

PHARMACOGNOSTIC AND PHYTOCHEMICAL STANDARDIZATION OF TINOSPORA CORDIFOLIA (WILLD.) HOOK. F. & THOMS. STEM: INSIGHTS FROM MICROSCOPY, PHYSICOCHEMICAL PARAMETERS, AND TLC ANALYSIS

Dr. Preeti Solanki^{1*}, Dr. Shiromani Mishra², Dr. Keshori Sharma³, Dr. Neha Yadav⁴, Dr. Shivkumar Mishra⁵, Dr. Sunita D. Ram⁶

¹MD scholar, Department of Dravyaguna Vigyana, Government Dhanwantari Ayurved Medical College, Ujjain (M.P.)
preetifly.solanki@gmail.com

²Professor & HOD, Department of Dravyaguna Vigyana, Government Dhanwantari Ayurved Medical College, Ujjain (M.P.),
shiromishra@gmail.com

³MD scholar, Department of Dravyaguna Vigyana, Government Dhanwantari Ayurved Medical College, Ujjain (M.P.),
dr.keshoris@gmail.com

⁴MD scholar, Department of Dravyaguna Vigyana, Government Dhanwantari Ayurved Medical College, Ujjain (M.P.),
yneh9810@gmail.com

⁵Associate Professor, Department of Dravyaguna Vigyana, Government Dhanwantari Ayurved Medical College, Ujjain (M.P.),
dr.shivkumarmishra88@gmail.com

⁶Professor, Department of Dravyaguna Vigyana, Government Dhanwantari Ayurved Medical College, Ujjain (M.P.),
drsunitadram72@gmail.com

*Corresponding author: Dr. Preeti Solanki, Email: preetifly.solanki@gmail.com

ABSTRACT

Introduction: *Tinospora cordifolia* (Willd.) Hook.f. and Thoms. (Guduchi) is an important medicinal plant extensively used in traditional medicine for its broad spectrum of therapeutic activities. The growing demand for herbal medicines necessitates standardized pharmacognostical evaluation to ensure the authenticity, quality, and purity of its stem used in herbal formulations.

Objectives: To evaluate the macroscopic, microscopic, and physicochemical characteristics of the stem of *Tinospora cordifolia* (Willd.) Hook.f. & Thoms. to establish diagnostic parameters for its identification, authentication, purity, and quality standardization.

Methods: The stem of *T. cordifolia* was subjected to pharmacognostical evaluation, including organoleptic, macroscopic, microscopic, physicochemical, and thin-layer chromatography (TLC) analyses using standard pharmacognostic procedures in accordance with the Ayurvedic Pharmacopoeia of India and ICMR guidelines.

Results: Phytochemical screening of *T. cordifolia* stem extract revealed the presence of major bioactive constituents, including alkaloids, diterpenoid lactones, glycosides, flavonoids, phenolic compounds. The physicochemical evaluation demonstrated acceptable quality parameters of the crude drug, with loss on drying of 5.46%, total ash value of 15.59%, acid-insoluble ash value of 2.96%, water-soluble extractive value of 12.90%, and alcohol-soluble extractive value of 4.61%. TLC fingerprinting of the stem extract showed a characteristic pattern of multiple bands under UV visualization and after anisaldehyde–sulphuric acid derivatization, confirming the presence of chemically diverse phytoconstituents. The obtained chromatographic profile with distinct R_f values provided a reproducible fingerprint pattern useful for authentication and preliminary standardization of *T. cordifolia* stem.

Conclusion: The present study focused on the pharmacognostical and phytochemical evaluation of *Tinospora cordifolia* (Guduchi) stem for identification, authentication, and quality standardization. Macroscopic and microscopic studies revealed key diagnostic features, while physicochemical and phytochemical analyses confirmed the presence of important secondary metabolites. TLC profiling provided a characteristic fingerprint for authentication and quality control. The established parameters support the standardization, safety, and quality assessment of Guduchi-based herbal formulations and highlight its therapeutic potential.

KEYWORDS: Guduchi, *Tinospora cordifolia* (Willd.) Hook.f. and Thoms., Amrita, Microscopic, Macroscopic, Pharmacognostical study, TLC

INTRODUCTION

Medicinal plants have served as a primary source of healthcare and therapeutic agents since ancient times. The growing global interest in herbal medicines has increased the need for proper identification, standardization, and quality control of medicinal plant materials. Pharmacognosy, the scientific study of crude drugs derived from natural sources, plays a vital role in establishing the authenticity, purity, and quality of herbal drugs.

Pharmacognostical evaluation provides comprehensive information regarding the macroscopic, microscopic, physicochemical, and phytochemical characteristics of medicinal plants, thereby ensuring their safe and effective use.¹

Guduchi (*Tinospora cordifolia* (Willd.) Hook.f. and Thoms.), belonging to the family Menispermaceae, is one of the most important medicinal climbers extensively used in traditional systems of medicine such as Ayurveda, Unani, and Siddha. Commonly known as Giloy, Amrita, and Heart-leaved Moonseed, Guduchi is widely distributed throughout tropical and subtropical regions of India. In Ayurvedic literature, it is revered as “Amrita,” meaning the nectar of immortality, due to its remarkable rejuvenating and therapeutic properties. The plant has been traditionally employed for the treatment of fever, diabetes, jaundice, rheumatism, urinary disorders, skin diseases, and various inflammatory conditions.

