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Original Article

Pharmacognostic, phytochemical, and antioxidant evaluation of ethanolic extract of *Tinospora cordifolia* using ABTS assay

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ABSTRACT

Objectives: *Tinospora cordifolia* is a key medicinal plant in Ayurveda, traditionally valued for its immunomodulatory, anti-inflammatory, and antioxidant properties. This study aimed to establish its pharmacognostic characteristics, phytochemical profile, and in vitro antioxidant potential, providing scientific support for its traditional therapeutic uses. **Methods**: The plant material was authenticated through macroscopic, microscopic, and powder microscopy examinations. Preliminary phytochemical screening was performed using standard qualitative tests. Thin-layer chromatography was employed to separate and identify major phytoconstituents. Antioxidant activity of ethanolic extract was assessed using the ABTS radical cation decolorization assay, with results expressed as percentage inhibition and compared to ascorbic acid as a standard. **Results**: Macroscopic and microscopic studies revealed characteristic features, including a heart-shaped leaf, cylindrical stem with prominent lenticels, and stellate trichomes, confirming the identity of *Tinospora cordifolia*. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, glycosides, tannins, phenolic compounds, and terpenoids. TLC showed multiple spots, with a prominent spot at Rf 0.6, suggestive of berberine or related isoquinoline alkaloids. The extract exhibited dose-dependent ABTS radical scavenging activity, with maximum inhibition of 55.11% at 2000 µg/ml compared to 92.55% by ascorbic acid at the same concentration. The established pharmacognostic standards ensure the authentic identification of *Tinospora cordifolia*. **Conclusion**: The presence of diverse bioactive secondary metabolites and moderate antioxidant activity scientifically support its traditional use as an immunomodulatory and therapeutic agent, warranting further pharmacological and clinical investigations to develop novel herbal therapeutics.

1. INTRODUCTION

Tinospora cordifolia (Guduchi, giloy), belonging to the Menispermaceae family, is a large, genetically diverse deciduous climbing shrub found at higher altitudes. It has been distributed throughout the tropical Indian subcontinent and China, where it ascends to an altitude of 300m. In Ayurveda, it has been used in Rasayanas to improve the immune system and the body's resistance to infections (Sharma et al., 2012). It has been widely used in Ayurvedic medicine for its anti-spasmodic, anti-arthritic, anti-inflammatory, anti-allergic, anti-periodic, and anti-diabetic properties. A range of bioactive compounds, including alkaloids,

steroids, aliphatics, glycosides, and diterpenoid lactones, have been isolated from various parts of the plant (Saha & Ghosh, 2012; Singh et al., 2003).

Tinospora cordifolia, a medicinal plant widely recognized in Ayurveda, belongs to the taxonomic classification within the kingdom Plantae, which encompasses all plants. As a member of the subkingdom Tracheophyte, it is characterized as a vascular plant with specialized tissues for water and nutrient transport. It falls under the Super-Division Spermatophyta, indicating its status as a seed-bearing plant, and within the Division Magnoliophyte, it is classified as a flowering plant. Further, *Tinospora cordifolia* is placed in the class Magnoliopsida, commonly known as dicotyledons, which are distinguished by having two seed leaves. Within the class, it belongs to the Subclass Polypetalae, where the petals of the flowers are free, not fused. The plant is classified in the Order Ranales and Family Menispermaceae, known as the moonseed family, which includes climbing plants with medicinal properties. Its genus is *Tinospora*, and the species is *cordifolia*, reflecting its heart-shaped leaves (Bharathi et al., 2018).

The plant is known by various vernacular names across different regions and languages, reflecting its widespread cultural and medicinal significance. In English, it is referred to as *Tinospora Gulancha* or Indian Tinospora. In Sanskrit, it is called Guduchi, Amritha, or Madhuparni, names that highlight its revered status in Ayurvedic tradition. In Bengali, it is known as Gulancha, while in Hindi, it is called Giloya or Guduchi. In Telugu, the plant is referred to as Tipaatigo, in Gujarati as Galo, and in Tamil as Shindilakodi, showcasing its diverse regional identities (Khan et al., 2016; Manohar & Paul, 2018).

