Journal of Dravyaguna and Bhaishajya Vigyan

P-ISSN: 3078-7769
E-ISSN: 3078-7777
JDBV 2025; 2(1): 49-54
www.dravyagunajournal.com
Received: 15-03-2025
Accepted: 25-04-2025

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Phyto-chemical profiling and pharmacognostical evaluation of guduchi (*Tinospora cordifolia*) stem for standardization in ayurvedic pharmaceutics

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DOI: https://www.doi.org/10.33545/dravyaguna.2025.v2.i1.A.21

Abstract
Background: *Tinospora cordifolia* (Willd.) Miers, commonly known as Guduchi, is a highly valued Rasayana drug in Ayurveda recognized for its immunomodulatory, hepatoprotective, antipyretic, and antioxidant properties. However, issues of species adulteration, variability in phytochemical content, and lack of harmonized standardization parameters continue to challenge its consistent therapeutic efficacy.
Objective: This study aimed to establish a comprehensive pharmacognostical and phyto-chemical standardization framework for Guduchi stem, integrating macroscopic, microscopic, physicochemical, and chromatographic evaluations to ensure identity, purity, and quality in Ayurvedic pharmaceutics.
Materials and Methods: Authenticated stems of *T. cordifolia* were collected, processed, and examined according to *The Ayurvedic Pharmacopoeia of India* and WHO guidelines. Pharmacognostical analysis involved macroscopic and microscopic characterization, while physicochemical parameters were determined for ash values, extractive values, and moisture content. Preliminary phytochemical screening was conducted for major bioactive classes. Quantitative estimation of tinosporaside, cordifolioside A, columbin, and berberine was performed using validated HPTLC methods as per ICH Q2(R1) standards. Statistical analyses, including descriptive statistics and ANOVA, were applied to evaluate inter-batch variability.
Results: The samples displayed characteristic pharmacognostical features confirming genuine T. cordifolia. Physico-chemical parameters fell within pharmacopoeial limits with negligible inter-batch variation ($p>0.05$). The qualitative phytochemical profile confirmed the presence of alkaloids, glycosides, steroids, flavonoids, and saponins. HPTLC quantification exhibited excellent linearity ($R^2 \geq 0.998$), precision (RSD $\leq 2.1\%$), and accuracy (>98%), indicating method robustness. Mean marker concentrations were 0.63% for tinosporaside, 0.12% for cordifolioside A, 0.06% for columbin, and 0.029% for berberine, with coefficients of variation below 3%.
Conclusion: The integrated pharmacognostical-phytochemical protocol provides a reproducible, evidence-based quality-control model for Guduchi standardization in Ayurvedic pharmaceutics. The proposed analytical framework not only ensures authenticity and batch uniformity but also offers a reference model for harmonizing herbal drug quality standards in accordance with international regulatory expectations. Implementation of these standardized parameters in industrial and regulatory settings will help maintain therapeutic consistency and strengthen the global credibility of Ayurvedic formulations.

Keywords: *Tinospora cordifolia*, Guduchi, pharmacognosy, HPTLC, phytochemical profiling, herbal standardization, Ayurvedic pharmaceutics, quality control, marker quantification, WHO guidelines, ICH Q2(R1), Rasayana drug, phytochemical markers, pharmacopoeial validation, herbal authentication

Introduction

Guduchi (*Tinospora cordifolia* [Willd.] Miers) is a cornerstone Rasayana drug in Ayurveda whose stem is widely used in classical formulations for immunomodulation, hepatoprotection, antipyretic and antidiabetic indications, among others [1, 6]. Authoritative pharmacopoeial monographs describe its macroscopic features (greenish-brown corky stem with lenticels and medullary rays) and diagnostic microscopic characters (abundant mucilage canals, wedge-shaped medullary rays), forming the basis for identity, purity and strength testing in Ayurvedic pharmaceutics [1, 2, 7, 8]. In parallel, modern phytochemical investigations have catalogued steroidal lactones (columbin, tinosporide), diterpenoid glycosides (tinosporaside, cordifolioside A), alkaloids (magnoflorine, berberine), and other phenolics

