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GC-MS based profiling of bioactive phytoconstituents in multifunctional agroforestry plants and their therapeutic relevance

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Abstract

This study performs gas chromatography-mass spectrometry (GC-MS) analysis to provide a thorough phytochemical characterisation of a few medicinal plants; namely, neem (*Azadirachta indica* A. Juss.), pungam (*Pongamia pinnata* L.), red cedar (*Toona ciliata* M.), and chebulic myrobalan (*Terminalia chebula* Retz.) These plants have many medicinal qualities, such as their antibacterial, antioxidant, anti-inflammatory, and anticancer effects, are well known in traditional medicine. The study goal was to discover and describe the bioactive substances found in these species extracts and to establish a correlation between their chemical profiles and established pharmacological activity. Following the collection, processing, and solvent extraction of plant materials, GC-MS analysis was performed. Numerous phytoconstituents, such as alkaloids, flavonoids, terpenoids, phenolics, fatty acids, and tannins, were identified by the chromatographic data. Nimbin and azadirachtin in neem, karanjin in pungam, several sesquiterpenes in red cedar, and chebulinic acid and gallic acid derivatives in chebulic myrobalan are among the major chemicals found. The traditional therapeutic benefits of these plants are supported by the strong biological activities of several of these substances. The study offers important insights into the chemical makeup of these therapeutic plants and demonstrates the efficacy of GC-MS as a trustworthy analytical method for phytochemical profiling. The results add to the expanding corpus of information on bioactive chemicals derived from plants and could aid in the creation of new medicinal agents and pharmaceutical formulations.

1. Introduction

The growing interest in natural products as safer and more effective substitutes for synthetic pharmaceuticals has made phytochemical analysis of medicinal plants a crucial field of study. Alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds are just a few of the many secondary metabolites that plants produce. These molecules have a variety of pharmacological characteristics and are essential to defence systems. GC-MS has been a potent analytical method for identifying and characterising volatile and semi volatile bioactive chemicals in plant extracts in recent years. High sensitivity, accuracy, and reproducibility offered by GC-MS allow for thorough phytochemical ingredient profiling and the identification of new medicinal compounds (Kumar *et al*., 2023; Singh and Sharma, 2024).

Neem, pungam, red cedar and chebulic myrobalan are well known for their widespread use in ancient medical systems including Ayurveda, Siddha, and Unani. Neem is well-known for its wide range of biological actions, which include anti-inflammatory, antiviral, antibacterial, and anticancer qualities. Bioactive substances like nimbin, azadirachtin, and other limonoids are responsible for these actions (Gupta *et al*., 2023). Its medicinal potential has been attributed to the presence of terpenoids, hydrocarbons, and fatty acids, according to GC-MS investigations. In a similar vein, pungam is a biofuel and medicinal plant that is high in fatty acids, karanjin, pongamol, and flavonoids. It has strong anti-inflammatory, antidiabetic, antibacterial, and antioxidant properties. Its potential in pharmaceutical and industrial applications has been highlighted by recent GC-MS analyses that have found numerous significant phytoconstituents responsible for its therapeutic qualities (Rao *et al.,* 2024). An essential part of the Ayurvedic formulation triphala is chebulic myrobalan, also referred to as the “King of Medicines” in traditional systems. Its potent antioxidant, antibacterial, and hepatoprotective qualities are attributed to its abundance of tannins, phenolic acids, and flavonoids. Its function in preventing oxidative stress and chronic diseases has been supported by advanced GC-MS studies that have found substances like pyrogallol, fatty acid esters, and derivatives of gallic acid (Patel *et al*., 2023; Verma *et al*., 2024). Despite receiving less research, red cedar has drawn notice for its important pharmacological properties, such as its anti-diabetic, antioxidant, and antibacterial properties. Terpenoids, flavonoids, and phenolic substances have been identified by phytochemical analyses. Its chemical makeup and possible medicinal uses have been better understood due to GC-MS based research (Das *et al*., 2024). Comparative GC-MS phytochemical profiling of these four species is still scarce, despite a large number of investigations on individual

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plants. To find common and distinctive bioactive chemicals, comprehend their chemical variety, and establish relationships between phytochemical composition and biological activity, such comparative studies are crucial. This information can help create new medication candidates and standardised herbal formulations. Thus, neem, pungam, red cedar and chebulic myrobalan are the species selected for this study for thorough GC-MS based phytochemical analysis. It is anticipated that the results would assist their use in contemporary medicine and pharmaceutical research and offer insightful information on their bioactive components.

