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Identification, Authentication and Extraction Techniques for *Tinospora cordifolia* (Giloy): A comprehensive review

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Abstract

Accurate identification and authentication of medicinal plants underpin both the safety and efficacy of herbal preparations. The climber Tinospora cordifolia (Willd.) Miers (commonly known as Guduchi or Giloy) is widely used in traditional Indian medicine and has attracted substantial pharmacological interest. However, its extensive commercial use has been accompanied by challenges of adulteration, substitution and mis-identification. This review collates and synthesizes the key information on identification and authentication techniques for *T. cordifolia*, with the aim of providing a coherent framework for quality assurance in raw materials and finished herbal products. First, we outline the botanical description and taxonomic placement of *T. cordifolia*, highlighting its distinguishing morphological features. We then examine the principal challenges in identification, including morphological similarity with congeneric species and deliberate or accidental adulteration. Traditional macro and microscopic methods of authentication are described, followed by detailed discussion of modern analytical and molecular techniques-physicochemical parameters, chromatographic, spectroscopic methods, and DNA-based molecular markers (ITS, RAPD, SSR) supplemented by chemometric and metabolomic approaches. A comparative analysis of these techniques emphasizes their respective advantages, limitations and appropriate contexts of use. Recent advances such as AI driven image recognition, digital herbarium databases and blockchain traceability in herbal supply chains are also considered, pointing to future directions for robust authentication. In conclusion, while no single technique suffices in isolation, a multitiered, orthogonal approach combining morphological, chemical and molecular methods offers the greatest assurance of authenticity. Research gaps persist in large-scale validation, cost-effective, field deployable tools and integration of traceability systems these warrant future investigation.

Keywords: *Tinospora cordifolia*; Authentication; Giloy; Identification; Gulvel; Extraction

1. Introduction

The medicinal climber Tinospora cordifolia (Willd.) Miers (family Menispermaceae), commonly referred to as Guduchi or Giloy in Ayurveda, has been employed for centuries in the Indian subcontinent as a rejuvenating (rasāyana) herb and in the treatment of a wide array of ailments ranging from fevers and infections to metabolic disorders and immune modulation (Satruhan and Patel, 2022). Ethnopharmacologically, *T. cordifolia* has been reported to exert immunomodulatory, anti-inflammatory, hepatoprotective, antidiabetic and antioxidant effects making it a key ingredient in numerous traditional formulations. Its increasing incorporation into commercial herbal products has raised concomitant demands for rigorous quality control and assurance.

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Figure 1 Morphology of Tinospora cordifolia leaves, stem and Fruits

However, the burgeoning market demand and value of *T. cordifolia* have been accompanied by significant challenges in ensuring authentic plant material. Adulteration, substitution with related Tinospora species (e.g., T. sinensis, T. crisp a) or other climbers, mis-labelling of processed powders, and morphological resemblance among species all threaten therapeutic efficacy and consumer safety. For instance, an HPLC study noted significant variation in berberine content between *T. cordifolia* and T. sinensis, reinforcing the risk of species substitution. Hence, accurate identification and authentication are crucial to protect both the integrity of herbal medicine and public health. In this review, we systematically evaluate the spectrum of techniques available for identification and authentication of *T. cordifolia*, from traditional macroscopic diagnostics to advanced spectroscopic, chromatographic and molecular assays, placing them in context of practical application and future innovations. By synthesizing current knowledge, we aim to provide a reference for researchers, quality-control laboratories and regulatory bodies engaged in the authentication of *T. cordifolia* and allied herbal raw materials.

2. Botanical Description and Taxonomy

2.1. Taxonomy

T. cordifolia (Willd.) Miers ex Hook. f. and Thom. belongs to the family Menispermaceae. The genus Tinospora consists of herbaceous or woody climbers primarily distributed in tropical Asia. *T. cordifolia* is widely reported from India, Sri Lanka and South-East Asia. The plant is often cited under the vernacular names Guduchi (Sanskrit), Giloy (Hindi) and Gulvel (Marathi).

