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Original Article

Comparative Analysis of Multi-Solvent *Tinospora cordifolia* Fresh and Dry Leaf Extract Using Advanced Analytical Approaches

KJ Sharmila[1,*], Nishalini Palani[1], Aarthy Sundaram[2], Santhiya Jayakumar[3]

[1]Department of Biotechnology, Dr. M.G.R. Educational and Research Institute, Maduravoyal, Chennai, Tamil Nadu, INDIA.
[2]Department of Microbiology, Saveetha Medical College and Hospital, Saveetha Nagar, Thandalam, Chennai, Tamil Nadu, INDIA.
[3]Department of Anatomy, Saveetha Medical College, Saveetha Nagar, Thandalam, Chennai, Tamil Nadu, INDIA.

ABSTRACT

Background: *Tinospora cordifolia* is used worldwide for its medicinal properties, but its bioactive phytoconstituents have been less explored. **Objectives:** The fresh and dry leaf extracts of *Tinospora cordifolia*, a traditional medicinal plant, were comparatively analysed for their phytochemical content and therapeutic potential. **Materials and Methods:** Qualitative methods such as Fourier Transform Infra-Red spectroscopy (FTIR), Gas Chromatography-Mass Spectrometry (GCMS), antimicrobial assay and antioxidant assays such as 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) and phosphomolybdenum test were employed to interpret functional groups, chemical constituents, bacterial and fungal antimicrobial properties, and radical scavenging activity, respectively. **Results:** Bioactive compounds were present more abundantly in *Tinospora cordifolia* dry leaf ethanol and aqueous extract. GC-MS chromatographic profiling identified about 189 bioactive compounds, while the FTIR analysis confirmed the presence of medicinally relevant phytoconstituents in *Tinospora cordifolia* leaves. The antioxidant assays, such as DPPH and phosphomolybdenum assays, of the dry leaf extract showed promising dose-dependent free radical scavenging activity of *Tinospora cordifolia*. **Conclusion:** Both fresh and dry leaf extracts showed broad-spectrum antimicrobial activity against bacteria and fungi. The dry leaf extract, which has more antioxidants and antimicrobial properties than the fresh leaves.

Keywords: Antimicrobial, Antioxidant, FTIR, GC-MS Analysis, *Tinospora cordifolia*.

Correspondence:
Prof. Sharmila KJ
Department of Biotechnology, Dr. M.G.R. Educational and Research Institute, Maduravoyal, Chennai-600095, Tamil Nadu, INDIA.
Email: sharmila.ibt@drmgrdu.ac.in

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INTRODUCTION

About 80% of the population uses traditional treatments using plant phytoconstituents, such as in the Ayurveda medical system, according to the World Health Organization (Yadav *et al*., 2022; Sushma *et al*., 2021). *T. cordifolia*, a tropical plant of the South Indian peninsular plains, has been used in Ayurvedic, Unani, and homoeopathic therapeutics and more commonly known as *Guduchi, Amrita*, and *Giloy*, referring to a divine elixir (Kirar *et al*., 2024), but has not received scientific attention (Nisal *et al*., 2024; Medha *et al*., 2021; Singh *et al*., 2024). Secondary plant metabolites, especially phenolic compounds, offer antioxidant, anticancer, antibacterial, anti-inflammatory, antifungal and other properties and are available affordably with few or no negative side effects and have gained a lot of attention in recent years (Alfarrayeh *et al*., 2024; Gautam *et al*., 2020) as they are produced using natural ingredients by eco-friendly protocols with higher economic value (Agrawal *et al*., 2018; Akhilraj *et al*., 2024; Singh *et al*., 2024; Gupta *et al*., 2024). In Ayurveda, the herb *T. cordifolia* is known as 'Rasayana' and is used as an efficient antimicrobial agent and also used for treating cancer, jaundice, chronic diarrhoea, urinary tract issues, and more (Tharani *et al*., 2023), as it increases macrophage activity (Kirar *et al*., 2024; Saini *et al*., 2022). In the present study, the phytoconstituents of *T. cordifolia* leaf have been explored and assessed for antioxidant and antimicrobial properties.