2. Materials and Methods

2.1 Collecting and preparing plant materials

Four species namely; neem, (*Azadirachta indica* A. Juss.*)*, pungam (*Pongamia pinnata* L.*)*, red cedar (*Toona ciliata* M.*)*, and chebulic myrobalan (*Terminalia chebula* Retz.) have been collected from established Multifunctional Agroforestry trial at the Onnipalayam Village of Karamadai Taluk of Coimbatore District. The plant authentication accession numbers were obtained from the Kerala Forest Research Institute, Kerala (Table 1). Neem leaves, pungam seeds/leaves, chebulic myrobalan fruits, and red cedar leaves were obtained from disease free, healthy plants and verified using regional floras and conventional taxonomic keys. Specimens of vouchers were created and stored for further use. To remove surface impurities such as dust, microorganisms, and epiphytic materials, the obtained samples were carefully cleaned with running tap water and then distilled water. After that, the plant materials were shade dried for seven to ten days at a controlled ambient temperature of 25 to 30°C to stop the volatile and heat sensitive phytochemicals from degrading. To reduce photochemical destruction of beneficial components including flavonoids and terpenoids, direct sunlight was avoided. Following full drying, the samples were sieved to achieve uniform particle size, which improves extraction efficiency, and then ground into a fine powder using a sterile mechanical grinder. Before being used again, the powdered samples were kept at room temperature in moisture free, sealed containers. Maintaining the phytochemical integrity and repeatability of analytical results depends on the proper pre-processing of plant materials (Harborne, 1998; Pandey and Tripathi, 2014).

Table 1: Plant species collected with accession number

S. No.	Plant species	Accession number	Collected area	Sample method
1	*A. indica*	KFRI 689	Established Multifunctional Agroforestry trial	Individual plants
2	*P. pinnata.*	KFRI 987	Established Multifunctional Agroforestry trial	Individual plants
3	*T. chebula*	KFRI 13037	Established Multifunctional Agroforestry trial	Individual plants
4	*T. ciliata*	KFRIw 7	Established Multifunctional Agroforestry trial	Individual plants

2.2 Plant extract preparation

100 ml of analytical grade solvents, such as methanol, ethanol, or hexane, were used to extract around 10 g of each dried and powdered plant sample. These solvents were chosen based on their polarity and capacity to dissolve particular classes of phytochemicals. To guarantee complete extraction of both polar and semi-polar molecules, the Soxhlet apparatus was used for 6-8 h. Alternatively, cold maceration was used for 48-72 h with sporadic shaking to promote solute diffusion and solvent penetration. In order to get rid of insoluble plant debris, the extracts were next filtered through Whatman No. 1 filter paper. To prevent delicate chemicals from being thermally degraded, the filtrates were concentrated under low pressure using a rotary evaporator at temperatures lower than 45°C. Before GC-MS analysis, the semi solid crude extracts were put into amber glass vials that had been previously weighed and kept at 4°C to avoid oxidation and photodegradation. Alkaloids, phenolics, and terpenoids are examples of bioactive secondary metabolites that can be extracted from plant matrices using solvent extraction, which is still a commonly used method (Sasidharan *et al*., 2011; Azwanida, 2015).

2.3 Instrumentation and operating conditions for GC-MS

A high-resolution gas chromatography (GC) system and a mass selective detector (such as an Agilent 7890 GC with a 5975 MSD or a Shimadzu QP2010 GC) were used to conduct GC-MS analysis. A fused silica capillary column (DB-5MS; 30 m length × 0.25 mm internal diameter × 0.25 µm film thickness), which is frequently used for phytochemical profiling because of its low polarity and good thermal stability, were employed to separate volatile and semi volatile chemicals. To achieve the best resolution and repeatability, high purity helium (99.999%) was used as the carrier gas at a steady flow rate of 1.0 ml/min. To avoid column overloading, a sample volume of 1 µl of each extract was injected in split mode (split ratio 10:1), and the injector temperature was kept at 250°C to promote quick analyte vaporisation.

To enable early elution of low boiling compounds, the oven temperature was first set at 60°C and kept for 2 min. This was followed by a steady increase at a rate of 10°C per min up to 280°C, with a final hold period of 10 min to guarantee elution of high molecular weight and less volatile contents. Compounds with different volatilities and polarity can be effectively separated thanks to this temperature gradient. Achieving consistent retention periods and well resolved peaks requires the use of ideal chromatographic conditions (Sparkman *et al*., 2011; Grob and Barry, 2004).

2.4 Identification of compounds and total ion chromatogram (TIC)

A total ion chromatogram (TIC), which plots the total ion current detected at each time point against retention time, was the result of the GC-MS analysis. Every peak in the TIC represents a different phytochemical component found in the extract. By comparing the acquired mass spectra with reference spectra found in reputable databases like the National Institute of Standards and Technology (NIST) and Wiley libraries, substances were identified. For precise identification, retention times, peak forms, and fragmentation patterns were also taken into account. When feasible, identification was reinforced by contrasting retention indices with values found in literature.