Guduchi in Ayurveda is a cornerstone ingredient in numerous herbal formulations listed in the National Formulary of Unani Medicine and The Ayurvedic Formulary of India, published by the Government of India. The entire plant is used medicinally, but the stem is recognized as the official medicinal part as per the Ayurvedic Pharmacopoeia of India (1989), likely due to its higher alkaloid content. Giloy is a rich source of berberine and other alkaloids, which contribute to its broad-spectrum antimicrobial and immunostimulant properties (Bisset & Nwaiwu, 1983). These attributes align with its traditional use as a tridoshic herb in Ayurveda, addressing all three doshas (Vata, Pitta, Kapha), and its application in treating fevers, infectious skin diseases, gastrointestinal disturbances, tuberculosis, bronchitis, syphilis, cancers, and malaria. Additionally, Giloy is used for conditions such as impotence, spermatorrhea, and general debility. Its juice is valued as a diuretic, an antidote to snakebite, and a treatment for chronic cough. Historically, it has been used in acute and chronic inflammation and to promote a balanced immune response (Yates et al., 2022). In modern research, *Tinospora cordifolia* has attracted significant attention as an immunomodulator over recent decades (Chaudhary et al., 2024; Singh & Chaudhuri, 2017; Spelman, 2001).

In Unani medicine, key products featuring giloy include Safoof-s-Dhayabitus for diabetes management, Arq Ma-ul-Laham Makoh Kasni Wala as a stomach and liver tonic, Qurs-e-Fizza for cardiac and cerebral weakness, Qurs-e-Hawamil to alleviate nausea during pregnancy, and Satt-e-Gilo as a purified extract for general debility. In Ayurvedic traditions, prominent formulations include Guduchyadi Churna for fever and skin disorders, Sanjivani Vati for fever, indigestion, and poisoning, Chyavanaprakash Avaleha as a rejuvenative tonic, Guduchi Ghrita for liver and eye ailments, Brihat Guduchi Taila for external application in joint pain, Amritarishta for fever and anemia, and Guduchi Sattva for diabetes and jaundice. These Formulations underscore Giloy's versatile role in promoting health and managing a wide range of ailments across both Unani and Ayurvedic systems (Khan et al., 2016; Sinha et al., 2004).

Although *Tinospora cordifolia* has been extensively studied for its pharmacognostic characteristics, phytochemical composition, and antioxidant potential, most investigations have addressed these aspects in isolation or have employed alternative radical-scavenging assays, such as the DPPH assay. To date, no single study has integrated detailed acroscopic, microscopic, and powder microscopy with comprehensive phytochemical screening, thin-layer chromatographic confirmation of berberine-like compounds (Rf=0.6), and quantitative evaluation of antioxidant activity using the ABTS assay on ethanolic extracts derived from authenticated plant material sourced from the Southern Indian State of Kerala. The present investigation, therefore, provides the first holistic Pharmacognostic – Phytochemical - ABTS antioxidant profile of *Tinospora cordifolia* from this specific geo-climatic region, establishing a robust scientific foundation for its traditional immunomodulatory and therapeutic claims while highlighting opportunities for extract purification to enhance radical-scavenging efficacy.

2. MATERIALS AND METHODS

2.1 Collection and identification of plant material

The plant *Tinospora cordifolia* was collected from Kattakada in Thiruvananthapuram district, Kerala, India, during November-December 2023, and was authenticated at JNTBGRI (Jawaharlal Nehru Tropical Botanic Garden and Research Institute), Thiruvananthapuram.

2.2 Macro and microscopic studies of stems

Macroscopic studies of stems were performed visually. For the microscopic studies, the stem has been cut into thin sections and stained with phloroglucinol and HCL (Patra et al., 2016). For powder analysis, the dried, powdered stems were stained using phloroglucinol and HCL (Choudhary et al., 2014; Khatoon et al., 2018).

2.3 Preparation of plant extract

The plant has been shade-dried, powdered, and extracted with 90% ethanol (350ml) in a Soxhlet extractor for 24-48 hours. The extract was concentrated to dryness by steam distillation at 60-70°C using a steam distillation apparatus (Figure 1). The extract was dark green in colour, a gummy solid (16gm). The extract was subjected to various phytochemical tests and the ABTS assay (Srivastav et al., 2024).