The stem of *T. cordifolia* is the most commonly utilized part for medicinal purposes and contains numerous bioactive constituents, including alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoids, polysaccharides, and phenolic compounds. These phytochemicals contribute to its diverse pharmacological activities such as immunomodulatory, antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, antimicrobial, anticancer, and adaptogenic effects. Owing to its extensive therapeutic applications and increasing commercial demand, adulteration and substitution of Guduchi in the herbal market have become significant concerns. Therefore, accurate identification and standardization of the crude drug are essential to ensure its quality, efficacy, and safety.

Pharmacognostical analysis serves as a fundamental tool for the authentication of medicinal plants. Macroscopic evaluation involves the examination of morphological features such as size, shape, color, texture, odor, and taste, while microscopic studies reveal diagnostic characteristics including epidermal cells, cork tissue, vascular bundles, medullary rays, starch grains, calcium oxalate crystals, and other cellular structures. In addition, physicochemical parameters such as ash values, extractive values, moisture content, and fluorescence analysis provide important standards for quality assessment. Preliminary phytochemical screening further aids in identifying the presence of biologically active secondary metabolites responsible for the therapeutic potential of the plant.

The pharmacognostical investigation of Guduchi stem is therefore essential for establishing reliable identification parameters and developing quality standards for herbal formulations containing this drug. Such studies contribute significantly to the prevention of adulteration, maintenance of batch-to-batch consistency, and compliance with regulatory requirements for herbal medicines. The present study aims to perform a comprehensive pharmacognostical evaluation of *T. cordifolia* stem through detailed macroscopic, microscopic, physicochemical, and phytochemical analyses, thereby providing scientific evidence for its authentication and quality control.²

1.1 Table no.1-Taxonomical classification.3

Kingdom	Plantae
Subkingdom	Tracheophyta
Subdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Polypetalae
Order	Ranunculaceae
Family	Menispermaceae
Genus	<i>Tinospora</i>
Species	<i>Cordifolia</i>

1.2 Vernacular Name⁴ :

- Sanskrit: Guduchi, Madhuparna, Amrita, Chhinnaruha, Vatsadani, Tantrika, Kundalini, Chakralakshanika
- Hindi: Giloy, Gudich, Gulneha, Gulbel, Guloh, Gurcha
- Bangali: Golancha, Giloe
- Malayali: Chittamritu, Amritu
- Punjabi: Gilo, Garham, Palo
- Gujrati: Gulvel, Galo
- Kannad: Amrutaballi, Yuganiballi, Madhuparni
- Tamil: Amrutavalli, Chintilikkoti, Chindil, Seendal, Seendil kodi
- Telugu: Thippateego, Amruta
- Arabi: Gilo
- Assam: Siddhilata, Amarlata
- Oriya: Gulochi, Gulancha
- Urdu: Gilo

1.3 Rasa Panchaka^{5,6}

- Rasa : Tikta, Kashaya

- Guna : Guru, Snigdha
- Virya :Ushna
- Vipaka :Madhura
- Doshaghata: Tridoshashamaka
- Rogaghata: Kushta, Vatarakta, Netraroga, Jwara, Grahani

1.4 Aims and Objectives

- To carry out a comprehensive pharmacognostical evaluation of the stem of *T. cordifolia* for establishing its identity, authenticity, purity, and quality standards.
- To evaluate the macroscopic and microscopic characteristics of the stem of *T. cordifolia* for its identification and authentication, including morphological features and diagnostic anatomical characters.
- To determine physicochemical parameters such as ash values, extractive values, and moisture content for quality assessment.

2.MATERIAL AND METHODS

Plant Collection : The plant material of Guduchi (*T. cordifolia*) used for the pharmacognostical study was collected from the Botanical Garden of Government Dhanvantari Ayurveda Medical College, Ujjain, Madhya Pradesh, India.

Literature Material: Relevant literature for the present review was compiled from classical Ayurvedic literature such as Samhitas and Nighantus, peer-reviewed scientific journals, contemporary and classical reference books, monographs, publications of the Ayurvedic Pharmacopoeia of India (API), ICMR quality control guidelines, and other reliable electronic databases and authenticated internet sources.

Methodology : The following pharmacognostical methods were employed for the standardization of Guduchi (*Tinospora cordifolia* (Willd.)Hook.f. & Thoms.).

- Organoleptic Characters
- Macroscopic Study
- Microscopic Study
- Physicochemical Parameters
- TLC

2.1 Table No.2- Organoleptic Characters of Guduchi stem (*Tinospora cordifolia* (Willd.)Hook.f. & Thoms.)^{7,8}

Drug	Color (Roopa)	Odor (Gandha)	Taste (Rasa)	Touch (Sparsa)	Fracture
<i>Tinospora cordifolia</i> (Willd.) Hook.f. & Thoms.	Young stem – green and smooth Older stem – light gray or creamish white	Characteristic or odorless	Bitter (Tikta))	Rough and slightly warty	Fibrous

2.2 Macroscopic Study

The stem of *T. cordifolia* is a perennial, succulent, glabrous climbing shrub that attains considerable length by twining over supporting trees and shrubs. Mature stems are cylindrical, fleshy, and exhibit a characteristic grayish-green to ash-colored appearance. The surface is marked by numerous raised, rounded to elongated lenticels, giving the stem a distinctly warty appearance. Longitudinal fissures and wrinkles are commonly observed on older stems. The stem diameter varies from approximately 0.5 to 3.5 cm depending on age and growing conditions. Young stems are smooth, green, and succulent, while older stems become woody and develop a corky outer layer. Aerial roots arise from the nodes and internodes of mature stems, particularly when the plant grows in humid environments⁹.

