

Identification of Bioactive Compounds from *Tinospora cordifolia* leaf through GC-MS Analysis

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Abstract

Tinospora cordifolia (Willd.) Hook.f. & Thomson. is a medicinally important plant known for its diverse phytochemical constituents. The present study was undertaken to characterize the phytochemical analysis of the methanolic leaf extract of *T. cordifolia* by gas chromatography–mass spectrometry (GC–MS). The methanolic extract was prepared from the leaf and subjected to GC–MS analysis for qualitative profiling of its chemical constituents. A total of six compounds were identified, among which 2-Heptenoic acid represented the major detected constituent (83.26%), followed by n-Hexadecanoic acid (7.39%) and Neophytadiene (3.96%). Other constituents occurred at comparatively lower abundances. The GC–MS profile demonstrates the presence of diverse chemical constituents in the leaf extract and provides preliminary evidence for its potential as a source of biologically relevant phytochemicals.

Introduction

Medicinal plants therefore remain important resources for the treatment and management of various health conditions and also have considerable potential for the development of indigenous and plant-based therapeutic products (Sarad *et al.*, 2017).

T. cordifolia widely referred Guduchi or Amrita, is a medicinal plant, large and glabrous deciduous climbing shrub belonging to the

moonseed family Menispermaceae. It holds a prominent history of use in traditional Ayurvedic medicine for the management of various ailments, such as diabetes, jaundice, rheumatism, urinary disorders, skin diseases, anemia, gout, inflammation, and allergic disorders, along with demonstrating anti-periodic and radioprotective properties (Sharma *et al.*, 2012; Sonkamble *et al.*, 2015).

T. cordifolia has a long history of use in

Ayurvedic and traditional healthcare practices in India. Various parts of the plant have been investigated for their diverse pharmacological properties, including immunomodulatory, antipyretic, anti-inflammatory, antioxidant, antidiabetic, antiallergic, and antiarthritic activities (Singh *et al.*, 2003). *T. cordifolia* possesses a diverse range of secondary metabolites, include alkaloids, glycosides, steroids, flavonoids, phenolic compounds, tannins, terpenoids, polysaccharides, essential oils, and fatty acids. Several characteristic constituents, such as β -sitosterol, clerodane-type furanoditerpenes, columbin, tinosporine, tinosporide, tinosporaside, cordifolide, cordifol, and heptacosanol, have been documented from different parts of the plant (Sharma *et al.*, 2019). Previous studies have indicated that the biological activities of *T. cordifolia* polysaccharides can vary depending on the extraction procedure employed. Methanolic extracts of the stem have been reported to exhibit higher antioxidant activity, whereas the methanolic leaf extract demonstrated stronger antiradical activity. In addition, the ethyl acetate stem extract showed notable antiradical potential (Jain *et al.*, 2010; Upadhyay *et al.*, 2013; Nithya *et al.*, 2025).

GC–MS is a widely employed analytical technique for the qualitative characterization of phytochemicals present in plant-derived extracts. The technique integrates the separation capability of gas chromatography with the

molecular identification capacity of mass spectrometry, enabling the detection and characterization of diverse chemical constituents. GC–MS is particularly useful for profiling compounds that are volatile or amenable to gas chromatographic analysis, including fatty acids, terpenoids, steroids, hydrocarbons and other secondary metabolites. The application of GC–MS in plant research therefore provides valuable information on the chemical composition and potential bioactive constituents of botanical extracts (Gautam *et al.*, 2020). In the present study, the methanolic leaf extract of *T. cordifolia* was subjected to GC–MS analysis to characterize its phytochemical constituents.

Materials and Methods

Sample collection

In this study, the leaf of *T. cordifolia* were collected from St. Jude's College Thoothoor, Kanniyakumari District, Tamil Nadu.

Sample preparation

The collected plant materials were thoroughly washed with tap water to remove adhering contaminants and were subsequently cut into small pieces. The materials were shade-dried under ambient conditions and then ground into a fine powder. The powdered samples were stored in airtight containers until further extraction.

