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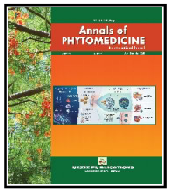

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# GC-MS chemical fingerprinting of diverse volatile organic compounds in *Tylophora indica* (Burm.f.) Merr. leaves

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**Abstract**

Low molecular weight molecules known as plant volatile organic compounds (VOCs) are among the most significant classes of plant metabolites. The presence of alkaloids, flavonoids, saponins, phenols, terpenoids, cardiac glycosides, and vitamin C was confirmed through phytochemical analysis of *Tylophora indica* (Burm. f.) Merr. leaf extracts. Gas chromatography-mass spectrometry (GC-MS), a potent method for component separation and identification, is used in VOC analysis. A combination of 38 bioactive compounds was identified using GC-MS chemical profiling of the methanolic extract of *T. indica* leaves. Aldehydes, fatty acids, ketones, and terpenoids have been shown to contribute to the total biological activity, whereas phthalates have drawn the most attention among them. The presence of specific bioactive compounds, such as 4H-pyran-4-one-2,3-dihydro-3,5-dihydroxy-6-methyl, 5-hydroxymethyl furfural, n-hexadecanoic acid, squalene, pluchidiol, neophytadiene, 9,12-octadecadienoic acid (Z, Z), phytol, dibutyl phthalate, 1,2-benzenedicarboxylic acid diethyl ester, and tetradecanoic acid were all clearly detected by the GC-MS result. The potential of *T. indica* leaves as a source of bioactive chemicals for a range of therapeutic uses, such as anti-inflammatory, antibacterial, and antioxidant activity, is highlighted by this investigation. The use of *T. indica* leaves in pharmaceutical formulations may be a subject of future investigation.

## 1. Introduction

There are several plants in nature that may treat sickness; thus, it is crucial to study these plants properly so that they can be used therapeutically (Gururani *et al*., 2020). A medicinal plant from the Asclepiadaceae family, *Tylophora indica* (Burm. f.) Merr. is in danger of extinction. Among its many global distributions are the islands of the Indian Ocean, Oceania, Ceylon, Malaysia, and Borneo. While in Tamil, it is known as Nancharuppan, in Ayurvedic terms it is known as Indian ipecacuanha, and according to Sunila and Priya (2012) and Nazar *et al*. (2020), it is also known as ananthamul and arkapani. With a long twinning stem and a maximum height of 1.5 m, this knotted climber is perennial and twinning. In shape and size, the leaves range from obovate-oblong to elliptic-oblong, with a width of 1.5-7 cm and a length of 3-10 cm. They are green in colour. Long and thick roots. The lateral cymes-arranged flowers have a light yellowish-purple colour (Gurav *et al*., 2011; Jaime *et al*., 2016).

According to Ignacimuthu and Ayyanar (2006) and Khare (2015), *T. indica* has a long history of medicinal usage in Ayurveda and Siddha medicine for a variety of conditions, including asthma, cough, dysentery, jaundice, cancer, rheumatism, gout, epilepsy, snake bites, and poisons. The plant's roots and leaves are rich in active phytochemicals including alkaloids, saponins, phytosterols, phenols, flavonoids, tannins, and main metabolites. There are 0.2-0.46% phenanthroindolizidine alkaloids in the plant's roots and leaves, which are essential for medicinal purposes (Ali and Bhutani, 1989; Gurav *et al*., 2011; Gururani *et al*., 2020). Isolation of phytochemicals from leaves was the norm. A number of pharmacological activities, such as anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, anticonvulsant, neuroinflammatory, anxiolytic, antitumor, antidiabetic, hepatoprotective, and diuretic effects, have been investigated in relation to *T. indica* extracts and isolated phyto-chemicals (Starlin *et al*., 2013; Gupta *et al*., 2020; Gururani *et al*., 2020; Nazar *et al*., 2020). Despite GC-MS's stellar reputation for detection and separation capabilities, very little is known about its use in secondary metabolite

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profiling of volatile organic compounds (VOCs). The use of formulations based on volatile organic compounds (VOCs) to protect crops and animals from different diseases has recently come to the fore. The purpose of this research was to use gas chromatography-mass spectrometry to determine which volatile organic chemicals were present in *T. indica* leaves; this information might shed light on the phytochemical substances that have traditional medicinal uses.