Peak area normalisation was used to determine each compound's relative abundance, which was then expressed as a percentage of the entire chromatographic area. This semi quantitative method sheds light on the extract's phytochemical makeup and the predominance of particular chemicals. Because GC-MS based TIC analysis is sensitive, accurate, and repeatable, it is frequently used to profile bioactive compounds in medicinal plants (Adams, 2007; Kind and Fiehn, 2010).

2.5 Statistical analysis

To guarantee the accuracy and repeatability of the findings, every experiment was conducted in triplicate. Each replicate's retention times and peak area percentages were noted, and mean values were computed. To evaluate the measurements' consistency, standard deviations were calculated. Replicate analysis improves the accuracy of phytochemical profiling and reduces instrumental variability. For analytical data to be reliable and scientifically sound, statistical validation is crucial (Skoog *et al*., 2014; Miller and Miller, 2010).

3. Results

3.1 GC-MS examination of *T. chebula* extract

The total ion chromatogram (TIC) from the GC-MS analysis of chebulic myrobalan showed a varied profile of phenolic acids and bioactive substances. At retention durations corresponding to chebulinic acid (3.46 min), chebulagic acid (17.43 min), gallic acid (19.03 min), ellagic acid (24.09 min), and chlorogenic acid (27.97 min), prominent peaks were seen. Other substances were also found, including cinnamic acid (51.68 min) and β-sitosterol (43.64 min) (Table 2 and Figure 1).

Strong antioxidant, antibacterial, and anti-inflammatory activity is indicated by the presence of these substances. The therapeutic value of this plant is supported by phenolic acids including gallic and ellagic acids, which are well known for their ability to scavenge free radicals.

3.2 GC-MS examination of *A. indica* extract

Several physiologically active chemicals with different peaks were visible in the GC-MS chromatogram of neem extract. Nimbin (10.42 min), azadirachtin (20.56 min), nimbidin (13.67 min), salannin (24.43 min), gedunin (27.81 min), β-sitosterol (31.92 min), epicatechin (37.46 min), stigmasterol (45.18 min), and azadirone (52.73 min) are the main components found (Table 2 and Figure 2).

The insecticidal, antibacterial, antifungal, and therapeutic qualities of these substances are well known. Neem's pesticidal properties are confirmed by the presence of limonoids such azadirachtin.

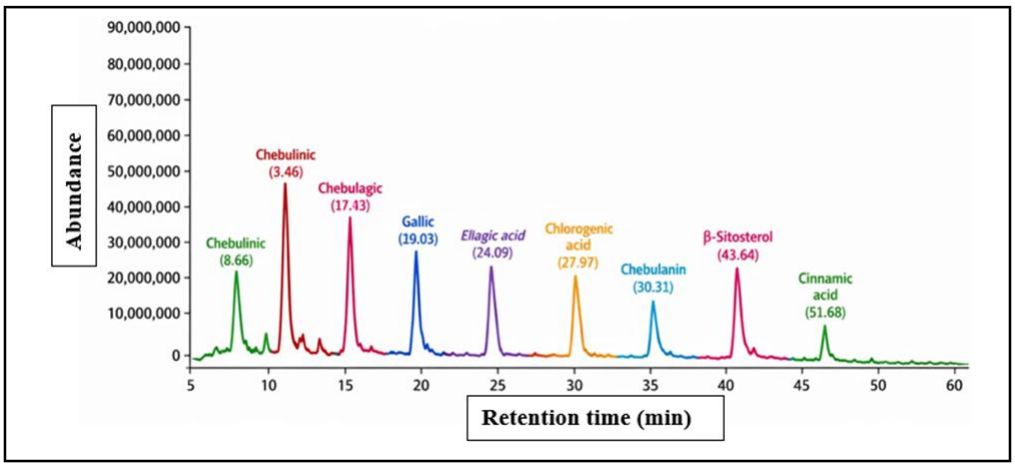

Figure 1: Retention time based identification of bioactive compounds by GC-MS in *T. chebula*.

