

# Comparative GCMS-based profiling of phytochemical constituents from *Azadirachta indica*, *Melia azedarach* and *Toona ciliata*

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## ABSTRACT

For countless generations, *Azadirachta indica*, *Melia azedarach* and *Toona ciliata* have been widely recognized for their ethnobotanical properties, which have been utilized for traditional and medicinal purposes. The extracts derived from the fruit, bark, and leaves of these species exhibit a heterogeneous mixture, including diverse chemical constituents. To ascertain the GC-MS-based metabolite profiling of bioactive constituents in various plant parts of selected tree species. It is often necessary to employ quick extract screening procedures for the analytical determination. In general, plants possess genetic regulation mechanisms that govern their metabolic profiles. However, quantifying these profiles can exhibit variability depending on the prevailing environmental conditions. The chosen three tree species were subjected to gas chromatography-mass spectrometry examination to assess the degree of variability in their volatile organic compounds. The leaves of *A. indica* were found to possess a total of eight bioactive compounds, whereas the bark and fruits of the plant had six and fifteen bioactive compounds, respectively. A total of eight bioactive chemicals were identified in the leaves of *M. azedarach*, while seven were found in the bark and 10 were detected in the fruits. A total of ten bioactive chemicals were detected in the leaves, eight in the fruits, and fourteen in the bark of *T. ciliata*. Hence, diverse chemical composition makes these tree species pharmaceutically relevant and forms a putative basis for further scientific work.

**Keywords:** Composition, Metabolites, Phytochemicals, Pharmaceutical, Trees.

## Highlights

- GC-MS has been done to analyze the phytochemical composition of different parts and identify the volatile bioactive compounds present in them.
- *Azadirachta indica* and *Melia azedarach* exhibited more complex profiles compared to *Toona ciliata*, which had a more straightforward profile dominated by fewer major compounds.
- All three species share some common compounds, such as various terpenes and flavonoids, but in differing concentrations and compositions.
- This research may serve as an incentive for a thorough assessment of the plant's efficacy as well as fill in any gaps in the literature currently in use.

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## INTRODUCTION

Traditional medicinal plants are frequently characterized by their affordability, local accessibility, and ease of use, either in their raw form or as uncomplicated therapeutic formulations. While the scientific testing of their efficacy and mechanisms of action is lacking in most instances, these uncomplicated pharmaceutical concoctions frequently facilitate advantageous responses as a result of their active chemical elements (Al-Nemari *et al.*, 2020). Plants that have been extensively utilized in traditional medicine throughout history constitute a substantial reservoir for the exploration and examination of novel therapeutic interventions within the field of pharmaceutical sciences (Balandrin *et al.*, 1988; Riyadi *et al.*, 2023). Tropical and subtropical regions are home to the Meliaceae family, which has more than 50 genera and about 1400 species, 12 of which are exclusive to India (Shilaluke *et al.*, 2022). Since the 19<sup>th</sup> century, Meliaceae has been the most important family for the expansion of forestry products in different parts of the world like Asia, tropical Africa and Latin America (Dougnon *et al.*, 2002). Phytochemical studies of various Meliaceae family members have revealed a range of natural products, including sesquiterpenoids, diterpenoids, triterpenoids, limonoids,

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lignans, and alkaloids. Additionally, plants from the Meliaceae family demonstrate a range of notable biological activities, such as cytotoxic, anti-plasmodial, antimicrobial, antidiabetic, antiviral, and anti-inflammatory effects (Riyadi *et al.*, 2023).

*Azadirachta indica* commonly known as neem, is one of the most important medicinal trees whose derivatives are among the plant products that have been claimed to be beneficial in the management of a range of plant diseases (Chhabra *et al.*, 2023). Additionally, the bark contains tannins, an essential oil, and significant carbohydrates like fructose, arabinose, and glucose. Neem seeds serve as a significant source of terpenoids and other biochemical components like lipids, proteins, sugars secondary metabolites etc. Phytochemical screening of *Melia azedarach* showed the presence of carbohydrates, proteins, terpenoids, alkaloids, saponins, flavonoids, steroids, amino acids, anthraquinones and tannins (Zhou *et al.*, 2005). *Toona ciliata* is commonly known as Indian mahogany and is a less explored tree in the family Meliaceae. Studies on phytochemistry revealed that *T. ciliata* species' leaves were abundant in flavonoids, alkaloids, phenols, terpenes, and anthraquinones (Kavitha and Satish, 2013). Chemical derivatives obtained from these tree species are among the products that have been claimed to be beneficial in the management of a range of human and plant diseases. Many decoctions obtained from the plant parts of these tree species were used in traditional medicine systems, viz., unani and sidha for the treatment of various diseases (D'Ambrosio and Guerriero, 2002). However, botanical extracts of species were also found to have antifungal activity against *Rhizoctonia solani* and *Bipolaris oryzae* in a study conducted by Chhabra *et al.*, (2023a). Botanical extracts are a rich source of bioactive compounds, which can act as natural biostimulants for improving plant growth and productivity. *A. indica* followed by *M. azedarach* are described in the scientific literature, while the information available on the bioactive constituents found in *T. ciliata* is sparse (Chhabra *et al.*, 2023b).

