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A Review on Molecular Identification of Sida cordifolia L. (Malvales: Malvaceae): Advances in DNA Barcoding and Molecular Authentication?

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Abstract: Sida cordifolia L. ("Bala"), a high-value medicinal plant in Ayurveda. It is widely recognized for its anti-inflammatory, analgesic, and rejuvenative properties. Yet, its close morphological resemblance to congeners (S. acuta, S. rhombifolia, S. spinosa) and unrelated taxa such as Abutilon indicum has led to frequent adulteration in the herbal raw drug trade. Conventional morphological and phytochemical methods fail to distinguish processed or powdered materials, underscoring the need for precise molecular authentication. Recent advances, including DNA barcoding (ITS2, psbA–trnH), sequence-characterized amplified region (SCAR) markers, high-resolution melting (HRM) analysis, and quantitative PCR (qPCR/ddPCR), have demonstrated high accuracy in differentiating S. cordifolia from its adulterants. In addition, next-generation sequencing (NGS) and emerging field-deployable methods, viz., LAMP and CRISPR-based assays, offer rapid and scalable solutions for quality control. This review synthesizes current molecular approaches, emphasizes the integration of SCAR and qPCR/ddPCR assays into regulatory frameworks. And identifies research gaps including reference database curation, development of mini-barcodes, and portable diagnostic kits. Strengthening these molecular authentication pipelines is essential to ensure therapeutic efficacy, consumer safety, and biodiversity conservation in the expanding global herbal market.

Keywords: Ayurveda; DNA barcoding; Herbal authentication; Malvaceae; Molecular authentication; qPCR; SCAR markers; Sida cordifolia

1. Introduction

Over 200 species of the genus Sida are found in tropical and subtropical parts of the world, with a notable variety in South America, Africa, and India [1]. Many of these species are recognized for their medicinal flexibility and are essential to ancient medical systems like Ayurveda, Unani, and Siddha. Commonly referred to as "Bala," "Flannel weed," "Country mallow," or "Heart-leaf sida," Sida cordifolia is a powerful Rasayana (rejuvenative drug) in Ayurveda.

S. cordifolia has a varied phytochemical profile that supports its medicinal potential. Its pharmacological richness is attributed to the presence of fatty acids, flavonoids, phytosterols, ephedrine-like alkaloids, and a variety of bioactive metabolites. Hepatoprotective, cardioprotective, neuroprotective, anti-inflammatory, antioxidant, analgesic, anti-obesity, and wound-healing qualities are among the reported actions [2]. Its alkaloid concentration is also noteworthy since it has been compared to ephedrine in terms of bronchodilatory and stimulating properties, extending its use beyond conventional systems to contemporary pharmacology.

S. cordifolia is used for musculoskeletal conditions, nervous system renewal, and systemic strengthening in traditional Ayurvedic formulations such Maharasnadi kwatha (polyherbal decoction), Bala taila (therapeutic oil), and Bala churna (powder). Numerous applications of the plant have been validated by contemporary pharmacological research, highlighting its significance as a link between ethnomedicine and evidence-based medicine.

The difficulty of guaranteeing authenticity in the herbal medication trade is shown in Table 1, which lists the overlapping traditional applications and phytochemical profiles of S. cordifolia and its prevalent adulterants. Closely related taxa like S. acuta and S. rhombifolia have similar morphologies but different phytoconstituents, with vasicine and cryptolepine-type alkaloids, respectively, while S. cordifolia, also known as "Bala," is valued in Ayurveda for its Rasayana qualities and rich alkaloid profile, which includes ephedrine and pseudoephedrine. In a similar vein, Abutilon indicum is commonly employed in traditional medicine despite lacking the alkaloid signature of S. cordifolia. This is because of its outward likeness. Reliable authentication techniques are essential for quality control and regulatory compliance since such substitutions not only jeopardize treatment effectiveness but also raise questions about safety and uniformity.

Field-level verification is made more difficult by the overlapping and sometimes unclear physical characteristics of S. cordifolia and its common adulterants, S. acuta, S. rhombifolia, and Abutilon indicum (Fig. 1). S. cordifolia is commonly identified by its globose schizocarp fruits, yellow flowers, and cordate (heart-shaped) leaf base. However, S. acuta sometimes gets confused for it because of its short, lanceolate leaves, which resemble yellow flowers and fruit form. While Abutilon indicum exhibits palmately lobed leaves and depressed globose fruits, which might appear to be superficially similar to those of S. cordifolia in herbal raw material form, S. rhombifolia is distinguished by rhombic leaves and disc-like fruits. Since leaf form and fruiting structures are broken in dried or powdered samples, the red circles highlight tiny diagnostic characteristics that are often lost or concealed. This figure underscores a central challenge: while morphological markers provide preliminary guidance, their overlapping nature and susceptibility to adulteration necessitate the integration of microscopy and molecular diagnostics for reliable identification of S. cordifolia in the herbal drug trade.

Taken together, the overlapping morphological and microscopic traits (Table 2) of S. cordifolia and its adulterants highlight the inherent limitations of conventional diagnostic approaches; this gap underscores the urgent need for molecular authentication pipelines—ranging from DNA barcoding and SCAR markers to qPCR, HRM, and next-generation sequencing-that can deliver species-level precision even in processed or powdered herbal materials.

