International Journal of Environment, Agriculture and Biotechnology
Vol-9, Issue-5; Sep-Oct, 2024
Peer-Reviewed International Journal
Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

FT-IR and GC-MS characterization of bioactive compounds from the root extract of *Anacyclus pyrethrum* Linn

Dr. S. P. Anand[1], R. Nagalakshmi[2*], S. Karthick[3], S. Vanathi[4]

[1,3,4]PG and Research Department of Botany, National college (Autonomous), Affiliated to Bharathidasan University, Trichy, India
[2*] Department of Botany, Srimad Andavan Arts and Science College (Autonomous), Affiliated to Bharathidasan University, Trichy, India
*Corresponding author: R. Nagalakshmi M.Sc., M.Phil., Ph.D., Department of Botany, Srimad Andavan Arts and Science College (Autonomous), Affiliated to Bharathidasan University, Trichy, India
Email: nagalakshmi.93botany@gmail.com

Received: 22 Aug 2024; Received in revised form: 23 Sep 2024; Accepted: 29 Sep 2024; Available online: 05 Oct 2024

Abstract— *Medicinal plants have long been considered for their ability to treat various human diseases owing to their potent phytochemical properties. However, isolating lead compounds from complex mixtures requires extensive knowledge, specialized equipment, and expertise. The emergence of novel diseases underscores the importance of accurately documenting the research on medicinal plants. This study focused on Anacyclus pyrethrum (L.) Lag, an ingredient in Kabasurakudineer. Although traditional and experimental evidence supports various claims regarding these plants, thorough evaluation and utilization of their potential are still necessary. Further research is required to elucidate the mode of action of these isolates. The primary objective of this study was to identify the bioactive compounds in the ethanolic root extract of Anacyclus pyrethrum using Fourier Transform-Infrared (FTIR) and Gas Chromatography-Mass Spectroscopy (GC-MS). FTIR analysis revealed the presence of hydroxyl groups, alcohols, carboxylic acids, aromatic compounds, nitro compounds, alkyl halides, and aryl halides with major peaks at 3740.03, 3281.04, 2903.30, 1612.69, 1402.96, 1242.33, 1023.12, 658.19, and 582.88, respectively, indicating phenols, flavonoids, tannins, and saponins. GC-MS analysis identified 60 compounds, with the major constituents being 4-Decadienamide, N-Isobutyl-, (E, E)-, and (2e,4e)-N-Isobutyldodeca-2,4-Dienamide, constituting 23.03% and 17.33% of the extract, respectively. This study confirmed that the roots of Anacyclus pyrethrum (L.) contain significant natural chemical compounds, validating its traditional use in various pharmacological activities.*

Keywords— ***Anacyclus pyrethrum, Bioactive compounds, Flavonoids, Gas chromatography, Hydroxyl compounds, Pharmacological activities***

I. INTRODUCTION

Medicinal plants are a valuable resource for drug development and synthesis. These plants play a crucial role in human culture and in the development of natural resources. Phytochemicals are biologically active compounds of plant origin that provide health benefits to humans [1]. These non-nutritive plant chemicals exhibit protective or disease-preventive properties, and may play a significant role in plant growth. Medicinal plants contribute substantially to the treatment and cure of numerous human diseases owing to their potent phytochemical constituents [2]. At present, more than 4,000 phytochemicals have been identified and classified according to their protective functions, physical characteristics, and chemical characteristics, with approximately 150 of them being studied in detail [3]. The process of isolating and identifying lead compounds from complex mixtures requires several resources, including comprehensive knowledge, specialized equipment, and expertise. The urgency for the discovery of

new agents arises from intractable factors including the emergence of novel pathogenic diseases. There is a significant need and ethical imperative to accurately document investigation findings regarding plants used for health purposes. Globally, the ongoing pursuit of an effective drug to combat the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has not been successful. Indian traditional medicines, particularly polyherbal formulations such as Nilavembu Kudineer and Kaba Sura Kudineer of the Siddha system of medicine, have been employed as public health interventions for controlling viral epidemics such as dengue and chikungunya. Traditional therapies are safe, effective, and widely used. Kabasurakudineer is a compound formulation comprising fifteen ingredients [4]. In this study, we selected one of the ingredients of Kabasurakudineer *Anacyclus pyrethrum* (L.) Lag. roots, commonly known as pellitory roots or Akarkara in Indian trade. In traditional medicine, the roots of *A. pyrethrum* are recommended for the treatment of toothache, salivary secretion, angina, digestive problems, lethargy, female infertility, and paralysis of the tongue and limbs. They are utilized in the form of cream-based animal fats to treat gout and sciatica, and to prevent illness. Other pharmacological and biological properties of *A. pyrethrum* roots have been reported in the literature, including sialagogue aphrodisiac [5,6], immunostimulant [7,8], antidepressant[9], antimicrobial [10,11], anesthetic [12], anti-inflammatory [13,14], anticonvulsant1 [5,16], antioxidant [17], antidiabetic [18], reproductive [19], and memory enhancer [20] properties. Although there is traditional and experimental evidence to support the various claims and benefits of these plants, proper evaluation and exploitation are required. Further investigations are necessary to assess the mode of action of the isolates in various activities. Therefore, this plant was selected for this study. The aim of the present study was to analyze the phytochemicals present in the roots of *Anacyclus pyrethrum* using gas chromatography and mass spectroscopy.

II. MATERIALS & METHODS

2.1 Study species

In this study, we selected *Anacyclus pyrethrum* L., a medicinal plant belonging to the Asteraceae family. It is a perennial herb found in North Africa, India, and other Arabian countries. The medicinal application of this plant is utilized in Ayurvedic, Unani, and herbal medicines globally for the treatment of male disorders, common cold, toothache, and pyorrhea, and plays a crucial role in preventing and controlling epilepsy and seizures [9,15].

2.2 Study area

Anacyclus pyrethrum L. was collected from Kollimalai in February, 2022. Kollimalai is situated west of Pachaimalais in the Namakkal district of Tamil Nadu, India. It comprises a compact block of hills with a total area of 490 square kilometers and altitude ranging from 1000 to 1300 m. The average annual precipitation is approximately 1200 mm.

2.3 Sample preparation

The collected plant roots were washed with water to remove soil and debris. The plant material was subsequently dried in the shade for four–five days and subsequently reduced to small pieces. It was then pulverized into a coarse powder. The resultant powder was used for extraction of the active compounds. Plant roots were extracted using a previously described method. The dried powders were immersed separately in ethanol (1:4, w/v) at room temperature for 24 h. The extracts were suction-filtered through a Whatman No.1 filter paper. This process was repeated for two additional days, and similar extracts were pooled, concentrated, and vacuum-evaporated using a rotary evaporator at a temperature of 40°C [2,4].