## 2. Materials and Methods

### 2.1 Cultivation of *Tylophora indica*

The seedlings were originally taken from the plain area of the Dharmapuri District in Tamil Nadu. The Department of Pharmacognosy at the Siddha Central Research Institute in Arumbakkam, Chennai, Tamil Nadu, India, obtained the fresh *T. indica* and verified its validity. The specimen was subsequently put in the herbarium (Voucher No: V28224011). The seedlings of *Tylophora indica* (Burm. f.) Merr. (Figure 1) were planted and cultivated at Veterinary Clinical Complex, Veterinary College and Research Institute, Thanjavur, Tamil Nadu, India.

### 2.2 Preparation of plant extract

The *T. indica* leaves were gathered fresh, rinsed with distilled water, and then let to dry naturally in the shade. We used a blender to pulverize and homogenize the dry leaves. After soaking 50 g of homogenized powder in 250 ml of methanol for the night, the mixture was agitated for 8 h at 200 rpm using a magnetic stirrer. Whatman filter paper No. 1 was used to filter the sample. The filtrate was then dried using a rotary vacuum evaporator and kept at -20ºC for further analysis.

**Figure 1: *Tylophora indica* (Burm. f.) Merr. plant.**

### 2.3 Qualitative phytochemical screening

The methanolic extract of *T. indica* leaves was analysed qualitatively for phytochemical components using the method outlined by Trease and Evans (1989). These components include alkaloids, terpenoids, flavonoids, phenols, saponins, tannins, cardiac glycosides, carbohydrates, proteins, and ascorbic acid.

### 2.4 Chemical fingerprinting

To ensure complete dissolution, 1.0 g of *T. indica* leaf methanolic extract was vortexed with 10 ml of HPLC grade methanol for 10 min and samples were passed through a 0.22 µm PTFE syringe filter. Gas chromatography-mass spectrometry (GC-MS) was then used for the chemical fingerprinting of volatile organic compounds.

#### 2.4.1 Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical components of methanolic crude extracts of *T. indica* leaves were determined by employing a gas chromatograph (GC-2010 plus) interfaced with a triple quadrupole mass spectrometer (GCMS-TQD 8050) analyser from Shimadzu, Japan. The Rxi-5 Sil MS capillary column, with dimensions of 30 m length, 0.25 mm i.d. and a film thickness of 0.25 mm, was used for this purpose. The carrier gas employed was helium, which has a purity level of 99.999%, and the flow rate was 1.31 µl/min. An autosampler (AOC 20S, Shimadzu, Japan) was used to inject one microlitre of the extract in splitless mode. At 250°C, the injector port was set, while the interface and ion source were each set at 230°C. The GC column oven was preheated to 80°C for 2 min, then increased to 280°C at a rate of

10°C/min, and finally maintained at that temperature for 5 min. With an electron ionization (EI) mode set at 70 eV and a scan interval of 0.3 s, the mass spectrometer was run. The instrument was run in full scan mode across a 50-500 m/z range. As part of the quality control process, the system's performance is confirmed by analysing a blank methanol solution. A strong analysis was guaranteed by repeatability tests, which confirmed the data' dependability.

### 2.4.2 VOCs identification

In order to identify volatile organic compound (VOC) detection, we used conventional GC-MS techniques to compare the retention durations and mass spectra of the identified peaks to those in the NIST (National Institute of Standards and Technology) and Wiley reference libraries. To find out the unknown components, we compared their mass fragmentation patterns to those of known components held in the Wiley and NIST libraries. All of the test materials' constituent names, molecular weights, and structures were determined. By comparing the average peak area of each chemical with the overall chromatographic peak area, we were able to establish their relative abundance. The supplier-provided software, Shimadzu GC-MS solution (Version 2.5), was used for system control and data acquisition.

## 3. Results

### 3.1 Qualitative phytochemical analysis

The qualitative phytochemical analysis results of the *T. indica* leaf methanolic extract are shown in Table 1. The phytochemicals found in the methanol extract were rather numerous in *T. indica* leaves, including alkaloids, flavonoids, terpenoids, saponins, phenols, cardiac glycosides, and vitamin C. The phytochemical analysis of the methanolic extract revealed the absence of proteins, carbohydrates, and tannins. The methanol extracts of *T. indica* included phenolic chemicals, which are known for their powerful antioxidant capabilities and wide range of biological activities.