MATERIALS AND METHODS

Preparation of extracts

The plant material was collected from Tiruverkadu, authenticated by a botanist and deposited in the department under voucher name KJ20261 for future reference. The cleaned and shade-dried leaves of *T. cordifolia* were broken into tiny bits and ground into a fine powder and utilised further (Sutariya *et al*., 2024). Fresh and dried extracts were prepared by adding leaf extract to solvent in a 1:10 ratio for ethanol and hexane and a 1:5 ratio for aqueous solution.

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Phytochemical qualitative analysis

The dry and freshly prepared ethanol, aqueous and hexane leaf extracts were subjected to typical phytochemical examination and indicated high phytochemical content (Gupta *et al.*, 2024).

DPPH radical scavenging assay

Fresh and dry plant extracts from all solvents at concentrations ranging from 50 to 300 µg/mL were subjected to a 0.1 mM DPPH solution in methanol at a 1:1 ratio, vortexed, and kept in the dark at room temperature for 30 min, and absorbance was measured using a UV-vis spectrophotometer at 517 nm and compared to the DPPH methanol control and distilled water blank (Sinha and Biswas, 2016). The antioxidants flavonoids and phenolics donate electrons or hydrogen and scavenge free radicals by donating electrons or hydrogen atoms, denoted by a decrease in absorbance (Patel *et al.*, 2011).

$$DPPH\ radical\ inhibition\ \% = \frac{control - sample}{control} \times 100$$

Phosphomolybdenum reduction assay

In the phosphomolybdenum reduction assay, the extract (20-120 µg/mL) was mixed with a reagent containing ammonium molybdate, sodium phosphate, and sulphuric acid at 90°C for 30 min. The extract's antioxidants reduce Mo (VI) to Mo(V), resulting in a green complex, which is measured at 695 nm compared to a distilled water blank (Pooja, 2023 and Prieto *et al.*, 1999).

$$\text{Phosphomolybdenum reduction } \% = \frac{\text{control} - \text{sample}}{\text{control}} \times 100$$

Antimicrobial activity

The antibacterial and antifungal potential of fresh and dry *T. cordifolia* leaf extracts (hexane, ethanol and aqueous) was assessed by testing their antimicrobial activity against gram-positive *S. aureus*, gram-negative *E. coli* and *Candida albicans*, respectively, by measuring distinct zones of inhibition (Shikha *et al.*, 2024; Kumar *et al.*, 2013).

FTIR analysis

The FTIR spectra decode functional groups and chemical bonds within molecules by analysing their infrared absorption patterns (Ashokkumar *et al.*, 2014; Priya *et al.*, 2017).

GC-MS Analysis

The dried leaf extract was soaked in ethanol (1:10) for 6 hr at room temperature in a shaker incubator, filtered and subjected to GC-MS analysis for identifying and quantifying bioactive compounds and other phytochemicals responsible for therapeutic effects (Suresh *et al.*, 2017).

Statistical analysis

The experimental results were triplicated and presented as mean±standard deviation for all assays, and the results were analysed using OriginPro and Microsoft Excel, and significant differences were noted using one-way ANOVA, and a value of $p<0.05$ was considered statistically notable.

RESULTS

Phytochemical Qualitative Analysis

The qualitative phytochemical screening of the ethanol and aqueous *T. cordifolia* leaf extracts showed pharmacologically significant alkaloids, flavonoids, tannins, glycosides, and steroid compounds. Hexane leaf extracts have shown only limited phytochemicals when compared to ethanol and hexane extracts. The dry leaf extracts have more bioactive phytoconstituents compared to the fresh sample, as the polar solvents in the fresh sample influence the efficiency of the compounds, while the dry solvent has more phytochemical efficiency, as it has concentrated forms of bioactive chemicals and fewer polar compounds. Thus, the comparative analysis of the phytochemicals of fresh and dry *T. cordifolia* revealed the potential of ethanolic extract in pharmacological analysis (Table 1).

