STANDARDIZATION OF *TINOSPORA CORDIFOLIA* (WILLD.) USING MACROSCOPIC AND MICROSCOPIC ASPECTS

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Abstract

Tinospora cordifolia (Willd.), commonly known as Guduchi or Giloy, is a deciduous climbing shrub belonging to the family Menispermaceae. Highly revered in traditional systems of medicine like Ayurveda, this plant possesses deep therapeutic potentials. This research article provides a structured compilation of its taxonomical classification, regional nomenclature, botanical morphology, Ayurvedic properties, and a comprehensive step-by-step pharmacognostical protocol for tissue fixation, microtomy, and staining required for microscopic analysis.

Key words: Giloy, microtomy.

1. Introduction & Botanical Classification

Tinospora cordifolia is widely distributed throughout the tropical regions of India, ascending to altitudes of 300 meters, as well as parts of China, Burma, and Sri Lanka (where it ascends up to 1200 meters).

Taxonomical Hierarchy

- **Kingdom:** Plantae
- **Division:** Magnoliophyta
- **Class:** Magnoliopsida
- **Order:** Ranunculales
- **Family:** Menispermaceae
- **Genus:** *Tinospora*
- **Species:** *cordifolia*

Vernacular Nomenclature

The plant is known by various regional names across the Indian subcontinent:

- **Hindi:** Giloe / Giloya
- **Sanskrit/Oriya:** Guduchi
- **Kannada:** Amritaballi
- **Marathi:** Gulvel
- **Tamil:** Seendal / Seendil kodi
- **Telugu:** Tippateega

2. Macromorphological Description

Tinospora cordifolia is an extensively spreading, large deciduous climber characterized by elongated twining branches.

Floral Organ	Morphological Characteristics
Inflorescence	Axillary or on old leafless stems, pseudo-racemose cymes measuring 4–15 cm in length.
Flowers	Unisexual, small, appearing greenish-yellow when the plant is leafless. Male flowers are clustered, whereas female flowers are solitary.
Perianth	Sepals are 6 in 2 series of 3 (outer ones smaller). Petals are 6, smaller than sepals, free, and membranous.
Fruits & Seeds	Drupes are ovoid or globose, turning bright red when ripe (5–7 mm across). Seeds are uniquely curved, a diagnostic trait giving the family *Menispermaceae* its name.

Below are the macroscopic representations of the fresh plant body and gathered crude material:

Fig. 1: GUDUCHI PLANT

Fig. 2: GUDUCHI STEM

Guduchi Plant climbing habit and dense foliage.

![Stem of Guduchi]

(349a0ede-7405-445e-bba4-d8d04114cd63_Fig.2 Stem of Guduchi.png)

Collected segments of Guduchi stem showing surface character.

3. Pharmacognostical Standardization

Macroscopic and microscopic analysis serves as the primary tool for validating the

authenticity of the crude drug sample.

A. Macroscopic Standardization

- **Dimensions:** Dried drug pieces exhibit varying thicknesses ranging from 0.6 to 5 cm in diameter.
- **Surface Texture:** Young stems appear green with a smooth surface and distinct swelling at the nodes. Older stems display a light brown surface marked by warty protuberances due to circular lenticels.

- **Fracture/Cross Section:** Transversely smoothed surfaces reveal a radial structure characterized by prominent, conspicuous medullary rays traversing a highly porous tissue matrix.
- **Organoleptic Taste:** Strongly bitter.

B. Microscopic Standardization

A transverse section (T.S.) of the stem reveals an outer layer of cork, differentiating into an outer zone of thick-walled, brownish, compressed cells, and an inner zone comprised of 3–4 rows of thin-walled, colourless, tangentially arranged cells.

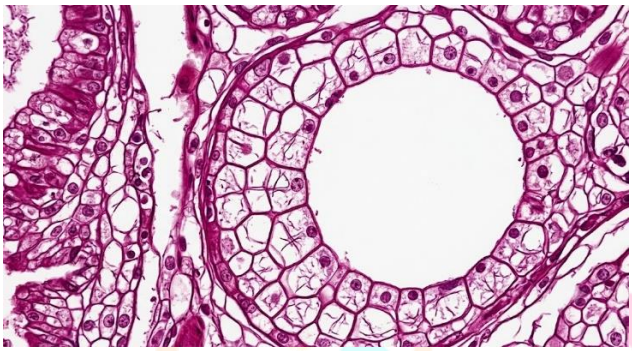

Fig. 3 (Top): Transverse section (T.S.) of a leaf.