2.4 Fourier-transform infrared spectroscopy (FTIR) analysis

FTIR is a chemical analytical method that measures infrared intensity versus the wavelength or wave number of light. It was employed to analyze potential biomolecules and bonding interactions between them. IR spectroscopy was used to determine the vibrational characteristics of the chemical functional groups in the samples. When infrared light interacts with matter, chemical bonds undergo stretching, contraction, and bending. This chemical functional group tends to adsorb infrared radiation in a specific wavenumber range within the structure of the rest of the molecule. The functional groups of the plant extracts were characterized by FTIR analysis, and the spectra were scanned in the range 4000–400 cm−1 [21] at a resolution of 4 cm−1. FTIR analysis of *Anacyclus pyrethrum* L. powdered root samples was conducted using FTIR spectroscopy (PERKIN ELMER, IR) at the National College Instrumentation Facility, National College Trichy.

2.5 GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) is a technique used to analyze and quantify organic volatile and semi-volatile compounds. Gas Chromatography (GC) was used to separate the mixtures into individual components using temperature-controlled capillary columns. GC-MS (Shimadzu QP-2020) was performed using a DB-WAX column containing a polar stationary phase. A sample injection volume of 1µl with a split ratio of

10:1, was used. The initial injector temperature was 250°C. The linear velocity to control the flow was maintained at 39.7 cm/sec, Pressure: 68.1 k Pa, total flow: 16. 2 mL/min, Column flow: 1. Twenty mL/min, ion-source temperature: 200°C, and interface temperature: 250 °C. The oven temperature was varied from 50 °C to 280°C for 2 min. ACQ Mode range from 50m/z to 500m/z. Mass spectra were examined by electron impact ionization at 70 eV.The gas chromatography (GC) run time for each sample was 40 minutes. Mass spectral range interpretation in gas chromatography-mass spectrometry (GC-MS) was conducted using NIST and WILEY library databases [22]. GC-MS analysis of the ethanolic extracts of *A. pyrethrum* L. roots was performed using gas chromatography-mass spectrometry (Shimadzu QP-2020) at the Heber Analytical Instrumentation Facility (HAIF), Bishop Heber College, Trichy.

III. RESULTS AND DISCUSSION

3.1 FT-IR spectral data interpretation

Fourier transform infrared (FTIR) spectroscopy is a vibrational spectroscopic technique that employs infrared radiation to induce vibrations in molecular bonds within a sample [23]. The FTIR spectrum was utilized to identify the functional groups of the active components present in the extract based on the peak values in the IR region. The FTIR spectra of the plant extracts (prepared in ethanol) of *A. pyrethrum* are presented in Fig 1. The peak values and probable functional groups present in the root extracts are summarized in TABLE 1. FTIR analysis results demonstrated that the root extract of *A. pyrethrum* contained hydroxyl groups, alcohols, carboxylic acids, aromatic compounds, nitrocompounds, alkyl halides, and aryl halides, which exhibited major peaks at 3740.03, 3281.04, 2903.30, 1612.69, 1402.96, 1242.33, 1023.12, 658.19, and 582.88, respectively. These peaks indicate the presence of phytochemical compounds such as phenols, flavonoids, tannins, and saponins. *Clitoria ternatea* was investigated for bioactive compounds in the leaf and flower parts used in this study. Fourier-transform infrared spectroscopy of the leaf extract revealed eight bands, indicating the presence of alcohols, carboxylic acids, and aromatic compounds, whereas the flower extract showed eight bands, suggesting the presence of nitro compounds and alkanes. Upon analyzing the results of this study, it is evident that they partially corroborate the previously mentioned output [24]. FTIR analysis of the four types of *Wedelia trilobata* extracts revealed the presence of carboxylic acids, alkenes, and nitro compounds, which aligns with the aforementioned output [25]. The FTIR analysis of *Rivinia humilis* and *Diplazium esculentum* samples at 4000–500 cm-1 demonstrated the presence of alkanes and aromatic compounds, which is consistent with the previously discussed results [26]. A subsequent study was initiated on *Allium sativum* and Nymphaea lotus samples, wherein multiple peaks were identified in different extractions. Among these peaks, alcohols, carboxylic acids, nitro compounds, alkanes, and aromatic compounds were identified, which is consistent with the previously mentioned results [27].