Antioxidant Assay

DPPH assay

The *T. cordifolia* leaf aqueous extracts showed the highest radical scavenging activity in both fresh (up to 90.66±1.12%) and dry (up to 95.49±0.96%) samples. Ethanolic extracts showed moderate activity in fresh samples up to 72.34±1.45%, while dry samples exhibited 80.12±1.21%, while hexane extracts showed the lowest activity for both fresh, up to 33.87±0.98%, and dry, up to 34.65±1.05%. Aqueous extracts were the most effective antioxidants. All triplicate analyses were expressed as mean±standard deviation values, with ANOVA showing notably higher activity for the aqueous extract as well as the dry leaf sample ($p<0.05$) compared to the ethanol, hexane and fresh leaf extracts (Figure 1).

Phospho-molybdenum assay

The radical reduction of dry and fresh *T. cordifolia* leaf extracts was determined using the phospho-molybdenum reduction method. Aqueous extracts showed the highest antioxidant activity, with fresh samples reaching up to 97.02±0.88% inhibition and dry leaves around 95.18±1.03%, while the ethanol extracts showed moderate activity for both fresh, with 94.52±1.12%, and dry, with 96.34±1.08%, while the hexane extracts showed lower activity for both fresh samples, with 90.85±1.05%, and dry samples, with 88.67±1.21%. The aqueous and ethanol extracts typically showed higher radical reduction activity ($p<0.05$) compared to the hexane extract, and notably no differences were noted between fresh and dry aqueous extracts ($p>0.05$) (Figure 2).

Antimicrobial activity

The fresh and dry leaf ethanolic extract subjected to antimicrobial sensitivity was quantitatively assessed by measuring the diameter of the inhibition zones formed on the Petri dish cultures. The maximum zone of inhibition of the fresh sample for *Escherichia coli* was 14±0.82 mm at a dose of 1000 µg/mL, while the dry sample for *Candida albicans* was 12±0.75 mm at the same dose. These differences may be due to changes in the concentration and stability of secondary metabolites during the drying process. Antimicrobial analysis was performed using ethanolic extracts; hence, solvent-wise comparison was not carried out for this assay. Since only the most effective ethanolic extract was used, solvent-wise statistical antimicrobial comparison was not performed. An antimicrobial assay graphical representation was provided (Figure 1).

FTIR analysis

The fresh ethanolic leaf extract has the presence of aliphatic primary amines (3346.50 cm^{-1}), alkanes (2974.23 cm^{-1} and 2889.37 cm^{-1}), and thiocyanates (2416.81 cm^{-1}), which represent the strong absorption bands, and isothiocyanates (2158.35 cm^{-1}) indicate the sulphur-containing chemicals. The presence of nitriles (2025.26 cm^{-1}) represents the derivation of cyanide. The presence of aromatic compounds (1975.11 cm^{-1}) and imines (1651.07 cm^{-1}) identifies the conjugated ring structures and nitrogen-containing functional groups. The aldehydes (1388.75 cm^{-1}) and phenols (1327.03 cm^{-1}) indicate the presence of oxygenated organic molecules. Secondary alcohols (1085.92 cm^{-1}) and aromatic amines (1278.81 cm^{-1}) define the sample complexity, while the anhydrides (1043.49 cm^{-1}) show the probable carboxyl derivatives. Finally, the 1,3-disubstituted aromatic molecules (877.61 cm^{-1}) indicate the presence of substituted benzene derivatives. These functional groups can vary in intensity, from weak to strong, revealing the fresh leaf sample's vast chemical variety (Figure 3).

The dry leaf extract showed a large absorption band at 3325.28 cm^{-1}, which represents the presence of the primary amines' N-H stretching group.

The peaks at 2972.31 cm^{-1} and 2885.51 cm^{-1} indicate the presence of C-H stretching in alkanes. The absorption at 2023.33 cm^{-1} and 2160.27 cm^{-1} represents the isothiocyanate groups and thiocyanate, while the peak at 1975.11 cm^{-1} belongs to the allene functionalities.