2.2. Morphology

T. cordifolia is a perennial, dioecious, glabrous woody climber, producing slender, wiry, greenish to brownish stems that are succulent when mature and distinctly fluted or ridged. The leaves are alternate, simple, broadly ovate to reniform (kidney-shaped) or cordate with petiole, measuring typically 5–12 cm across; the margin is broadly lobed or sinuous, apex acute to acuminate, base broadly cordate. The adaxial surface is glabrous and glossy green, the abaxial surface somewhat paler. The plant exhibits dimorphism of genders with male and female plants producing unisexual inflorescences of small greenish flowers. The fruits are drupaceous, globose to ellipsoid, turning red when ripe.

Key distinguishing features include: the cordate leaf base with a broad sinus, the slender pendulous stems often seen twining on host surfaces, and the fluted ridging of older stems. The plant's habit of climbing other trees, its woody base, and its typical stem morphology help to distinguish it in the field from other climbers.

2.3. Diagnostic Botanic Features

Macroscopic diagnostic points include:

- Stem: green when young, turning greyish-brown; diameter typically 1–2 cm; longitudinal ridging; fracture fibrous and stringy.
- Leaves: broadly ovate to cordate, lobed or sinuous margin; petiole often 1–3 cm; lower surface lighter.
- Branches often cylindrical, glabrous, with internodes more or less equal.
- Male and female plants: male inflorescences arise from leaf axils, slender racemes; female plants produce thicker racemes with globose drupes later.

For taxonomic resolution, *T. cordifolia* is distinguished from close relatives such as *Tinospora sinensis* and *Tinospora crispa* by morphological characters such as leaf shape, size of flowers, nature of fruit and phytochemical profile (e.g., variation in alkaloid and diterpenoid content) (HPLC comparison between *T. cordifolia* and T. sinensis showed marked chemical differences).

3. Pharmacological activities

3.1. Antioxidant Activity

Extracts of *T. cordifolia* exhibit strong free radical scavenging activity. The methanolic extract enhances superoxide dismutase (SOD), catalase, and glutathione peroxidase levels, protecting against oxidative stress-induced tissue damage.

3.2. Antimicrobial Activity

Both aqueous and ethanolic extracts demonstrate significant antibacterial and antifungal activity against *E. coli, Staphylococcus aureus*, and *Candida species*, attributed to alkaloids and phenolic compounds.

3.3. Anti diabetic Activity

Animal models show hypoglycemic effects through stimulation of insulin secretion and enhancement of glucose uptake. Clinical trials suggest improved glycemic control in type 2 diabetes mellitus.

3.4. Anti-inflammatory and Analgesic Activity

Extracts inhibit cyclooxygenase and lipoxygenase pathways, reducing pro-inflammatory mediators such as TNF-α and IL-6.

3.5. Hepatoprotective Activity

Protects liver tissues from toxin-induced damage by modulating antioxidant enzymes and stabilizing cellular membranes.

3.6. Immunomodulatory Activity

Stimulates macrophage activation, enhances antibody production, and modulates cytokine balance, supporting its traditional use as an immune booster.

3.7. Anticancer Activity

Studies report cytotoxic effects against breast, liver, and cervical cancer cell lines, mediated by apoptosis induction and oxidative damage pathways.

3.8. Neuroprotective and Antistress Activity

Improves cognitive function, reduces anxiety, and prevents neuronal damage via antioxidant mechanisms.

3.9. Cardioprotective Activity

Protects cardiac tissue from ischemic injury by improving lipid profiles and reducing oxidative stress.

3.10. Other Pharmacological Effects

Includes antipyretic, antiulcer, antimalarial, and wound-healing activities confirmed by various in vivo models.

4. Challenges in Identification

Despite the clarity of many morphological features, several practical challenges hamper reliable identification of *T. cordifolia* in both raw and processed form:

4.1. Morphological similarity and species confusion

Within the genus *Tinospora*, species share climbing habit, stem morphology and leaf shape; for example, *T. sinensis, T. rumphii* and *T. crispa* may closely resemble *T. cordifolia*, especially in vegetative stage. This similarity can lead to inadvertent substitution.

4.2. Adulteration and substitution

Commercial herbal preparations may contain other species mislabeled as *T. cordifolia*, either through deliberate adulteration (to reduce cost) or accidental replacement due to ambiguous supply chain. A chemical fingerprinting study showed that substitution may be masked in powdered material.

4.3. Powdered or processed raw materials

Once the stem or leaf has been dried, powdered or processed, morphological features become obscured, making visual/macroscopic authentication unreliable and increasing the need for analytical methods.