Figure 1: Soxhlet extraction of *Tinospora cordifolia*

2.4 Preliminary phytochemical screening

The ethanolic extract of the plant was subjected to preliminary phytochemical screening for the presence of various chemical groups of compounds using standard identification methods (Kaur et al., 2016; Khan et al., 2016).

2.4.1 Test for alkaloids

Mayer's Test: The extract is treated with Mayer's reagent (Potassium mercuric iodide solution), the formation of a whitish yellow coloured precipitate, indicating the presence of alkaloids.

Wagner's Test: The extract is treated with Wagner's reagent (Saturated solution of iodine in potassium iodide), forming a brown/reddish-brown precipitate, indicating the presence of alkaloids.

Dragendorff's test: Extract treated with Dragendorff's reagent (saturated solution of potassium bismuth iodide). Formation of a red precipitate indicates the presence of alkaloids.

Hager's Test: Extract treated with Hager's reagent (saturated solution of picric acid). Formation of a yellow-coloured precipitate indicates the presence of alkaloids.

2.4.2 Test for saponins

Froth test: Extracts were diluted to 20 mL with distilled water and shaken in graduated cylinders for 15 minutes. The formation of a foam 1cm high indicates the presence of saponins.

2.4.3 Test for glycosides

Borntrager's test: The extract was hydrolysed with dilute sulphuric acid, filtered, and to the filtrate, chloroform was added. It was shaken well, and ammonia was added. In the ammoniacal layer, a pink to red colour was observed, indicating the presence of anthraquinone glycosides.

Baljet test: The extract was added to 1 ml of sodium picrate solution. The formation of a yellow to orange colour indicates the presence of glycosides.

Keller-Killiani Test: To the extract, glacial acetic acid containing drops of $FeCl_3$ was added. Formation of a brown colour ring indicates the presence of glycosides.

2.4.4 Test for carbohydrates and sugar

Fehling's test: The Extract was treated with equal quantities of Fehling solutions A and B. Upon heating, a brick-red precipitate indicates the presence of reducing sugars.

2.4.5 Test for amino acid

Millon's Test: Extract is added to Millon's reagent; on heating, the formation of a white precipitate changes to a red precipitate, indicating the presence of amino acids.

Test for flavonoids: Heat the solution with Zn dust and HCL, the formation of red colour indicates the presence of flavonoids.

2.4.6 Test for tannins and phenolic compounds

Lead acetate test: The extract was treated with a basic lead acetate solution, resulting in the formation of a white precipitate, indicating the presence of tannins.

Braymer's test: The Extract was treated with 10% alcoholic FeCl3; the formation of blue-black or green colour indicates the presence of tannins.

Ferric chloride test: Extract treated with a 5% ferric chloride solution; formation of dark blue or greenish-black products indicates the presence of phenol.

Gelatin test: To the extract was added 1% gelatin solution containing 10% sodium chloride solution, which resulted in the formation of a white precipitate, indicating the presence of tannins.

2.4.7 Test for terpenoids

Salkowski test: The Extract was treated with chloroform, and a few drops of concentrated H_2SO_4 were carefully added to form a layer. A reddish-brown colouration at the interface showed the presence of terpenoids.

2.5 Thin-layer chromatography

Thin-layer chromatography was performed on a glass slide coated with a thin layer of adsorbent material, silica gel, which serves as the stationary phase. After the application of the sample, the plate was developed to a distance of 9 cm with 10 ml of toluene: chloroform: methanol (5:4:1) mobile phase. After removal from the chamber, the plates were dried entirely in air at room temperature and documented (Kareppa et al., 2025).

2.6 ABTS assay (*In-vitro* studies)

The antioxidant activity of *Tinospora cordifolia* was evaluated using the ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) assay, which relies on the generation of a blue chromogen ABTS radical monocation through the oxidation of ABTS with potassium persulfate. The radical is reduced in the presence of hydrogen-donating antioxidants, enabling measurement of antioxidant capacity. A 20mM ABTS solution (Solution 1) and a 17mM potassium persulfate solution (Solution 2), both prepared in water, were combined by adding 0.3 ml of Solution 2 to 50ml of Solution 1. The resulting reaction mixture was left to stand at room temperature overnight in the dark to ensure radical formation before use.