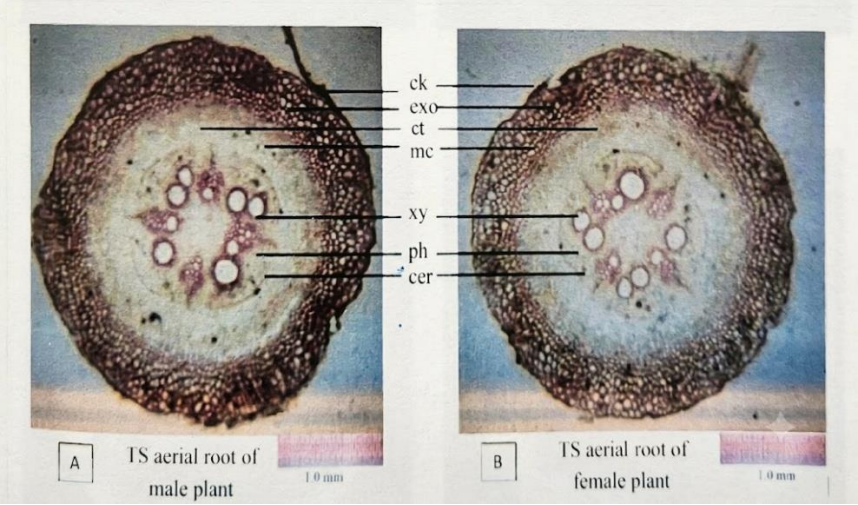

Fig. 4 : Comparative anatomical transverse sections of aerial roots from male (A) and female (B) plants, highlighting structural landmarks: cork (ck), exodermis (exo), cortex (ct), mucilage cell (mc), xylem (xy), phloem (ph), and cambiform element/ray (cer).

4. Materials and Methodology

Fresh stems and leaves were procured from the medicinal plant garden of the Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University (BHU), Varanasi, Uttar Pradesh.

To prepare permanent slides for histomorphological assessment, the tissue specimens underwent a rigorous fixation and processing sequence:

Specimen Preparation & Softening

Hard tissues (such as fruit, seed, or bark) were immersed in water for 2–3 days to achieve sufficient softening before being sectioned using a sharp razor or knife.

Fixation and Dehydration Sequence

The tissue fragments were processed through graded reagent solutions to prevent cellular collapse:

1. **FAA Fixative:** Formaldehyde + Glacial Acetic Acid (1:1 ratio) $\rightarrow$ **24 hours**.

2. **Graded Alcohol Dehydration:** 50% Alcohol (**24 hrs**) $\rightarrow$ Safranin stain (**24 hrs**) $\rightarrow$ Blotting/cleaning $\rightarrow$ 90% Alcohol (**24 hrs**) $\rightarrow$ Absolute Alcohol (**24 hrs**).

3. **Clearing (Alcohol/Chloroform ratios):** Absolute Alcohol + Chloroform (2:1 ratio) for **24 hrs** $\rightarrow$ Absolute Alcohol + Chloroform (1:2 ratio) for **24 hrs** $\rightarrow$ Pure Chloroform I (**24 hrs**) $\rightarrow$ Pure Chloroform II (**24 hrs**).

Block Preparation & Microtomy

The cleared tissues were placed into melted paraffin wax (62°C). The wax was changed three times until effervescence fully ceased. Tissues were left in fresh wax for 2–3 hours before casting the paraffin block, which was then mounted onto a wooden block for structural stability. Sections were cut using a rotary microtome knife at a thickness ranging from $5\ \mu\text{m}$ to $12\ \mu\text{m}$. The thin ribbons were floated onto egg albumin-coated slides and fixed firmly using a laboratory hot plate.

Deparaffinization and Counter-Staining

To distinguish differential cell layers under light microscopy, the prepared slides were subjected to a reverse-hydration and double-staining cycle (Safranine and Fast Green):

[Slides in Xylene (to remove wax)]

↓

[Absolute Alcohol (30 min)] → [95% Alcohol (30 min)] → [70% Alcohol (30 min)] → [50% Alcohol (30 min)]

↓

[Double Distilled Water Wash (2 changes)]

↓

[Safranine Stain (1 hour)]

↓

[Double Distilled Water Wash]

↓

[30% Alcohol (30 min)] → [50% Alcohol (30 min)] → [70% Alcohol (30 min)] → [95% Alcohol (30 min)]

↓

[Fast Green Counter-Stain (1 hour)]

↓

[Absolute Alcohol I, II, III (30 min each)]

↓

[Xylene Clearing I, II, III (30 min each)]

↓

[Air Dry & Mount in DPX Synthetic Medium] → [Microscopic Observation]

(Detailed timeline adapted from experimental procedures).

5. Ayurvedic Properties & Clinical Formulations

In Ayurvedic pharmacology (Dravyaguna), the medicinal attributes of *T. cordifolia* are categorized as follows:

- **Rasa (Taste):** *Tikta* (Bitter), *Kasaya* (Astringent)
- **Guna (Physical Property):** *Laghu* (Light)
- **Virya (Potency):** *Usna* (Hot)
- **Vipaka (Post-digestive effect):** *Madhura* (Sweet)
- **Karma (Action):** *Tridosasamaka* (balancing all three doshas), *Samgrathi*, *Balya* (tonic), *Dipana* (appetizer), *Rasayana* (rejuvenator), *Raktasodhaka* (blood purifier), and *Jvaraghna* (antipyretic).

Therapeutic Indications & Dosage

Guduchi is indicated for clinical management of *kustha* (skin diseases), *vatarakta* (gouty arthritis), *jvara* (fever), *kamala* (jaundice), *pandu* (anemia), and *prameha* (diabetes). Popular classic formulations include *Amrtarisa*, *Amrtottara kvatha curna*, *Guduchi taila*, *Guducyadi curna*, *Guduchi sattav*, and *Chinnodbhavadi kvatha curna*.

- **Powdered Form:** 3–6 g
- **Decoction (Kwath):** 20–30 g of crude drug

6. Conclusion

The comprehensive anatomical and macro-morphological standards outlined here provide unmistakable identification keys for *Tinospora cordifolia*. Given its vast application in traditional pharmacy, structural validation through sequential double-staining tissue fixation protocols guarantees purity, minimizing risks of adulteration in commercial crude drug batches.

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