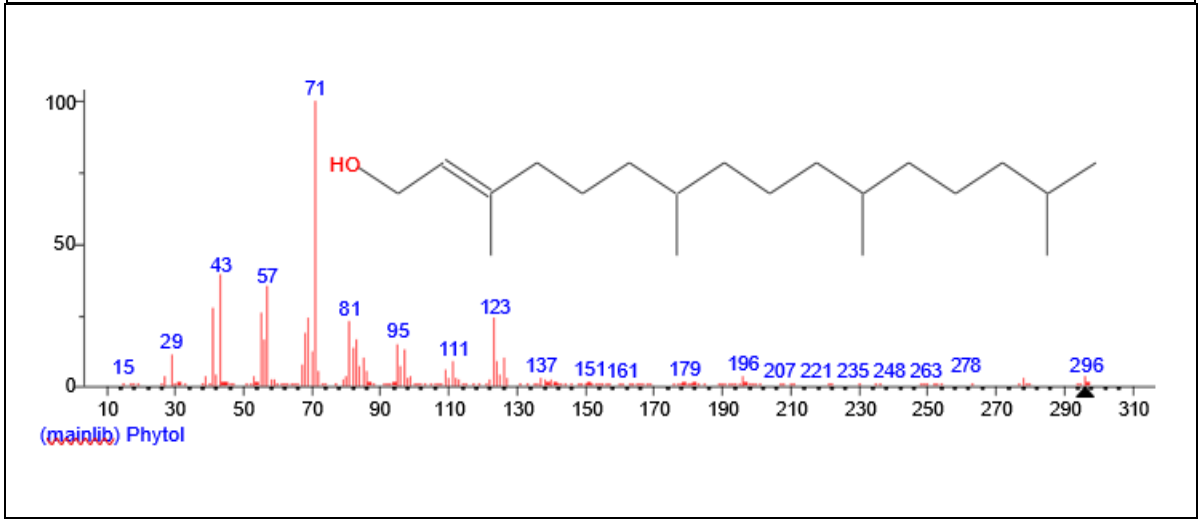
Fig 1: GC – MS Chromatogram of acetone extract of *Melia azedarach*, L. leaves




(mainlib) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
(mainlib) 2-Furancarboxaldehyde, 5-(hydroxymethyl)-
(mainlib) 2-Methoxy-4-vinylphenol


(mainlib) Hydroquinone


(mainlib) D-Allose
(mainlib) 3,7,11,15-Tetramethyl-2-hexadecen-1-ol
(mainlib) n-Hexadecanoic acid