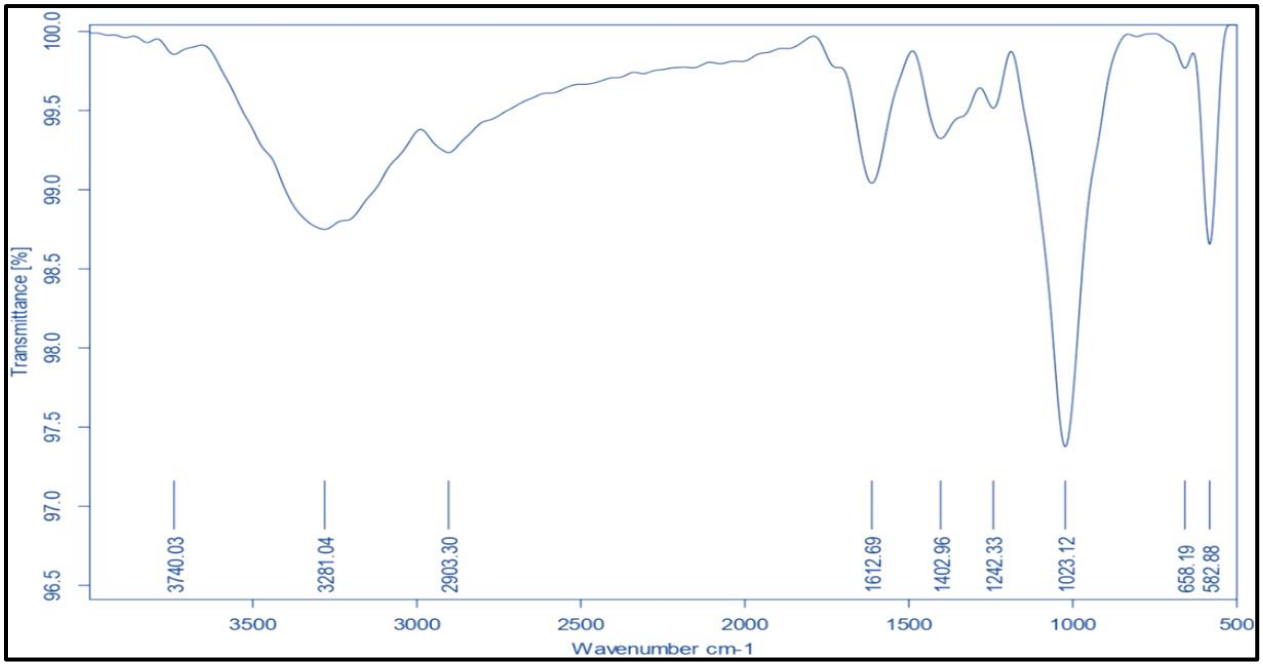


Fig 1. FTIR spectrum of A. pyrethrum

Table 1. FTIR functional groups of compounds in root extract of A. pyrethrum

Frequency range	Molecular motion	Intensity	Functional group
3740.03	O-H(Non-bonded)	W	Hydroxyl group
3281.04	O-H stretch	Br	Alcohol
2903.30	O-H stretch	Br	Carboxylic Acid
1612.69	C-H stretch	M-W	Aromatic compound
1402.96	NO_2 stretch	S	Nitro compound
1242.33	C-F stretch	M-W	Alkyl & Aryl Halides
1023.12	C-F stretch	S	Alkyl & Aryl Halides
658.19	C-l stretch	S	Alkyl & Aryl Halides
582.88	C-l stretch	S	Alkyl & Aryl Halides

3.2 Gas chromatography mass spectrometry

The ethanolic root extract of A. pyrethrum was subjected to gas chromatography-mass spectrometry (GC-MS) analysis, wherein the mass spectra of the compounds identified in the extract were compared with the National Institute of Standards and Technology (NIST) library[28]. Sixty compounds exhibiting various phytochemical activities were identified in the ethanolic root extracts of A. pyrethrum. The chromatogram is presented in Fig. 2, and the chemical constituents and their retention time (RT), molecular formula, molecular weight, and concentration (%) in the root extract of *A. pyrethrum* are presented in TABLE 2 and some structures are in TABLE 3. Fatty amides were the predominant chemical constituents. The major components were 2,4-Decadienamide, N-Isobutyl-,(E,E)- (23.03%) , (2E,4E)-N-Isobutyldodeca-2,4-dienamide (17.33%), Tetrapentacontane, and N-Hexadecanoic Acid (3.22%). A study was conducted to evaluate phytochemical screening and GC-MS analysis for the presence of secondary metabolites, including alkaloids, flavonoids, terpenoids, steroids, and tannins. Extracts of numerous plant species have gained popularity in recent years, and efforts to characterize their bioactive properties for various pharmaceutical applications [29]. *Erigeron canadensis* was subjected to infrared and mass spectroscopy analyses, yielding 20 peaks, among which alcohols, phenols, and carboxylic acids were the most prevalent. Mass spectroscopy was used to identify 23 bioactive compounds [30]. Subsequently, *Chenopodium album* was used to investigate its antifungal activity using mass spectroscopy, resulting in the identification of six compounds [31]. *Senna occidentalis* was analyzed via GC-MS and infrared spectroscopy, leading to the isolation of nine compounds, including fatty acids, using a separation technique [32]. The roots of *Cassia siberiana* produced eight strong and four weak peaks, and mass spectroscopy was used to isolate 18 compounds [33]. The root extract of *Asparagus racemosus* was subjected to FTIR, yielding the expected peaks along with carboxylic acids, whereas mass spectroscopy was utilized to isolate the methyl group [34]. The bioactive compounds present in *Solanum khasianum* were investigated using gas chromatography-mass spectrometry. A total of 48 compounds were identified, 13 in the leaves and 32 in the roots. These results demonstrated that similar compounds were observed in this study [35]. *Eichornia crassipes* leaves were used for phytochemical profiling, and FTIR analysis revealed 16 peaks, including those for aromatic, alcohol, phenol, and nitro compounds. GC-MS analysis revealed several peaks, the most prevalent of which were those of the five compounds [36]. *Senna tora* were subjected to phytochemical characterization and GC-MS, several major peaks were identified in the samples along with phenolic compounds [37]. The leaf extracts of *Tamarindus indica* were subjected to infrared and mass spectroscopy, which provided evidence to support these results. Infrared analysis revealed 26 peaks from the ethanol and water samples with alcohol, alkene, and carboxylic groups, whereas mass spectroscopy identified 60 compounds with hexadecanoic acid, which is consistent with the findings of the aforementioned study. These results provide crucial evidence to support the aforementioned findings [38]. The present study utilized *Mentha spicata* for phytochemical investigations. GCMS analysis revealed six peaks indicative of the presence of alcohol and phenol groups [39]. Phytochemical investigations were conducted on *Ruellia tuberosa*. Infrared analysis revealed 13 peaks corresponding to phenols and alkene compounds, whereas GC-MS identified 16 distinct compounds [40].