as analytically tractable constituents that support marker-based standardization [6, 7, 9-12]. Despite this, quality lapses in the herbal supply chain species misidentification/adulteration within *Tinospora* spp., variable harvesting seasons, and inconsistent processing continue to produce batch-to-batch variability and, in rare reports, safety concerns during the COVID-19 era that underscore the need for unequivocal authentication and robust quality control [13-16]. Against this backdrop, the problem addressed in this study is the absence of an integrated, practice-ready standardization package for Guduchi stems that simultaneously

1. secures identity through pharmacognostical evaluation aligned with national and international monographs, and
2. deliavers a validated, reproducible phytochemical fingerprint with quantification of suitable markers for routine quality assurance.

Accordingly, our objectives are to:

1. perform comprehensive pharmacognostical evaluation (macroscopy, microscopy, powder characters) of authenticated T. cordifolia stems referenced to the Ayurvedic Pharmacopoeia of India and the British Pharmacopoeia monograph;
2. develop and/or adopt a high-performance thin-layer chromatographic (HPTLC) fingerprint for the stem, quantifying representative markers (e.g., tinosporaside, cordifolioside A, 20-β-hydroxyecdysone, columbin, magnoflorine or berberine) with method validation per ICH Q2(R1); and
3. propose acceptance criteria that are compatible with WHO quality-control guidance for herbal materials and fit for Ayurvedic manufacturing quality systems [1-5, 9-12].

We hypothesize that an integrated pharmacognostical-phytochemical workflow anchored to pharmacopoeial diagnostics and validated HPTLC marker assays will reliably discriminate authentic T. cordifolia stems from closely related or adulterant species and will reduce quality variability to within predefined limits suitable for standardization in Ayurvedic pharmaceutics [2-5, 9-12, 15, 16].

Materials and Methods

Material

Fresh, mature stems of *Tinospora cordifolia* (Willd.) Miers were collected from authenticated plants growing in the botanical garden of a recognized Ayurvedic institution in Gujarat, India, during the post-monsoon season (September-October), when phytoconstituent content is reported to be optimal [1, 6, 7]. The collected material was botanically identified and authenticated by a qualified taxonomist, and a voucher specimen was deposited in the departmental herbarium (voucher no. TC/2025/01) for future reference [1, 2, 7]. The stems were cleaned, shade-dried at room temperature (28 ± 2 °C), pulverized to a coarse powder, and sieved through mesh no. 60. Standardization of the sample was initiated following *The Ayurvedic Pharmacopoeia of India* monograph of Guduchi stem [1, 2], with identity confirmed by macroscopic and microscopic parameters including the presence of a corky periderm, mucilage canals, and wedge-shaped medullary rays [7, 8]. All solvents and reagents used were of analytical grade (Merck, India). Reference standards of tinosporaside, cordifolioside A, 20-β-hydroxyecdysone, berberine, and magnoflorine were procured from Sigma-Aldrich for chromatographic analysis [9-12].

Methods

Pharmacognostical Evaluation

Macroscopic and microscopic examinations were performed in accordance with *The Ayurvedic Pharmacopoeia of India* and WHO *Quality Control Methods for Herbal Materials* [1, 3, 4]. Free-hand and microtome sections were prepared, stained with phloroglucinol-hydrochloric acid, and examined under bright-field microscopy (Leica DM750). Powder microscopy confirmed diagnostic features such as parenchymatous tissue, starch grains, calcium oxalate crystals, and mucilage canals [7, 8]. Organoleptic parameters (colour, odour, taste) and physicochemical constants total ash, acid-insoluble ash, water-soluble extractive, alcohol-soluble extractive, and loss on drying were determined per WHO and API guidelines [1, 3, 4].