A band at 1654.92 cm^{-1} indicates the presence of an imine or oxime. Other prominent peaks indicate the alkane vibrations in the range 1415.75 cm^{-1} and the aldehyde stretching at 1384.89 cm^{-1}. The band at 1327.03 cm^{-1} shows the phenol absorption. The prominent band at 1280.73 cm^{-1} indicates aromatic amines. The anhydrides and secondary alcohols can be identified at the peaks at 1045.42 cm^{-1} and 1046.42 cm^{-1}.

The presence of alkenes (920.05 cm^{-1}), 1,3-disubstituted alkenes (879.54 cm^{-1}), and halo compounds (792.74 cm^{-1}) identifies the structural makeup of the material. The fresh and dry extracts showed the presence of several organic functional groups, implying a complex molecular structure.

GCMS analysis

GC-MS analysis of the ethanol leaf extract of *T. cordifolia* is shown in Table 2. The GCMS results concluded the presence of 189 compounds in the sample. The GCMS analysis showed the presence of some major volatile compounds found in the extract may be responsible for its healing properties, supporting its traditional use in medicine. The bioactive compounds such as fatty acid methyl esters, phthalates, aldehydes, hydrocarbons, and steroidal derivatives indicate the presence of lipid-derived

Table 1: Qualitative phytochemical screening of Fresh (F) and Dry (D) leaf extracts of *Tinospora cordifolia* using ethanol, hexane, and aqueous solvents.

Phytochemicals	Eth-F	Eth-D	Hex-F	Hex-D	Aq.-F	Aq.-D
Alkaloids	1	1	1	0	1	1
Terpenoids	0	1	1	0	0	1
Phenolic Compounds	0	1	1	0	0	1
Tannins	1	1	0	0	1	1
Flavonoids	1	1	0	1	1	1
Saponins	0	1	0	0	0	1
Carbohydrates	1	1	0	0	1	1
Proteins	1	0	0	0	1	0
Glycosides	1	1	0	0	1	1
Quinones	1	1	0	0	1	1
Steroids	1	1	0	0	1	1

Note: 1 indicates presence and 0 indicates absence; Eth- Ethanol; Hex- Hexane; Aq- Aqueous; F- Fresh; D-Dry.

phytoconstituents with metabolic diversity and therapeutic significance. This study has highlighted the traditional use of plants and its application in pharmaceutical and nutraceutical development (Figure 4).

DISCUSSION

The current investigation highlights the significant phytochemical diversity and biological potential of *Tinospora cordifolia* leaf extracts, thereby reinforcing its relevance in traditional therapeutics. The consistent detection of flavonoids, alkaloids, tannins, saponins, and terpenoids across all extracts indicates a broad spectrum of secondary metabolites, which are widely associated with pharmacological activities. Similar phytochemical profiles have been reported in recent studies, where the presence of phenolic-rich compounds contributed significantly to the antioxidant potential (Boro *et al.*, 2025).

The antioxidant potential observed through DPPH and phosphomolybdenum assays confirms the ability of *T. cordifolia* extracts to scavenge free radicals effectively. This activity is primarily linked to phenolic and flavonoid constituents, which act as electron donors and reduce oxidative stress. Previous studies have demonstrated comparable concentration-dependent antioxidant activity in *T. cordifolia*, particularly in polar solvent extracts, supporting the findings of the present work (Parimi *et al.*, 2026). The improved activity in dry leaf extracts observed in this study may be attributed to reduced enzymatic degradation and increased concentration of active compounds during dehydration.