On transverse cutting, the stem exhibits a creamy-white to yellowish-green internal tissue with a mucilaginous texture. The fracture is fibrous due to the presence of vascular strands. The odor is indistinct or faintly characteristic, while the taste is distinctly bitter, a feature attributed to the presence of diterpenoid lactones and alkaloids. Stem pieces used as crude drugs are usually cylindrical, slightly curved, and display characteristic circular scars and lenticels on the outer surface¹⁰.



Fig. 1



Fig.2

2.3 Microscopic Study

The transverse section (T.S.) of the stem of *T. cordifolia* reveals a circular to slightly irregular outline with a distinctly undulating outer margin. The outermost region consists of a multilayered cork composed of tangentially elongated, compactly arranged cells forming the protective tissue. The cortex is broad and consists predominantly of thin-walled parenchymatous cells.

The vascular tissue is arranged in a ring as numerous collateral, open vascular bundles separated by conspicuous broad medullary rays. Each vascular bundle comprises phloem towards the periphery, a distinct cambial zone, and lignified xylem towards the center with vessels of varying diameters. The medullary rays are broad, uniseriate to multiseriate, extending radially between the vascular bundles into the central region. The pith is large and composed of thin-walled, polygonal parenchymatous cells, with a few scattered internal vascular strands evident within the medullary region. These anatomical features are characteristic of the mature stem of *T. cordifolia*11.

Figure legend

Figure 3,4 &5. Transverse section of *T. cordifolia* stem showing multilayered cork (Co), cortex (C), collateral open vascular bundles (VB), phloem (Ph), cambium (Ca), xylem vessels (Xy), broad medullary rays (MR), and central parenchymatous pith (P).



Fig.3



Fig. 4

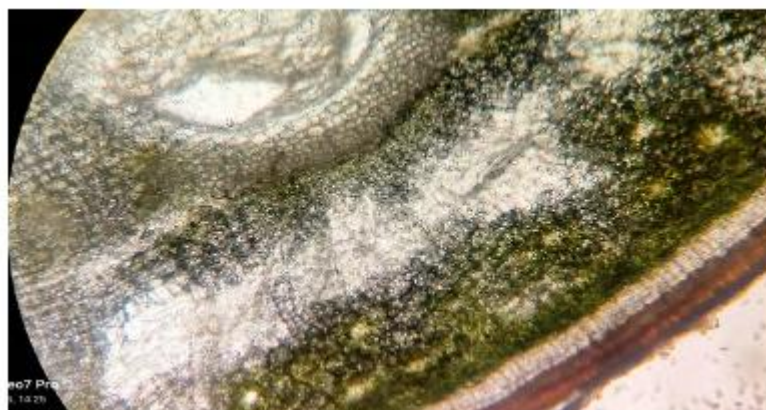


Fig 5

2.4 Phyto-chemistry

The stem of *T. cordifolia* is a rich source of structurally diverse phytoconstituents that contribute to its broad spectrum of pharmacological activities. Phytochemical investigations have revealed the presence of alkaloids, diterpenoid lactones, glycosides, phenolic compounds, flavonoids. Among these, diterpenoid lactones and alkaloids are considered the major bioactive constituents of the stem. Alkaloids identified from the stem include berberine, magnoflorine, palmatine, jatrorrhizine, choline, and tembetarine¹². These compounds have been associated with antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities. Berberine and magnoflorine, in particular, have attracted considerable attention due to their therapeutic potential in metabolic and inflammatory disorders¹³.

Diterpenoid lactones represent the characteristic phytochemical markers of *T. cordifolia*. Important diterpenoids isolated from the stem include tinosporide, columbin, cordifolide, cordifol, tinocordifolin, tinosporol, and tinosporon. These compounds belong predominantly to the clerodane diterpene class and are believed to contribute significantly to the immunomodulatory, hepatoprotective, and anti-inflammatory properties of the plant. Several glycosides have also been reported from the stem, including cordifoliosides A–E, cordioside, syringin, and tinocordioside. These glycosidic constituents exhibit notable antioxidant and immunostimulatory activities. Cordifolioside A has been particularly recognized for its role in enhancing immune responses and reducing oxidative stress¹⁴.

The stem further contains phytosterols such as β -sitosterol, stigmasterol, ecdysterone, and giloinsterol. These compounds are known for their anti-inflammatory and membrane-stabilizing effects. In addition, phenolic acids and flavonoids including gallic acid, ellagic acid, ferulic acid, quercetin, rutin, luteolin, and apigenin contribute substantially to the antioxidant capacity of the plant¹⁵.

Water-soluble polysaccharides and arabinogalactans isolated from the stem have demonstrated significant immunomodulatory activity through macrophage activation and stimulation of cytokine production. These macromolecules are considered important contributors to the adaptogenic and immune-enhancing properties of Guduchi.

