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Research Article

GC-MS Based Phytochemical Profiling of *Tinospora cordifolia* Ethanolic Extract: Identification of Bioactive Compounds

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ABSTRACT

Tinospora cordifolia (Giloy) is a renowned medicinal plant in traditional Indian medicine, recognized for its immunomodulatory, antioxidant, anti-inflammatory, hepatoprotective, and antidiabetic properties. These therapeutic effects are attributed to its diverse secondary metabolites, including alkaloids, diterpenoid lactones, glycosides, and steroids. This study aimed to perform GC-MS based phytochemical profiling of the ethanolic leaf extract of Tinospora cordifolia to identify and characterize its bioactive volatile constituents. Fresh leaves of T. cordifolia were collected from Latur, Maharashtra, shade-dried, and powdered. The powdered material (50 g) was subjected to maceration with 90% ethanol for 72 hours. The concentrated extract was analyzed using gas chromatography-mass spectrometry, and compounds were identified by comparing retention times and mass spectral fragmentation patterns with library data. The GC-MS analysis revealed major bioactive compounds. These bioactive compounds exhibit anti-inflammatory, immunomodulatory, antioxidant, antimicrobial, and anticancer properties. The findings provide scientific validation for the traditional Ayurvedic uses of Tinospora cordifolia in treating fever, diabetes, inflammatory disorders, and immune deficiencies. Further in vitro studies are recommended to evaluate the mechanisms of action of the individual bioactive constituents.

1. Introduction

Tinospora cordifolia (Giloy) is a well-known medicinal plant in traditional Indian medicine, recognized for its diverse therapeutic properties including immunomodulatory, antioxidant, and hepatoprotective activities (Saha & Ghosh, 2012). Its immunomodulatory properties have been extensively documented, supporting its traditional use in managing inflammatory and immune-related disorders (Yates, Bruno, & Yates, 2022). The plant exhibits multifarious pharmacological paradigms including immunomodulatory, antioxidant, anti-inflammatory, hepatoprotective, and antidiabetic activities (Gupta, Gupta, & Bajpai, 2024). Its therapeutic significance is attributed to a rich reservoir of secondary metabolites such as alkaloids, diterpenoid lactones, glycosides, and steroids.

A comprehensive review encompassing both Ayurvedic and modern perspectives highlights its physiological, medicinal, and safety aspects, demonstrating its efficacy in various disease conditions (Ninama et al., 2022). Studies have demonstrated that the ethanolic extract of its leaves exhibits potent antioxidant and hepatoprotective activities, as evidenced by both in vitro and in vivo investigations (Hussien et al., 2023). These pharmacological properties are attributed to the presence of diverse bioactive secondary metabolites. Gas chromatography-mass spectrometry (GC-MS) is a reliable analytical technique for phytochemical profiling.

Tinospora cordifolia is a medicinally important plant recognized for its rich repository of bioactive phytochemicals. Gas chromatography-mass spectrometry (GC-MS) serves as a powerful analytical tool for exploring these compounds, with significant implications for drug development and functional food applications (Reddy, Sampathkumar, & Godishala, 2025). The identification and characterization of these phytochemicals are essential for understanding the plant's pharmacological properties. This study aims to perform GC-MS based phytochemical profiling of the ethanolic extract of *T. cordifolia* to identify and characterize its bioactive volatile constituents.

2. Materials and Methods

2.1 Plant Material Collection, Drying, and Powder Preparation

Fresh, healthy, and disease-free leaves of *Tinospora cordifolia* (Giloy) were collected from Latur, Maharashtra, India. The

Table 1. Number of mutations and unique mutations observed across the SARS-CoV-2 genome

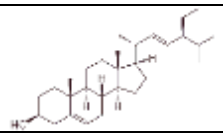
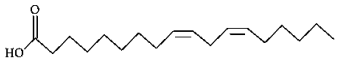
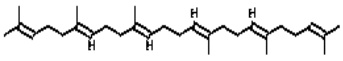
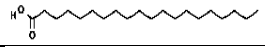
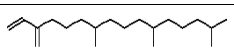
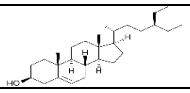
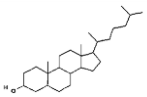
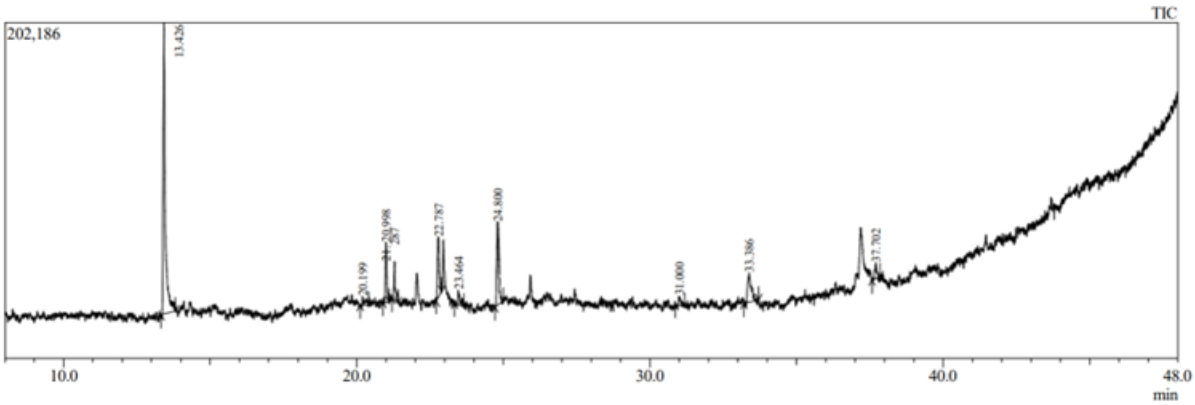
Sr. No.	Name of Compound	Retention time	Molecular weight	Molecular formula	Molecular structure
1	Stigmasterol	13.35	412.69	C29H48O	
2	9,12-Octadecadienoic acid	20.25	280.45	C18H32O2	
3	Squalene	21.52	410.72	C30H50	
4	Tetradecanoic acid	22.81	228.37	C14H28O2	
5	Eicosanoic acid	23.5	312.53	C20H40O2	
6	Neophytadiene	25.2	278.52	C20H38	
7	β-Sitosterol	31.11	414.71	C29H50O	
8	Cholestanol	34.06	388.67	C27H48O	