For the assay, 1ml of distilled water was mixed with 0.2 ml of various concentrations of the standard, ascorbic acid (125-2000µg/ml, prepared from a 10 mg/ml stock solution), followed by the addition of 0.6 ml of the ABTS solution, bringing the final volume to 1.36 ml. A control sample, containing an equivalent amount of distilled water but no test compound, was also prepared. The absorbance of each sample was measured at 734 nm using a UV-Visible spectrophotometer (SHIMADZU-UV-1900i) after a 20-minute incubation period, enabling the quantification of the antioxidant activity by comparing the reduction in absorbance to the control (Chamandy et al., 2022; Pachaiappan et al., 2018; Polu et al., 2017).

3. RESULTS AND DISCUSSION

3.1 Macroscopic studies

Stems are filiform and tend to climb. The bark is deeply left spirally and is first creamy white and then grey. These macroscopic characteristics are typical of *Tinospora cordifolia*, a climbing shrub widely used in Ayurvedic medicine. The filiform nature and climbing tendency suggest an adaptation for structural support in its natural habitat. At the same time, the spiral bark pattern and colour transition from creamy white to grey are distinctive features that aid in its identification. These traits align with previous botanical descriptions of the species and facilitate its differentiation from other plants in traditional and pharmacological contexts (Modi et al., 2020).

3.2 Microscopic studies

The epidermis is layered with a thick cuticle and a collenchymatous hypodermis. Vascular bundles are bicollateral, open, and arranged in a ring, with well-developed phloem consisting of sieve tubes. Xylem consists of vessels, tracheids, fibres, etc. Starch grains are abundant in all parenchymatous cells. Mucilage canals are found to be scattered in the cortical, phloem, and pith regions. Pith is thin-walled and