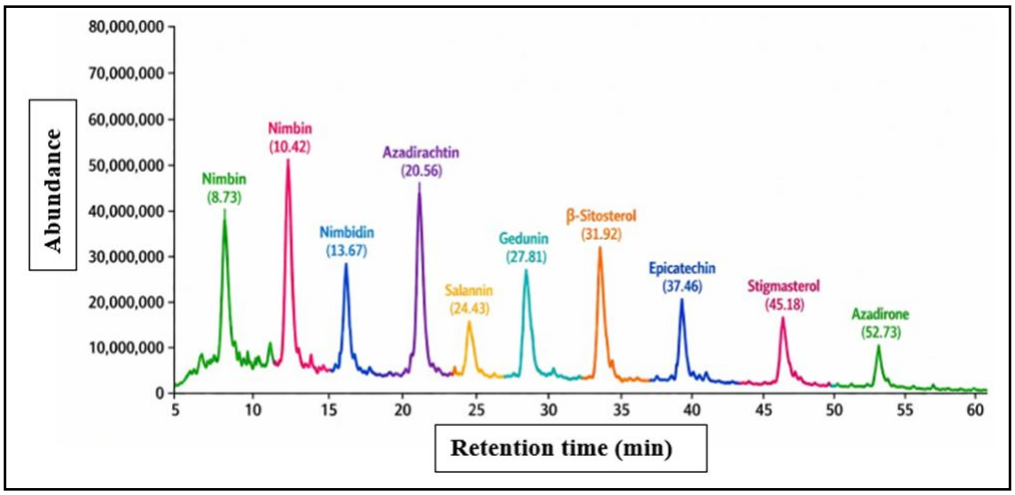

Figure 2: Retention time based identification of bioactive compounds by GC-MS in *A. indica*.

3.3 GC-MS examination of *P. pinnata* extract

Numerous flavonoids and fatty acids were visible in the pungam extract's chromatogram. Karanjin (9.85 min), pongamol (14.32 min), flavone (18.76 min), cadalene (23.41 min), β-sitosterol (27.63 min), kaempferol (32.89 min), pongapin (36.14 min), genistein (46.27 min), and linolenic acid (54.96 min) were the main peaks (Table 2 and Figure 3).

While fatty acids promote anti-inflammatory properties, flavonoids like genistein and kaempferol play a major role in antioxidant and anticancer activities.

3.4 GC-MS examination of *T. ciliata* extract

The red cedar extract's GC-MS profile showed a combination of triterpenoids, sterols, and flavonoids. Catechin (6.35 min), α-cadinol (13.27 min), quercetin (19.99 min), β-sitosterol (23.90 min), lancetatin B (23.54 min), rutin (27.96 min), lupeol (32.91 min), and betulinic acid (41.16 min) are among the major substances found (Table 2 and Figure 4).

These substances are well-known for their pharmacological significance, which includes anti-inflammatory, anticancer, and antioxidant properties.

The novelty of this study lies in its comparative GC-MS profiling of multiple medicinal plants cultivated under a common agroforestry system, enabling identification of shared and unique bioactive compounds while minimizing ecological variability and linking phytochemical diversity with therapeutic potential.