In phytoscience, researchers face a significant challenge due to the presence of multiple bioactive compounds within a single plant. Identifying these bioactive constituents and understanding their biological properties is essential for assessing toxicity, determining optimal dosages, and selecting the most effective extraction methods (Thakur *et al.*, 2023). Plants from the Meliaceae family, for instance, produce a variety of metabolites that can be difficult to differentiate using ultraviolet spectroscopy alone; however, mass spectrometry (MS) offers a more effective means of identification (Siddiqui *et al.*, 2009). Plant extract screening is a valuable approach for elucidating the chemical composition of various species. Gas chromatography-mass spectrometry (GC-MS) is a critical technique for analyzing phytochemicals and conducting chemotaxonomic studies of medicinal plants with physiologically active components. GC-MS is widely used for determining and identifying chemical constituents within botanical extracts (Olivia *et al.*, 2021). Despite its potential, the full benefits of Meliaceae extracts remain underexplored due to insufficient research, lack of standardized extraction methods, and incomplete identification of bioactive components. This study aims to comprehensively identify, quantify, and differentiate the distribution of bioactive chemicals in different parts (fruit, leaf, and bark) of selected Meliaceae tree species using GC-MS. By identifying phytochemical-rich plant species, this research could enhance their utilization and promote crop diversification (Arunachalam *et al.*, 2024). Moreover, our findings underscore the need for an

in-depth investigation of sesquiterpenoid compounds in the Meliaceae family, given their unique structures and diverse biological activities. This exploration is vital for discovering new compounds with potential drug development applications and could provide valuable opportunities for validating traditional medicinal claims and developing safe, effective treatments.

## MATERIAL AND METHODS

The experiment was conducted at Punjab Agricultural University (PAU), located in Ludhiana (30°54'26" N and 75°47'38" E). This region has a subtropical to tropical climate with three distinct seasons: a hot and dry summer (April to June), a hot and humid monsoon (July to September), and a cold winter (December to February). Frost is rare in this area. The average annual rainfall is 680 mm, predominantly occurring in July, August, and September. The leaves and bark of the three chosen species, *A. indica*, *M. azedarach* and *T. ciliata* were gathered from a variety of trees growing at the Research Farm, Department of Forestry and Natural Resources, PAU, between March and August. These trees ranged in age from 11 to 13 years. While the fruits of *A. indica* were harvested in July and August during the peak fruiting season, the completely developed fruits of *M. azedarach* and *T. ciliata* were taken in May and June. The fresh samples (1000 g) were subjected to drying the samples in an oven and were finely ground into a powder with a particle size of 0.25 mm using an electric blender. To determine the composition of the fruits, leaves, and bark of selected tree species, samples were collected from the research farm of the Department of Forestry and Natural Resources (PAU), Ludhiana. The 0.5 g of dried powder was extracted using a soxhlet apparatus in HPLC-grade methanol procured from Sigma Aldrich, USA. All the chemicals used for this quantification were of analytical purity. The following HPLC conditions were maintained:

Column conditions	TRACE 1300 GC and a TSQ 8000 TRIPLE QUADRUPOLE MS equipped with a TG 5MS (30m X 0.25 mm, 0.25 m) and an S/SL injector
Injector temperature	250°C
MS transfer line temperature	300°C
Ion source temperature	230°C
Column temperature	60–280°C at a rate of 10°C/min
Carrier gas	Helium (99.999%) at a rate of 1-mL/min
Injection volume	1.0 µL in DMSO with a split flow rate of 1-mL/min
Mass spectra	75 eV with a mass scan range of m/z 40–500 amu

The mass spectrum was analyzed using the National Institute of Standards and Technology (NIST) database. The spectra of the unknown components were compared with the reference spectra of known substances in the NIST library. Additionally, the PubChem Bioassay database, part of the USA National Institutes of Health (NIH) Molecular Libraries Roadmap Initiative, served as a public resource for information on chemical substances and their biological activities (Kim *et al.*, 2016). Compound identification was further supported by comparing findings with published literature and consulting peer-reviewed research

articles for bioassay data on the identified compounds. The Sophisticated Analytical Instrumentation Facility (SAIF), Central Instrumentation Laboratory, Panjab University, Chandigarh, India, performed the GCMS spectral analysis.