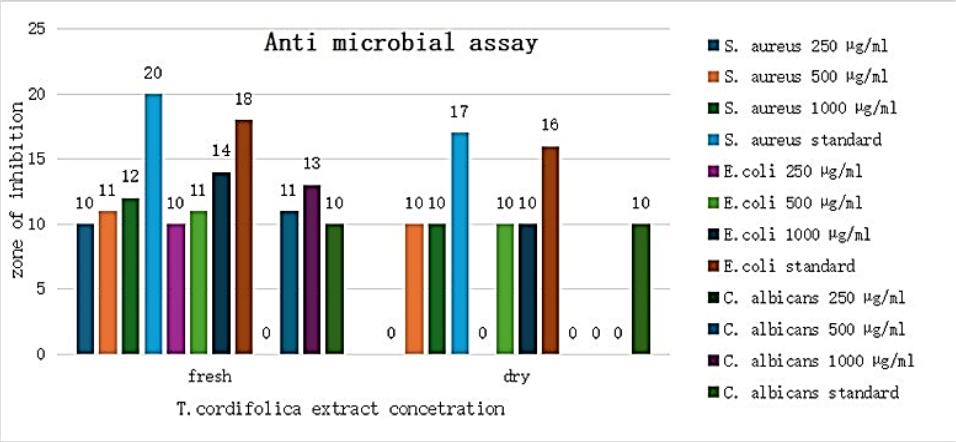


Figure 1: Antimicrobial activity of fresh and dry *T. cordifolia* leaves.

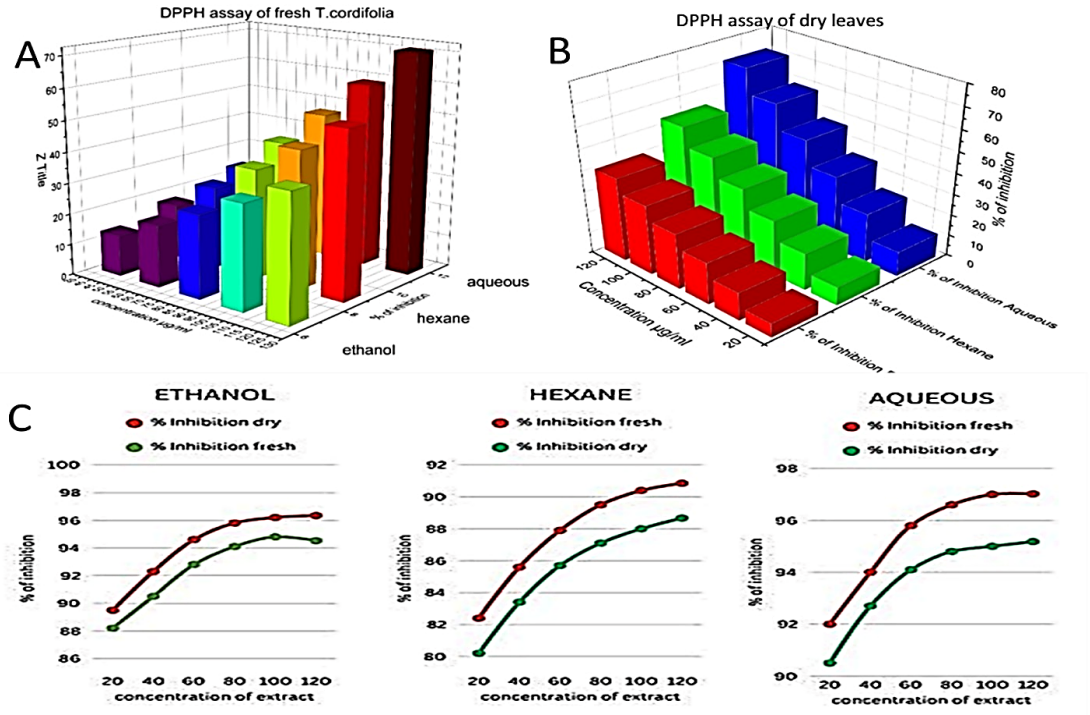
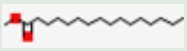
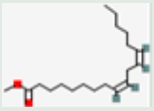
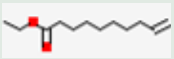
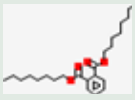
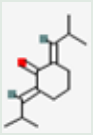
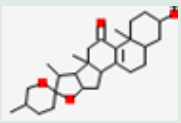


Figure 2: (A-B) DPPH assay and (C) Phosphomolybdenum assay of fresh and dry *T. cordifolia* leaves.