(mainlib) Phytol

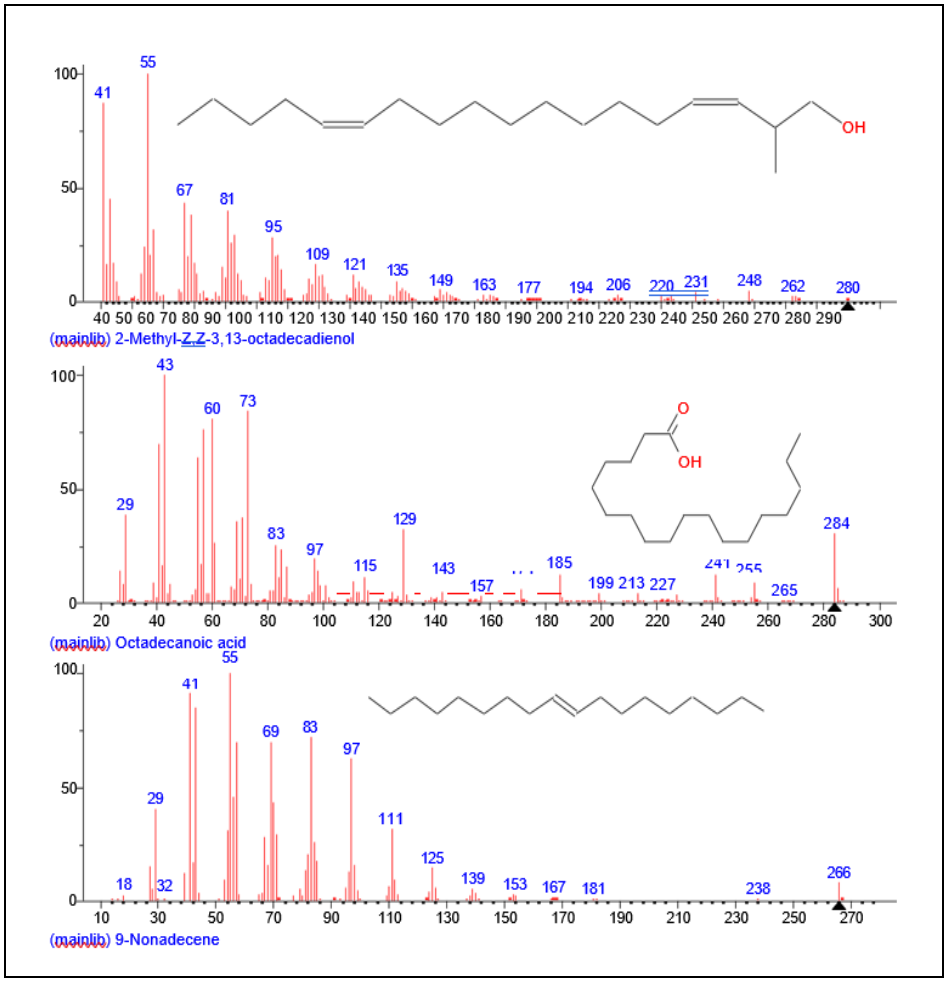


Fig 2: Mass spectra of major phytoconstituents of acetone leaf extract of *Melia azedarach*, L.