**Table 1: Qualitative phytochemical analysis of *T. indica* leaves**

| S. No. | Name of the phytochemical | Name of the test | Test result |
|---|---|---|---|
| 1. | Alkaloids | Dragendroff test | Positive |
| 2. | Terpenoids | Salkowski test | Positive |
| 3. | Flavonoids | Shinoda test | Positive |
| 4. | Tannin | Braymer test | Negative |
| 5. | Saponin | Foam test | Positive |
| 6. | Phenols | Ferric chloride test | Positive |
| 7. | Cardiac glycosides | Keller-Kiliani test | Positive |
| 8. | Carbohydrates | Benedict's test | Negative |
| 9. | Proteins | Millon's test | Negative |
| 10. | Vitamin C | Ascorbic acid test | Positive |

### 3.2 Optimization of GC-MS instrument parameters

In order to separate and identify volatile organic compounds (VOCs) from the leaf samples, many critical gas chromatography-mass spectrometry (GC-MS) parameters were fine-tuned to attain high resolution, sensitivity, and accuracy. In order to achieve accurate VOC separation and identification, the following instrumental conditions were fine-tuned: temperature gradient, carrier gas flow, injection technique, and scan parameters. In a single GC run, 38 volatile organic compounds (VOCs) were identified using baseline separation under the optimized oven conditions (Figure 2). The first eluting compound, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, occurred at 8.298 min, and the last, squalene, occurred at 49.111 min, indicating excellent resolution across a wide retention time range. The analysis was also affected by the flow rate of the carrier gas. Although, higher flow rates shorten run durations, they decrease the analytes' separation efficiency. The VOCs profiling in this investigation was carried out using a column flow rate of 1.3 ml/min. Due to the limited sample volume that reaches the column in the split injection mode, not all chemicals may be fully eluted. To guarantee that all potential chemicals were delivered onto the column for analysis with improved sensitivity, the 1µl injection volume splitless high-speed injection mode was used. Operating in the same manner as a conventional full scan, the quadruple ionization source tracked a wide mass range of m/z 50-500. Searching for lower mass pieces (below 50 m/z) is often discouraged because it might amplify background noise caused by airborne interferences like nitrogen (m/z 28). To get the best peak resolution, we adjusted the MS scan speed at 0.3 s. The separation of 38 volatile chemical components in *T. indica* leaves was therefore enhanced by optimizing the instrument settings.

### 3.3 Chemical fingerprinting of VOCs in *T. indica* leaves

The GC-MS analysis of the *T. indica* methanolic fraction revealed many peaks that were indicative of phthalates, fatty acids, and oxygenated esters, ketones, and aldehydes, among other derivatives. Using GC-MS, a wide range of chemical groups were able to be detected, leading to the identification of at least 38 VOCs. Table 2 shows that more than 75% of the volatile content was made up of the following components: diethyl phthalate derivatives (42.05%), 5-hydroxymethylfurfural (26.57%), and n-hexadecanoic acid (10.08%). Some of the other important components were found to include dodecanoic acid (1.16%), bis(2-ethylhexyl) phthalate (1.59%), 3,5-dimethoxy acetophenone (1.65%), tetradecanoic acid (2.76%), 9,12-octadecadienoic acid (Z,Z) (2.90%),octadecanoic acid (0.99%), 4H-pyran-4-one-2,3-dihydro-3,5-dihydroxy-6-methyl (0.96%), 1-(4-hydroxyphenyl)ethenone (0.95%), vaccenic acid

(0.93%), phytol (0.76%), apocynin (0.70%), ethyl-9cis-11-trans-octadecadienoate (0.64%), benzoic acid-2-hydroxy-methyl ester (0.54%), hexadecanoic acid ethyl ester (0.54%), squalene (0.38%), and ethyl oleate (0.35%).