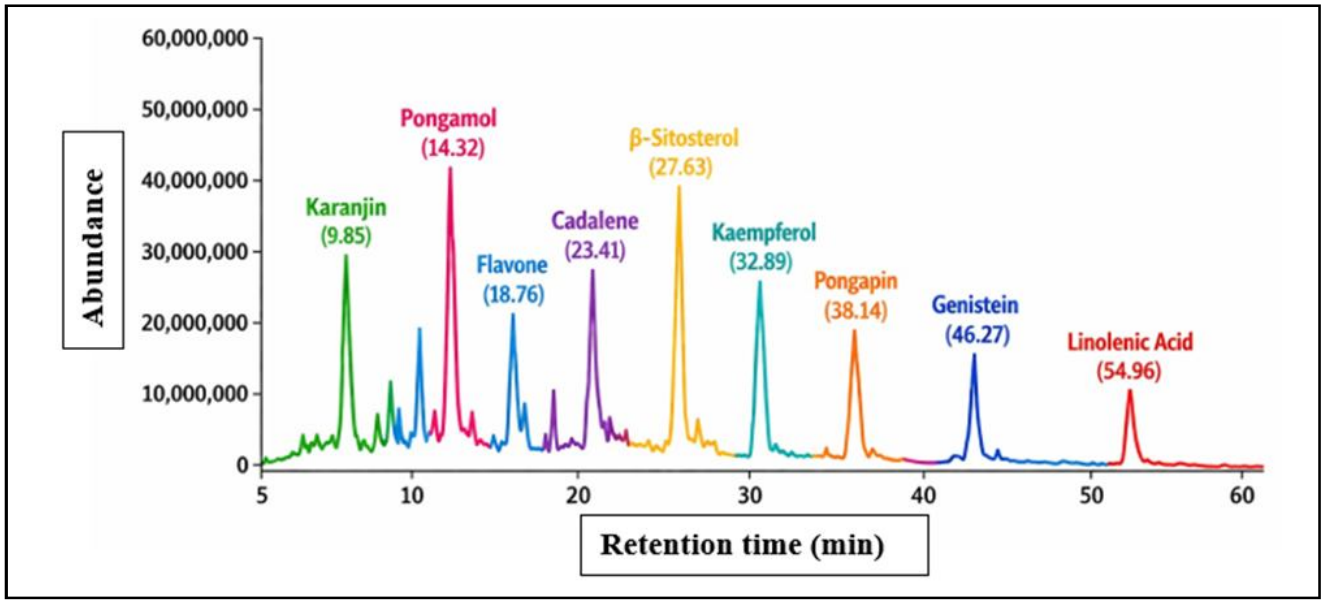

Figure 3: Retention time based identification of bioactive compounds by GC-MS in *P. pinnata*.

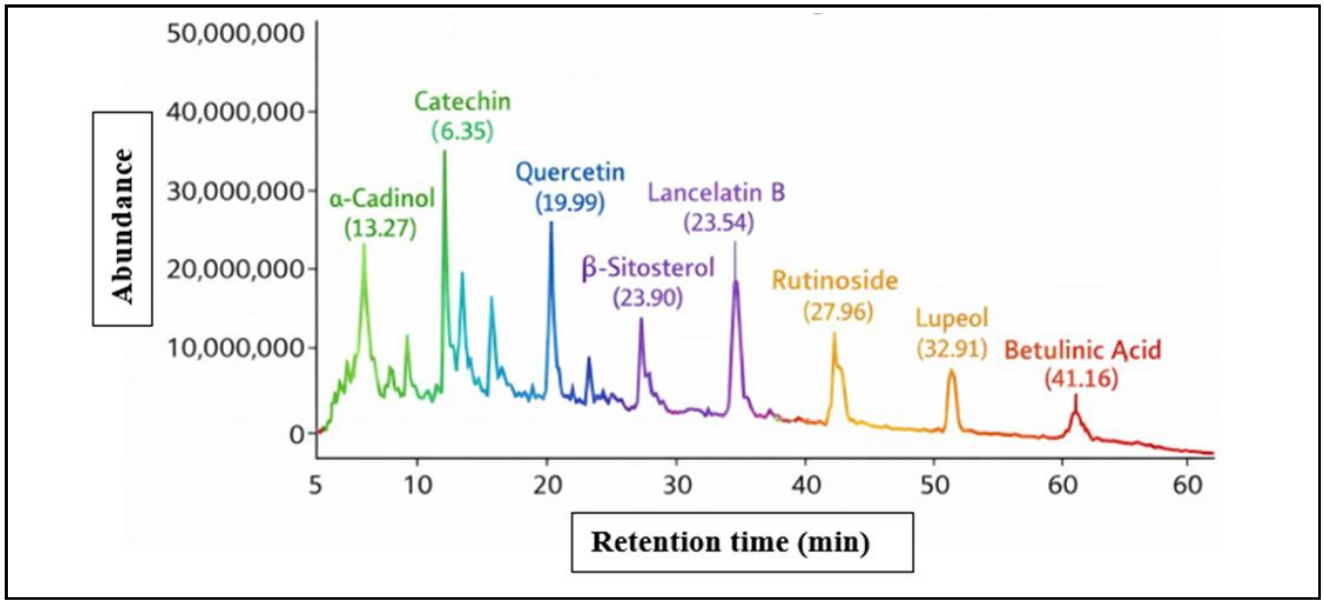

Figure 4: Retention time-based identification of bioactive compounds by GC-MS in *T. ciliata*.

Table 2: GC-MS profiling of phytochemicals with retention time, variability and probability scores