## RESULTS AND DISCUSSION

Depending on their origin and characteristics, a range of chemicals, including organic acids, fatty acids, and essential volatile oils, were identified by GC-MS analysis. These compounds were then sorted in order of increasing retention time. In various species, the same chemicals exhibited varying retention durations and regions. The longer retention time is determined by the compound's interaction with the stationary phase. Analyzing the retention duration under particular conditions can provide a qualitative identification of the type of component present in a sample. The changes in concentration of known sample mixtures were calculated using the percentage peak area in the GCMS tables (Olivia *et al.*, 2021).

Mevalonic acid (MVA) is the source of the majority of volatile compounds (i.e., triterpenoids and sesquiterpenoids). However, plastidial methylerythritol phosphate is used in the biosynthesis of monoterpenoids, diterpenoids, and tetraterpenoids (MEP) (Sawai and Saito 2011). A total of eight bioactive compounds were detected in the leaves of neem viz., acetaldehyde, acetone, butanal, n-hexane, cyclopropyl methyl carbinol, 2-methyl butanal, N-methyl-7-azabicyclo (2,2,1) hept-2-ene and trifluoroacetyl- $\alpha$ -terpineol (Table 1). The NIST database searches of these chemicals indicated that they were documented for antimicrobial, antioxidant as well as fungicidal properties. Phytochemicals such as carbinol are important chiral building blocks in the synthesis of natural plant products (Honda 2012)

Data presented in Table 2 revealed the presence of ethyl iso-allochololate, 1-monolinoleoylglycerol trimethylsilyl ether, 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, 9,12,15-Octadecatrienoic acid, ergosta-5,22-dien-3-ol acetate (3 $\acute{a}$ ,22E), and 3-pyridinecarboxylic acid in the bark of neem tree. Maximum area% was observed for 9,10-secocholesta-5,7,10(19)-triene-3, 24, 25-triol, whereas the minimum was observed for 9,12,15-octadecatrienoic acid. 9,12,15-octadecatrienoic acid is a fatty acid popular as an antioxidant, antimicrobial, anticancer, anti-inflammatory, antiasthmatic, antiarthritic and diuretic compound.

A perusal of data from Table 3 revealed the occurrence of 13 compounds, viz., acetaldehyde, trimethyl-oxirane, dichloroacetaldehyde, 2-methyl-propanal, n-Hexane, 2,2-dimethyl-Butanoic acid, cyclopropyl methyl carbinol, allyl ethyl ether,  $\alpha$ -pinene, octamethyl-cyclotetrasiloxane, 2,6-bis[(trimethylsilyl)oxy]-trimethylsilyl ester benzoic acid, caryophyllene, 9,12,15-octadecatrienoic acid, 1-monolinoleoylglycerol trimethylsilyl ether and ethyl iso-allochololate in fruits of neem. The fruits of neem trees contained the most phytochemicals, followed by the leaves and bark. *A. indica* is an important medicinal plant traditionally known to possess antimicrobial ability owing to its rich source of various types of bioactive ingredients (Al-Marzoqi *et al.*, 2015).  $\beta$ -Pinene is a naturally occurring substance with antimicrobial potential and can participate in many chemical reactions (Jassal *et al.*, 2021). The chemical modification enables the manufacture of a