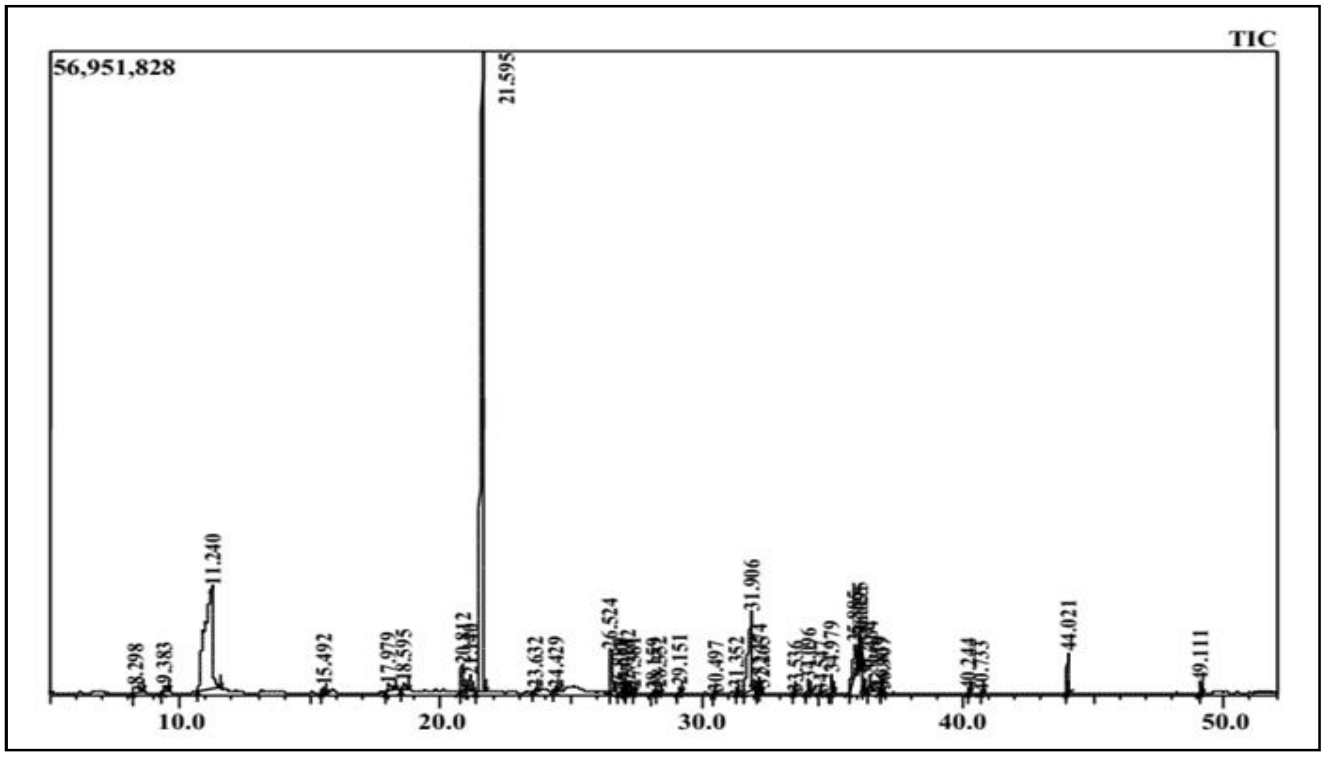


**Figure 2:** Gas chromatography-mass spectrometry chromatogram of methanolic extract of *T. indica* leaves.

**Table 2:** Chemical profiling of *T. indica* leaves by GC-MS

| S. No. | RT (min) | Area (%) | Name of the compound | Chemical class | Molecular formula | Molecular weight (g/mole) |
|---|---|---|---|---|---|---|
| 1. | 8.298 | 0.96 | 4H-pyran-4-one-2,3-dihydro-3,5 -dihydroxy-6-methyl | Pyranone | $C_6H_8O_4$ | 144.12 |
| 2. | 9.383 | 0.54 | Benzoic acid- 2-hydroxy- methyl ester | Benzoate esters | $C_{10}H_{12}O_3$ | 180.2 |
| 3. | 11.240 | 26.57 | 5-hydroxymethylfurfural | Aldehyde | $C_6H_6O_3$ | 126.11 |
| 4. | 15.492 | 0.29 | Tetradecane | Alkane | $C1_4H_{30}$ | 198.39 |
| 5. | 17.979 | 0.95 | 1-(4-hydroxyphenyl)ethenone | Alkyl phenyl ketones | $C_8H_8O_2$ | 136.15 |
| 6. | 18.595 | 0.70 | Apocynin | Alkyl phenyl ketones | $C_9H_{10}O_3$ | 166.17 |
| 7. | 20.812 | 1.65 | 3,5-dimethoxy acetophenone | Ketone | $C1_0H_{12}O_3$ | 180.20 |
| 8. | 21.140 | 1.16 | Dodecanoic acid | Fatty acid | $C_{12}H_{24}O_2$ | 200.32 |
| 9. | 21.595 | 42.05 | 1,2-benzenedicarboxylic acid diethyl ester | Phthalate | $C_{12}H_{14}O_4$ | 222.24 |
| 10. | 23.632 | 0.11 | 3-buten-2-ol-4-(2,6,6-tri methyl -1-cyclohexen-1-yl) | Terpenoid alcohol | $C_{13}H_{20}O$ | 192.29 |
| 11. | 24.429 | 0.15 | 1-{2-[3-(2-Acetyl-oxiran-2-yl)-1, 1-dimethyl-propyl] -cycloprop-2 -enyl} ethenone | Ketone | $C_{14}H_{20}O_3$ | 236.31 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 12. | 26.524 | 2.76 | Tetradecanoic acid | Fatty acid | $C_{14}H_{28}O_2$ | 228,37 |
| 13. | 26.880 | 0.12 | 6-hydroxy-4,4,7a-tri methyl-5,6, 7,7a-tetrahydro benzofuran-2(4H) -one | Benzofuran | $C_{11}H_{16}O_3$ | 196.24 |
| 14. | 27.071 | 0.08 | Benzaldehyde 3,4-dimethoxy -methylmonoacetal | Aromatic aldehyde | $C_{10}H_{14}O_4$ | 198.22 |
| 15. | 27.162 | 0.31 | Heneicosane | Alkane | $C_{21}H_{44}$ | 296.57 |
| 16. | 27.380 | 0.22 | Pluchidiol | Sesquiterpene | $C_{13}H_{20}O_2$ | 208.00 |