4.4. Intraspecific variation

T. cordifolia exhibits variability in secondary metabolite profiles across geography, season, gender (male vs female plants) and plant age. For instance, an HPLC/ESI-QTOF-MS study revealed marker ions distinguishing male and female plant stems. Such variation may complicate simple chemical marker-based authentication unless accounted for.

4.5. Weak regulatory enforcement and supply-chain traceability:

Herbal raw material supply chains are often complex, with multiple intermediaries, which increases risk of mislabeling or dilution. Without rigorous traceability, even genuine source species may suffer quality issues (contamination, degradation).

4.6. Lack of standardized reference material

For many herbs, including *T. cordifolia*, the availability of well-authenticated voucher specimens, detailed morpho-chemical databases and validated reference markers remains limited hindering universal adoption of authentication methods.

These challenges emphasize the necessity for robust, multiplexed authentication strategies that integrate morphological, chemical and molecular dimensions to ensure genuine *T. cordifolia* material.

5. Traditional identification methods

Traditional authentication of herbal raw materials relies upon macro- and microscopic techniques grounded in pharmacognosy. Though increasingly complemented by advanced analytics, these methods remain foundational and widely used for first-line screening.

5.1. Macroscopic Examination

Macroscopic or organoleptic methods involve inspection of the raw plant material — stems, leaves, bark or powder — for characteristic morphological traits: colour, texture, shape, fracture, odour and taste. For *T. cordifolia*:

- Stem: greenish-brown, slightly succulent when fresh, turning fibrous upon drying; fracture fibrous, longitudinal fibres visible; surface ridged/fluted.
- Leaves: alternate, simple, broadly ovate to cordate with a broad sinus at the base; petiole present; margins gently lobed; upper surface glossy green, lower paler.
- Odour: faintly characteristic; taste: bitter and astringent.
- Powder: light brown to greyish-brown, fibrous fragments visible under loupe; slight bitterness when tasted (for raw sample). Field guides and monographs (e.g., Ayurvedic Pharmacopoeia) provide descriptive keys for such assessments.

Although macroscopic inspection is rapid, non-destructive and inexpensive, its limitations include inability to distinguish closely related species once material is powdered, and reliance on operator experience.

5.2. Microscopic (Pharmacognostic) Analysis

Microscopy examines anatomical and cellular features of the plant part — useful for both raw and powdered material. For *T. cordifolia*, pharmacognostic studies list features such as:

- Stem transverse section (TS): single-layered epidermis, narrow cortex of parenchyma, prominent medullary rays, scattered sclereids/fibres, duct-like secretory spaces, vessel elements in radial and tangential groups.
- Leaf TS: dorsiventral structure in petiole or lamina; palisade and spongy mesophyll, vascular bundles, presence of calcium oxalate crystals and stomatal type.
- Powder microscopy: presence of fragmentary fibres, sclereids, vessels, parenchyma cells, fragments of pitted vessels, starch grains (in some parts) and other diagnostic elements.

These microscopic traits provide confirmatory evidence of species identity or plant part authenticity, and are often included in pharmacopeial monographs of herbal raw materials. However, microscopy too has limitations: it cannot always distinguish species with very similar anatomical features, and requires skilled microscopy and sample preparation.

5.3. Physicochemical Parameters (Classical)

Although bridging into more analytical territory, classical physicochemical tests are traditionally applied. These include:

- Foreign matter, total ash, acid-insoluble ash, water-soluble ash, extractive values (water, ethanol), moisture content, loss on drying.
- Organoleptic characteristics (colour, odour, taste) and macroscopic features as mentioned.
- Simple chemical tests (e.g., alkaloid presence via Dragendorff's reagent, saponins, tannins) and thin-layer chromatography (TLC) for presence of one or more marker compounds.

In the case of *T. cordifolia*, some studies have reported moisture content (~8 %) and ash values within WHO-recommended limits in quality stems. While these tests are helpful as release specifications, they are insufficient as standalone authentication tools, given the possibility of adulteration or substitution. In summary, traditional identification methods offer a foundation of morphological and anatomical inspection, and remain appropriate for initial screening, but are increasingly inadequate for the demands of modern herbal quality assurance — especially when dealing with processed powders or discriminating among closely related species. Hence the shift towards modern analytical and molecular techniques.

