PHYTOCHEMICAL PROFILING AND SPECTROSCOPIC CHARACTERIZATION OF METHANOLIC EXTRACT OF *TINOSPORA CORDIFOLIA*

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Abstract:

The aim of the current research was to determine the phytochemical profile and describe the methanolic stem extract of Tinospora cordifolia through Soxhlet extraction. Methanol, based on its polarity and effectiveness in extracting a broad spectrum of bioactive compounds, produced an extractive value of 19.41 percent, which signified high recovery of secondary metabolites. The initial phytochemical screening showed the presence of alkaloids, flavonoids, glycosides, steroids, triterpenoids, tannins, phenols, saponins, terpenoids, cardiac glycosides and quinones whereas carbohydrates were not found. This affirms the selectivity of methanol to secondary metabolites. The UV-Visible spectroscopic analysis revealed that it had strong absorption peak at 280 nm, which demonstrated that it contains phenolic and flavonoid compounds. The existence of functional groups hydroxyl (O-H), carbonyl (C=O), alkane (C-H) and aromatic (C=C) further confirmed the presence of the identified phytochemical constituents by FTIR spectroscopy. These results confirm the T. cordifolia use as a herbal medicine and give a chemical reason to its pharmacological effects. These findings justify the feasibility of the methanolic extract as a source of therapeutically active constituents and provide the foundation of future standardization and bioactivity-directed fractionation in pharmaceutical use.

Keywords: *Tinospora cordifolia*, methanolic extract, Soxhlet extraction, phytochemical screening, secondary metabolites, UV-Visible spectroscopy, FTIR analysis

1. Introduction

Tinospora cordifolia commonly called Guduchi or Giloy, Miers is often used in classical Ayurvedic practice because it shows a range of benefits such as lowering fever, fighting inflammation, acting as an antioxidant, improving the immune system, *T. cordifolia* and controlling diabetes. is found in the tropical parts of India and has long been used to help treat fever, diabetes, and problems of the liver [1]. Analyzing the composition of medicinal plants helps us reveal which chemicals in them make them medicinally beneficial. Methanolic extraction is highly preferred for separating various phytoconstituents, including alkaloids, flavonoids, terpenoids, tannins, and phenolics, because it is a polar solvent and can easily get through cell membranes [2]. A number of useful secondary metabolites in methanolic extracts of *T. cordifolia* are known to shape its pharmacological activity. These compounds assist in removing free radicals, adjust the immune response, and block microbial activity [3].

With the aid of modern analytical techniques like thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas

chromatography–mass spectrometry (GC-MS), FTIR and UV analysis it is now possible to study the important phytocomponents found in traditional medicines [4]. These approaches both prove the past uses of *T. cordifolia* and provide support for its role in new drugs. The significance of these studies is that they can prove traditional knowledge with science and reveal extra ingredients that may be used for medicine. Because of the rise in antimicrobial resistance, chronic illnesses, and lifestyle disorders, people are looking for safe, effective, and plant-based therapies. Applying methanolic extraction and modern profiling techniques to *T. cordifolia* may discover new details on what makes it effective and help make drugs from plants. For this reason, the research carried out in this study explores the chemicals in Tinospora cordifolia and its effects on the body, reinforcing its traditional use and assisting its inclusion in the field of modern medicine.

2. Literature Review

Pushpa *et al.* (2013) found that methanol was a powerful solvent for getting antioxidant substances from the stem of *Tinospora cordifolia*. Since methanol is very polar, it can dissolve many phytochemicals and is thus used to extract bioactive parts from plants. It was found that the methanolic extract of *T. cordifolia* demonstrated strong antioxidant activity in different in vitro studies, and this increased with higher doses. It has been found that this plant can minimize oxidative stress, stop the breakdown of lipids, and combat free radicals. For isolation of certain antioxidant compounds, the methanol extract was divided into fractions with silica gel chromatography. Despite having lower polyphenol levels than most others, one of the tested fractions turned out to be a strong antioxidant in the DPPH assay. Upon further examination by HPLC and mass spectrometry, the group found (−)-epicatechin, a flavonoid noted for its strong antioxidant and anti-viral effects. The presence of (−)-epicatechin in *T. cordifolia* may explain its longtime use in health treatments and for promoting immunity.