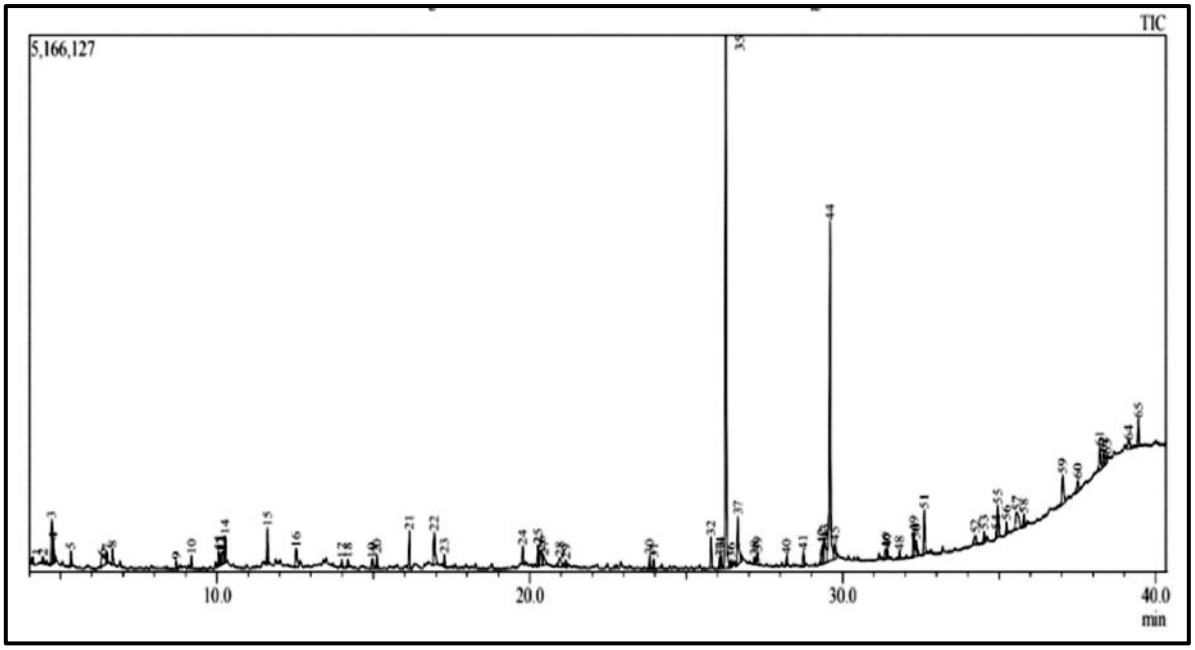


Fig 2: Chromatogram of A. pyrethrum

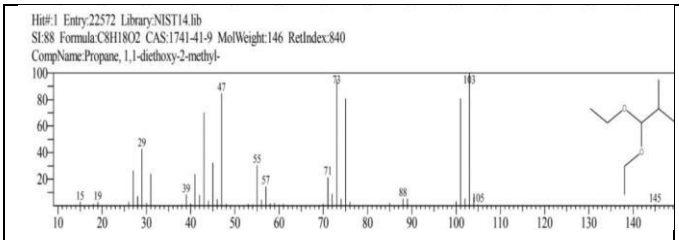
Table 2: Phytochemical Components in the root extract of A. pyrethrum by GC-MS

Peak	R.t (Min)	Area(%)	Compound name	Molecular weight	Molecular formula
1.	4.114	0.25	Propane, 1,1-Diethoxy-2-Methyl-	146	$C_8H_{18}O_2$
2.	4.438	0.33	1-Butanol, 3-Methyl-, Acetate	180	$C_7H_{14}O_2$
3.	4.718	2.79	1-Butanol, 2-Amino-3-Methyl- (+/-.)-	103	$C_5H_{13}NO$
4.	4.77	0.84	Acetic Acid, 1-Ethyl-2-Methylpropyl Ether	144	$C_8H_{16}O_2$
5.	5.33	0.55	1,2-Cyclooctanedione	98	$C_5H_6O_2$
6.	6.335	1.51	Glycerin	92	$C_3H_8O_3$
7.	6.475	0.49	2,4-Dihydroxy-2,5-Dimethyl-3(2h)-Furan-3-One	144	$C_6H_8O_4$
8.	6.662	0.68	Formic Acid, 2-Propenyl Ester	86	$C_4H_6O_2$
9.	8.706	0.35	Uracil, 1-methyl-	126	$C_5H_6N_2O_2$
10.	9.186	0.51	1,1,3-Triethoxybutane	190	$C_{10}H_{22}O_3$
11.	10.061	0.81	Cyclopentasiloxane, Decamethyl-	370	$C_{10}H_{30}O_5Si_5$
12.	10.13	0.67	N,N-Dimethyl-O-(1-Methyl-Butyl)-Hydroxylamine	131	$C_7H_{17}NO$
13.	10.165	1	2-[(Dimethylamino) Oxy]Pentane	131	$C_7H_{17}NO$
14.	10.265	1.32	4h-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-	144	$C_6H_8O_4$
15.	11.617	1.55	Dodecane	170	$C_{12}H_{26}$
16.	12.529	1.25	3-Acetoxy-3-Hydroxypropionic Acid, Methyl Ester	162	$C_6H_{10}O_5$
17.	13.99	0.32	Cyclohexasiloxane, Dodecamethyl-	444	$C_{12}H_{36}O_6Si_6$
18.	14.18	0.45	2-Methoxy-4-Vinylphenol	150	$C_9H_{10}O_2$
19.	14.967	0.53	Phenol, 2,6-Dimethoxy-	154	$C_8H_{10}O_3$
20.	15.113	0.61	Eugenol	164	$C_{10}H_{12}O_2$

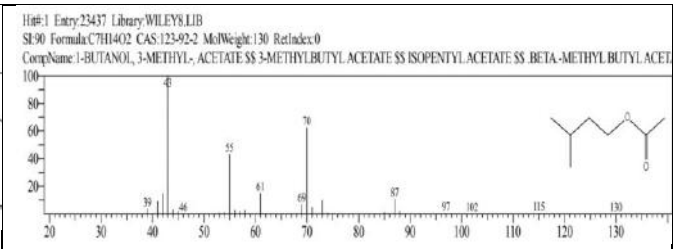
21.	16.157	1.55	Tetradecane	198	$C_{14}H_{30}$
22.	16.946	1.87	Benzaldehyde, 2-Hydroxy-4-Methyl-	136	$C_8H_8O_2$
23.	17.272	0.49	1,6,10-Dodecatriene, 7,11-Dimethyl-3-Methylene-, (E)-	204	$C_{15}H_{24}$
24.	19.777	0.8	Lavandulyl Propionate	210	$C_{13}H_{22}O_2$
25.	20.261	0.83	Hexadecane	226	$C_{16}H_{34}$
26.	20.37	1.23	1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic Acid	192	$C_7H_{12}O_6$
27.	20.455	0.97	4-Methylmannitol	196	$C_7H_{16}O_6$
28.	20.957	0.96	Beta.-D-Glucopyranose, 4-O-.Beta.-D-Galactopyranosyl-	342	$C_{12}H_{22}O_{11}$
29.	21.159	0.44	Acetic Acid, Trifluoro-, Octahydro-4-Hydroxy-1,5-Methano-1h-Inden-1-Yl Ester	264	$C_{12}H_{15}F_3O_3$
30.	23.826	0.58	9,11Dimethyltetracyclo[7.3.1.0(2.7).1(7.11)]Tetradecane	218	$C_{16}H_{26}$
31.	23.96	0.38	Tetradecane	198	$C_{14}H_{30}$
32.	25.793	1.27	1-(3-Phenyl-Bicyclo[1.1.1]Pent-1-Yl)-Propan-1-One	200	$C_{14}H_{16}O$
33.	26.075	0.38	Methyl 14-Methylpentadecanoate	270	$C_{17}H_{34}O_2$
34.	26.106	0.68	1h-Indene, 1-Ethylideneoctahydro-7a-Methyl-, Cis-	164	$C_{12}H_{20}$
35.	26.258	23.03	2,4-Decadienamide, N-Isobutyl-, (E,E)-	223	$C_{14}H_{25}NO$
36.	26.435	0.53	7-Hexadecyn-1-Ol	238	$C_{16}H_{30}O$
37.	26.652	3.22	N-Hexadecanoic Acid	256	$C_{16}H_{32}O_2$
38.	27.182	0.32	Hexadecanoic Acid, Ethyl Ester	284	$C_{18}H_{36}O_2$
39.	27.274	0.69	[1,1'-Biphenyl]-2-Ol	170	$C_{12}H_{10}O$
40.	28.207	0.46	Naphthalene, Decahydro-1,1-Dimethyl-	166	$C_{12}H_{22}$
41.	28.741	0.64	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester	294	$C_{19}H_{34}O_2$
42.	29.341	1.23	9,12-Octadecadienoic Acid (Z,Z)-	280	$C_{18}H_{32}O_2$
43.	29.419	2.26	(Z,Z)-6,9-Cis-3,4-Epoxy-Nonadecadiene	278	C19H34O
44.	29.597	17.33	(2e,4e)-N-Isobutyldodeca-2,4-Dienamide	251	$C_{16}H_{29}NO$
45.	29.759	0.97	N-Propyl 9,12-Octadecadienoate	322	$C_{21}H_{38}O2$
46.	31.381	0.65	N-(2-Methylbutyl)Undeca-(2e,4z)-Diene-8,10-Diynamide	243	$C_{16}H_{21}NO$
47.	31.43	0.38	9-Tricosene, (Z)-	322	$C_{23}H_{46}$
48.	31.813	0.3	Eicosane	282	$C_{20}H_{42}$
49.	32.271	1.14	N-Isobutyl-(2e,4z,8z,10e)-Dodecatetraenamide	247	$C_{16}H_{25}NO$
50.	32.345	0.83	(2e,4e,10e)-N-Isobutylhexadeca-2,4,10-Trienamide	305	$C_{20}H_{35}NO$
51.	32.608	2.05	(2e,4e)-N-Isobutyltetradeca-2,4-Dienamide	279	$C_{18}H_{33}NO$
52.	34.246	0.81	Stigmasta-3,5-Diene	396	$C_{29}H_{48}$
53.	34.52	0.45	Octadecane	254	$C_{18}H_{38}$
54.	34.905	0.29	1,2-Benzenedicarboxylic Acid	390	$C_{24}H_{38}O_4$
55.	34.957	1.58	5,8,11-Eicosatriynoic Acid, Methyl Ester	314	$C_{21}H_{30}O_2$
56.	35.232	0.55	5,8,11,14-Icosatetraynoic Acid	296	$C_{20}H_{24}O_2$