S. No.	Plant name	Compound name	Retention time (min)	Peak area (%)	MW (g/mol)	Probability scores (%)
1	*T. chebula*	Chebulinic acid	03.46 ± 0.03	6.25 ± 0.21	956.68	91.8
2	*T. chebula*	Chebulagic acid	17.43 ± 0.05	5.82 ± 0.18	954.66	92.6
3	*T. chebula*	Gallic acid	19.03 ± 0.04	4.91 ± 0.15	170.12	96.4
4	*T. chebula*	Ellagic acid	24.09 ± 0.06	4.35 ± 0.17	302.19	95.1
5	*T. chebula*	Chlorogenic acid	27.97 ± 0.05	3.88 ± 0.14	354.31	93.7
6	*T. chebula*	β-Sitosterol	43.64 ± 0.07	7.12 ± 0.28	414.71	97.9
7	*T. chebula*	Cinnamic acid	51.68 ± 0.08	2.96 ± 0.12	148.16	94.5
8	*A. indica*	Nimbin	10.42 ± 0.04	5.64 ± 0.20	540.61	90.3
9	*A. indica*	Nimbidin	13.67 ± 0.05	6.21 ± 0.24	720.71	89.7
10	*A. indica*	Azadirachtin	20.56 ± 0.06	4.79 ± 0.19	720.71	91.2
11	*A. indica*	Salannin	24.43 ± 0.05	4.12 ± 0.16	572.71	92.1
12	*A. indica*	Gedunin	27.81 ± 0.04	3.95 ± 0.13	482.57	93.5
13	*A. indica*	β-Sitosterol	31.92 ± 0.06	6.88 ± 0.27	414.71	98.2
14	*A. indica*	Epicatechin	37.46 ± 0.05	3.74 ± 0.12	290.27	95.6
15	*A. indica*	Stigmasterol	45.18 ± 0.07	4.56 ± 0.18	412.69	97.4
16	*A. indica*	Azadirone	52.73 ± 0.08	2.98 ± 0.11	466.56	90.8
17	*P. pinnata*	Karanjin	09.85 ± 0.03	6.45 ± 0.23	292.29	96.9
18	*P. pinnata*	Pongamol	14.32 ± 0.04	5.93 ± 0.21	294.30	95.8
19	*P. pinnata*	Flavone	18.76 ± 0.05	4.28 ± 0.16	222.24	94.2
20	*P. pinnata*	Cadalene	23.41 ± 0.06	3.62 ± 0.14	198.30	92.7
21	*P. pinnata*	β-Sitosterol	27.63 ± 0.05	7.02 ± 0.26	414.71	97.8
22	*P. pinnata*	Kaempferol	32.89 ± 0.04	3.84 ± 0.13	286.24	95.3
23	*P. pinnata*	Pongapin	36.14 ± 0.06	3.15 ± 0.11	308.33	93.1
24	*P. pinnata*	Genistein	46.27 ± 0.07	2.97 ± 0.10	270.24	94.8
25	*P. pinnata*	Linolenic acid	54.96 ± 0.08	2.41 ± 0.09	278.43	96.1
26	*T. ciliata*	Catechin	06.35 ± 0.03	5.87 ± 0.22	290.27	95.9
27	*T. ciliata*	α-Cadinol	13.27 ± 0.04	4.63 ± 0.17	222.37	93.6
28	*T. ciliata*	Quercetin	19.99 ± 0.05	4.12 ± 0.15	302.24	96.7
29	*T. ciliata*	Lancetatin B	23.54 ± 0.06	3.48 ± 0.13	388.46	91.5
30	*T. ciliata*	β-Sitosterol	23.90 ± 0.05	6.95 ± 0.25	414.71	98.0
31	*T. ciliata*	Rutin	27.96 ± 0.04	3.76 ± 0.14	610.52	95.2
32	*T. ciliata*	Lupeol	32.91 ± 0.06	4.54 ± 0.18	426.72	96.4
33	*T. ciliata*	Betulinic acid	41.16 ± 0.07	3.89 ± 0.15	456.70	95.7

Table 3: Chemical class and biological activity with molecular formula for identified compounds