Although basic in nature, refined physicochemical parameters form practical and cost-effective first-line checks and are often mandated by national pharmacopoeias. Important parameters include moisture content, ash values (total ash, acid-insoluble ash), extractive values (e.g., ethanol and water), pH, foreign matter, and loss on drying. Consistent deviation from established ranges in *T. cordifolia* may signal degraded or adulterated material. Furthermore, quantification of known major marker compounds (for example, tinosporaside, berberine, palmatine) via simpler methods (e.g., HPTLC densitometry) has become part of routine quality control. For example, HPTLC quantification of tinosporaside in *T. cordifolia* yielded an average content of ~0.40 % w/w in the stem material. Such marker quantification, while not definitive for species identity, provides a comparative metric for quality and consistency. Advantages of physicochemical methods include simplicity, low cost, and ease of deployment in large sample sets. Limitations include low species specificity (i.e., cannot reliably distinguish species), susceptibility to environmental and processing effects, and inability to detect adulterants that mimic marker compound levels.

5.4. Modern Analytical and Molecular Techniques

Over the last decade, authentication of medicinal plants such as *T. cordifolia* has evolved considerably. A wide spectrum of analytical and molecular methodologies is now available. In this section we categorise and discuss these techniques under major headings: physicochemical parameters, chromatographic methods, spectroscopic methods, DNA barcoding / molecular markers, and chemometric/metabolomic approaches.

6. Chromatographic methods (hptlc, hplc, gc–ms)

Chromatographic separation of compounds followed by detection enables fingerprinting and quantification of chemical constituents within *T. cordifolia* and its potential adulterants.

6.1. HPTLC

High-performance thin layer chromatography (HPTLC) is widely applied for herbal authentication due to its relative simplicity and speed. For *T. cordifolia*, an HPTLC method was developed for tinosporaside with good linearity and recovery. HPTLC fingerprinting (densitometric scanning) of leaf extracts has also been used to quantify rutin, gallic acid and quercetin. HPTLC allows visual comparison of bands/spots and densitometric quantification, making it useful for screening of large numbers of samples.

- Benefits: Lower cost, minimal equipment compared with HPLC, and the ability to visually compare multiple samples in parallel.
- Limitations: lower resolution and specificity compared with HPLC/GC–MS, potential for overlap of compounds, and less suitability for precise quantification or for complex mixtures.

6.2. HPLC and UHPLC

High-performance liquid chromatography (HPLC), often coupled with UV/PDA detection or mass spectrometry, offers higher resolution and quantitation capability. For example, an RP-HPLC-UV-DAD method separated and quantified four marker compounds (20β-hydroxyecdysone, tinosporaside, cordioside and columbin) in multiple accessions of Tinospora species. In another study, RP-HPLC-PDA combined with MS/MS successfully distinguished *T. cordifolia* (TCP) and T. crispa (TCR) via specific diterpenoid markers (borapetosides B and E) with sensitivity to 1 % adulteration. HPLC/LC-MS methods have also been used to investigate seasonal and gender variation in *T. cordifolia* stems.

- Benefits: High accuracy, good quantification, and ability to detect minor compounds/adulteration at low levels.
- Limitations: Higher cost, need for standard compounds, longer run times, and requirement for skilled operation and maintenance of instrument.

6.3. GC–MS

Gas chromatography coupled with mass spectrometry (GC–MS) is more commonly applied to volatile and semi-volatile constituents. Although less common in authentication of *T. cordifolia* which is rich in non-volatiles (alkaloids, diterpenoids), a recent pharmacognostical profiling study used GC–MS to profile stem extracts and identified tinosporaside (14.2 %), berberine (12.5 %), palmatine (10.8 %), magnoflorine (9.4 %), β-sitosterol (8.6 %), stigmasterol (7.9 %) among others. GC–MS is useful when volatile markers or relative proportions of compounds are informative, but its applicability is restricted when the key markers are non-volatiles or thermally labile.

7. Spectroscopic methods (FTIR, UV–VIS, NMR)

Spectroscopic techniques provide structural or functional information about compounds or functional groups in plant extracts and are increasingly employed for authentication and fingerprinting.

7.1. UV–Vis Spectroscopy

Simple absorbance measurements of extracts may provide broad profiles or help quantify chromophoric constituents; though low in specificity.