57.	35.559	2.3	Tetrapentacontane	758	$C_{54}H_{110}$
58.	35.795	0.58	Tetrapentacontane	758	$C_{54}H_{110}$
59.	37.031	2.14	Tetrapentacontane	758	$C_{54}H_{110}$
60.	37.51	0.84	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	390	$C_{24}H_{38}O_4$
61.	38.215	1.28	Tetrapentacontane	758	$C_{54}H_{110}$
62.	38.332	1.01	1-Methyl-1h-Imidazole-4,5-Dicarboxamide #	168	$C_6H_8N_4O_2$
63.	38.44	0.72	Silikonfett	9999	-
64.	39.141	0.79	Stigmasta-5,22-Dien-3-Ol	412	$C_{29}H_{48}O$
65.	39.448	1.37	Tetrapentacontane	758	$C_{54}H_{110}$

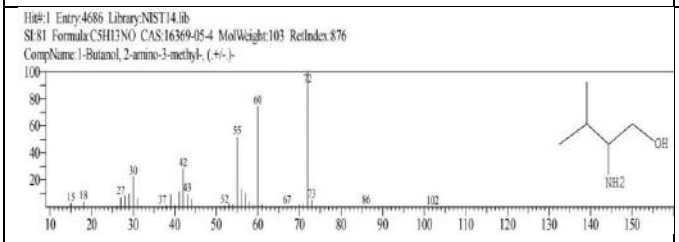
Table 3: The structure of some identified compounds



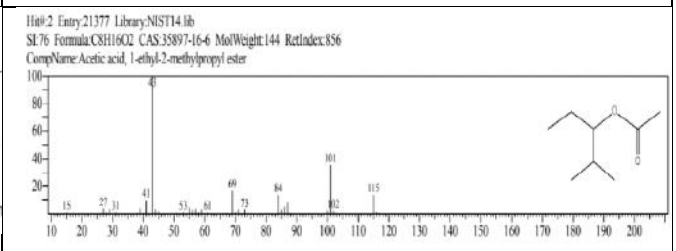
Peak 1: Propane, 1,1-Diethoxy-2-Methyl-



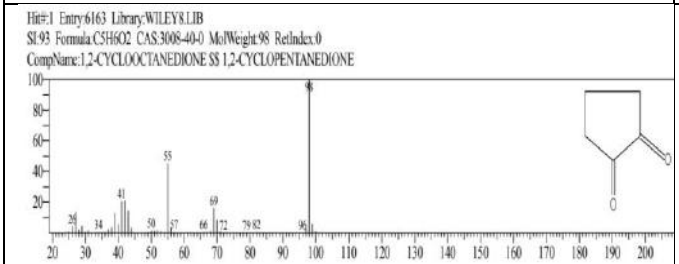
Peak 2: 1-Butanol, 3-Methyl-, Acetate



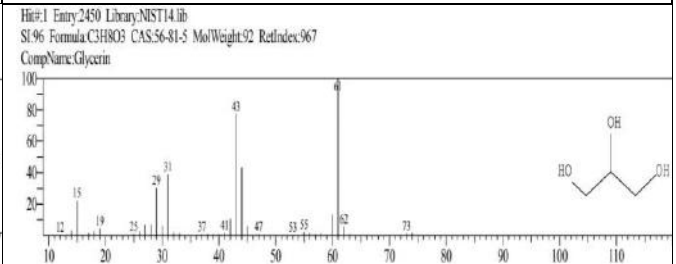
Peak 3:1-Butanol, 2-Amino-3-Methyl-



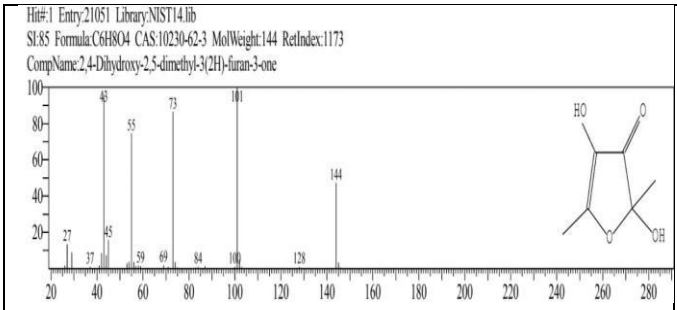
Peak 4:Acetic Acid, 1-Ethyl-2-Methylpropyl Ester