| 17. | 28.159 | 0.15 | Neophytadiene | Diterpene | $C_{20}H_{38}$ | 278.50 |
| 18. | 28.352 | 0.19 | 2-pentadecanone- 6,10,14 -trimethyl | Sesquiterpenoid | $C_{18}H_{36}O$ | 268.48 |
| 19. | 29.150 | 0.28 | Pentadecanoic acid | Fatty acid | $C_{15}H_{30}O_2$ | 242.40 |
| 20. | 30.497 | 0.05 | Hexadecanoic acid methyl ester | Fatty acid | $C_{17}H_{34}O_2$ | 270.45 |
| 21. | 31.352 | 0.23 | Dibutyl phthalate | Phthalate | $C_{16}H_{22}O_4$ | 278.34 |
| 22. | 31.906 | 10.08 | n-hexadecanoic acid | Fatty acid | $C_{16}H_{32}O_2$ | 256.42 |
| 23. | 32.174 | 0.54 | Hexadecanoic acid ethyl ester | Fatty acid ester | $C_{18}H_{36}O_2$ | 284.47 |
| 24. | 32.265 | 0.10 | Eicosane | Alkane | $C_{20}H_{42}$ | 282.50 |
| 25. | 33.536 | 0.08 | 9-octadecenoic acid | Fatty acid | $C_{18}H_{34}O_2$ | 282.50 |
| 26. | 34.541 | 0.07 | 4-decenoic acid ethyl ester | Fatty acid ester | $C_{12}H_{22}O_2$ | 198.30 |
| 27. | 34.979 | 0.76 | Phytol | Diterpene alcohol | $C_{20}H_{40}O$ | 296.50 |
| 28. | 35.885 | 2.90 | 9,12-octadecadienoic acid (Z,Z) | Fatty acid | $C_{18}H_{32}O_2$ | 280.40 |
| 29. | 36.023 | 0.93 | Vaccenic acid | Fatty acid | $C_{18}H_{34}O_2$ | 282.50 |
| 30. | 36.095 | 0.64 | Ethyl 9 cis 11-trans octade- cadienoate | Fatty acid | $C_{20}H_{36}O_2$ | 308.50 |
| 31. | 36.230 | 0.38 | Ethyl oleate | Fatty acid esters | $C_{20}H_{38}O_2$ | 310.50 |
| 32. | 36.464 | 0.99 | Octadecanoic acid | Fatty acid | $C_{18}H_{36}O$ | 284.47 |
| 33. | 36.839 | 0.08 | Octadecanoic acid ethyl ester | Fatty acid esters | $C_{20}H_{40}O_2$ | 312.53 |
| 34. | 36.907 | 0.03 | 11-methyltricosane | Alkane | $C_{24}H_{50}$ | 338.65 |
| 35. | 40.244 | 0.09 | 4,8,12,16-tetramethyl heptadecan -4-olide | isoprenoid β-lactone | $C_{21}H_{40}O_2$ | 324.5 |
| 36. | 40.733 | 0.07 | Ethyl 14-methyl-hexadecanoate | Fatty esters | $C_{19}H_{38}O_2$ | 298.50 |
| 37. | 44.020 | 1.59 | Bis (2-ethylhexyl) phthalate | Phthalates | $C_{24}H_{38}O_4$ | 390.55 |
| 38. | 49.111 | 0.45 | Squalene | Triterpene | $C_{30}H_{50}$ | 410.70 |