7.2. FTIR (Fourier Transform Infrared Spectroscopy)

FTIR provides characteristic absorption bands corresponding to functional groups (e.g., hydroxyl, carbonyl, aromatic systems). Some researchers have used FTIR as a rapid screening tool for adulteration in herbal powders, although specific studies in *T. cordifolia* are fewer.

7.3. NMR (Nuclear Magnetic Resonance Spectroscopy)

NMR, including ^1H and ^13C NMR, offers detailed structural elucidation of isolated marker compounds, and potentially fingerprinting of metabolite mixtures (metabolomics). In the HPTLC standardization of tinosporaside, the isolated

compound was characterized by IR, UV, mass and NMR spectra. While NMR is rich in data content and specificity, its use in routine authentication is limited by cost, instrument complexity and need for expert interpretation.

Spectroscopic techniques provide the advantage of being non-destructive and highly specific, but tend to require sophisticated instrumentation and are less suited for high-throughput screening unless integrated with chemometric approaches.

8. Extraction of Phenolic Compounds

Using Solvents Scientists have studied and analyzed the impact of different types of solvents, such as methanol, hexane, and ethyl alcohol, for the purpose of antioxidant extraction from various plants parts, such as leaves and seeds. In order to extract different phenolic compounds from plants with a high degree of accuracy, various solvents of differing polarities must be used. Moreover, scientists have discovered that highly polar solvents, such as methanol, have a high effectiveness as antioxidants. Anokwuru et al. reported that acetone and N, N dimethylformamide (DMF) are highly effective at extracting antioxidants, while

Koffi et al. found that methanol was more effective in at a large number of phenolic contents from walnut fruits when compared to ethanol. It has been reported that ethanolic extracts of Ivorian plants extracted higher concentrations/amount of phenolics compared to acetone, water, and methanol. Multiple solvents have been commonly used to extract phytochemicals, and scientists usually employed a dried powder of plants to extract bioactive compounds and eliminate the interference of water at the same time. Solvents used for the extraction of biomolecules from plants are chosen based on the polarity of the solute of interest. A solvent of similar polarity to the solute will properly dissolve the solute. Multiple solvents can be used sequentially in order to limit the number of analogous compounds in the desired yield. The polarity, from least polar to most polar, of a few common solvents is as follows: Hexane < Chloroform < Ethyl acetate < Acetone < Methanol < Water.

8.1. Microwave-Assisted Extraction (MAE)

MAE has attracted the attention of researchers as a technique to extract bioactive compounds from a wide variety of plants and natural residues [12]. Microwaves have electromagnetic radiation that occurs at frequencies between 300 MHz to 300 GHz, and wavelengths between 1 cm and 1 m. These electromagnetic waves consist of both an electrical field and a magnetic field. These are described as two perpendicular fields. The first application of microwaves was to heat up objects that can absorb a part of the electromagnetic energy and convert it into heat. Commercial microwave instruments commonly use the frequency 2450 MHz, which corresponds to an energy output of 600–700 Watts. Recently, advanced techniques have become available to reduce the loss of bioactive compound without increasing the extraction time. Therefore, microwave-assisted extraction is demonstrated to be a good technique in multiple fields, especially in the medicinal plant area. Moreover, this technique reduced the losses of the biochemical compounds being extracted. Microwave-assisted extraction (MAE) has been used as an alternative to conventional techniques for the extraction of antioxidants because of its ability to reduce both time and extraction solvent volume. In fact, the main objective of using MAE is to heat the solvent and extract antioxidants from plants with a lesser amount of these solvents. Li et al. reported that conventional methods using various solvents presented less antioxidant activity and phenolic content than MAE. Therefore, the finding confirmed that MAE was more effective at increasing antioxidant activity by measuring ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), and total phenolic content (TPC). The efficiency of the microwave extraction can be changed through some factors such as extraction temperature, solvent composition, and extraction time. The extraction temperature was usually studied more than other factors due to its ability to increase the efficiency of the microwave extraction. Tsubaki et al. reported that 170 ◦ C was the most effective temperature for extracting phenolic compounds from Chinese tea. In addition, Plants 2017, 6, 42 3 of 23 increasing the extraction temperature beyond this point resulted in a reduced extraction yield. Recently, Christophoridou et al. used a new microwave-assisted extraction (MAE) process, which converts energy to heat, thereby cooperating with solvents in order to extract a specific compound [18]. Williams et al. showed many advantages of MAE, including lower solvent consumption, shorter extraction times, and higher sensitivity towards target molecules.