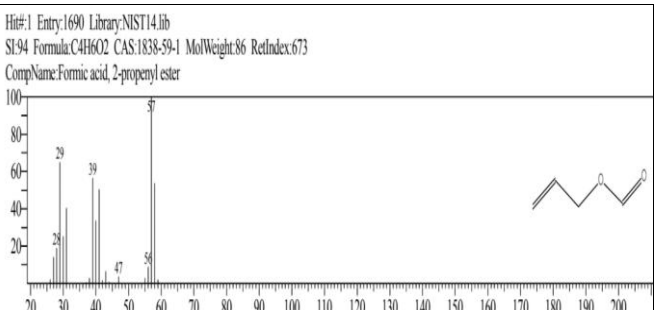
Peak 5:1,2-Cyclooctanedione



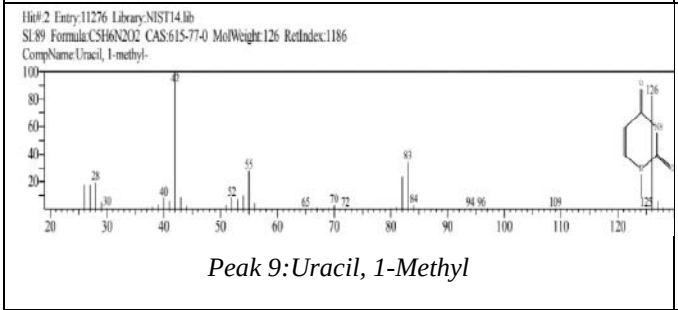
Peak 6:Glycerin



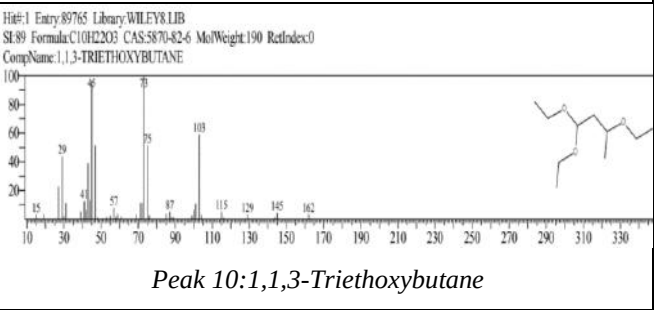
Peak 7:2,4-Dihydroxy-2,5-Dimethyl-3(2h)-Furan-3-One



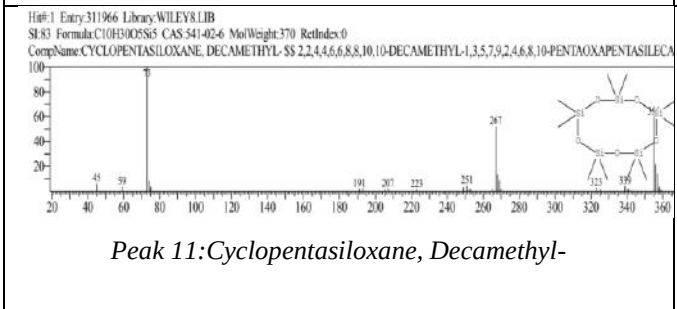
Peak 8:Formic Acid, 2-Propenyl Ester



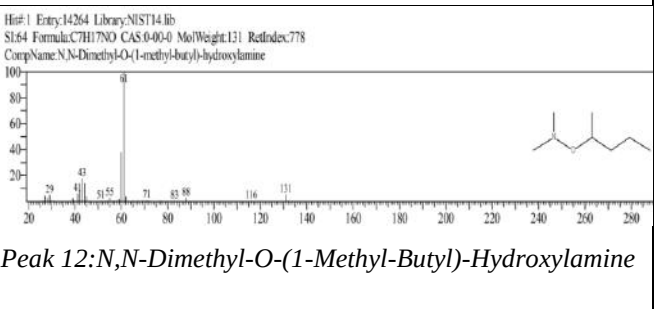
Peak 9:Uracil, 1-Methyl



Peak 10:1,1,3-Triethoxybutane



Peak 11:Cyclopentasiloxane, Decamethyl-



Peak 12:N,N-Dimethyl-O-(1-Methyl-Butyl)-Hydroxylamine

IV. CONCLUSIONS

In conclusion, this investigation offers significant insights into the phytochemical composition of *Anacyclus pyrethrum* (L.) Lag root extract, corroborating its traditional applications in various pharmacological contexts. Fourier-transform infrared spectroscopy (FTIR) analysis revealed the presence of essential functional groups, including hydroxyl groups, alcohols, carboxylic acids, and aromatic compounds, indicating the presence of phenols, flavonoids, tannins, and saponins. Gas chromatography-mass spectrometry (GC-MS) analysis identified 60 bioactive compounds, with 4-Decadienamide, N-Isobutyl-, (E, E)-, and (2e,4e)-N-Isobutyldodeca-2,4-Dienamide as the predominant constituents. These findings are consistent with those of previous studies on various medicinal plants, which demonstrated the presence of similar functional groups and bioactive compounds. The identification of these phytochemicals provides a scientific foundation for the traditional use of *A. pyrethrum* in the treatment of various ailments and supports its potential for pharmaceutical development. Additional research is necessary to elucidate the specific pharmacological activities of the identified compounds and their mechanisms of action. The isolation and individual testing of these bioactive constituents could potentially lead to the discovery of novel therapeutic agents. Furthermore, in vivo studies and clinical trials are required to fully understand the efficacy and safety of *A. pyrethrum* extracts in medical applications. This study contributes to the expanding body of knowledge on medicinal plants and underscores the importance of continued ethnopharmacological research. The results highlight the potential of *A. pyrethrum* as a valuable resource for phytopharmaceutical development and emphasize the need for the conservation and sustainable utilization of medicinal plants.

ACKNOWLEDGEMENTS

All the authors wish to acknowledge to Dr. D. Saravanan and Mrs. Kavitha, National College Instrumental Facility (NCIF), National College (Autonomous), Tiruchirappalli for their support and guidance during the FT-IR analysis.

5.1 Author contribution

All the authors contributed in the design of the study. All the authors contributed in the collection and fractionation of the plant extract. All the authors contributed in FT-IR evaluation of the plant sample, and in the interpretation of the results. All the authors contributed in preparing the manuscript. All the authors read and approved the manuscript

REFERENCES

[1] Nasim, N., Sandeep, I. S., & Mohanty, S. (2022). Plant-derived natural products for drug discovery: current approaches and prospects. *The Nucleus*, 65(3), 399-411. DOI: 10.1007/s13237-022-00405-3

[2] Nostro, A., Germanò, M. P., D'Angelo, V., Marino, A., & Cannatelli, M. A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters in applied microbiology*, 30(5), 379-384. DOI:10.1046/j.1472-765x.2000.00731.x

[3] Kumar, A., P, N., Kumar, M., Jose, A., Tomer, V., Oz, E., & Oz, F. (2023). Major phytochemicals: recent advances in health benefits and extraction method. *Molecules*, *28*(2), 887. DOI:10.3390/molecules28020887

[4] Srivastava, A., Rengaraju, M., Srivastava, S., Narayanan, V., Gupta, V., Upadhayay, R., ... & AarthiVelmurugan. (2021). Efficacy of two siddha polyherbal decoctions, Nilavembu Kudineer and Kaba Sura Kudineer, along with standard allopathy treatment in the management of mild to moderate symptomatic COVID-19 patients—a double- blind, placebo-controlled, clinical trial. *Trials*, 22(1), 570 . DOI:10.1186/s13063-021-05478-0.