**Table 1:** Different volatile compounds in the leaves of *A. indica* analyzed through GC-MS

S. No.	IUPAC name of the chemical	Chemical formula	Retention time	Area %
1	Acetaldehyde	C <sub>2</sub> H <sub>4</sub> O	1.63	14.74
2	Acetone	C <sub>3</sub> H <sub>6</sub> O	1.79	24.05
3	Butanal	C <sub>4</sub> H <sub>8</sub> O	2.00	14.61
4	n-Hexane	C <sub>6</sub> H <sub>14</sub>	2.19	4.33
5	Cyclopropyl methyl carbinol	C <sub>5</sub> H <sub>10</sub> O	2.59	14.29
6	2- methyl butanal	C <sub>5</sub> H <sub>10</sub> O	2.67	9.16
7	N-Methyl-7-azabicyclo (2,2,1) hept-2-ene	C <sub>7</sub> H <sub>11</sub> N	3.48	8.22
8	Trifluoroacetyl- $\alpha$ -terpineol	C <sub>12</sub> H <sub>17</sub> F <sub>3</sub> O <sub>2</sub>	8.08	10.61

**Table 2:** Different volatile compounds in the bark of *A. indica* analyzed through GC-MS

S. No.	IUPAC name of the chemical	Chemical formula	Retention time	Area %
1	Ethyl iso-allochololate	C <sub>26</sub> H <sub>44</sub> O <sub>5</sub>	25.98	3.42
2	1-Monolinoleoylglycerol trimethylsilyl ether	C <sub>27</sub> H <sub>54</sub> O <sub>4</sub> Si <sub>2</sub>	26.17	5.26
3	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,	C <sub>27</sub> H <sub>44</sub> O <sub>3</sub>	26.37	7.08
4	3-Pyridinecarboxylic acid	C <sub>32</sub> H <sub>39</sub> NO <sub>10</sub>	26.96	6.71
5	Ergosta-5,22-dien-3-ol, acetate, (3 $\acute{a}$ ,22E)-	C <sub>30</sub> H <sub>48</sub> O <sub>2</sub>	27.36	4.25
6	9,12,15-Octadecatrienoic acid	C <sub>27</sub> H <sub>52</sub> O <sub>4</sub> Si <sub>2</sub>	27.79	1.91

**Table 3:** Different volatile compounds in the fruits of *A. indica* analysed through GC-MS

S. No.	IUPAC name of the chemical	Chemical formula	Retention time	Area %
1	Acetaldehyde	C <sub>2</sub> H <sub>4</sub> O	1.62	2.52
2	Trimethyl- oxirane	C <sub>5</sub> H <sub>10</sub> O	1.79	20.66
3	Dichloroacetaldehyde	C <sub>2</sub> H <sub>2</sub> Cl <sub>2</sub> O	1.91	4.14
4	2-methyl-propanal	C <sub>4</sub> H <sub>8</sub> O	2.00	11.53
5	n-Hexane	C <sub>6</sub> H <sub>14</sub>	2.19	2.54
6	2,2-dimethyl-butanoic acid	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	2.29	8.13
7	Cyclopropyl methyl carbinol	C <sub>5</sub> H <sub>10</sub> O	2.59	5.70
8	Allyl ethyl ether	C <sub>5</sub> H <sub>10</sub> O	2.67	4.31
9	$\alpha$ -pinene	C <sub>10</sub> H <sub>16</sub>	6.45	3.68
10	Octamethyl-Cyclotetrasiloxane	C <sub>8</sub> H <sub>24</sub> O <sub>4</sub> Si <sub>4</sub>	7.21	1.16
11	2,6-bis[(trimethylsilyl)oxy]-trimethylsilyl ester Benzoic acid	C <sub>16</sub> H <sub>30</sub> O <sub>4</sub> Si <sub>3</sub>	9.67	2.89
12	Caryophyllene	C <sub>15</sub> H <sub>24</sub>	13.91	5.70
13	9,12,15-Octadecatrienoic acid,	C <sub>27</sub> H <sub>52</sub> O <sub>4</sub> Si <sub>2</sub>	25.01	3.10
14	1-Monolinoleoylglycerol trimethylsilyl ether	C <sub>27</sub> H <sub>54</sub> O <sub>4</sub> Si <sub>2</sub>	25.15	3.57
15	Ethyl iso-allochololate	C <sub>26</sub> H <sub>44</sub> O <sub>5</sub>	25.89	3.53

considerable quantity of  $\beta$ -pinene derivatives, several of which have demonstrated notable antimicrobial efficacy.