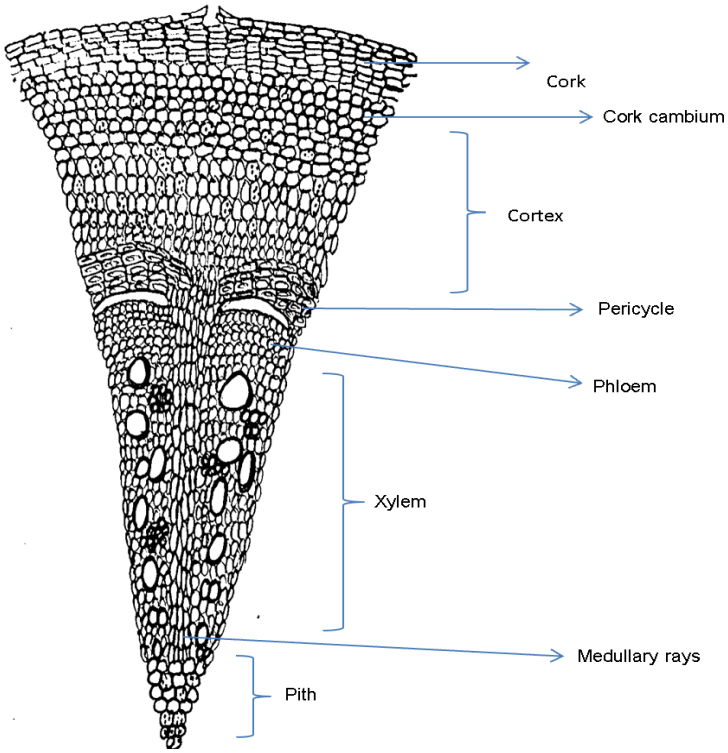

Figure 2: Transverse section of stem of *Tinospora cordifolia*

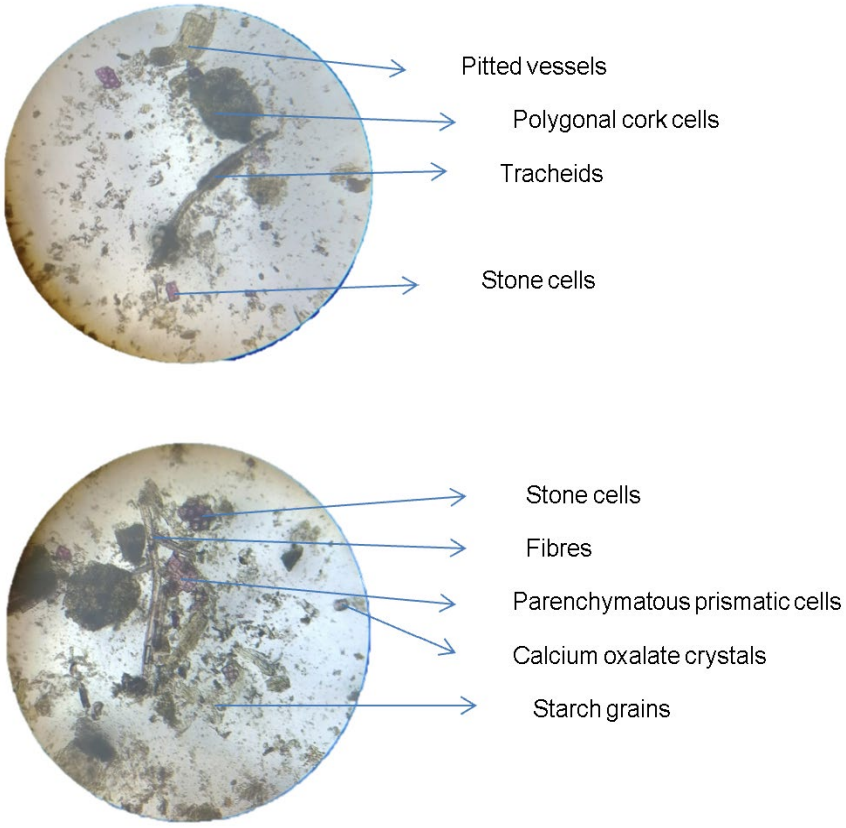

Figure 3: Powder microscopy of the stem of *Tinospora cordifolia*

parenchymatous (Figure 2). The microscopic features reveal a structurally robust stem adapted for efficient nutrient transport and mechanical support. The thick cuticle and collenchymatous hypodermis indicate adaptations for environmental protection and structural integrity. The bicollateral vascular bundles and well-developed phloem and xylem suggest efficient conduction systems, essential for the plant's growth and survival. The abundance of starch grains in parenchymatous cells points to significant energy storage, while mucilage canals may contribute to water retention or defense mechanisms. These anatomical characteristics are consistent with prior studies on *Tinospora cordifolia* and confirm its suitability for pharmacological applications, given its stable structural matrix (Patra et al., 2016).

3.3 Powder microscopy

Cork cells are polygonal with colouring matter associated with collenchymatous cells of the cortex. Fibres are lignified, thick-walled, with blunt ends. Xylem vessels are cylindrical, lignified, and pitted-walled. Sclerenchymatous cells are also lignified, forming thick-walled groups. Calcium oxalate crystals are prismatic and seen in clusters. Starch is simple and compound (Figure 3). The powder microscopy findings corroborate the microscopic observations, highlighting the lignified nature of cork cells, fibers, xylem vessels, and sclerenchymatous cells, which contribute to the plant's structural rigidity and resistance to environmental stress. The prismatic calcium oxalate crystals and starch grains are characteristic of Tinospora cordifolia and may play roles in defense and energy storage, respectively (Mohammad & Malik, 1963). These features support the use of these plants in herbal medicines, as the lignified components likely protect bioactive compounds during processing. At the same time, the presence of starch and crystals may contribute to their therapeutic properties, such as anti-inflammatory effects.

3.4 Phytochemical screening

The phytochemical analysis of the ethanolic extract of *Tinospora cordifolia* confirms the presence of a diverse array of bioactive compounds, consistent with its traditional use for various therapeutic purposes. The positive tests for alkaloids, saponins, glycosides, flavonoids, tannins, phenolic compounds, and terpenoids suggest that these constituents may contribute to the plant's reported antioxidant, anti-inflammatory, and immunomodulatory properties. The absence of carbohydrates, sugars, and amino acids indicates that the extract's bioactivity is likely driven by secondary metabolites rather than primary metabolic products. (Table 1) The diverse array of secondary metabolites identified in the ethanolic extract aligns with the plant's traditional use in treating conditions such as fever, diabetes, and infections. Alkaloids, flavonoids, and phenolic compounds are particularly notable for their antioxidant, anti-inflammatory, and immunomodulatory properties, which likely highlight the plant's therapeutic efficacy. The absence of primary metabolites, like carbohydrates and amino acids, suggests that the extract's bioactivity is driven by secondary metabolites, which are often more potent in pharmacological applications. These findings provide a chemical basis for the plant's traditional use and highlight its potential for further exploration in drug development (Wani et al., 2011).