[5] Zaidi, S. M. A., Pathan, S. A., Singh, S., Jamil, S., Ahmad, F. J., & Khar, R. K. (2013). Anticonvulsant, anxiolytic and neurotoxicity profile of Aqarqarha *(Anacyclus pyrethrum)* DC (Compositae) root ethanolic extract. *Pharmacology & Pharmacy*, 4(07), 535. DOI:10.4236/pp.2013.47077

[6] Haghmorad, D., Mahmoudi, M. B., Haghighi, P., Alidadiani, P., Shahvazian, E., Tavasolian, P., ... & Mahmoudi, M. (2019). Improvement of fertility parameters with *Tribulus Terrestris* and *Anacyclus Pyrethrum* treatment in male rats. *International brazilian journal of urology*, 45(5), 1043-1054. DOI:10.1590/S1677-5538.IBJU.2018.0843.

[7] Bendjeddou, D., Lalaoui, K., & Satta, D. (2003). Immunostimulating activity of the hot water-soluble polysaccharide extracts of *Anacyclus pyrethrum*, *Alpinia galanga* and *Citrullus colocynthis*. *Journal of ethnopharmacology*, 88(2-3), 155-160. DOI:10.1016/S0378-8741(03)00226-5.

[8] Sharma, V., Thakur, M., Chauhan, N. S., & Dixit, V. K. (2010). Immunomodulatory activity of petroleum ether extract of *Anacyclus pyrethrum*. *Pharmaceutical biology*, 48(11), 1247-1254. DOI:10.3109/13880201003730642.

[9] Elazzouzi, H., Fadili, K., Cherrat, A., Amalich, S., Zekri, N., Zerkani, H., ... & Zair, T. (2022). Phytochemistry, biological and pharmacological activities of the *Anacyclus pyrethrum* (L.) lag: a systematic review. *Plants*, 11(19), 2578. DOI:10.3390/plants11192578

[10] Baslam, A., Aitbaba, A., Aboufatima, R., Agouram, F., Boussaa, S., Chait, A., & Baslam, M. (2023). Phytochemistry, Antioxidant Potential, and Antibacterial Activities of *Anacyclus pyrethrum:* Promising Bioactive Compounds. *Horticulturae*, 9(11), 1196. DOI: 10.3390/horticulturae9111196

[11] Amine, D., Mohamed, B., Jamal, I., & Laila, N. (2017). Antibacterial activity of aqueous extracts of *Anacyclus pyrethrum* (L) Link and *Corrigiola telephiifolia* Pourr. from the middle atlas region-Morocco. *Euroupian Scientific Journal ESJ*, 13, 116. 30;13:116. Doi: 10.19044/esj.2017.v13n33p116

[12] Muralikrishnan, K., Asokan, S., Priya, P. G., Ahmed, K. S. Z., & Ayyappadasan, G. (2017). Comparative evaluation of the local anesthetic activity of root extract of *Anacyclus pyrethrum* and its interaction at the site of injection in guinea pigs. *Anesthesia Essays and Researches*, 11(2), 444-448. DOI: 10.4103/0259-1162.194568

[13] Manouze, H., Bouchatta, O., Gadhi, A. C., Bennis, M., Sokar, Z., & Ba-M'hamed, S. (2017). Anti-inflammatory, antinociceptive, and antioxidant activities of methanol and aqueous extracts of *Anacyclus pyrethrum* roots. *Frontiers in pharmacology*, 8, 598. DOI: 10.3389/fphar.2017.00598

[14] Selles, C., Dib, M. E. A., Djabou, N., Beddou, F., Muselli, A., Tabti, B., ... & Hammouti, B. (2013). Antimicrobial activity and evolution of the composition of essential oil from Algerian *Anacyclus pyrethrum* L. through the vegetative cycle. *Natural product research*, 27(23), 2231-2234. DOI:10.1080/14786419.2013.811409

[15] Manouze, H., Bouchatta, O., Bennis, M., Sokar, Z., & Ba-M'hamed, S. (2019). Anticonvulsive and neuroprotective effects of aqueous and methanolic extracts of *Anacyclus pyrethrum* root in kainic acid-induced-status epilepticus in mice. *Epilepsy Research*, 158, 106225. DOI:10.1016/j.eplepsyres.2019.106225

[16] Yousef, B. A., Awad, Z., Adam, S., Abdelgadir, S., & Mergani, A. (2021). Assessment of Anticonvulsant activities of Petroleum Ether Extract of *Anacyclus pyrethrum* roots on experimental rats. *Pharmaceutical and Biomedical Research*, 7(1), 47-54. DOI:10.18502/pbr.v7i1.7356

[17] Pahuja, M., Mehla, J., Reeta, K. H., Tripathi, M., & Gupta, Y. K. (2013). Effect of *Anacyclus pyrethrum* on pentylenetetrazole-induced kindling, spatial memory, oxidative stress and rho-kinase II expression in mice. *Neurochemical research*, 38, 547-556. DOI: 10.1007/s11064-012-0947-2

[18] Nadira, H., Benslama, O., Benbott, A., Mosbah, C., Daoui, H., Chbilli, L., & Arhab, R. (2022). Study of the antioxidant and antidiabetic effect of ethanol and aqueous extracts of *Anacyclus pyrethrum* L. roots. *South Asian Journal of Experimental Biology*, 12(5), 735-744. DOI: 10.38150/sajeb.12(5).p735-744

[19] Sharma, V., Thakur, M., Chauhan, N. S., & Dixit, V. K. (2009). Evaluation of the anabolic, aphrodisiac and reproductive activity of *Anacyclus pyrethrum* DC in male

rats. *Scientia pharmaceutica*, 77(1), 97-110. DOI: 10.3797/scipharm.0808-14

[20] Zaidi, S. M. A., Pathan, S. A., Jain, G. K., Ahmad, F. J., Jamil, S., Singh, S., & Khar, R. K. (2009). Anticonvulsant and neuropharmacological studies of *Anacyclus pyrethrum* root extract. *Neuroscience Research*, (65), S250. DOI:10.1016/j.neures.2009.09.1423

[21] Sumathi, R., & Anuradha, R. (2016). FT-IR Spectroscopic Studies on Flowers of *Allamanda neriifolia* Hook. *International Journal of Current Microbiolgy and Applied Sciences*, 56, 287-291. DOI: 10.20546/ijcmas.2016.506.032

[22] Sparkman, O. D. (2005). Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy Robert P. Adams: Allured Carol Stream, IL 60188, USA ISBN 0-931710-85-5 2001, Book 175,469pp; BookandDisk, 625 DOI: 10.1016/j.jasms.2005.07.008.