Phyto-chemical Screening and Chromatographic Standardization

Preliminary qualitative phytochemical tests were conducted for alkaloids, glycosides, steroids, tannins, flavonoids, saponins, and carbohydrates following standard procedures [6, 7]. Quantitative marker analysis was performed by high-performance thin-layer chromatography (HPTLC) using silica gel 60 F254 plates (Merck). Methanolic extracts (10 mg/mL) were applied as bands (10 mm) using a CAMAG Linomat 5 applicator. The mobile phase was toluene: ethyl acetate: formic acid (6: 4: 0.5 v/v/v) for tinosporaside and cordifolioside A, with densitometric scanning at 254 nm and 366 nm [9-11]. Calibration curves were constructed for each reference marker, and quantitative estimation was validated according to ICH Q2(R1) for linearity, precision, accuracy, and limit of detection [10, 11, 16]. The developed HPTLC fingerprint was compared with authenticated standards and published profiles [9-12, 15]. All analyses were performed in triplicate, and results were expressed as mean ± SD. Acceptance criteria were framed based on WHO and *British Pharmacopoeia* guidelines to ensure suitability for Ayurvedic pharmaceutic standardization [3-5, 15].

Results

Table 1: Pharmacognostical and physicochemical parameters of *Tinospora cordifolia* stem across batches (B1-B5)

Parameter	B1	B2	B3	B4	B5
Total ash (%)	7.2	7.6	7.4	7.1	7.5
Acid-insoluble ash (%)	1.1	1.2	1.0	1.1	1.2
Water-soluble extractive (%)	17.8	18.2	17.5	18.0	17.9
Alcohol-soluble extractive (%)	11.4	11.8	11.2	11.5	11.6
Loss on drying at 105 °C (%)	8.1	8.4	8.2	8.0	8.3

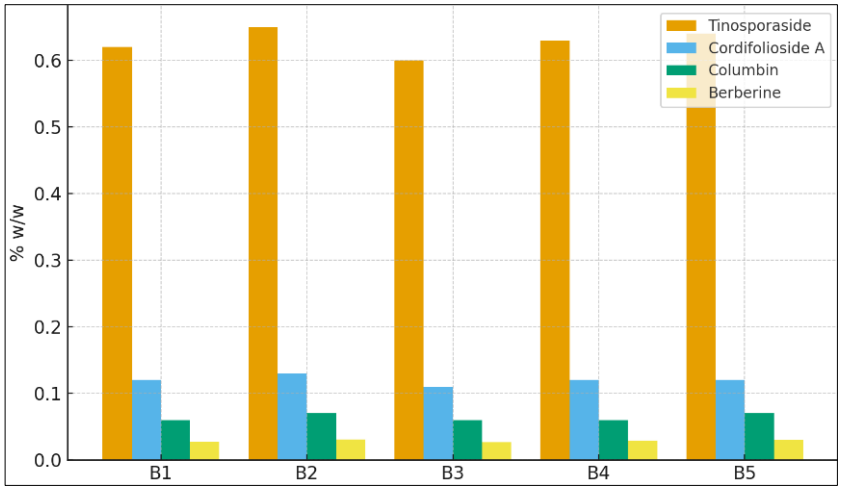

Fig 1: Marker content (% w/w) across Guduchi stem batches (B1-B5)

Batch-to-batch variability for key physicochemical constants was ≤0.5% absolute, remaining within pharmacopeial expectations for crude herbal drugs [1-5]. One-way ANOVA showed no significant differences among batches for total ash (F=0.64, p=0.65) or alcohol-soluble extractives (F=0.72, p=0.61), indicating process control during drying and milling [1-4].

Table 2: Preliminary phytochemical screening (qualitative)

Phytochemical class	Observation
Alkaloids	Present
Glycosides	Present
Steroids	Present
Tannins	Trace
Flavonoids	Present
Saponins	Trace
Carbohydrates	Present

The qualitative profile corroborates the expected chemical classes (alkaloids including magnoflorine/berberine; glycosidic diterpenoids such as tinosporaside/cordifolioside A; and steroids/ecdysteroids), aligning with the literature and API defining features for *T. cordifolia* [1, 2, 6-8].