Table 1: Results obtained in Phytochemical screening

Phytochemical test	Results
Test for alkaloids	+ve
Test for saponins	+ve
Test for glycosides	+ve
Test for carbohydrates and sugars	-ve
Test for amino acids	-ve
Test for flavonoids	+ve
Test for tannins and phenolic compounds	+ve
Test for terpenoids	+ve

3.5 Thin-layer chromatography

The TLC analysis of *Tinospora cordifolia* extract was conducted using silica gel G as the stationary phase and a mobile phase of toluene, chloroform, and methanol (5:4:1). The TLC plate showed multiple spots, indicating the presence of various phytochemical constituents. The primary spot was centrally located and light yellowish-brown, suggesting the presence of a major compound. Additional faint spots were visible, indicating the presence of minor constituents. The Rf value of the principal spot was found to be 0.6, which is equivalent to the Rf values of berberine and other markers. The presence of multiple spots on the TLC plates confirms the chemical complexity of *Tinospora cordifolia* extract, with the principal spot (Rf=0.6) suggesting berberine as a major constituent. Berberine, a well-documented alkaloid, is known for its antimicrobial, anti-inflammatory, and antioxidant properties, which likely contribute

significantly to the plant's pharmacological activities. The faint spots indicate minor compounds, potentially other alkaloids, flavonoids, or terpenoids identified in the phytochemical screening. These results underscore the need for further chromatographic studies to isolate and characterize these minor constituents, which could enhance the understanding of their contributions to the plant's bioactivity (Solanki & Rodric, 2022; Kareppa et al.,2025).

3.6 ABTS assay

The ABTS assay results demonstrate that both the standard and the *Tinospora cordifolia* ethanolic extract exhibit significant antioxidant activity, as evidenced by the reduction in absorbance with increasing concentrations, correlating with higher percentage inhibition. The standard, likely a known antioxidant, i.e., ascorbic acid, displayed superior radical scavenging activity, achieving a maximum inhibition of 92.55% at 2000µg/mL. This suggests a highly efficient antioxidant capacity, serving as a positive control for the assay.

In contrast, the *Tinospora cordifolia* extract showed a moderate effect, with a maximum inhibition of 55.10% at 2000µg/mL. This indicates that the extract possesses antioxidant properties; its efficacy is lower than that of the standard. The dose-dependent trend observed in the extract aligns with the presence of phytochemicals such as alkaloids, flavonoids, and phenolics, which are known to contribute to antioxidant activity. The lower inhibition percentage compared to the standard may be attributed to the complex mixture of compounds in the crude extract, in which not all constituents may be equally effective as free radical scavengers, or to the presence of inactive components that dilute the overall effect.

The IC50 value for the standard appears to be below 125 µg/mL, given the 36.70213% inhibition at this concentration. For Tinospora cordifolia, it likely falls between 500 and 1000 µg/mL, based on the 32.87234% and 46.54255% inhibition at these concentrations, respectively (Figures 4 and 5). This difference highlights the potency of the standard and suggests that further purification or optimisation of the *Tinospora cordifolia* extract could enhance its antioxidant potential. These findings support the traditional use of *Tinospora cordifolia* as an antioxidant agent, though its efficacy is lower than that of the standard (Table 2).