[23] Baker, M. J., Gazi, E., Brown, M. D., Shanks, J. H., Gardner, P., & Clarke, N. W. (2008). FTIR-based spectroscopic analysis in the identification of clinically aggressive prostate cancer. *British journal of cancer*, 99(11), 1859-1866. DOI: 10.1038/sj.bjc.6604753

[24] Lakshmi, C. H. N. D., Raju, B. D. P., Madhavi, T., & Sushma, N. J. (2014). Identification of bioactive compounds by FTIR analysis and in vitro antioxidant activity of *Clitoria ternatea* leaf and flower extracts. *Indo American Journal of Pharmacy and Research*, *4*(9), 3894-3903. DOI: 10.1044/1980-iajpr.14941

[25] Ubhale, A. V., Madavi, S. S., Wagh, S. D., Lawane, V. P., Chaudhary, P. H., Burange, P. J., & Ruikar, D. B. Qualitative and Quantitative Phytochemical Analysis Of Wedelia Trilobata Linn. 2(6), 428-441. DOI:10.5281/zenodo.11520151

[26] Saragih, W. S., Purba, E. D. I. S. O. N., & Basyuni, M. (2021). The Fourier transform infrared spectroscopy from *Diplazium esculentum* and *Rivina humilis* analysis to reveals the existence of necessary components in oil palm plantations of *Ganoderma boninense* control. *Biodiversitas: Journal of Biological Diversity*, 22(9). DOI: 10.13057/biodiv/d220902

[27] Jesse, A. O. (2020). Fourier transform infrared spectroscopy analysis of *Allium sativum* L. and *Nymphaea lotus* L. *Asian Journal of Applied Chemistry Research*, 6(2), 7-24 DOI: 10.9734/AJACR/2020/v6i230154.

[28] Stan, H. J. (2005). GC-MS. I: Basic principles and technical aspects of GC-MS for pesticide residue analysis. In *Comprehensive Analytical Chemistry* (Vol. 43, pp. 269-337). Elsevier. DOI: 10.1016/S0166-526X(05)80026-1.

[29] Prejeena, V., Suresh, S. N., & Varsha, V. (2017). Preliminary Phytochemical Screening And Gas Chromatography Mass Spectrometry Analysis Of *Costus Pictus* D. Don, *World Journal of Pharmaceutical Research*, Volume 6, Issue 10, 865-873. DOI: 10.20959/wjpr201710-9385

[30] Mugendhiran, S., & Sheeja, B. D. (2021). Evaluation of phytoconstituents of *Erigeron canadensis* L. by FTIR and GC-MS analysis. *International Journal of Pharmaceutical Sciences and Research*, 12(5), 2823-2834. DOI:10.13040/IJPSR.0975-8232.12(5).2823-34

[31] Alkooranee, J. T., Al-khshemawee, H. H., Al-badri, M. A. K., Al-srai, M. S., & Daweri, H. H. (2020). Antifungal activity and GC-MS detection of leaves and roots parts of *Chenopodium album* extract against some phytopathogenic fungi. *Indian Journal of Agricultural Research*, 54(1), 117-121 .DOI:10.18805/IJARe.A-433.

[32] Amako, N. F., Mgbemena, M. A. N., & Odo, S. P. (2023). GC-MS profile and antimicrobial activities of extracts from root of *Senna occidentalis* Linn. *Ovidius University Annals of Chemistry*, 34(2), 63-71.DOI: 10.2478/auoc- 2023-0009

[33] Aguree, S., Abagale, S. A., & Sackey, I. (2024). Chemical Characterization of Crude Aqueous Extracts of *Kigelia Africana* Leaves and *Cassia Sieberiena* Root as Elephants' Feeds Using GC-MS and FTIR, *Advanced Journal of Chemistry: Section B*, 6, 166-181 DOI: 10.48309/AJCB.2024.437414.1228.

[34] Oli, S., Kumar Chauhan, H., Kumar Bisht, A., Agnihotri, S., & Dobhal, P. (2023). Bioactive compound, polyphenol content, and antioxidant activity of *Asparagus racemosus* Linn. root extract. *Natural Product Research*, 1-6. DOI: 10.1080/14786419.2023.2272029

[35] Chirumamilla, Pavani, Sunitha Bai Dharavath, and Shasthree Taduri. "GC–MS profiling and antibacterial activity of *Solanum khasianum* leaf and root extracts." *Bulletin of the National Research Centre* 46.1 (2022): 127. DOI: 10.1186/s42269-022-00818-9

[36] Shukla, A., Tyagi, S., Gupta, V., Jain, P., Kanai, T., & Tripathi, R. (2022). FT-IR, GC-MS, and HPLC profiling of the bioactive constituents of ethyl acetate fraction of *Eichhornia crassipes* as a hepatoprotectant. *Letters in Applied NanoBioScience*, 12, 96. DOI: 10.33263/LIANBS124.096

[37] Fathalla, N., Bishr, M., Nasser Singab, A., & Salama, O. (2019). GC-MS and LC-MS Identification of the Phenolic Compounds Present in the ethyl Acetate Fraction Obtained from *Senna tora*, L. Roxb. seeds. *Natural product research*, 33(19), 2878-2881 DOI: 10.1080/14786419.2018.1508138.

[38] Mehdi, M. A. H., Alawi, A. H., Thabet, A. Z. A., Alarabi, F., Omar, G. M. N., & Pradhan, V. (2021). Analysis of bioactive chemical compounds of leaves extracts from *Tamarindus indica* using FT-IR and GC-MS spectroscopy. *Asian Journal of Research in Biochemistry*, 8(1), 22-34. DOI: 10.9734/AJRB/2021/v8i130171

[39] Mohammed, J., Oba, O. A., & Aydinlik, N. P. (2023). Preliminary Phytochemical Screening, Gc-Ms, Ftir Analysis Of Ethanolic Extracts Of *Rosmarinus officinalis*, *Coriandrum sativum* L And *Mentha spicata*. *Hacettepe Journal of Biology and Chemistry*, 51(1), 93-102 DOI: 10.15671/hjbc.1073300.

[40] Harika, M. N. L. C., & Radhika, P. (2019). Phytochemical Analysis of *Ruellia tuberosa* Tuber Ethanolic Extract Using UV-VIS, FTIR and GC-MS Techniques. *International Journal of Pharmacy and Biological Sciences*, 9(1), 889-892. DOI: 10.21276/ijpbs.2019.9.1.113