Kaur *et al.* (2016) performed a full analysis of the stem of *Tinospora cordifolia* to find out if it has therapeutic benefits. The focus of the study was on making phytochemicals and testing them for antibacterial and antioxidant effects. Using Soxhlet, the plant's stem material was soaked with different solvents and subjected to an initial screening of phytochemicals. There were found a group of key bioactive compounds, such as carbohydrates, glycosides, flavonoids, phenols, tannins, and amino acids. The antibacterial power of the extracts was evaluated in vitro using the agar well dilution method on *Staphylococcus aureus* and *Escherichia coli* bacteria. It was found that the activity showed a correlation with the dose, and the methanolic extract showed the best antibacterial results among all the doses used. The methanolic extract was found to be strong in antioxidant activity by scavenging free radicals well. The evidence indicates that *T. cordifolia* can be used to treat bacterial infections and oxidative stress naturally. It indicates that *T. cordifolia* contains various ingredients that can be useful in medicine.

Kumar *et al.* (2018) carried out an extensive study on what substances are in the hydro-ethanolic extract from *Tinospora cordifolia*, and its impact on biological processes. Several main bioactive components, such as tinocordioside, cordifolide A, palmatine, quercetin, β-sitosterol, heptacosanol, and syringin, were found through their quantitative and qualitative studies. Even though the extract did not show strong antibacterial or antifungal activities, it was very effective in several antioxidant tests, scoring 60–80% effectiveness in DPPH radical scavenging, metal chelating, ferric reducing antioxidant power (FRAP), and superoxide and nitric oxide radical scavenging. A significant positive relation was found between the antioxidant activity and the presence of high total phenolic and total flavonoid substances. When tested for protein binding, the result was as strong as that for aspirin, indicating that the molecule may play an important role in the body. Also, the extract with water and ethanol showed potent anti-inflammatory effects, suggesting its use for treating inflammatory diseases as practiced in culture. The study proves the potential of this herb, reflecting its ability to fight free radicals and reduce levels of inflammation, which makes it a good candidate for natural health products.

3. Research Methodology

Preparation of Stem Extract

Using a mortar and pestle, we ground small amounts of herbs to make a fine powder. Stems of *Tinospora cordifolia* were obtained from the nearby markets. Soil and dirt were first removed from the collected plant material by washing it with tap water, and next, it was rinsed with distilled water to keep it clean. After cleaning, the stems were left in the shade to preserve the important plant compounds from degradation. When the stems were dried, they were reduced to smaller pieces and ground into coarse powder using a grinder. The dried powder from the stems was placed in an airtight container to be used for later extraction steps [8].

Preparation of Stem Extract Using Hot Continuous Methanol Extraction

A Soxhlet extractor was used to process 750 g of the dried and ground stem with methanol being the solvent. The process of extracting the compound continued for 48 hours without interruption. After completing the extraction, the liquid was filtered through Whatman No. 1 filter paper prior to concentration on a water bath, leaving a dark reddish-brown extract [9]. The final formula used to determine the percentage yield was calculated.

Phytochemical Profiling of *Tinospora cordifolia*

Major bioactive constituents in the stem extract of *Tinospora cordifolia* were identified by a qualitative phytochemical screening according [6]

i. Alkaloids – Dragendorff's Reaction

A sample of the extract was mixed with Dragendorff's reagent (potassium bismuth iodide). When a reddish-brown precipitate formed, it indicated that alkaloids, with their pharmacological effects, were present.

ii. Carbohydrates – Molisch's Reaction

The extract was treated with α-naphthol, and then concentrated sulfuric acid was added. The existence of a purple-violet ring highlighted in the interface revealed that there were carbohydrates, as sugars were dehydrated and produced furfural derivatives that reacted with α-naphthol.