The ABTS assay results confirm that *Tinospora cordifolia* extract possesses moderate antioxidant activity, consistent with the presence of flavonoids, phenolics, and alkaloids identified in the phytochemical screening. The dose-dependent inhibition trend aligns with the known free radical-scavenging properties of these compounds. However, the extract's lower efficacy (55.10638% at 200µg/ml) compared to the standard (92.5533319% at 2000µg/ml) may be due to the crude nature of the extract, which contains a mix of active and inactive compounds, potentially diluting its overall antioxidant capacity. The higher IC_{50} value of the extract (500-1000µg/ml) compared to the standard (<125µg/ml) further highlights the standard's superior potency. These findings support the traditional use of *Tinospora cordifolia* as an antioxidant agent but suggest that purification or fractionation of the extract could enhance its efficacy, potentially by isolating compounds like berberine or flavonoids. The results are consistent with prior studies on the plant's antioxidant properties and provide a foundation for optimizing its therapeutic potential (Boro et al., 2024).

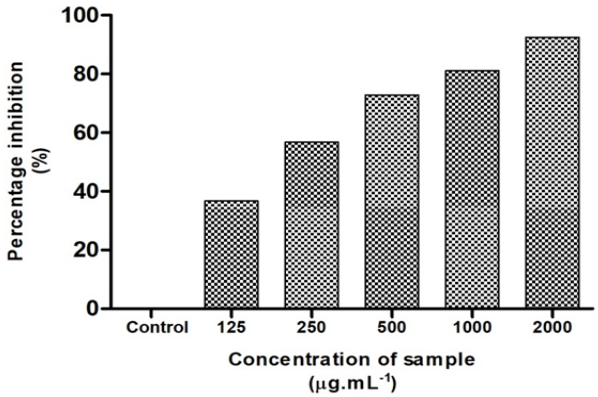

Figure 4: Graphical illustration of ABTS radical scavenging activity of standard (Ascorbic acid)

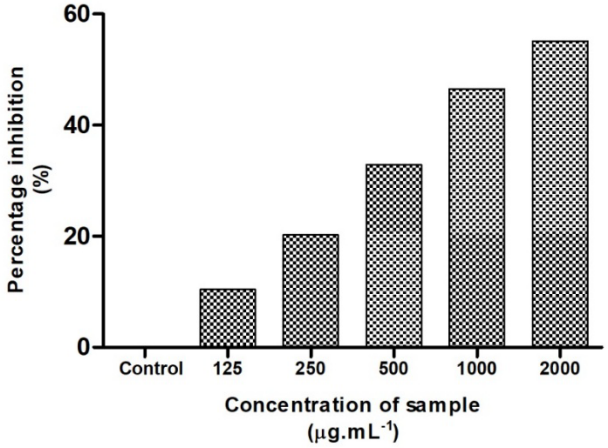

Figure 5: Graphical illustration of ABTS radical scavenging activity of *Tinospora cordifolia*

Table 2: Observations obtained on ABTS Assay

Concentration (µg/mL)	Absorbance	Percentage of inhibition
Control	0.188	00
Standard		
125	0.119	36.70213
250	0.0813	56.75532
500	0.051	72.87234
1000	0.0355	81.11702
2000	0.014	92.55319
Tinospora cordifolia		
125	0.1684	10.42553
250	0.15	20.21277
500	0.1262	32.87234
1000	0.1005	46.54255
2000	0.0844	55.10638

IC_{50} Value-Standard: 207.89µg/mL (Calculated using ED50 PLUS V1.0 Software).
IC_{50} Value- *Tinospora cordifolia*: 1403.72µg/mL (Calculated using ED50 PLUS V1.0 Software).

4. CONCLUSION

The pharmacognostic, phytochemical, and antioxidant evaluation of the ethanolic extract of *Tinospora cordifolia* provides robust evidence supporting its traditional medicinal applications. The macroscopic and microscopic studies confirmed the plant's distinct anatomical characteristics, while phytochemical screening identified a diverse array of bioactive secondary metabolites, including alkaloids, flavonoids, and phenolics, which likely contribute to its therapeutic efficacy. The TLC analysis further corroborated the presence of key compounds, potentially including berberine. The ABTS assay demonstrated moderate antioxidant activity, though less potent than the standard ascorbic acid, suggesting that the crude extract's efficacy could be enhanced through purification. These results affirm *Tinospora cordifolia*'s role as a valuable medicinal plant with significant antioxidant properties, warranting further studies to isolate and optimise its bioactive constituents for potential therapeutic applications.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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