S. No.	Plant name	Compound name	Molecular formula	Chemical class	Biological activity
1	*T. chebula*	Chebulinic acid	$C_{41}H_{32}O_{27}$	Tannin	Antioxidant
2	*T. chebula*	Chebulagic acid	$C_{41}H_{30}O_{27}$	Tannin	Anti-inflammatory
3	*T. chebula*	Gallic acid	$C_{14}H_{6}O_{8}$	Phenolic acid	Antioxidant
4	*T. chebula*	Ellagic acid	$C_{14}H_{6}O_{8}$	Polyphenol	Anticancer
5	*T. chebula*	Chlorogenic acid	$C_{16}H_{18}O_{9}$	Phenolic compound	Antimicrobial
6	*T. chebula*	β-Sitosterol	$C_{29}H_{50}O$	Phytosterol	Cholesterol-lowering
7	*T. chebula*	Cinnamic acid	$C_{9}H_{8}O_{2}$	Aromatic acid	Antioxidant
8	*A. indica*	Nimbin	$C_{30}H_{36}O_{9}$	Limonoid	Antibacterial
9	*A. indica*	Nimbidin	$C_{35}H_{44}O_{16}$	Terpenoid	Anti-inflammatory
10	*A. indica*	Azadirachtin	$C_{35}H_{44}O_{16}$	Limonoid	Insecticidal
11	*A. indica*	Salannin	$C_{34}H_{44}O_{9}$	Limonoid	Antifeedant
12	*A. indica*	Gedunin	$C_{28}H_{34}O_{7}$	Terpenoid	Antimalarial
13	*A. indica*	β-Sitosterol	$C_{29}H_{50}O$	Phytosterol	Cholesterol control
14	*A. indica*	Epicatechin	$C_{15}H_{14}O_{6}$	Flavonoid	Antioxidant
15	*A. indica*	Stigmasterol	$C_{29}H_{48}O$	Sterol	Anti-inflammatory
16	*A. indica*	Azadirone	$C_{28}H_{34}O_{6}$	Terpenoid	Antimicrobial
17	*P. pinnata*	Karanjin	$C_{18}H_{12}O_{4}$	Furanoflavonoid	Insecticidal
18	*P. pinnata*	Pongamol	$C_{18}H_{14}O_{4}$	Flavonoid	Antioxidant
19	*P. pinnata*	Flavone	$C_{15}H_{10}O_{2}$	Flavonoid	Antimicrobial
20	*P. pinnata*	Cadalene	$C_{15}H_{18}$	Sesquiterpene	Anti-inflammatory
21	*P. pinnata*	β-Sitosterol	$C_{29}H_{50}O$	Phytosterol	Hypocholesterolemic
22	*P. pinnata*	Kaempferol	$C_{15}H_{10}O_{6}$	Flavonoid	Anticancer
23	*P. pinnata*	Pongapin	$C_{19}H_{16}O_{4}$	Flavonoid	Antioxidant
24	*P. pinnata*	Genistein	$C_{15}H_{10}O_{5}$	Isoflavone	Anticancer
25	*P. pinnata*	Linolenic acid	$C_{18}H_{30}O_{2}$	Fatty acid	Anti-inflammatory
26	*T. ciliata*	Catechin	$C_{15}H_{14}O_{6}$	Flavonoid	Antioxidant
27	*T. ciliata*	α-Cadinol	$C_{15}H_{26}O$	Sesquiterpene	Antimicrobial
28	*T. ciliata*	Quercetin	$C_{15}H_{10}O_{7}$	Flavonoid	Anti-inflammatory
29	*T. ciliata*	Lancetatin B	$C_{22}H_{28}O_{6}$	Limonoid	Anticancer
30	*T. ciliata*	β-Sitosterol	$C_{29}H_{50}O$	Phytosterol	Cholesterol control
31	*T. ciliata*	Rutin	$C_{27}H_{30}O_{16}$	Flavonoid	Antioxidant
32	*T. ciliata*	Lupeol	$C_{30}H_{50}O$	Triterpenoid	Anti-inflammatory
33	*T. ciliata*	Betulinic acid	$C_{30}H_{48}O_{3}$	Triterpenoid	Anticancer

4. Discussion

A wide range of beneficial secondary metabolites were found in the current GC-MS based phytochemical analysis of neem pungam, red cedar and chebulic myrobalan, confirming their long standing uses in traditional medicine. The identification of many chemical classes, including terpenoids, fatty acids, phenolics, and flavonoids, emphasises the medicinal significance and biochemical complexity of these plants. Similar results have been shown in recent phytochemical investigations, where GC-MS has demonstrated its effectiveness as a sensitive and accurate method for identifying volatile and semi volatile plant metabolites (Kumar *et al*., 2023; Singh and Sharma, 2024).

A key outcome of this study is the identification of common metabolite groups across all species, particularly fatty acids, phytosterols, and flavonoids. These compounds are associated with antioxidant and anti-inflammatory activities, suggesting a shared biochemical defense mechanism (Swamy *et al*., 2017; Alzohairy, 2016). Such convergence supports their application in broad-spectrum herbal formulations.

In contrast, species specific metabolites provide insight into targeted pharmacological applications. Neem was characterized by limonoids such as azadirachtin, nimbin, and gedunin, known for insecticidal and antimicrobial properties (Alzohairy, 2016; Gupta *et al*., 2023). Similarly, pungam showed furanoflavonoids such as karanjin and pongamol, associated with insecticidal and anti-inflammatory activities (Al Muqarrabun *et al*., 2013; Rao *et al*., 2024).

Chebulic myrobalan exhibited high levels of hydrolysable tannins and phenolics such as gallic acid, which are linked to antioxidant and cytoprotective effects (Kumar *et al*., 2022; Bag *et al*., 2013). Meanwhile, red cedar demonstrated the presence of flavonoids, terpenoids, and triterpenoids, suggesting anti-inflammatory and anticancer potential (Das *et al*., 2024; Chen *et al*., 2023)

Phytol, n-hexadecanoic acid, and other fatty acid derivatives found in neem are consistent with previous GC-MS research showing the plant's antibacterial, antioxidant, and anti-inflammatory properties (Gupta *et al*., 2023; Alzohairy, 2016). Particularly, phytol has been shown to have antioxidant, antibacterial, and anticancer qualities, and palmitic acid (n-hexadecanoic acid) helps reduce inflammation (Swamy *et al*., 2017). Together, these substances support neem's widespread usage in the treatment of inflammatory diseases and infections.