Table no. 3 Phytochemical parameters of *Tinospora cordifolia* stem sample¹⁶

Sl no.	Name of the test	Guduchi
1	Foreign matter	0.2%
2	Loss on drying at 1050C	5.46%
3	Total Ash	15.59%
4	Acid Insoluble Ash	2.96%
5	Alcohol soluble extractive	4.61%
6	Water soluble extractive	12.9%

3. Chemical Constituents

3.1 TLC Profile of Guduchi Stem (*Tinospora cordifolia* (Willd.) Miers)

Thin-Layer Chromatography (TLC)

Thin-layer chromatography (TLC) was carried out to establish the chromatographic fingerprint profile of the ethanolic stem extract of *T. cordifolia* for authentication and quality evaluation. TLC is a simple and reproducible chromatographic technique based on the differential migration of phytochemical constituents between a stationary phase and a mobile phase, producing characteristic band patterns useful for herbal standardization^{17,18}.

Materials and Reagents

- TLC plates: Precoated silica gel 60 F₂₅₄ plates.
- Sample: Ethanolic extract of powdered *T. cordifolia* stem.
- Extraction solvent: Ethanol.
- Partition solvent: Chloroform.

- Mobile phase: Chloroform:methanol (9:1, v/v).
- Basifying agent: Ammonia solution.
- Detection methods: UV light at 254 nm and 366 nm.
- Derivatizing reagent: Anisaldehyde–sulphuric acid reagent.

Preparation of Test Solution

The powdered stem material of *T. cordifolia* was extracted with ethanol by maceration. The extract was concentrated and diluted with distilled water. The aqueous extract was basified with ammonia solution and subjected to chloroform partitioning using a separating funnel. The chloroform fraction was collected, concentrated, and reconstituted in chloroform to prepare the test solution for TLC analysis.

TLC Procedure

TLC analysis was performed on precoated silica gel 60 F₂₅₄ plates. The prepared test solution was applied onto the plates and developed using chloroform:methanol (9:1, v/v) as the mobile phase in a saturated chromatographic chamber.

After completion of development, the plates were removed, air-dried, and examined under ultraviolet light at 254 nm and 366 nm. The plates were subsequently derivatized with anisaldehyde–sulphuric acid reagent and heated to visualize additional phytochemical bands.

The retention factor (R_f) values were calculated using the formula 19:

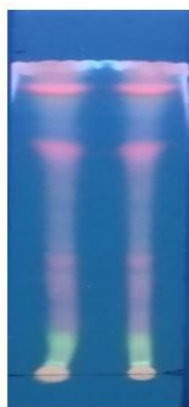
$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

3.RESULTS

The TLC fingerprint profile of *T. cordifolia* stem extract revealed multiple characteristic bands under UV light (254 nm and 366 nm) and after anisaldehyde–sulphuric acid derivatization. Different phytochemical constituents showed distinct migration patterns with varying R_f values and colour responses. The observed R_f values and band appearances are presented in Table 4.

Table no. 4 . TLC fingerprint profile of *T. cordifolia* stem extract under different visualization conditions.

Detection condition	R _f value	Band appearance
UV 366nm	0.11	Green fluorescent
	0.27	Red fluorescent
	0.32	Red fluorescent
	0.75	Red fluorescent
	0.95	Violet fluorescent
UV 254nm	0.13	Dark quenching band
	0.25	Dark quenching band
	0.33	Dark quenching band
	0.55	Dark quenching band
	0.73	Dark quenching band
	0.79	Dark quenching band
	0.93	Dark quenching band
Anisaldehyde- sulphuric acid	0.25	Brown
	0.32	Brown
	0.68	Violet
	0.73	Violet
	0.80	Violet
	0.87	Dark violet
	0.93	Dark purple



4. Significance

The TLC fingerprint profile established in this study provides a characteristic chromatographic pattern for *T. cordifolia* stem extract. The presence of multiple resolved bands under different visualization conditions reflects the complex phytochemical composition of the plant material. The developed fingerprint can serve as a useful qualitative parameter for authentication, standardization, and quality control of *T. cordifolia* raw material and herbal formulations.

6. Qualitative Chemical Analysis

Table no. 5

Sl.no.	Components	Name of tests	<i>Tinospora cordifolia</i>
1	Carbohydrate	Benedict's test	+
2	Protein	Biuret's test	+
3	Tanin	Ferric chloride test	+
4	Saponin	Forth formation test	+
5	Diterpenoids	Salkowski's Test	+
6	Glycosides	Legals test	+
7	Flavonoids	Alkaline reagent test	+
8	Alkaloids	Mayers test	+



6.1 Therapeutic uses of Guduchi (*Tinospora cordifolia* (Willd.) Hook.f. & Thoms.) Stem:

1. Immunomodulatory Activity

Guduchi stem is renowned for enhancing the body's immune response. It stimulates macrophage function, improves resistance to infections, and supports overall immune health. Therefore, it is commonly used in recurrent infections and conditions associated with weakened immunity²⁰.

2. Antipyretic (Fever-Reducing) Effect

Traditionally, Guduchi stem is used in the management of various fevers, including chronic and recurrent fevers. Its antipyretic action helps reduce elevated body temperature and promotes recovery²¹.

3. Anti-inflammatory and Analgesic Properties

The stem exhibits significant anti-inflammatory activity by reducing inflammatory mediators. It is useful in managing inflammatory conditions such as arthritis, gout, and musculoskeletal disorders, while also providing relief from pain²².