iii. Glycosides – Keller-Killiani Reaction

Glacial acetic acid, ferric chloride, and concentrated sulfuric acid were mixed with the extract. When a reddish-brown layer was followed by a bluish-green layer, it showed the presence of cardiac glycosides, which are sugar-containing chemicals.

iv. Steroids and Triterpenoids - Liebermann-Burchard Test

The extract was combined with acetic anhydride and concentrated sulfuric acid. The appearance of a green color pointed to steroids, while the presence of deep red color marked triterpenoids. Both are involved in hormone production and have biochemical actions.

v. Flavonoids - Shinoda Test

Turnings of magnesium and strong hydrochloric acid were mixed into the extract. The presence of a red or pink pigment in the extract indicated flavonoids, which are polyphenolic compounds that have antioxidant properties.

vi. Tannins and Phenolic Compounds - Ferric Chloride Test.

The extract was mixed with a few drops of ferric chloride solution. Formation of a blue-green or dark precipitate in the test showed that tannins or phenolic compounds are present in the plant, which have both antimicrobial and antioxidant value.

vii. Saponins – Froth test

Vigorous shaking was used to combine the extract with water. The presence of saponins, surfactant glycosides that have hemolytic and immunomodulatory effects, was confirmed by the appearance of a stable froth.

viii. Quinones – Alkaline Reagent Test

The sodium hydroxide and copper acetate solution were added to the extract. Quinones, which are aromatic compounds with antimicrobial and antioxidant effects, were revealed by the blue-green or red tint.

Characterization of the Extract

i. UV-Visible Spectroscopy

UV-Visible spectral analysis of the extracts was carried out by dissolving 1 gram of the dried extract in 10 ml of the respective solvent, as per the method described by previous studies . The prepared solution was scanned over a wavelength range of 200–800 nm using a Shimadzu UV-1800 PC spectrophotometer (Japan). Characteristic absorption peaks were recorded to assess the presence of specific phytoconstituents [11].

ii. Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR analysis was performed to identify the functional groups present in the methanol extract of *Tinospora cordifolia*. About 100 mg of the dried extract was finely ground and mixed with potassium bromide (KBr) to form a pellet suitable for analysis. The sample was then scanned in the range of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} using a Perkin Elmer FT-IR spectrophotometer. The infrared absorption spectrum was interpreted based on the characteristic peaks corresponding to various chemical bonds and functional groups, providing insights into the molecular structure of the plant constituents [11].

4. Result and Discussion

The Soxhlet extraction method was employed to extract bioactive compounds from the stem of *Tinospora cordifolia* using methanol as the solvent. The percentage yield of the methanolic extract was found to be 19.41%, indicating a significant recovery of phytoconstituents. The choice of methanol as the solvent was strategic, owing to its polarity and ability to dissolve a wide range of bioactive compounds including phenolics, flavonoids, alkaloids, glycosides, and saponins [11]. Methanol's efficiency in extracting both polar and moderately non-polar compounds makes it a preferred solvent in phytochemical investigations [12].

A percentage yield of 19.41% reflects a good concentration of plant metabolites and is comparable to values reported in earlier studies involving *T. cordifolia* and other medicinal plants. Upadhyay et al. (2010) [13] reported yield values ranging from 15–22% for methanolic extracts of *T. cordifolia* stems, which supports the efficacy of the extraction conditions used in the present study. The yield is influenced by multiple factors such as particle size of the plant material, duration of extraction, temperature, and the polarity of the solvent. In Soxhlet extraction, the cyclic heating and condensation of the solvent improve the contact between the plant matrix and solvent, enhancing extraction efficiency [11]. The high yield in this study also highlights the richness of *T. cordifolia* stems in extractable constituents, supporting its extensive use in traditional and modern herbal medicine. The plant is known for a broad range of pharmacological activities including antioxidant, immunomodulatory, anti-inflammatory, antidiabetic, and anticancer effects, all of which are attributed to its diverse phytochemical profile [1]. The significant extractive value obtained here justifies further analysis through phytochemical screening and bioactivity assays to explore these therapeutic potentials.