The leaves of *M. azedarach* recorded a total of eight diverse compounds, viz., acetaldehyde, acetone, isobutylene epoxide, n-hexane, 2-(2-propenyloxy)-ethanol, allyl ethyl ether, 4,4-dimethyl-cyclopentene, and 1-methyl-1H-pyrrole (Table 4). Acetaldehyde is a naturally occurring volatile compound produced mostly during fruit ripening. It is reported to exhibit antimicrobial properties against postharvest pathogens, inhibiting their germination and growth (Saini *et al.*, 2023). Acetaldehyde-fumigated fruits showed reduced fungal decay and conidial germination. This volatile compound is also known to inhibit postharvest microorganisms found in fruits and vegetables because the aldehyde vapors are found to be most lethal to fungal pathogens (Safaei-Ghomi *et al.*, 2010).

A total of seven compounds were detected in the bark of *M. azedarach*, namely acetaldehyde, acetone, methyl ester acetic acid, butanal, n-hexane, pentanal and allyl ethyl ether (Table 5). Many compounds reappeared in this analysis and were quite similar to *A. indica* compounds. Ten compounds presented in Table 6 were observed like acetaldehyde, acetone, methylene chloride, butanal, n-hexane, pentane, allyl ethyl ether,  $\alpha$ -pinene, octamethyl-cyclotetrasiloxane and cedrene. The maximum and minimum percentage area of 33.10 is covered by acetone and covered by n-hexane (4.11), respectively. Octamethyl-cyclotetrasiloxane is a silicon-based polymer having antimicrobial properties (Gavrilov *et al.*, 2010).

**Table 4:** Different volatile compounds in the leaves of *M. azedarach* analysed through GC-MS

S. No.	IUPAC name of the chemical	Chemical formula	Retention time	Area%
1	Acetaldehyde	C <sub>2</sub> H <sub>4</sub> O	1.63	18.97
2	Acetone	C <sub>3</sub> H <sub>6</sub> O	1.80	26.35
3	Isobutylene epoxide	C <sub>4</sub> H <sub>8</sub> O	2.01	8.19
4	n-Hexane	C <sub>6</sub> H <sub>14</sub>	2.20	4.55
5	2-(2-propenyloxy)- Ethanol	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	2.60	10.36
6	Allyl ethyl ether	C <sub>5</sub> H <sub>10</sub> O	2.68	6.54
7	4,4-dimethyl-cyclopentene	C <sub>7</sub> H <sub>12</sub>	3.02	5.26
8	1-methyl-1H-Pyrrole	C <sub>5</sub> H <sub>7</sub> N	3.49	19.79

**Table 5:** Different volatile compounds in the bark of *M. azedarach* analysed through GC-MS

S. No.	IUPAC name of the chemical	Chemical formula	Retention time	Area %
1	Acetaldehyde	C <sub>2</sub> H <sub>4</sub> O	1.63	11.60
2	Acetone	C <sub>3</sub> H <sub>6</sub> O	1.79	33.10
3	Methyl ester acetic acid	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	1.90	5.29
4	Butanal	C <sub>4</sub> H <sub>8</sub> O	2.00	11.80
5	n-Hexane	C <sub>6</sub> H <sub>14</sub>	2.19	4.11
6	Pentanal	C <sub>5</sub> H <sub>10</sub> O	2.59	22.17
7	Allyl ethyl ether	C <sub>5</sub> H <sub>10</sub> O	2.67	11.93

Previously, *Melia dubia* revealed the occurrence of total of 42 phytochemicals rich in unsaturated fatty acids, phenolic derivatives, terpenoids (diterpenes and sesquiterpenes) and lipophilic organic compounds (Safaei-Ghomi *et al.*, 2010). According to this study, there are volatile compounds that belong to steroids, esters, alcohols, hydroxyimine derivatives, isothiocyanates, aromatics, halocompounds, cyanides, unsaturated alkene, alkyne, and indol groups, but they are primarily rich in fatty acids. Thus, these phytochemicals of *M. azedarach* may be suitable candidates for further experimental analysis and are renowned for ethnobotanical and therapeutic applications.

Compounds (Table 7) from leaves of *T. ciliata* detected include namely ethanol, dimethyl sulphide, butanal, acetic anhydride, 3-methyl-butanal, 4-methyl-, trans-cyclohexanol, levomenthol, isocaryophyllene, caryophyllene and humulene. Caryophyllene is a multifunctional sesquiterpene known for its diverse benefits, such as anti-inflammatory, analgesic, antioxidant, and antimicrobial properties. Found in essential oils and numerous plants, it highlights its potential uses in pharmaceuticals, cosmetics, and food preservation (Mulani *et al.*, 2022). Many compounds reappeared in this analysis and were quite similar to each other in various parts of the tree.