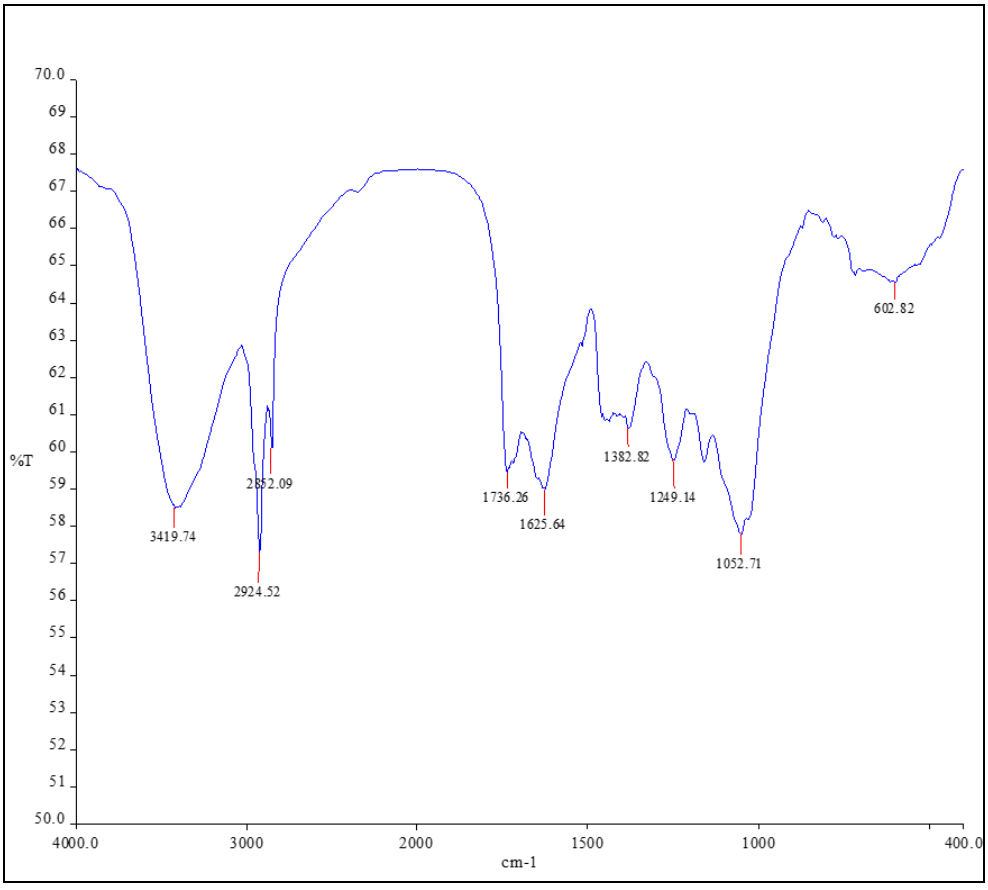


Fig 3: FT-IR Spectrum of acetone extract of *Melia azedarach*, Linn. leaves

Conclusion

The results of the present work authenticates that, acetone leaf extract of *M. azedarach* possesses certain important bioactive compounds which are identified through GC-MS and FTIR analysis. These compounds may acquire important medicinal properties in combating various diseases. This proof gives a supportive base and outline for further study in understanding the nature of active principles subjecting to biological activity; isolation, identification and validation of bioactive compound present in the selected plant. Finally, it is concluded that, *M. azedarach* contains various phytocompounds and it is recommended as a plant of pharmaceutical importance.

Acknowledgement

The authors are extremely thankful to the Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, NH67, Thirumalaisamudram, Thanjavur, Tamil Nadu for providing the facilities to carry out this research work.

Conflict of Interest

The authors declare no conflict of interest.

Abbreviation USED

% T: Transmittance Percentage
C: Carbon
cm^{-1}: reciprocal wavelength
Da: Dalton atomic units
EI: Electron Ionization
eV: electronvolt
FTIR: Fourier transform- infrared
g: gram
GC-MS: Gas chromatography-mass spectrometry
H: Hydrogen
I: Iodine
KBr: Potassium Bromide
m: metre
mm: millimetre
NIST: National Institute Standard and Technique
O: Oxygen
^{o}C: Celsius

References

1. Azam MM, Mamun-Or-Rashid ANM, Towfique NM, Sen MK, Nasrin S. Pharmacological potentials of *Melia azedarach* L. – A Review. American Journal of Bioscience,2013:1(2):44-49.
2. Vedha pal JS, Ramya N, Senthilkumaran K, Senthilnathan B, Murugan N. Phytochemical and qualitative analysis of leaves of *Melia azedarach* and seeds of *Piper longum.* J Chem Pharm Res,2018:10(2): 25-31.
3. Cox P, Balick M. The ethnobotanical approach to drug discovery. Sci. American, 1994, 82.
4. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr.J.Biotechnol,2005:4:685-688.
5. Kaushik JC, Arya Sanjay, Tripathi NN, Arya S. Antifungal properties of some plant extracts against the damping of fungi of forest nurseries. Indian J. Forest,2002:25:359-361.
6. Chaman Lal, Verma LR. Use of certain bioproducts for insect- pest control. Indian J. Trad. Knowl,2006:5(1):79-82.
7. De-Fatima A, Modolo LV, Conegero LS, pilli RA, Ferreira CV, Kohn LK *et al*. Lactones and their derivatives: biological activities, mechanism of action and potential leads for drug design. Curr. Med. Chem,2006:13:3371-3384.
8. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plant extracts. Afr. J. Tradit. Complement Altern. Med,2011:8(1):1-10.
9. Roberts Hites JKM, Xia JH. Methods in Cell biology,1995:49:245-258.
10. Ronald Hites A. Handbook of Instrumental Techniques for analytical chemistry (Settle, FA. Ed.), 1997, 609-626.
11. Koduru S, Grierson DS, Afolayan A. Antimicrobial activity of *Solanum aculeastrum*. Pharm. Biol,2006.44:283-286.
12. Movasaghi Z, Rehman S, Rehman IU. Fourier transform infrared spectroscopy of biological tissues. Appl. Spec. Rev,2008:43:134-179.
13. Liebler DC, Burr JA, Philips L, Ham AJL. Gas Chromatography- mass spectrometry analysis of vitamin E and its oxidation products. Anal. Biochem,1996.236:27-34.
14. Murugaesan S, Senthilkumar N, Rajesh Kannan, Vijayalakshmi KB. Phytochemical characterization of *Melia dubia* for their biological properties. Pelagia Research Library,2013:4(1):36-40.