Methanolic extraction has been shown to yield a higher quantity of total phenolics and flavonoids compared to aqueous or other organic solvents [14]. These secondary metabolites are known to contribute to the biological activity of *T. cordifolia*, particularly its antioxidant and anticancer properties [15]. Therefore, the 19.41% yield obtained suggests a promising foundation for isolating such bioactive constituents. In terms of standardization, the extractive value obtained can also be used as a benchmark for quality control in herbal formulations. Consistency in yield and phytochemical

content is essential to ensure reproducibility in pharmacological studies and therapeutic efficacy. Moreover, a high extraction yield with methanol may reduce the requirement of large quantities of plant material, making the process more economical and sustainable, especially when scaled up for commercial production.

Phytochemical Screening

A wide variety of bioactive compounds were found in the initial phytochemical screening of the methanolic stem extract of *Tinospora cordifolia*, showing how promising the plant is for ethnomedical and pharmacological studies. Various test results confirmed that the methanolic extract includes several major phytochemical groups, for example, alkaloids, glycosides, flavonoids, steroids, triterpenoids, tannins, phenols, saponins, terpenoids, cardiac glycosides, and quinones. But all three carbohydrate-specific tests Molisch's, tests did not show any presence of simple or reducing sugars. When tested with Dragendorff's tests the alkaloids yielded a positive reaction. Lots of people recognize alkaloids for helping in pain relief, fighting malaria, treating cancer, and controlling high blood sugar [16]. Since alkaloids are found in great quantity in the extract, it can contribute to treating inflammatory and infectious illnesses as suggested by Ayurvedic medicine.

The lack of carbohydrates means that methanol, a polar organic solvent, is only able to pull out secondary metabolites and not primary metabolites like sugars. This observation corresponds with previous work showing that polyphenols, alkaloids, and terpenoids are the main constituents extracted using methanol from *T. cordifolia*, instead of saccharides [1]. Using this technique makes methanol more efficient for extracting the medicinally relevant components from herbs. Keller-Killiani tests indicated that glycosides were present in the extract. Some glycosides, especially the cardiac group, have powerful effects on heart muscles and are often used for heart failure and arrhythmias [17]. These elements may play a role in the traditional use of the plant to improve heart health. Based on positive Liebermann-Burchard tests, steroids and triterpenoids have been reported to play a role in anti-inflammatory, anti-diabetic, and hepatoprotective effects. These results agree with the reported traditional Ayurvedic uses of *T. cordifolia* as a general tonic and enhancer of immunity [16]. The existence of steroid also implies a possibility of hormonal and metabolic regulatory action.

Flavonoids, which gave a positive response in Shinoda test are well-known antioxidants. They contribute to scavenge free radicals, and they have protective action against oxidative stress-related diseases like cancer, neurodegeneration, and aging [18]. Their presence indicates the major marker of antioxidant potential of the extract and provides value to conduct further pharmacological studies. The ferric chloride test also indicates the presence of tannins and phenolic compounds that also substantiate antioxidant and antimicrobial activity. They are polyphenols which have been known to inhibit pathogen growth and are protective in chronic disease prevention [19]. The tentative combination

of tannins, phenols and flavonoids contributes to the bio efficacy of the extract and suggests the possibilities of use in herbal formulations against infection, inflammation and degenerative diseases. Saponins, which were positive to frothing test, add immune-modulatory, cholesterol-lowering and anticancer effects. They also possess surfactant qualities and therefore have the ability to increase the absorption of other active compounds [20]. Quinones, which test positive in the sodium hydroxide test, are bioactive aromatic ketones that have been associated with antimicrobial and anticancer activities because of their capability to generate reactive oxygen species. In general, the present study agrees with the phytochemical diversity reported previously on *T. cordifolia* that supports its status as a medicinal plant [15, 1]

Characterization of the Extract

UV-Visible spectral analysis of the methanolic stem extract of Tinospora cordifolia showed a broad absorption maximum at 280 nm (Figure 11), which is characteristic of conjugated compounds, in particular, aromatic and phenolic compounds. The highest absorbance at this wavelength is associated with the electronic transitions that are common to phenolic compounds and flavonoids, which are phenomena known to contain a benzene ring and hydroxyl groups that can absorb UV radiation in this area. The absorbance spectrum shows that it gradually increases between 200nm to 280nm where there is a sharp peak then it gradually decreases. This typical absorption profile is usually attributable to $\pi \rightarrow \pi$ electronic transitions in aromatic systems [3]. The occurrence of such transitions indicates the presence of important amounts of aromatic phytoconstituents such as flavonoids, tannins and phenolic acids in methanolic extract, which is in agreement with the results of the preliminary phytochemical screen.