When all the substances were classified according to their chemical properties, the order was as follows: phthalates (43.87%), aldehydes (26.65%), fatty acids (21.27%), ketones (3.65%), and terpenes (2.13%). This information is shown in Figure 3. There were also other chemicals found, including pyranones (0.96%), alkanes (0.73%), and esters (0.54%). The GC-MS investigation revealed the presence of pluchidiol (0.22%), a benzofuran molecule (6-hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one),which accounted for 0.12%. Table 3 shows the biologically active organic chemical components found by volatile organic compound (VOC) profiling utilizing gas chromatography-mass spectrometry in the current investigation.

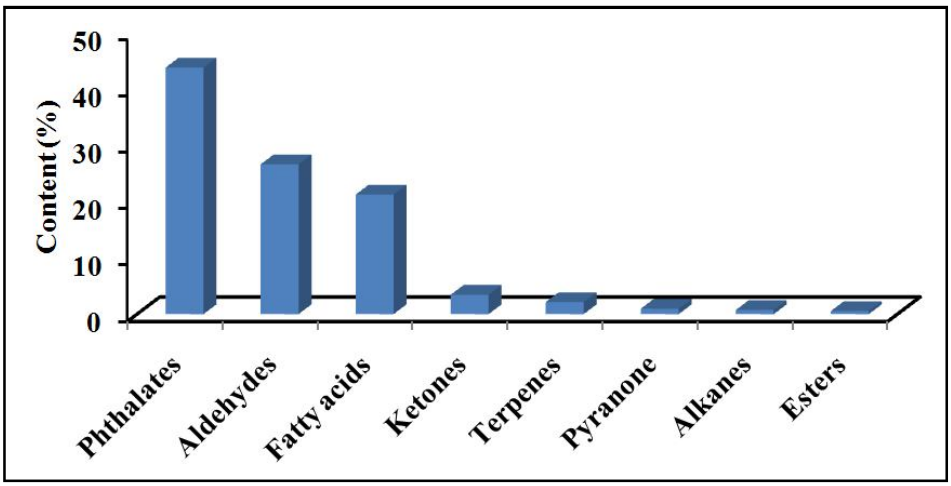


**Figure 3: Chemical group of volatile organic compounds in *T. indica* leaves by GC-MS.**

**Table 3: Biological activity of phytocomponents in the methanolic extract of *T. indica* leaf**

| Name of the compounds | Biological activity |
|---|---|
| 4H-Pyran-4-one- 2,3-dihydro-3, 5-dihydroxy-6-methyl, 5-hydroxymethylfurfural, n-hexadecanoic acid, squalene, pluchidiol and neophytadiene. | Antioxidant |
| 5-Hydroxymethylfurfural, n-hexadecanoic acid, 9,12-octadecadienoic acid (Z, Z), phytol, pluchidiol and neophytadiene. | Anti-inflammatory |
| Dibutyl phthalate, 1,2-benzenedicarboxylic acid diethyl ester, squalene, 9,12-octadecadienoic acid (Z,Z), phytol, tetradecanoic acid and neophytadiene. | Antimicrobial |