15. Mohammed SB, Azhari NH, Mashitah YM, Abdurahman NH, Mazza S. In vitro antimicrobial activity and GC-MS analysis of medicinal plant *Swietenia macrophylla*. J. Chem. Pharm. Res,2015:7(11):519-524.
16. Ali Hussein Al – Marzoqi, Imad Hadi Hameed, Salah Ali Idan. Analysis of Bioactive chemical components of two medicinal plants (*Coriandrum sativum* and *Melia azedarach*) leaves using gas chromatography-mass spectrometry (GC-MS). Afr. J. Biotech,2015:14(40):2812-2830.
17. Praveen Kumar P, Nandhini AR, Subhapriya P, Gowri Shankar BA. Analysis of volatile compounds in the sap of *Azadirachta indica* (neem) using gas chromatography mass spectrometry. Intl. Res. J. Pharmacy,2018:9(3):38-43.
18. Coates J. Interpretation of infrared spectra: A practical approach – Encyclopedia of analytical chemistry. R.A. Meyers (Ed) John Wiley and sons Ltd. Chichester, 2000, 1085-1087.
19. Wang W, Saho Z. Enzymes and genes involved in aerobic alkane degradation. Front Micrbiol,2013:4:116.
20. Eberhardt TL, Li X, Shupe TF, Hse CY. Chinese TallowTree (*Sapium sebiferum*) utilization: characterization of extractives and cell wall chemistry. Wood Fiber Sci,2007:39:319-324.
21. Hazra KM, Roy RN, Sen SK, Laska S. Isolation of antibacterial pentahydroxy flavones from the seeds of *Mimusops elengi* Linn. Afr.J.Biotechnol,2007:6(12):1446-1449.
22. Rutkowska E, Pajak K, Jozwiak, K. Lipophilicity-methods of determination and its role in medicinal chemistry. Acta Pol Pharm,2013:70(1):3-18.
23. Elfotoh MAA, Shams K, Anthony KP, Shahat AA. Lipophilic constitutents of *Rumex vesicarius* L. and *Rumex dentatus* L. Antioxidants,2013:2:167-180.
24. Sapana S, Sujata B, Amruta T, Kalal KM. GC-MS study of hydrocarbons – a renewable biofuel with high calorific value from ariel roots of *Ficus benghalensis* L. Elec. J. Env. Agri. Food Chem,2008:7(14):2743-2748.
25. Omoyeni OA, Aterigbade E, Akinyeye RO, Olowu RA. Phytochemical screening, nutritional/anti-nutritional and amino acid compositions of Nigeria *Melanthera scandens*. Sci. Rev. Chem. Commun,2012:2(1):20-30.
26. Rajan S, Suvetha P, Thirunalasundari T, Jeeva S. Anti-enteric bacterial of the traditional medicinal plants of Kanyakumari coast, Tamil Nadu. J. Coast Life Med,2015:3(8):640-4.
27. Nguta JM, Appiah-Opong R, Nyarko AK, Yeboah – Manu D, Addo PG. Medicinal plants used to treat TB in Ghana. Intl J Mycobacteriol,2015:4(2):116-23.
28. Vishnukanta AC. *Melia azedarach*: A phytopharmacological review. Pharmcogn. Rev: Plant Rev. 2008. 2(3):173-179.
29. Hariprasad PS, Ramakrishnan N. GC-MS analysis of *Rumex vesicarius* L. Int.J.Drug Dev. Res,2011:3(2):272-279.
30. Sen A, Batra A. *Melia azedarach.* A paradise tree. J. Funct. Environ. Bot,2011:1(1):59-69.