Table 2: GC-MS analysis of ethanol leaf extract of dried *T. cordifolia*.

Sl. No.	Compound Name	RT	MF	MW	MS
1.	2-Amino-4-methyl-4-pentenoic acid	6.9	$C_6H_{11}NO_2$	129.16	
2.	Hexadecanoic acid, methyl ester	21.8	$C_{17}H_{34}O_2$	270.45	
3.	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	23.9	$C_{19}H_{34}O_2$	294.47	
4.	Methyl stearate	24.3	$C_{19}H_{38}O_2$	298.51	
5.	Ethyl 9-decenoate	25.4	$C_{12}H_{22}O_2$	198.30	
6.	13-Tetradecenal	26.2	$C_{14}H_{26}O$	210.35	
7.	Nonadecane, 9-methyl-	27.3	$C_{20}H_{42}$	282.55	
8.	Hexadecanal	27.7	$C_{16}H_{32}O$	240.43	
9.	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	28.2	$C_{57}H_{104}O_6$	885.43	
10.	Di-n-octyl phthalate	29.02	$C_{24}H_{38}O_4$	390.56	
11.	Nonadecane, 9-methyl-	29.4	$C_{20}H_{42}$	282.5	
12.	Cyclohexanone, 2,6-bis(2-methylpropylidene)-	30.03	$C_{14}H_{22}O$	206.32	
13.	2-Methylhexacosane	30.37	$C_{27}H_{56}$	380.73	
14.	Pentacosanoic acid, methyl ester	30.74	$C_{26}H_{52}O_2$	396.69	
15.	Spirost-8-en-11-one, 3-hydroxy-, (3. beta.,5. alpha.,14. beta.,20. beta.,22. beta.,25R	31.14	$C_{27}H_{40}O_4$	428.6	

Note: RT: Retention Time; MF: Molecular Formula; MS: Molecular structure; MW: Molecular weight.

FTIR analysis confirmed the presence of hydroxyl and carbonyl functional groups, which are characteristic of phenolic and bioactive molecules involved in redox reactions. Additionally, GC-MS profiling revealed multiple bioactive constituents, including esters, alkaloids, and fatty acids. Recent GC-MS studies have also reported similar phytochemical diversity, highlighting the presence of compounds with antimicrobial and antioxidant properties (Saxena *et al.*, 2025).

The antimicrobial activity observed in this study further validates the therapeutic potential of *T. cordifolia*. The extracts demonstrated inhibitory effects against bacterial strains, which may be attributed to the disruption of microbial membranes and interference with metabolic processes by phenolic and terpenoid compounds. Comparable antimicrobial efficacy has been reported in recent studies, where *T. cordifolia* extracts exhibited significant antibacterial and bioactive effects (Ezhilarasu *et al.*, 2023). Moreover, advanced phytochemical investigations have emphasised that the biological activity of *T. cordifolia* is governed by the synergistic interaction of multiple compounds rather than a single constituent (Arthanari, 2024).

An important observation of the present study is the superior performance of dry leaf extracts compared to fresh samples. This suggests that drying enhances phytochemical stability and concentration, possibly due to reduced moisture content and