4. Antioxidant Activity

The stem of *T. cordifolia* possesses potent antioxidant properties due to its rich content of phenolics, flavonoids, alkaloids, and diterpenoid lactones. These bioactive compounds scavenge free radicals, enhance endogenous antioxidant defense systems, and protect cells against oxidative stress, thereby reducing the risk of oxidative damage associated with various chronic diseases²³.

5. Hepatoprotective Effect

The stem of *Tinospora cordifolia* exhibits significant hepatoprotective activity by protecting hepatocytes against toxin-induced injury, reducing oxidative stress, and enhancing antioxidant defense mechanisms. These properties

contribute to improved liver function and support its traditional use as an adjunct in the management of liver disorders²⁴.

6. Antidiabetic Activity

The stem of *Tinospora cordifolia* exhibits significant antidiabetic activity by improving glucose metabolism, enhancing insulin secretion and sensitivity, and reducing oxidative stress. Experimental and clinical studies suggest that it helps regulate blood glucose levels and may serve as a supportive therapeutic agent in the management of diabetes mellitus²⁵.

7. Gastroprotective and Digestive Benefits

Tinospora cordifolia stem has traditionally been used as a digestive tonic in Ayurveda. Its bitter principles and phytoconstituents are reported to stimulate digestive functions, improve appetite, and alleviate symptoms associated with indigestion and gastric discomfort. The presence of bioactive compounds such as alkaloids, glycosides, and polyphenols may contribute to its beneficial effects on gastrointestinal health²⁶.

8. Cardioprotective Effects

Its antioxidant and anti-inflammatory actions may contribute to cardiovascular health by reducing oxidative stress and supporting healthy circulation²⁷.

9. Adaptogenic and Rejuvenative Action

As a Rasayana herb, Guduchi helps the body adapt to physical and mental stress, enhances vitality, improves endurance, and supports overall well-being²⁸.

6.2 Pharmacological Research and clinical evidence :

1. Medhya Rasayana Activity: The aqueous extract of Guduchi stem administered to the AlCL3-treated wistar rats for 42 days. The EMP test revealed that treatment with Guduchi extract showed marked improvement of memory status in rats along with reduced oxidative stress and a marked modulation of the AChE inhibition and expression of AChE tubulin proteins²⁹.

2. Analgesic activity: A preclinical study in albino rats found that *Tinospora cordifolia* (Guduchi) extract significantly reduced pain in both the hot plate and abdominal writhing tests ($p < 0.05$). These findings suggest that Guduchi possesses analgesic activity through both central and peripheral mechanisms, supporting its potential as a natural pain-relieving agent³⁰.

3. Anti-inflammatory Activity: A preclinical study using an indomethacin-induced ulcerative colitis rat model demonstrated that Guduchi (*Tinospora cordifolia*) possesses significant anti-inflammatory activity. Oral administration of Guduchi (300 mg/kg for 16 days) significantly reduced inflammatory markers (ESR and CRP), improved disease activity, normalized hematological abnormalities associated with inflammation, and decreased colonic inflammation. Histopathological examination further confirmed reduced inflammatory cell infiltration, improved mucosal architecture, and enhanced tissue healing, supporting the therapeutic potential of Guduchi in inflammatory bowel disease through its anti-inflammatory, antioxidant, and immunomodulatory properties³¹.

4. Antioxidant Activity: An in vitro study evaluated the antioxidant activity of Guduchi (*Tinospora cordifolia*) leaf extracts using DPPH radical scavenging, reducing power, phosphomolybdenum, and metal chelating assays. The methanolic leaf extract exhibited the highest antioxidant activity, with 92.09% DPPH radical scavenging at 250 µg/mL, 81.62% metal chelating activity, and the greatest total antioxidant capacity, correlating with its high phenolic (44.36 mg/g) and flavonoid (10.31 mg/g) contents. These findings demonstrate that the antioxidant potential of Guduchi is largely attributed to its phenolic constituents, which effectively scavenge free radicals and reduce oxidative stress³².

5. Anti-diabetic Activity: Researchers evaluated different Ayurvedic formulations of Guduchi (*Tinospora cordifolia*) prepared using the traditional Bhavana process to determine whether this processing method enhances its antidiabetic activity. The formulations were assessed through enzyme inhibition assays and glucose tolerance tests in experimental mice. Among all the formulations, Svarasa Bhavita Guduchi Churna (SBGC) demonstrated the strongest inhibition of α -amylase and α -glucosidase enzymes and produced the greatest reduction in post-glucose blood sugar levels. It also showed the highest berberine content, indicating that the Bhavana process may improve the antidiabetic efficacy of Guduchi³³.

6. Oral health & Anti-microbial effect: study evaluated the effectiveness of Guduchi decoction as a mouthwash in 17 participants for 21 days. The decoction was prepared by boiling Guduchi nursery in water and was used twice daily after a pilot study confirmed its 12-hour substantivity. Clinical and microbiological parameters such as plaque score, gingival score, and oral bacterial count were assessed before and after use. The results showed a significant reduction in plaque and gingival scores along with a decrease in *Streptococcus mutans* and gram-negative bacterial counts. The study suggested that Guduchi decoction may have beneficial antimicrobial effects and could support oral hygiene³⁴.