The phenolic compounds usually exhibit an absorption maximum of 270 290 nm because of the benzene ring, whereas flavonoids have a broad absorption in both UV-A and UV-B regions [21]. The high absorption at 280 nm in the present work agrees with the previous literature that underlines the abundance of polyphenols and flavonoids in *T. cordifolia* [15]. These substances have been described to have antioxidant, anti-inflammatory, and antimicrobial effects which are the foundation of the extensive therapeutic uses of the plant in traditional medicine. It was further observed that UV absorption profile could be used as a quality control/standardisation fingerprint of herbal extracts. The intensive and sharp peak is also an indicator of the purity of the extract and the good choice of the solvent since methanol has been reported to be a good solvent to polar phenolic compounds [22].

The functional groups contained in the methanolic stem extract of *Tinospora cordifolia* were determined by Fourier-transform infrared (FTIR) spectroscopy. As seen in the FTIR spectrum (Figure 2), a broad and strong peak at around 3480 cm^{-1} corresponds to O-H stretching frequencies of hydroxyl functional groups. Such absorption is usually assigned to alcohols and phenolic compounds,

indicating the high proportion of such components in the extract [11]. A sharp peak at around 2920 -2850 $^{-1}$ cm corresponds to CH stretching vibrations of aliphatic -CH_2 and -CH_3 groups which usually signify the presence of alkanes or fatty acid chains [22]. These peaks indicate the presence of long-chain hydrocarbons or triterpenoids which are renowned in *T. cordifolia*.

The sharp absorption band at around 1650 $^{-1}$ cm is attributes to C=O stretch vibrations of amides or carbonyl compounds which are characteristic of proteins or flavonoids with carbonyl functional groups. This could also be assigned to C=C stretching of aromatic rings found in flavonoids as well as other polyphenolic compounds [23]. A high at about 1380 cm $^{-1}$ has been assigned to C -H bending vibrations and is usually indicative of methyl groups due to alkanes or carboxylic acids. Moreover, the peaks below 1000 cm -1, especially those in the range of 600800 cm $^{-1}$, may be assigned to C -Cl or C -Br stretching, which may be a characteristic of halogenated compounds or complicated aromatic systems [24]. The results of the phytochemical screening supported by the spectrum showed the presence of phenols, flavonoids, saponins, steroids, and glycosides. Therefore, FTIR confirms the occurrence of major bioactive functional groups that attribute to the pharmacological actions of *T. cordifolia* such as antioxidant, anti-inflammatory, and immunomodulatory effects. FTIR spectral analysis proves that methanolic extract of *Tinospora cordifolia* comprises numerous biologically relevant functional groups, such as hydroxyls, carbonyls, alkanes, and aromatic rings.

5. Figures and Tables

Table 1: Results of Phytoprofiling of Methanolic Extracts of *T.cordifolia*

Phytochemical Constituents	Test	Result
Alkaloids	Dragondroff's	+
Carbohydrates	Molisch's Test	-
Glycosides	Keller-killiani	+
Steroids and Triterpenoids	Liberman-Burchard's Test	+
Flavonoids	Shinoda Test	+
Tannins and Phenolic Compounds	Ferric chloride Test	+
Saponins	Frothing Test	+
Quinones	Sodium Hydroxide Test	+