**Table 6:** Different volatile compounds in the fruits of *M. azedarach* analysed through GC-MS

Sr. no.	IUPAC name of the chemical	Chemical formula	Retention time	Area %
1	Acetaldehyde	C <sub>2</sub> H <sub>4</sub> O	1.63	10.63
2	Acetone	C <sub>3</sub> H <sub>6</sub> O	1.80	17.61
3	Methylene chloride	CH <sub>2</sub> Cl <sub>2</sub>	1.92	5.20
4	Butanal	C <sub>4</sub> H <sub>8</sub> O	2.01	28.92
5	n-Hexane	C <sub>6</sub> H <sub>14</sub>	2.20	4.74
6	Pentanal	C <sub>5</sub> H <sub>10</sub> O	2.60	7.49
7	Allyl ethyl ether	C <sub>5</sub> H <sub>10</sub> O	2.68	8.87
8	$\alpha$ -Pinene	C <sub>10</sub> H <sub>16</sub>	6.45	7.77
9	Octamethyl- Cyclotetrasiloxane	C <sub>8</sub> H <sub>24</sub> O <sub>4</sub> Si <sub>4</sub>	7.21	2.80
10	Cedrene	C <sub>15</sub> H <sub>24</sub>	13.90	2.13

**Table 7:** Different volatile compounds in the leaves of *T. ciliata* analysed through GC-MS

S. No.	IUPAC name of the chemical	Chemical formula	Retention time	Area %
1	Ethanol	C <sub>2</sub> H <sub>6</sub> O	1.79	2.99
2	Dimethyl sulfide	C <sub>2</sub> H <sub>6</sub> S	1.86	3.72
3	Butanal	C <sub>4</sub> H <sub>8</sub> O	2.04	8.89
4	Acetic anhydride	C <sub>4</sub> H <sub>6</sub> O <sub>3</sub>	2.19	10.54
5	Butanal, 3-methyl-	C <sub>5</sub> H <sub>10</sub> O	2.62	25.23
6	4-methyl-, trans- cyclohexanol	C <sub>7</sub> H <sub>14</sub> O	3.02	4.37
7	Levomenthol	C <sub>10</sub> H <sub>20</sub> O	10.43	6.73
8	Isocaryophyllene	C <sub>15</sub> H <sub>24</sub>	13.70	1.80
9	Caryophyllene	C <sub>15</sub> H <sub>24</sub>	13.91	32.64
10	Humulene	C <sub>15</sub> H <sub>24</sub>	14.37	3.09

**Table 8:** Different volatile compounds in the fruits of *T. ciliata* analysed through GC-MS

S. No.	IUPAC name of the chemical	Chemical formula	Retention time	Area %
1	Acetaldehyde	C <sub>2</sub> H <sub>4</sub> O	1.63	25.99
2	Acetone	C <sub>3</sub> H <sub>6</sub> O	1.80	20.74
3	Methylene chloride	CH <sub>2</sub> Cl <sub>2</sub>	1.92	4.87
4	Butanal	C <sub>14</sub> H <sub>24</sub> O	2.01	21.80
5	n-Hexane	C <sub>6</sub> H <sub>14</sub>	2.20	3.93
6	Cyclopropyl methyl carbinol	C <sub>5</sub> H <sub>10</sub> O	2.60	8.31
7	Allyl ethyl ether	C <sub>8</sub> H <sub>16</sub> O	2.68	9.43
8	Caryophyllene	C <sub>15</sub> H <sub>24</sub>	13.91	4.93