## 4. Discussion

The phytochemicals found in the methanol extract were rather numerous in *T. indica* leaves, including alkaloids, flavonoids, terpenoids, saponins, phenols, cardiac glycosides, and vitamin C. This finding is quite congruent with what Maheshwari and Vijayarengan (2020) found. The results show that *T. indica* leaves has a complex phytochemical makeup. According to various studies (Starlin *et al*., 2013; Gupta *et al*., 2020; Gururani *et al*., 2020; Vani,2021; Nazar *et al*., 2020), alkaloids have a variety of pharmacological effects, such as anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, anticonvulsant, neuroinflammatory, anxiolytic, antitumor, antidiabetic, hepatoprotective, and diuretic activities. The methanol extracts of *T. indica* included phenolic chemicals, which are known for their powerful antioxidant capabilities and wide range of biological activities. According to research by Barbosa (2014) and Roy *et al*. (2022), the saponin and flavonoid chemicals found in this extract have antibacterial, anti-tumour, and anti-inflammatory capabilities.

According to earlier research, *T. indica* produces a wide variety of bioactive chemicals with antimicrobial, antifungal, and antiphytopathogenic properties. Numerous studies have documented the antioxidant properties of volatile metabolites, including 4H-pyran-4-one-2,3-dihydro-3,5-dihydroxy-6-methyl, 5-hydroxymethylfurfural, n-hexadecanoic acid, squalene, and neophytadiene (Yu *et al*., 2013; Starlin *et al*., 2013; Sindhu *et al*., 2024; Shapla *et al*., 2018; Aparna *et al*., 2012; Beulah *et al*., 2018; Kavitha, 2021; Rajeshwaran and Rajan, 2025). Phytol, pluchidiol, neophytadiene, 5-hydroxymethylfurfural, and 9,12-octadecadienoic acid (z, z) have all been reported to have anti-inflammatory properties (Aparna *et al*., 2012; Kim *et al*.,2020; Beulah*et al*., 2018; Kavitha, 2021; Rajeshwaran and Rajan, 2025).

Numerous studies have indicated that volatile organic compounds with antimicrobial properties include squalene, 1,2-benzenedicarboxylic acid diethyl ester, dibutyl phthalate, tetradecanoic acid, neophytadiene, 9,12-octadecadienoic acid (Z, Z), and phytol (Javid *et al*., 2020; Beulah *et al*., 2018;Rajeshwaran and Rajan, 2025; Hu *et al*., 2021; Manjusha *et al*.,2024). Spanova and Daum (2011) and Kim and Karadeniz (2012) found that squalene, a triterpene molecule, had antioxidant and anticancer actions, among its various biological characteristics. Grabarczyk *et al*. (2015) found that the benzofuran molecule 6-hydroxy-4,4,7a-tri methyl-5,6,7,7a-tetrahydro benzofuran-2(4H)-one possesses antioxidant, antibacterial, antifungal, and anti-cancer biological characteristics, whereas Kumar *et al*. (2023) found that pluchidiol exhibits antioxidant capabilities. Genetic variability, environmental conditions (such as temperature, light, soil nutrients, and water availability), developmental phases, herbivory, and harvesting time are some of the several factors that affect the composition of active chemicals in plants, leading to diversity in volatile organic compounds (VOCs).

## 5. Conclusion

There has been a shift in focus away from chemical methods of disease control and towards biological control in recent years. To determine the components of the volatile organic compounds (VOCs), GC-MS was used on the methanolic extract of *T. indica* leaves. The extracts were found to include a variety of components, including phthalates, aldehydes, fatty acids, ketones, terpenes, and other trace

components. These mostly contribute to the strong biological activity against different types of infections. These results raise the possibility that volatile organic compounds (VOCs) produced by *T. indica* might be useful in the field of sustainable disease control for both livestock and crops.

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## Conflict of interest

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

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