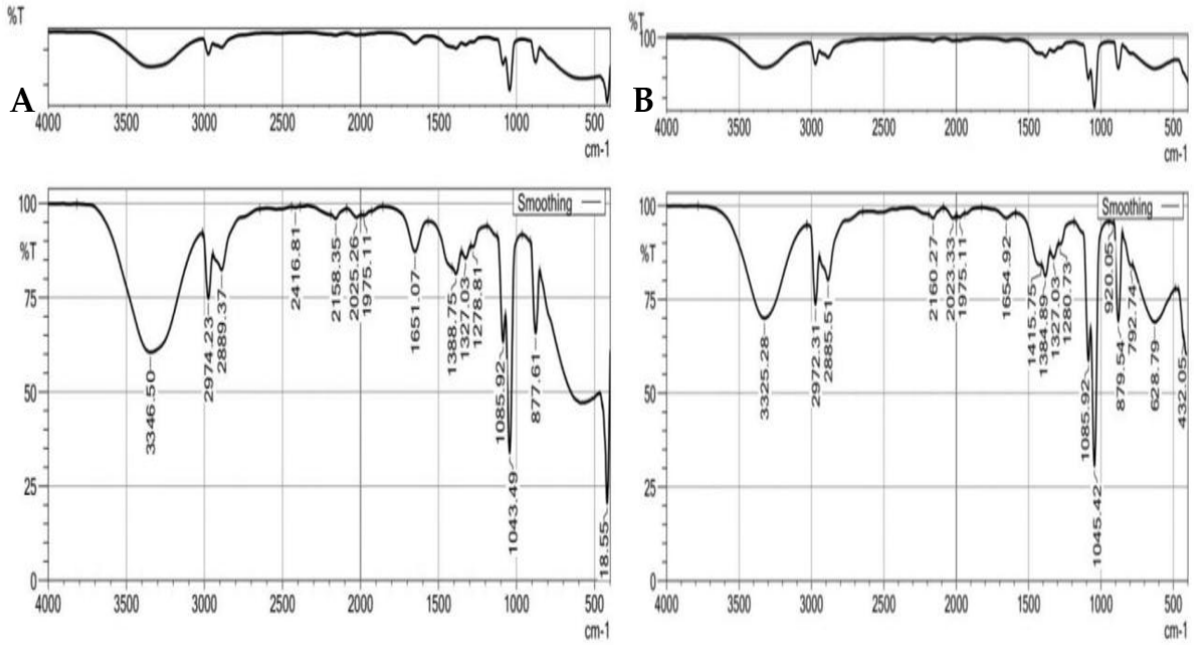


Figure 3: FTIR spectra of the ethanol extract of (A) fresh and (B) dry *T. cordifolia* leaves.

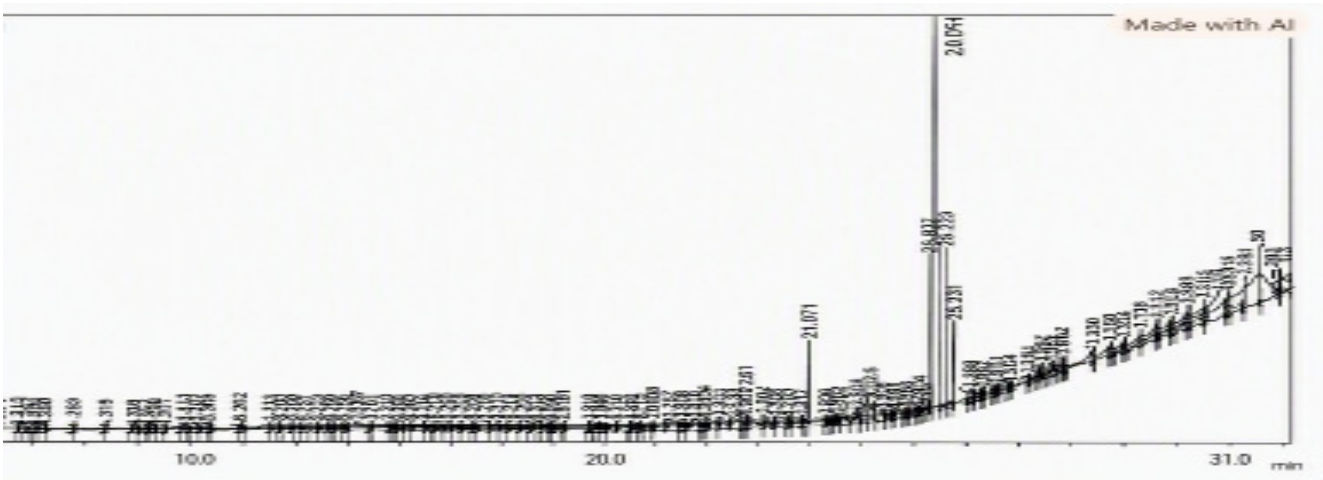


Figure 4: GC-MS chromatogram of ethanol leaf extract of dried *T. cordifolia*.

minimized enzymatic degradation. Similar findings have been reported in recent phytochemical studies, where processing conditions significantly influenced the yield and bioactivity of plant extracts (Sun *et al.*, 2025).