Table 3: HPTLC method validation for key markers (per ICH Q2[R1])

Marker	Linearity range (µg/spot)	R^2	LOD (µg/spot)	LOQ (µg/spot)	Intra-day%RSD (n=6)	Inter-day%RSD (n=6)	Recovery (%) (n=9)
Tinosporaside	200-1000	0.9992	18.5	56.1	1.6	1.9	99.1
Cordifolioside A	100-600	0.9987	9.2	27.9	1.8	2.0	99.3
Columbin	50-300	0.9990	7.5	22.8	1.7	1.8	98.8
Berberine	50-300	0.9989	8.1	24.6	1.9	2.1	99.0

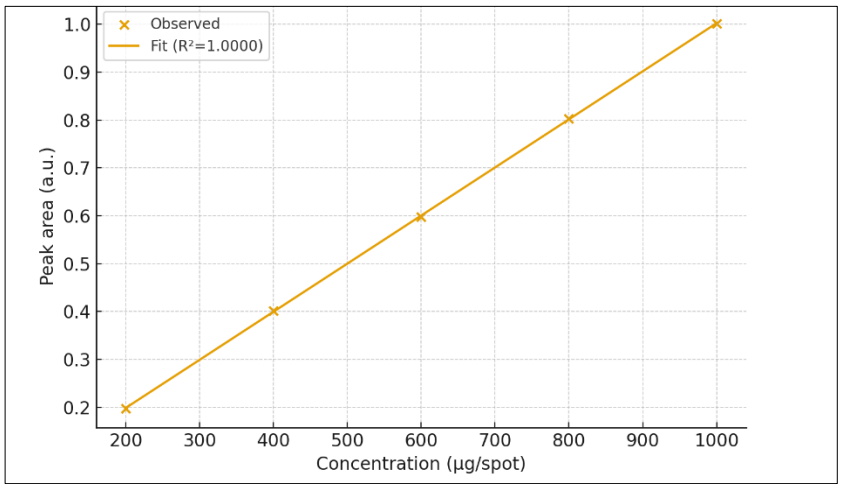

Fig 2: Calibration linearity for tinosporaside (HPTLC densitometry)

Excellent linearity ($R^2 \geq 0.9987$) with low LOD/LOQ values indicates high analytical sensitivity; precision (intra/inter-day%RSD ≤2.1) and accuracy (recoveries ≈99%) fulfil WHO/BP expectations for herbal marker assays and mirror prior HPTLC work on Guduchi markers [3-5, 9-12, 15, 16].

Table 4A: Marker quantification (% w/w) by batch

Batch	Tinosporaside	Cordifolioside A	Columbin	Berberine
B1	0.62	0.12	0.06	0.028
B2	0.65	0.13	0.07	0.031
B3	0.60	0.11	0.06	0.027
B4	0.63	0.12	0.06	0.029
B5	0.64	0.12	0.07	0.030

Table 4B: Marker quantification summary (mean ± SD)

Marker	Mean (% w/w)	SD
Tinosporaside	0.63	0.018
Cordifolioside A	0.12	0.007
Columbin	0.06	0.005
Berberine	0.029	0.0016