Sample Extraction

For solvent extraction, 1 g of accurately weighed *T. cordifolia* leaf sample was placed in a 25 mL test tube and mixed with 5 mL of analytical-

grade methanol. The mixture was homogenized using a vortex mixer for 2 min. Subsequently, the sample was subjected to extraction in a mini rotary flask shaker (MAC-MFRS-450 × 450, Macro Scientific Works Pvt. Ltd., Delhi, India) at 150 rpm for 14–16 h at room temperature. After extraction, the mixture was passed through Whatman No. 1 filter paper to obtain a clear filtrate. Where necessary, further purification of the extract was carried out using sodium phosphate buffer. The resulting extract was transferred to suitable containers and maintained under refrigerated conditions until GC–MS analysis.

GC–MS Analysis

The prepared methanolic extract was transferred into GC–MS vials and stored under refrigerated conditions prior to analysis. GC–MS analysis was performed using an Agilent GC–MS system equipped with an Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas at a flow rate of 1 mL min⁻¹. An aliquot of 2 μL of the sample was injected at 280 °C using a 10:1 split ratio. The oven temperature was initially maintained at 110 °C for 3.5 min, followed by an increase to 200 °C at a rate of 10 °C min⁻¹ without an additional hold. The temperature was subsequently raised to 280 °C at 5 °C min⁻¹ and maintained for 12 min, giving a total run time of 40.5 min. Mass spectral detection was carried out in electron ionization mode at 70 eV, with the mass range scanned from 50 to 550 amu. The ion source and transfer

line temperatures were maintained at 250 °C and 290 °C, respectively. The phytochemical constituents were identified by comparing the obtained mass spectra with those available in the National Institute of Standards and Technology (NIST) Mass Spectral Library and by referring to standard spectral interpretation methods (Stein 1994; Mayorov *et al.*, 2017 and 2018; Mikaia *et al.*, 2022).

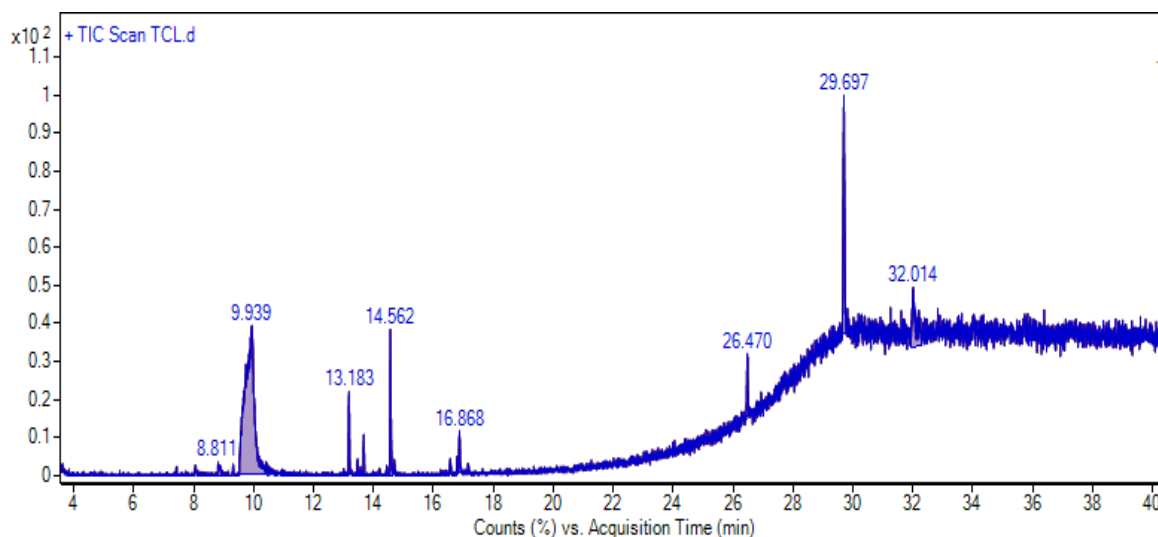
Result and Discussion

GC–MS analysis of methanolic extract of *T. cordifolia* leaf sample revealed the presence of six compounds, with retention times ranging from 9.93 to 16.86 min. The identified compounds, their molecular weights, molecular formulae, retention times, and corresponding peak area percentages are presented in Table 1. Among the detected compounds, 2-Heptenoic acid was the predominant constituent, showing the highest peak area of 83.26% at a retention time of 9.93 min. The molecular weight of 2-Heptenoic acid was 128, with the molecular formula C₇H₁₂O₂.