7. DISCUSSION

7.1 Macroscopic Discussion

The macroscopic characteristics of the stem of *T. cordifolia* observed in the present study agree with the standard pharmacognostic descriptions reported in the literature. The cylindrical, succulent stem with a grayish-green surface, prominent lenticels, longitudinal fissures, and aerial roots are distinctive features useful for identification. The creamy-white mucilaginous internal tissue and characteristic bitter taste further support its authenticity and

indicate the presence of important phytoconstituents responsible for its medicinal properties. These morphological features serve as valuable parameters for the identification and quality assessment of the crude drug.

7.2 Microscopic Discussion

The microscopic characteristics observed in the present study are consistent with the established pharmacognostic descriptions of *T. cordifolia* and confirm the identity of the investigated stem. The presence of a multilayered cork, broad parenchymatous cortex, collateral open vascular bundles with a distinct cambial zone, broad medullary rays, and a large parenchymatous pith are typical anatomical features of the mature stem reported in previous studies and standard pharmacopoeial references.

The well-developed cork provides mechanical protection, while the extensive cortical and pith parenchyma serve as storage tissues for reserve materials and bioactive metabolites. The distinct cambial zone and lignified xylem indicate active secondary growth and efficient vascular conduction. Broad medullary rays facilitate the radial transport of water and nutrients and represent an important diagnostic feature for species identification. Furthermore, the occurrence of medullary (intraxylary) vascular bundles within the pith is a characteristic anatomical marker of *T. cordifolia* and is considered valuable for differentiating the species from related taxa and potential adulterants.

Overall, the anatomical features observed in this study agree with published pharmacognostic reports and support the use of microscopy as a reliable tool for the authentication and quality standardization of *T. cordifolia*. These diagnostic characters provide a scientific basis for ensuring the identity and quality of crude drug materials used in herbal formulations and may serve as reference standards for future pharmacognostic and quality-control investigations.

7.3 Discussion of Phytochemical and Physicochemical Findings

The present study highlights the rich phytochemical composition and acceptable physicochemical characteristics of *T. cordifolia* stem. The presence of alkaloids, diterpenoid lactones, glycosides, flavonoids and phenolic compounds supports the wide range of pharmacological activities reported for the plant, including antioxidant, anti-inflammatory, immunomodulatory, hepatoprotective, and antidiabetic effects.

The physicochemical parameters obtained indicate good quality of the crude drug. The low loss on drying (5.46%) suggests suitable moisture content for storage, while the total ash (15.59%) and acid-insoluble ash (2.96%) values indicate acceptable inorganic content. The higher water-soluble extractive value (12.9%) compared to the alcohol-soluble extractive value (4.61%) suggests the predominance of polar bioactive constituents, supporting the traditional use of aqueous preparations of *T. cordifolia*. Overall, the findings are consistent with previous reports and support the quality and therapeutic potential of the stem as a valuable medicinal plant.

7.4 TLC Fingerprinting and Chromatographic Characterization

The TLC fingerprint profile developed for *T. cordifolia* stem extract demonstrated a characteristic chromatographic pattern with multiple resolved bands under different visualization conditions, indicating the presence of chemically diverse phytoconstituents. The observed variation in band profiles under UV illumination and after derivatization reflects differences in the polarity, concentration, and chemical properties of constituents present in the extract. The enhanced visualization after anisaldehyde–sulphuric acid derivatization suggests the presence of compounds belonging to different classes of secondary metabolites that may not be detectable under UV conditions alone.

The occurrence of multiple bands with distinct *R_f* values supports the complex phytochemical nature of *T. cordifolia*, which is reported to contain various bioactive constituents, including alkaloids, diterpenoids, steroids, glycosides, and other secondary metabolites. The developed chromatographic pattern provides a characteristic fingerprint that can be used for comparative evaluation of plant material and may assist in maintaining the identity and consistency of Guduchi-based herbal preparations. Although TLC fingerprinting alone does not allow definitive identification of individual compounds, the obtained band pattern and *R_f* values provide valuable qualitative information for authentication and routine quality assessment. The reproducibility of characteristic bands can be useful for detecting possible variations arising from factors such as geographical origin, harvesting conditions, processing methods, or adulteration of raw materials. Therefore, the established TLC profile represents a simple, rapid, and economical approach for preliminary standardization of *T. cordifolia* stem. This chromatographic fingerprint can serve as a supportive quality control parameter and provides a foundation for further chemical characterization using advanced analytical techniques such as HPTLC, HPLC, or LC–MS.

8. Standardization and future prospects

Standardization of *T. cordifolia* stem is essential to ensure its identity, purity, quality, safety, and therapeutic efficacy. With the increasing demand for Guduchi in herbal medicine, the possibility of adulteration, substitution, and variation in phytochemical composition due to geographical and environmental factors has become a significant concern. Therefore, establishing reliable pharmacognostical and phytochemical standards is crucial for maintaining the quality of the crude drug and its formulations.

The present study provides comprehensive standardization parameters based on macroscopic, microscopic, physicochemical, phytochemical, and chromatographic evaluations. Diagnostic morphological characters such as the succulent cylindrical stem, prominent lenticels, aerial roots, fibrous fracture, and bitter taste, along with characteristic microscopic features including multilayered cork, broad cortex, collateral open vascular bundles, medullary rays, and intraxylary vascular bundles, serve as dependable criteria for botanical authentication.

Physicochemical constants such as loss on drying, ash values, and extractive values provide quantitative standards for evaluating purity and detecting contamination or adulteration. Furthermore, the TLC fingerprint profile developed in this study establishes a characteristic chromatographic pattern that can be used as a routine qualitative marker for the identification and quality assessment of *T. cordifolia* stem.