Fig 1. Total Ion Chromatogram (TIC) of GC–MS analysis of the ethanolic leaf extract of *Tinospora cordifolia*



collected leaves were thoroughly washed under running tap water for 5–10 min to remove soil, dust, and surface debris, followed by two successive rinses with distilled water to eliminate contaminants. The cleaned leaves were shade-dried at room temperature (25–30°C) for 10 days with periodic turning to ensure uniform drying and prevent microbial growth. The completely dried leaves were coarsely powdered using a mortar and pestle. The powder was stored in an airtight container at room temperature in a dry, dark place for further use.

2.2 Preparation of Ethanolic Extract

The powdered plant material 50g was subjected to extraction using 90% ethanol as the solvent via maceration. The powder was placed in a clean, dry conical flask and soaked with 500 mL of ethanol. The flask was sealed with aluminium foil and kept at room temperature with occasional shaking (every 6-8 hours) for 72 hours (3 days) to facilitate maximum extraction of bioactive compounds. After the maceration period, the mixture was filtered through muslin cloth, then with Whatman No. 1 filter paper. All filtrates were concentrated using a rotary vacuum evaporator under reduced pressure at 40-50°C. The concentrated extract was then air-dried and stored for further use.

3. Results and Discussion

3.1 GC–MS Chromatogram

GC-MS was performed at Infinite Biotech, Sangli, Maharashtra. The analysis was carried out using a gas chromatography-mass spectrometry instrument. The sample was analyzed with an injection volume of 1.00 µL. The sample was passed through column and retention time was recorded. The chromatogram revealed the presence of multiple peaks corresponding to various bioactive compounds. Each peak was identified by comparing its retention time with the provided library. The retention time, molecular formula, molecular weight and compound name were recorded for each detected constituent. The analysis demonstrated that the ethanolic extract contains a

diverse range of phytochemicals including fatty acids, sterols, terpenoids, and other secondary metabolites.

3.2 Identified Compounds

The identification of bioactive components in the extract was performed by comparing their retention indices, peak area percentages, and mass spectral fragmentation patterns with data provided by Infinite Biotech, Sangli, Maharashtra. The name, molecular weight, molecular formula and structure of each identified compound were subsequently determined.

The GC-MS analysis of the ethanolic leaf extract of *Tinospora cordifolia* revealed bioactive compounds, including phytosterols, fatty acids, and terpenoids, supporting the plant's therapeutic potential. The identification of stigmasterol, β-sitosterol, and cholestanol is significant, as phytosterols possess anti-inflammatory and immunomodulatory properties. β-Sitosterol induces apoptosis in cancer cells and modulates immune responses, while stigmasterol exhibits neuroprotective and anti-diabetic effects (Adeboye et al., 2026; Namthabad et al., 2014). The presence of 9,12-octadecadienoic acid (linoleic acid), an essential omega-6 fatty acid, shows the extract's nutritional value through its anti-inflammatory and cardioprotective roles. The saturated fatty acids, tetradecanoic acid and eicosanoic acid, contribute antimicrobial activities (Cortes-López et al., 2021). Among terpenoids, squalene is recognized for its potent antioxidant and immunomodulatory activities, including its use as a vaccine adjuvant (Lou-Bonafonte et al., 2018). Neophytadiene enhances the anti-inflammatory profile by inhibiting pro-inflammatory enzymes such as lipoxygenase and cyclooxygenase (Phytomedicine, 2025; Lunavath et al, 2013; Swapna et al, 2024). The phytochemical diversity observed provides a molecular basis for the traditional Ayurvedic uses of T. cordifolia in treating fever, diabetes, inflammatory disorders, and immune deficiencies. Future studies employing HPLC-MS and in vivo bioassays are recommended to validate the pharmacological activities of the identified compounds. Overall, these findings support *Tinospora cordifolia* as a valuable source of natural therapeutic agents.

4. Conclusion

The GC-MS analysis of the ethanolic leaf extract of *Tinospora cordifolia* successfully identified major bioactive compounds: stigmasterol, 9,12-octadecadienoic acid, squalene, tetradecanoic acid, eicosanoic acid, neophytadiene, β-sitosterol, and cholestanol. These phytosterols, fatty acids, and terpenoids exhibit significant anti-inflammatory, immunomodulatory, antioxidant, antimicrobial, and anticancer properties, providing scientific validation for the traditional Ayurvedic uses of *Tinospora cordifolia* in treating fever, diabetes, inflammatory disorders, and immune deficiencies. The findings confirm that *T. cordifolia* is a rich source of pharmacologically active natural compounds. Further *in vitro* and *in vivo* studies are recommended to elucidate the mechanisms of action and to explore the therapeutic potential of the individual bioactive constituents.

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Competing interests:

The authors declare that they have no competing interests

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