Pungam phytochemical profile revealed the presence of several bioactive substances with well-established pharmacological properties, including karanjin, pongamol, and fatty acid esters. According to reports, the furanoflavonoid karanjin has insecticidal, anti-inflammatory, and antioxidant qualities (Rao *et al*., 2024; Al Muqarrabun *et al*., 2013). Furthermore, the existence of long chain hydrocarbons and esters that support antibacterial activity has been verified by GC-MS analysis in recent research (Elanchezhian *et al*., 2022). These results confirm the increasing interest in pungam as a source of bioactive chemicals for use in agriculture and medicine.

Pyrogallol, derivatives of gallic acid, and chemicals related to tannins were among the several phenolic substances that red cedar displayed. These results are in line with earlier research showing its potent antioxidant and free radical scavenging qualities (Patel *et al*., 2023; Verma *et al*., 2024). Chronic disorders can be prevented by phenolic substances like gallic acid and chebulinic acid, which are known to decrease oxidative stress and prevent cellular damage (Kumar *et al.,* 2022). Furthermore, chebulic myrobalan's high tannin content has been linked to its antibacterial effectiveness by interfering with microbial cell membranes and enzyme systems (Bag *et al*., 2013).

Terpenoids, phenolic chemicals, and fatty acid derivatives have been identified in red cedar in accordance with recent metabolomic and GC-MS research (Das *et al*., 2024; Chen *et al*., 2023). These substances are well known for their antidiabetic, antioxidant, and antibacterial properties. Red cedar has demonstrated encouraging pharmacological potential despite being relatively less studied, especially because it contains limonoids and triterpenoids, which are said to have cytotoxic and anti-inflammatory properties (Roy and Saraf, 2006). The present research adds important information to the scant literature on this species.

Comparative analysis of the four plant species reveals both common and distinct phytochemical components. All samples contained common chemicals including fatty acids and their esters, indicating a fundamental involvement in antibacterial and anti-inflammatory properties. However, plant specific substances that emphasise their unique medicinal characteristics include azadirachtin in neem, karanjin in pungam, and high tannin concentration in chebulic myrobalan (Alzohairy, 2016; Al Muqarrabun *et al*., 2013). These changes could result from variations in extraction techniques, environmental factors, and genetic composition (Singh and Sharma, 2024).

GC-MS is useful for detecting volatile chemicals, but it is not as good at detecting non-volatile, high molecular weight phytoconstituents such glycosides and complex polyphenols. For thorough phytochemical profiling, supplementary methods like LC-MS and HPLC are advised (Kumar *et al*., 2023; Wolfender *et al*., 2019).

From an agroforestry perspective, neem and pungam can function as biological pest regulators, while chebulic myrobalan and red cedar contribute to high-value non-timber forest products (NTFPs), enhancing system sustainability (Singh and Sharma, 2024). Furthermore, synergistic interactions among shared and unique metabolites may support the development of polyherbal formulations for pharmaceutical and nutraceutical applications (Wolfender *et al*., 2019)

5. Conclusion

In summary, the current work effectively showed how GC-MS analysis can be used to profile the various phytochemical components of a few multifunctional agroforestry species, including neem (*A. indica)*, pungam (*P. pinnata)*, red cedar (*T. ciliata)*, and chebulic myrobalan (*T. chebula*). Flavonoids, phenolics, terpenoids, fatty acids, and tannins are only a few of the many bioactive chemicals that have been identified, demonstrating the tremendous chemical variety and medicinal potential of these plants. Important pharmacological characteristics, such as antioxidant, antibacterial, anti-inflammatory, and anticancer activity, were linked to key chemicals such azadirachtin, karanjin, chebulinic acid, quercetin, and betulinic acid. Both common and distinct phytoconstituents across the species were identified by the comparative investigation, highlighting their complimentary functions in both conventional and contemporary medical systems. These results confirm the

ethnopharmacological significance of these plants and bolster their potential use in herbal formulation development and medication discovery. Although, GC-MS has been shown to be a sensitive and dependable analytical method for volatile and semi volatile substances, more research utilising sophisticated methods like LC-MS and HPLC is advised for thorough metabolite profiling.

The results highlight the enormous potential of these agroforestry species as environmentally benign, economical, and sustainable sources of novel bioactive compounds, greatly advancing the fields of phytochemistry, pharmacology, and natural product-based medication discovery.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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