Overall, the study confirms that *Tinospora cordifolia* is a potent source of natural antioxidants and antimicrobial agents. The integration of phytochemical screening, FTIR analysis, GC-MS profiling, and biological assays provides a comprehensive understanding of its therapeutic properties. The findings emphasise the importance of extraction methods and processing conditions in enhancing bioactivity. However, further investigations focusing on compound isolation, mechanistic pathways, and *in vivo* validation are required to establish its clinical applicability.

CONCLUSION

This study examined the phytochemical composition and biological activities of *T. cordifolia using* fresh and dry leaf extracts prepared with ethanol, hexane, and water. The goal was to validate its traditional medicinal uses by analysing its chemical profile, antioxidant capacity, and antimicrobial effects. Phytochemical screening revealed the consistent presence of key secondary metabolites flavonoids, alkaloids, saponins, tannins, and terpenoids across all extracts. These compounds are known for their pharmacological properties, including antioxidant, antimicrobial, and anti-inflammatory activities. Antioxidant assays showed that all extracts had strong free radical scavenging abilities, indicating potential to counter oxidative stress linked to chronic illnesses. FTIR spectroscopy confirmed functional groups such as hydroxyl and carbonyl, typically found in biologically

active molecules. Antimicrobial testing demonstrated that both fresh and dry extracts effectively inhibited the growth of selected bacterial strains. Notably, GC-MS analysis of the ethanol extract from dry leaves revealed a wide range of bioactive compounds associated with antibacterial and antioxidant properties. Overall, dry leaf extracts consistently outperformed fresh ones, showing higher phytochemical content, stronger antioxidant activity, and better antimicrobial efficacy. This suggests that drying enhances the concentration and stability of active compounds. Overall, the findings highlight *Giloy* promise as a natural source of therapeutic agents. Further studies, particularly those isolating individual compounds and conducting *in vivo* tests, are recommended to explore its full medicinal potential.

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ABBREVIATIONS

FTIR: Fourier Transform Infrared Spectroscopy; **GC-MS:** Gas Chromatography-Mass Spectrometry; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **T. cordifolia:** *Tinospora cordifolia*; **RT:** Retention Time; **M.W.:** Molecular Weight; **MF:** Molecular Formula; **UV-vis:** Ultraviolet-visible; **Eth:** Ethanol; **Hex:** Hexane; **Aq:** Aqueous; **F:** Fresh; **D:** Dry.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed to this work. The concept and design of the study, acquisition of data, analysis and interpretation of data, materials and methodology, concept, design, definition of intellectual content, literature search, clinical studies, experimental studies, data acquisition, data analysis, statistical analysis, and analysis were performed by [Nishalini Palani] and [Sharmila K.J.]. Drafting the article or revising it critically for important intellectual content, manuscript preparation, manuscript editing, manuscript review, and drafting were performed by [Santhiya Jayakumar] and [Aarthy Sundaram], ORCID ID: 0009-0003-4124-3973, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Guarantor - [Sharmila K.J., Santhiya Jayakumar].

DECLARATION STATEMENT

The submitted work is original and has not been published elsewhere in any form or language (partially or in full), unless the new work concerns an expansion of previous work. Results were presented clearly, and no manipulation was done. All the authors mentioned have read and understood and have complied, as applicable, with the statement on "Ethical responsibilities of Authors" as found in the "Instructions for Authors" in the journal guidelines.

SUMMARY

A pharmacology study of *T. cordifolia* leaves bioactive phytoconstituents showed presence of fatty acid methyl esters, phthalates, phenolics and more, which was confirmed using GC-MS. The compounds were verified with the NIST library and past publications; this study supports the traditional medicinal usage of plants and indicates its potential pharmacological applications. This study highlights the phytochemical diversity of *T. cordifolia* leaf extracts and provides a foundation for further biological and therapeutic investigations.

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