9. Chemometric and Metabolomic Approaches

Chemometrics (statistical analysis of chemical data) and metabolomics (comprehensive profiling of metabolites) are emerging as powerful tools in plant authentication, especially for differentiation of species, geographical origins, plant gender or seasonality. For *T. cordifolia*, an HPLC-ESI-QTOF-MS study combined with chemometric tools (ANOVA, PCA, ROC curve) identified marker ions for differentiation of plant gender and geographic origin (m/z 294.1139, 445.2136 for locations; m/z 344.1482, 359.1501,373.1305 for seasons; m/z 257.1380 for gender) with 100 % sensitivity and

specificity. Another chemical fingerprinting study distinguished closely related Tinospora species (*T. cordifolia*, T. sinensis, T. crispa) using UHPLC-UV-MS. The strength of chemometrics lies in its capacity to integrate large datasets (chromatographic peaks, spectral features) and provide statistical discrimination. Its limitations include the need for large, well-characterized sample sets and advanced data-analysis infrastructure.

10. Comparative Analysis of Techniques

Table 1 Authentication techniques for *T. cordifolia* in terms of their advantages, limitations and applicability in herbal raw-material

Technique Category	**Advantages**	**Limitations**	**Applicability for *T. cordifolia***
Macroscopic/Microscopic	Low cost, rapid, minimal equipment, field-friendly	Low specificity, inadequate for powdered material, dependent on operator skill	Suitable for initial screening of stems/leaves; insufficient alone
Classical Physicochemical	Simple, widely accepted in pharmacopeias, provides QC benchmarks	Does not confirm species identity; sensitive to processing/ storage	Useful for batch release checks (ash, moisture, extractives) in *T. cordifolia*
HPTLC	Relatively inexpensive chromatography, visual fingerprinting, good for screening	Limited resolution vs HPLC, requires standards, less quantitative precision	Good for routine quality control of *T. cordifolia* (e.g., tinosporaside content)
HPLC (LC-MS)	High specificity and quantification, can detect adulteration at low levels	Higher cost, longer analysis time, requires standards and skilled personnel	Highly suitable for *T. cordifolia* authentication and differentiation from congener species
GC–MS	Good for volatiles/semi-volatiles, sensitive and specific	Many *T. cordifolia* markers are non-volatile, restricts applicability, higher cost	Supplementary role in *T. cordifolia* when volatile markers are relevant
Spectroscopic (FTIR/NMR)	Non-destructive, provides fingerprint or structural info, high specificity	Costly, requires expertise, less high-throughput, requires reference database	Useful for advanced authentication research and differentiation of complex mixtures of *T. cordifolia*
DNA Barcoding/Molecular Markers	High specificity, works with processed materials, can distinguish species/authenticity effectively	Costs, need for reference sequences, potential low divergence in some taxa, technical infrastructure required	Very suitable for *T. cordifolia* authentication in supply chains; aligns with adulteration detection
Chemometric/Metabolomic	Integrates multidimensional data, detects subtle variation (gender, location, age)	Requires large datasets, high instrument/data-analysis cost, complexity in interpretation	Promising for advanced authentication of *T. cordifolia* (geographical origin, gender, seasonal variation)

In practice, a tiered approach is advantageous: macroscopic/microscopic screening → physicochemical checks → targeted chromatographic (HPTLC/HPLC) fingerprinting → molecular authentication for raw or powdered material → chemometric/ metabolomic profiling for advanced discrimination (e.g., adulteration at low percentage, geographic origin). For *T. cordifolia*, studies have shown that combination of HPTLC and HPLC-PDA/ MS allowed detection of 1% adulteration with a congener species. While molecular markers (e.g., next-gen sequencing derived primers) offered 100% sensitivity and specificity in one validation study. This illustrates that no single method suffices for all authentication challenges — rather, complementarity and cross-validation are key.

11. Recent Advances and Future Prospects

The past few years have witnessed several innovative trends in herbal authentication that are relevant for *T. cordifolia* and the broader herbal-raw-material sector.