**Table 9:** Different volatile compounds in the bark of *T. ciliata* analysed through GC-MS

Sr. no.	IUPAC name of the chemical	Chemical formula	Retention time	Area %
1	Acetaldehyde	C <sub>2</sub> H <sub>4</sub> O	1.63	12.44
2	Allyl acetate	C <sub>5</sub> H <sub>8</sub> O <sub>2</sub>	1.80	12.22
3	Methylene chloride	CH <sub>2</sub> Cl <sub>2</sub>	1.92	4.13
4	2-ethyl-butanal	C <sub>6</sub> H <sub>12</sub> O	2.01	12.39
5	n-Hexane	C <sub>6</sub> H <sub>14</sub>	2.20	3.94
6	Trichloromethane	CHCl <sub>3</sub>	2.36	2.62
7	Cyclopropyl methyl carbinol	C <sub>5</sub> H <sub>10</sub> O	2.60	11.88
8	Allyl ethyl ether	C <sub>5</sub> H <sub>10</sub> O	2.68	7.14
9	2,6-bis [(trimethylsilyl) oxy]-trimethylsilyl ester benzoic acid	C <sub>16</sub> H <sub>30</sub> O <sub>4</sub> Si <sub>3</sub>	9.67	2.29
10	Caryophyllene	C <sub>15</sub> H <sub>24</sub>	13.91	1.62
11	Ethyl iso-allochololate	C <sub>26</sub> H <sub>44</sub> O <sub>5</sub>	25.05	3.42
12	2-Bromotetradecanoic acid	C <sub>14</sub> H <sub>27</sub> BrO <sub>2</sub>	25.49	2.21
13	1-Monolinoleoylglycerol trimethylsilyl ether	C <sub>27</sub> H <sub>54</sub> O <sub>4</sub> Si <sub>2</sub>	25.63	3.19
14	Acetic acid	C <sub>25</sub> H <sub>36</sub> O <sub>5</sub>	26.03	2.02

Table 8 represents the data of fruits of *T. ciliata* showed the presence of 8 peaks which are related to relative abundance, namely acetaldehyde, acetone, methylene chloride, butanal, n-Hexane, cyclopropyl methyl carbinol, allyl ethyl ether and caryophyllene in order of their appearance. In plants, butanal plays multiple roles as a volatile organic compound, contributing to aroma, defence, and stress responses. Acetaldehyde recorded the maximum area % while n-Hexane recorded the lowest. Bajpai *et al.*, (2008) in mass spectrometry of *Silene armeria* identified similar compounds of methyl derivatives and oxides (caryophyllene oxide) with remarkable effects against various phytopathogens.

The data recorded in Table 9 resulted in a GC-MS chromatogram of the bark of *T. ciliata*, revealing the presence of various phytochemicals with different retention times. A total of 14 compounds were identified mainly as acetaldehyde,

allyl acetate, methylene chloride, 2-ethyl-butanal, n-Hexane, trichloromethane, cyclopropyl methyl carbinol, allyl ethyl ether, 2,6-bis [(trimethylsilyl) oxy]-trimethylsilyl ester benzoic acid, caryophyllene, ethyl iso-allochololate, 2-bromotetradecanoic acid, 1-monolinoleoylglycerol trimethylsilyl ether and acetic acid. 1-monolinoleoylglyceroltrimethylsilyl ether and ethyl iso-allochololate are alkaloid compounds.

While some of these compounds, such as methylene chloride and trichloromethane, are not naturally occurring in plants however, their presence can also be part of the plant's response to different environmental conditions. Yang *et al.*, (2020) conducted an investigation which focused on the examination of the properties exhibited by various compounds, which were then classified into three distinct components that undergo notable alterations. The initial segment exhibits a retention time of less than 5 minutes and primarily consists of small organic acids. In contrast, the subsequent segment displays a retention time ranging from 5 to 25 minutes and is primarily composed of diverse ketone compounds. Notably, the majority of furans and cyclopentenone compounds in the latter segment are derived from hemicellulose. Phenols and their derivatives are mostly generated during the pyrolysis of lignin and other extractives when the retention duration exceeds 25 minutes. The bark had the highest concentration of phytochemicals, with the leaves and fruits following suit. The bark of *T. ciliata* contains a higher concentration of chemical ingredients, which presents an opportunity for scientific exploration and potential utilisation of the bark and wood in various industrial applications.

## CONCLUSION

Chemical profiling of various tree species often uncovers significant genetic variability, with differences in phytochemistry potentially arising from interactions between genotype and environment, as well as inherent genetic differences. Positive outcomes are anticipated by isolating specific phytochemical components and examining their biological activity. Our research involved comparing the methanolic fractions of these tree species using GC-MS to elucidate the similarities and differences in their chemical profiles under the Punjab region. The findings suggest that these tree species possess notable phytopharmaceutical value due to the diverse array of bioactive compounds they contain. However, further research is needed to explore their bioactivity and toxicity profiles in greater detail.

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## AUTHOR CONTRIBUTION

Chhabra R. collected the data and wrote the preliminary draft of this manuscript. Sharma R gave excellent insights during the investigation and acted as supervisor. Sharma P. critically revised the manuscript. Kaur A. and Thakur S. also assisted in writing the paper. All the authors have critically read and approved the drafted manuscript for publication.

## CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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