To achieve comprehensive quality assurance, these pharmacognostical parameters should be complemented with advanced analytical techniques such as HPTLC, HPLC, LC–MS, and DNA barcoding. Adherence to the standards prescribed by the Ayurvedic Pharmacopoeia of India (API), WHO guidelines, Good Agricultural and Collection Practices (GACP), and Good Manufacturing Practices (GMP) will further ensure batch-to-batch consistency, safety, and efficacy of Guduchi-based herbal formulations. Such an integrated approach will strengthen the scientific validation and global acceptance of this important medicinal plant.

Future Perspectives and Research Directions of *Tinospora cordifolia* (Willd.) Hook.f. & Thoms.

Tinospora cordifolia possesses considerable potential for future drug development owing to its diverse pharmacological activities. Further research should focus on the isolation of bioactive marker compounds, standardization of extracts, and well-designed clinical trials to establish efficacy and long-term safety. Integration of advanced analytical techniques such as HPTLC, HPLC, LC–MS, metabolomics, and DNA barcoding will improve quality control and minimize adulteration. In addition, studies on molecular mechanisms, pharmacokinetics, herb–drug interactions, and sustainable cultivation practices will strengthen the scientific validation and global acceptance of Guduchi as an evidence-based medicinal plant.

9. CONCLUSION

The present study provides a comprehensive pharmacognostical evaluation of the stem of *T. cordifolia* (Guduchi), establishing important parameters for its identification, authentication, and quality standardization. The macroscopic and microscopic characteristics, including the succulent cylindrical stem, prominent lenticels, multilayered cork, broad cortex, vascular bundles, medullary rays, and intraxylary vascular tissues, serve as reliable diagnostic features for confirming the identity of the crude drug. Physicochemical analysis revealed acceptable quality parameters, while phytochemical screening confirmed the presence of important bioactive constituents such as alkaloids, terpenoids, glycosides, tannins, saponins, and other secondary metabolites. The TLC fingerprint profile further provided a characteristic chromatographic pattern useful for routine authentication and quality control.

The findings support the medicinal importance of *T. cordifolia* stem and highlight its potential therapeutic properties, including immunomodulatory, antioxidant, anti-inflammatory, hepatoprotective, and antidiabetic activities. The established pharmacognostical and phytochemical standards can serve as valuable references for preventing adulteration and ensuring the quality, safety, and efficacy of Guduchi-based herbal formulations. Further advanced analytical studies and clinical investigations are recommended to strengthen its scientific validation and therapeutic applications.

REFERENCES

1. World Health Organization. Quality Control Methods for Medicinal Plant Materials. Geneva: World Health Organization; 2011.
2. Saha S, Ghosh S. *Tinospora cordifolia*: One plant, many roles. *Ancient Science of Life*. 2012;31(4):151–159. doi:10.4103/0257-7941.107344.
3. Kirtikar KR, Basu BD. *Indian Medicinal Plants*. Vol. I. Dehradun: International Book Distributors; 2005.
4. Government of India, Ministry of Health and Family Welfare.
5. The Ayurvedic Pharmacopoeia of India. Part I, Volume I. New Delhi: Department of AYUSH; 1986. Monograph: Guduchi (*Tinospora cordifolia* (Willd.) Miers).
6. Government of India, Ministry of AYUSH. The Ayurvedic Pharmacopoeia of India. Part 1, Volume 1. New Delhi: Department of AYUSH, Ministry of Health and Family Welfare, Government of India; 1986. Monograph: Guduchi (*Tinospora cordifolia* (Willd.) Miers).
7. Database On Medicinal Plants Used In Ayurveda, volume 3, Central council for research in Ayurveda and Siddha, Deptt. Of ISM & H, Ministry of Health and Family Welfare, Govt. of India.
8. Khandelwal KR. *Practical Pharmacognosy: Techniques and Experiments*. 30th ed.
9. Trease GE, Evans WC. *Trease and Evans Pharmacognosy*. 16th ed. London: Saunders Elsevier; 2009. Pune: Nirali Prakashan; 2022.
10. Indian Council of Medical Research. Quality Standards of Indian Medicinal Plants. Vol. 1. New Delhi: Indian Council of Medical Research; 2003. p. 212–221.
11. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B, Ghosh AC. Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi). *Indian Journal of Pharmacology*. 2003;35(2):83–91.
12. Government of India, Ministry of Health and Family Welfare. The Ayurvedic Pharmacopoeia of India. Part I, Vol. I. New Delhi: Department of AYUSH; 2001. Monograph: Guduchi (*Tinospora cordifolia* (Willd.) Hook. f. & Thomson).
13. Singh D, Chaudhuri PK. Chemistry and pharmacology of *Tinospora cordifolia*. *Natural Product Communications*. 2017;12(2):299–308.