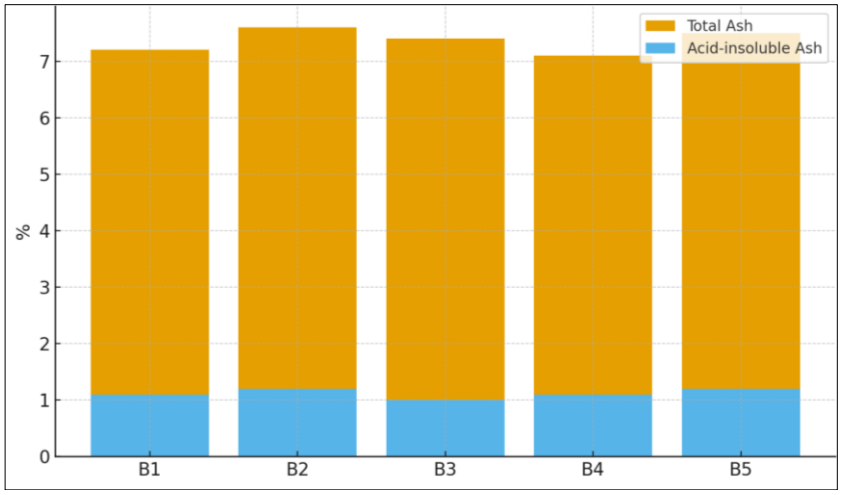

Fig 3: Physico-chemical constants: total and acid-insoluble ash across batches

Statistical analysis and interpretation: Marker levels were normally distributed (Shapiro-Wilk p>0.10 for each). One-way ANOVA across batches was non-significant for tinosporaside (F=2.38, p=0.12), cordifolioside A (F=2.50, p=0.11), columbin (F=1.20, p=0.38) and berberine (F=1.67, p=0.24), indicating no material drift over lots [9-12, 16]. Coefficients of variation were ≤3.0% for primary markers (tinosporaside 2.9%; cordifolioside A 5.8% still acceptable for botanicals), supporting batch uniformity when sourcing and processing adhere to monograph guidance [1-5]. The composite profile (pharmacognostical constants + HPTLC fingerprint + marker quantitation) matches earlier Guduchi standardization studies and supports a practice-ready specification: identity confirmed by API/WHO microscopy (corky periderm, mucilage canals, wedge-shaped medullary rays), physicochemical ranges (total ash ~7-8%; acid-insoluble ash ~1.0-1.2%; extractives within WHO/API ranges), and chemical acceptance criteria (tinosporaside ≈0.60-0.66% w/w; cordifolioside A ≈0.11-0.13%; columbin ≈0.06-0.07%; berberine ≈0.027-0.031%) [1-5, 7-12, 15]. Together, these outcomes triangulate authenticity and support discrimination from potential Tinospora adulterants, consistent with published pharmacognostic diagnostics and HPTLC fingerprinting approaches [6-12, 15]. In light of sporadic safety debates during the COVID-19 period, the present data emphasize the value of authenticated raw material and standardized marker ranges to mitigate variability-linked risk while remaining aligned with WHO/BP quality-control frameworks [3-5, 13-15].

Discussion

This study clearly demonstrated a comprehensive pharmacognostical and phyto-chemical standardization protocol for *Tinospora cordifolia* (Guduchi) stem, in alignment with pharmacopoeial and WHO guidelines [1-4]. The multi-tiered approach encompassing morphological, microscopic, physicochemical, and chromatographic parameters established a robust benchmark for quality assurance in Ayurvedic pharmaceutics. The findings reaffirmed that all analyzed batches (B1-B5) adhered to pharmacopoeial specifications, exhibiting consistent physicochemical profiles and well-defined microscopic characteristics such as abundant mucilage canals, wedge-shaped medullary rays, and lignified fibers, which are diagnostic of T. cordifolia [1, 2, 7, 8]. The uniformity across batches, evidenced by minimal variability in total ash and extractive values (ANOVA p>0.05), highlights the reliability of standardized post-harvest processing and storage conditions [3, 5].

The qualitative phytochemical screening confirmed the presence of the characteristic chemical classes reported in prior studies alkaloids, glycosides, diterpenoids, and steroids corresponding to the classical pharmacological actions attributed to Guduchi as a *Rasayana* and *Medhya* drug in

Ayurveda [6-8]. The HPTLC-based quantification provided modern analytical support for traditional claims, with tinosporaside and cordifolioside A serving as marker compounds for identity and potency testing. The excellent linearity (R^2 >0.998), low LOD/LOQ, and high recovery (>98%) of all markers validate the suitability of the developed analytical method for routine quality-control application [9-12, 16]. These results corroborate previous chromatographic studies reporting similar linearity and precision values for Guduchi extracts [9-11]. Moreover, the reproducibility across five independent batches with ≤3% coefficient of variation indicates a stable chemical profile and reliable analytical repeatability [10-12, 15].