The other compounds detected included n-Hexadecanoic acid (7.39%), Neophytadiene (3.96%), Cyclobarbitol (2.81%), Citronellol (1.67%), and 1,2-Dioxaspiro [4.5] decan-3-one, 4-methylene- (0.90%). Among these, 1,2-Dioxaspiro [4.5] decan-3-one, 4-methylene- showed the lowest relative abundance with the molecular formula C₉H₁₂O₃.

leaf extract**Table 1. GC MS profile of *T. cordifolia***

R.T	Compound Name	Molecular Weight	Formula	Peak Area %
9.93	2-Heptenoic acid	128	C ₇ H ₁₂ O ₂	83.26
13.18	Neophytadiene	278	C ₂₀ H ₃₈	3.96
13.67	Citronellol	156	C ₁₀ H ₂₀ O	1.67
14.56	n-Hexadecanoic acid	256	C ₁₆ H ₃₂ O ₂	7.39
14.68	1,2-Dioxaspiro [4.5] decan-3-one, 4-methylene-	168	C ₉ H ₁₂ O ₃	0.90
16.86	Cyclobarbital	236	C ₁₂ H ₁₆ N ₂ O ₃	2.81

Figure 1. GC-MS chromatogram of *Tinospora cordifolia* extract

The phytochemical constituents of *T. cordifolia* include diverse secondary metabolites such as terpenoids, alkaloids, phenolic compounds, steroids and fatty acids, which are associated with several biological and pharmacological activities (Papitha *et al.*, 2016; Sharma *et al.*, 2019). In the present study, GC–MS analysis of the

methanolic extract of *T. cordifolia* identified six compounds. Among these, 2-Heptenoic acid was the predominant constituent with a peak area of 83.26%, followed by n-Hexadecanoic acid (7.39%) and Neophytadiene (3.96%). Citronellol, Cyclobarbital and 1,2-Dioxaspiro [4.5] decan-3-one, 4-methylene- were detected at

1.67%, 2.81% and 0.90%, respectively.

Previous investigations have reported the occurrence of n-Hexadecanoic acid and Neophytadiene among the phytoconstituents of *T. cordifolia* (Siswadi *et al.*, 2021; Zhang *et al.*, 2010). n-Hexadecanoic acid has been reported to possess antioxidant and anti-inflammatory properties, while Neophytadiene has also been associated with various biological activities (Paniagua *et al.*, 2017). The detection of these compounds in the present study is in accordance with earlier reports. However, differences in the relative abundance and composition of the detected compounds may be attributed to variations in plant material, extraction solvent and experimental conditions (Sharma *et al.*, 2012; Paniagua *et al.*, 2017).

Neophytadiene was identified in the present methanolic extract of *T. cordifolia* with a peak area of 3.96%. A recent study reported

Conclusion

The present study demonstrated the presence of diverse phytochemical constituents in the methanolic extract of *T. cordifolia* leaf through GC–MS analysis. Six compounds were identified, with Neophytadiene and n-Hexadecanoic acid among the notable constituents. The observed phytochemical profile highlights the chemical diversity of *T. cordifolia* and

Neophytadiene among the phytoconstituents identified by GC–MS analysis of *T. cordifolia* leaf and stem extracts and also demonstrated antioxidant and antifungal activities of the extracts (Rao *et al.*, 2026). Earlier GC–MS investigations have likewise reported the occurrence of Neophytadiene in *T. cordifolia* extracts (Siswadi *et al.*, 2021; Zhang *et al.*, 2010; Umamaheshwar *et al.*, 2026). The detection of Neophytadiene in the present study is therefore consistent with previous reports and further supports the chemical diversity of the methanolic extract. Overall, the GC–MS analysis revealed a diverse phytochemical profile in the methanolic extract of *T. cordifolia* with Neophytadiene and n-Hexadecanoic acid among the notable identified constituents. The presence of these compounds is consistent with previous reports and highlights the potential chemical diversity of *T. cordifolia*

supports its potential as a source of biologically relevant natural compounds. Further studies are required to validate the biological activities of the identified constituents and to establish their potential applications.

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