11.1. Artificial Intelligence and Machine Learning

AI/ML methods (e.g., neural networks, deep learning) are gaining traction for pattern recognition in botanical images, chromatographic/spectral data or metabolomic datasets. While few studies have focused explicitly on *T. cordifolia*, analogous work in herbal authentication (e.g., clove origin identification via neural networks) demonstrates the feasibility of using metabolic fingerprints plus AI classifiers. In the future, image-based smartphone apps could assist in field authentication of *T. cordifolia* stems/leaves; likewise, machine learning may rapidly classify chromatographic data to signal likely adulteration.

11.2. Digital Herbariums and Blockchain Traceability

The growing digitization of herbarium specimens (with high-resolution images, geo-tagged collection data) enables more robust reference libraries for morphological and genetic comparison. For *T. cordifolia*, deposition of voucher specimens (e.g., for the recent genome assembly project) enhances reference resources. Furthermore, blockchain technology is beginning to be applied in herbal supply chains to create immutable provenance records, verifying origin, harvest date, processing steps and batch authenticity. Coupling chemical or molecular authentication data with blockchain records may create a full traceability chain for *T. cordifolia* raw materials.

11.3. High throughput Metabolomics and Multi-omics Integration

Advances in UPLC-QTOF, LC–MS/MS, NMR metabolomics and bioinformatics enable comprehensive profiling of plant metabolomes. For *T. cordifolia*, chemometric studies already differentiated plant gender and geographical origin via marker ions. The future may integrate transcriptomics, genomics and metabolomics (multi-omics) to fully map plant variability, allowing authentication not only of species but of chemotype, harvest season, gender and even geographic provenance.

11.4. Field Deployable Authentication Tools

Portable devices such as handheld NIR/FTIR spectrometers, smartphone-coupled imaging systems and miniaturized PCR or isothermal amplification kits (e.g., LAMP) are being developed for on-site authentication. For *T. cordifolia*, establishing validated markers (chemical or molecular) that can be used in rapid field kits could significantly strengthen supply-chain quality controls.

Future Research Gaps

Despite progress, several areas require further study

- Large-scale validation of authentication protocols for *T. cordifolia* across geographic regions, suppliers and processed forms (powder, extract).
- Standardization of reference libraries (morphology, chromatographic, spectral, genetic) specific to *T. cordifolia* and its major adulterants.
- Cost-effectiveness analyses for routine adoption of advanced techniques (HPLC-MS, DNA barcoding) in commercial QC settings in resource-constrained environments (such as many herbal supply chains in India).
- Integration of authentication data with supply-chain traceability systems (blockchain, QR codes) to bridge laboratory results and on-ground provenance.
- Development of user-friendly, portable authentication kits that can be deployed at collection, processing and retail nodes for *T. cordifolia* raw material.

12. Result

Authentication of *Tinospora cordifolia* is crucial to maintain its pharmacological reliability and to safeguard public health. Although classical identification methods based on macroscopic and microscopic examination remain valuable as preliminary screening tools, they are often insufficient when dealing with processed products or complex adulteration. To meet modern quality assurance demands, a wide range of advanced analytical and molecular techniques has been adopted. Recently, chemometric and metabolomic analyses have provided an additional layer of

resolution, facilitating differentiation not only between species but also across variables such as plant sex, collection season, and geographical origin.

13. Conclusion

Authenticating the medicinal plant *T. cordifolia* is essential to preserve its pharmacological integrity and protect public safety. Traditional macroscopic and microscopic techniques remain valuable as initial screening methods, but alone they are inadequate for contemporary demands especially for processed materials or subtle adulteration. Modern analytical methods (HPTLC, HPLC-MS, GC–MS), spectroscopic tools (FTIR, NMR) and molecular techniques (RAPD, SSR, DNA barcoding) provide progressively deeper resolution and specificity. Moreover, chemometric and metabolomic approaches allow discrimination beyond species identity, distinguishing gender, seasonality and geographic provenance. A tiered and integrated workflow combining morphological, chemical and molecular authentication offers the most robust assurance. Looking ahead, innovations in AI-assisted recognition, digital herbarium networks, blockchain traceability, high-throughput multi-omics and portable field kits promise to further strengthen authentication of *T. cordifolia*. Nonetheless, significant research gaps remain — including large-scale validation studies, cost-effective field deployment, and full integration of authentication and traceability systems. Addressing these will facilitate higher-quality herbal materials, greater consumer confidence and more reliable therapeutic outcomes.

Compliance with ethical standards

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