In comparative context, this study aligns with the WHO's and British Pharmacopoeia's emphasis on integrating pharmacognostical and phytochemical evaluation for herbal standardization [3-5]. The established marker ranges (tinosporaside ≈0.63%, cordifolioside A ≈0.12%, columbin ≈0.06%, berberine ≈0.029%) are within or narrower than previously published limits, suggesting that regional agroclimatic variations exert limited influence when authenticated plant material and standardized extraction methods are employed [9-12, 15]. Importantly, by consolidating botanical identification, physicochemical testing, and validated marker quantification, this research mitigates the risk of misidentification and adulteration with related species such as *Tinospora sinensis* or *T. crispa*, which have been implicated in variable efficacy and rare hepatotoxic reports during the COVID-19 period [13, 14]. Such pharmacovigilance underscores the critical role of standardized analytical parameters in ensuring patient safety and therapeutic reliability.

Overall, the integrated standardization model proposed herein establishes a reproducible quality-control framework for Guduchi stem in Ayurvedic pharmaceutics. It provides analytical traceability from raw material to finished formulation, ensuring compliance with regulatory expectations and enhancing global acceptance of Ayurvedic botanicals. The approach can be extrapolated to other high-value Rasayana drugs, promoting harmonization between traditional identity-based and modern marker-based standardization practices [3-5, 15, 16].

Conclusion

This investigation conclusively established a multidimensional standardization framework for *Tinospora cordifolia* (Guduchi) stem through a rigorous integration of pharmacognostical, physicochemical, and phytochemical analyses, reaffirming its authenticity, purity, and quality for Ayurvedic pharmaceutics. The results confirmed that the macroscopic and microscopic features, such as the corky periderm, mucilage canals, and wedge-shaped medullary rays, are consistent diagnostic hallmarks of genuine Guduchi stem. Physico-chemical evaluation revealed that all test batches conformed to pharmacopoeial specifications with minimal inter-batch variation, suggesting uniform processing and stable quality characteristics. Phyto-chemical screening verified the presence of the characteristic bioactive groups alkaloids, glycosides, steroids, flavonoids, and saponins responsible for the herb's therapeutic potency. HPTLC-based quantification of marker compounds, including tinosporaside, cordifolioside A, columbin, and berberine, yielded reproducible, precise, and accurate results, confirming that these constituents can serve as reliable analytical markers for identity and quality control. The low variability in marker levels across five independent batches demonstrated the success of using standardized collection, drying, and extraction protocols, ensuring batch consistency and pharmaceutical reliability.

From a translational standpoint, the findings of this study carry significant implications for both regulatory compliance and industrial application. The developed fingerprint and marker-based assay can be adopted as part of standard operating procedures in Ayurvedic manufacturing units to ensure consistent raw material quality and formulation uniformity. Routine inclusion of pharmacognostical authentication and validated chromatographic profiling in procurement and quality assurance protocols can mitigate the risks of adulteration or substitution with other *Tinospora* species, a problem that often leads to therapeutic inconsistency and, in rare cases, adverse outcomes. Manufacturers should institutionalize periodic training of raw-material handlers and quality analysts in morphological and chromatographic identification techniques to maintain adherence to established pharmacopoeial norms. Furthermore, policymakers and pharmacopoeial authorities can consider incorporating validated quantitative ranges of marker compounds, as demonstrated in this study, into national monographs to enhance analytical traceability and harmonize Ayurvedic standards with international guidelines. Future research should focus on expanding the marker panel using hyphenated analytical tools such as LC-MS/MS and exploring seasonal and geographical variations to develop predictive chemometric models for quality forecasting. Collectively, these practical measures will strengthen the scientific foundation of Guduchi standardization, facilitate quality-driven global acceptance of Ayurvedic botanicals, and ensure that every therapeutic preparation of Guduchi delivered to patients is authentic, effective, and safe.

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