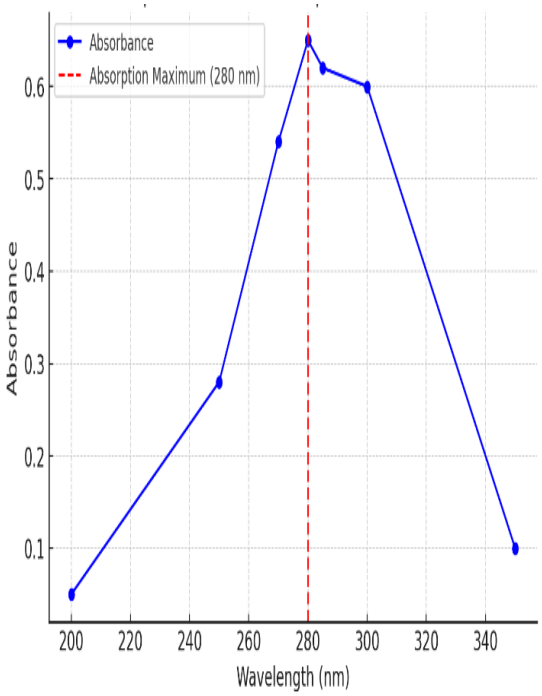

Figure 1: UV absorption peaks of methanolic stem extract of *T.cordifolia*

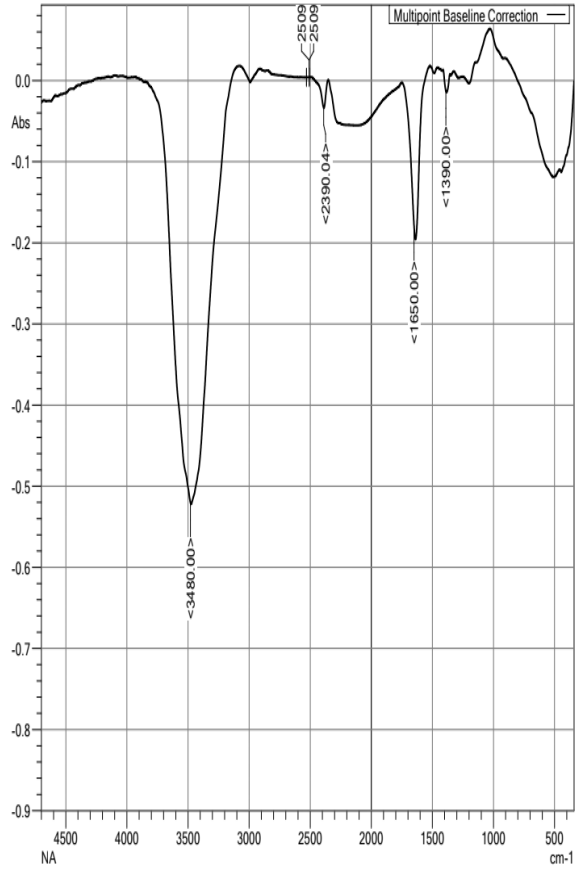

Figure 2: FTIR Spectrum of *Tinospora cordifolia* methanolic Extract

6. Conclusion

The current investigation offers a thorough analysis of the methanolic stem bark extract of *Tinospora cordifolia* which validates its suitability as a source of bioactive compounds. A relatively high percentage (19.41%) of extract was obtained in the Soxhlet extraction which showed that methanol was a good choice of solvent in the extraction of phytoconstituents. The phytochemical investigation initially showed the existence of different secondary metabolites, such as alkaloids, flavonoids, phenols, tannins, saponins, glycosides, and terpenoids, which are commonly attributed to therapeutic effects. These were supported by spectroscopic investigations. UV-visible examination revealed a powerful absorption maximum at 280 nm, which is an indicator of phenolic and flavonoid compounds. The phytochemical profile of the plant matched with the identification of major functional groups, including hydroxyl, carbonyl, and alkane groups by FTIR spectral analysis. These results justify the traditional medicinal uses of *T. cordifolia* and give a scientific background to its pharmacological potentiality. In general, the present research forms the basis of further research on isolation, structural characterization, and bioactivity of individual phytochemicals in *T. cordifolia* to enable its formulation into standardized herbal therapeutics.

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