14. Upadhyay AK, Kumar K, Kumar A, Mishra HS. *Tinospora cordifolia* (Willd.) Hook. f. and Thomson: A review of its ethnobotany, phytochemistry and pharmacology. *International Journal of Ayurveda Research*. 2010;1(2):112–121.
15. Pendse VK, Dadhich AP, Mathur R. Studies on diterpenoid constituents of *Tinospora cordifolia*. *Indian Journal of Chemistry*. 1981.
16. Sharma P, Dwivedee BP, Bisht D, Dash AK, Kumar D. *Tinospora cordifolia* (Giloy): Phytochemistry, ethnopharmacology, clinical application and conservation strategies. *Current Drug Metabolism*. 2020;21(11):897–930.
17. Ministry of AYUSH, Government of India. The Ayurvedic Pharmacopoeia of India. Part I, Vol. I. New Delhi: Government of India, Ministry of AYUSH.
18. Wagner H, Bladt S. *Plant Drug Analysis: A Thin Layer Chromatography Atlas*. 2nd ed. Berlin: Springer; 2009.
19. World Health Organization. *Quality Control Methods for Herbal Materials*. Geneva: WHO Press; 2011.
20. Mukherjee PK. *Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals*. New Delhi: Business Horizons; 2002. ISBN: 8190078844
21. Mathew S, Kuttan G. Immunomodulatory and antitumour activities of *Tinospora cordifolia*. *Fitoterapia*. 1999;70(1):35–43. doi:10.1016/S0367-326X(98)00017-3.
22. Hussain L, Akash MSH, Ain NU, Rehman K, Ibrahim M. The Analgesic, Anti-Inflammatory and Anti-Pyretic Activities of *Tinospora cordifolia*. *Adv Clin Exp Med*. 2015;24(6):957–964.
23. Alamgeer, Naeem M, Uttra AM, et al. The analgesic, anti-inflammatory and antipyretic activities of *Tinospora cordifolia*. *Clin Exp Pharmacol Physiol*. 2017;44(7):785–794. doi:10.1111/1440-1681.12754.
24. Upadhyay AK, Kumar K, Kumar A, Mishra HS. *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. (Guduchi): Validation of the Ayurvedic pharmacology through experimental and clinical studies. *Int J Ayurveda Res*. 2010;1(2):112–121. doi:10.4103/0974-7788.64405.
25. Prince, P. S. M., & Menon, V. P. (1999). Antioxidant action of *Tinospora cordifolia* root extract in alloxan diabetic rats. *Phytotherapy Research*, 13(1), 86–87.
26. Stanely, P., Prince, M., & Menon, V. P. (2000). Hypoglycaemic and other related actions of *Tinospora cordifolia* roots in alloxan-induced diabetic rats. *Journal of Ethnopharmacology*, 70(1), 9–15.
27. Upadhyay AK, Kumar K, Kumar A, Mishra HS. *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. (Guduchi): Validation of the Ayurvedic pharmacology through experimental and clinical studies. *Int J Ayurveda Res*. 2010;1(2):112–121. doi:10.4103/0974-7788.64405.
28. Kumar P, Kamle M, Mahato DK, Bora H, Sharma B, Rasane P, Bajpai VK. *Tinospora cordifolia* (Giloy): Phytochemistry, ethnopharmacology, clinical application and conservation strategies. *Curr Pharm Biotechnol*. 2020;21(12):1165–1175. doi:10.2174/1389201021666200430114547.
29. Sharma P, Dwivedee BP, Bisht D, Dash AK, Kumar D. The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*. *Heliyon*. 2019;5(9):e02437. doi:10.1016/j.heliyon.2019.e02437.
30. Jamadagni SB, Ghadge PM, Tambe MS, Srinivasan M, Prasad GP, Jamadagni PS, et al. Amelioration of ALC13-induced memory loss in the rats by an aqueous extract of Guduchi, a Medhya Rasayana. *Pharmacogn Mag*. 2023;19(2):231–243. doi:10.1177/09731296221145063.
31. Mandal P, Das AK, Mandal A, Biswas TK. A study to evaluate efficacy of *Tinospora cordifolia* (Guduchi) as analgesic agent using albino Wistar rats as an experimental animal model. *Int J Basic Clin Pharmacol*. 2020;9(2):242–246. doi:10.18203/2319-2003.ijbcp20200170.
32. Taj N, Saha J, Das P, Sen R, Gorain R, Sarkar S, et al. Exploring the therapeutic potential of *Tinospora cordifolia* (Guduchi) against indomethacin-induced ulcerative colitis in experimental animals: A preclinical study. *Afr J Biol Sci*. 2024;6(16):2299–2312. doi:10.48047/AFJBS.6.16.2024.2299-2312.
33. Praveen N, Thiruvengadam M, Kim HJ, Praveen Kumar JK, Chung IM. Antioxidant activity of *Tinospora cordifolia* leaf extracts through non-enzymatic method. *J Med Plants Res*. 2012;6(33):4790–4795. doi:10.5897/JMPR12.200.
34. Sharma R, Bolleddu R, Maji JK, Ruknuddin G, Prajapati PK. In-vitro α -amylase, α -glucosidase inhibitory activities and in-vivo anti-hyperglycemic potential of different dosage forms of Guduchi (*Tinospora cordifolia* [Willd.] Miers) prepared with Ayurvedic Bhavana process. *Front Pharmacol*. 2021;12:642300. doi:10.3389/fphar.2021.642300.
35. Nair S, Kakodkar P, Shetiya SH, Dharkar N, Jayashree C, Rajpurohit L. Efficacy of *T. cordifolia* (Guduchi) against plaque and gingivitis: A clinico-microbiological study. *Indian J Dent Res*. 2020;31(6):830–834. doi:10.4103/ijdr.IJDR_521_18.