The surging demand for herbal therapeutics. It has intensified concerns over adulteration and substitution. In the Indian herbal raw market, “Bala” is frequently substituted with look-alikes such as S. acuta, S. rhombifolia, and even unrelated species like A. indicum [3]. Such adulteration compromises product efficacy and safety, diminishes consumer trust, and jeopardizes the credibility and integrity of traditional medicine systems. The situation is compounded by widespread usage of powdered or processed forms of herbal drugs, where conventional morphological and macroscopic identification methods become ineffective.

Furthermore, the consumption of Sida roots in Indian Ayurvedic and related drug-manufacturing industries is strikingly high-estimated at 5,000–10,000 metric tonnes annually-reflecting both its commercial importance and the scale of demand [4]. This underscores the urgent need for robust authentication tools to ensure consistent quality, safety, and medicinal integrity.

Against this backdrop of escalating market demand and rising risks of adulteration, there is an imperative for scientific, species-level authentication methods. In this review, we critically examine contemporary molecular approaches-such as DNA barcoding, SCAR (Sequence Characterized Amplified Region) markers, quantitative real-time PCR (qPCR), and high-resolution melting (HRM) analysis-to authenticate S. cordifolia. By doing so, we aim to strengthen regulatory frameworks, fortify quality control strategies, and safeguard both

This systematic review aims to consolidate recent advancements in the molecular identification of S. cordifolia, with a focus on DNA barcoding and molecular authentication approaches, thereby providing a comprehensive understanding of its taxonomic resolution, authenticity verification, and implications for pharmacognosy and quality control.

2. Materials and Methods

The literature for this review was systematically compiled from Scopus, PubMed, ScienceDirect, and Google Scholar databases, covering the period from 2010 to 2025. The following keyword combinations were employed: “Sida cordifolia” AND “DNA

barcoding," "Sida cordifolia" AND "molecular identification," "Sida cordifolia" AND "authentication," and "Sida cordifolia" AND "Malvaceae."
An initial pool of 312 publications was retrieved. After screening for relevance to molecular identification and authentication of S. cordifolia, 87 articles were included in this review. Publications unrelated to the scope, such as general ethnobotanical or pharmacological reports without molecular components, were excluded.
To strengthen the contextual background, additional references from ethnobotanical surveys, phytochemical studies, and pharmacological reports were considered, even if they did not strictly meet the above search parameters. The curated literature provides a consolidated overview of advances in DNA barcoding, molecular markers, and authentication strategies for S. cordifolia within the family Malvaceae.

3. Necessity for Molecular Identification of Sida cordifolia

The authenticity of medicinal plant raw materials is fundamental to ensuring therapeutic efficacy, consumer safety, and compliance with pharmacopoeial standards. Sida cordifolia, a highly valued Ayurvedic drug. It is prone to frequent adulteration and substitution owing to its morphological resemblance to closely related congeners (Sida acuta, S. rhombifolia, S. spinosa) and even unrelated taxa such as Abutilon indicum [1]. In the herbal raw drug markets of India, surveys have reported that a substantial proportion of commercial samples labelled as "Bala" are misidentified, with substitution rates ranging from 60% to over 75% in some regions [5, 6].
Conventional morphological identification, though effective for whole-plant or fresh specimens, becomes impractical when materials are traded in powdered, cut, or processed forms [7]. Similarly, organoleptic and phytochemical profiling approaches, while useful for preliminary screening, cannot reliably discriminate between closely related Sida species with overlapping chemical profiles [8]. This creates a significant bottleneck in quality control, as adulterated or substituted materials may compromise pharmacological activity, introduce toxic constituents, and erode consumer trust in herbal medicines.
In this context, molecular identification methods-including DNA barcoding, SCAR (Sequence Characterized Amplified Region) markers, quantitative PCR (qPCR), and high-resolution melting (HRM) analysis—offer a reliable, reproducible, and rapid means of species authentication [9, 10]. DNA-based tools are particularly valuable because they are unaffected by environmental, seasonal, or processing-related variations, and can detect adulteration even in trace amounts within polyherbal formulations [11].
For S. cordifolia, validated barcode loci such as ITS2 and psbA–trnH have shown high discriminatory power from its adulterants [5]. Moreover, the derivation of species-specific SCAR markers from barcode sequences enables large-scale, low-cost screening in industrial quality control pipelines. Incorporating these molecular tools into routine pharmacognostic protocols is essential for safeguarding therapeutic integrity,

supporting biodiversity conservation, and aligning with WHO guidelines on herbal drug standardization [12].

3.1 Challenges in Conventional Authentication

The authentication of S. cordifolia using conventional pharmacognostic methods faces several limitations, particularly when dealing with raw drugs in their processed or powdered form.

1. **Morphological limitations** – Classical taxonomy relies on diagnostic features such as floral morphology, fruit shape, and leaf arrangement. However, these traits are absent in processed, fragmented, or powdered materials that dominate the herbal drug trade. Even in fresh specimens, S. cordifolia exhibits considerable phenotypic similarity to related species like Sida acuta and S. rhombifolia, making field-level discrimination challenging [1].

2. **Microscopy constraints**– Powder microscopy can detect species-specific anatomical characters, such as unicellular trichomes, stellate hairs, and characteristic epidermal patterns. Yet, these features are often obscured or destroyed during grinding, drying, or extraction processes. Furthermore, adulterants may share similar microanatomical traits, increasing the risk of misidentification [7].

3. **Phytochemical variation** – Chemical profiling, based on secondary metabolites like alkaloids, flavonoids, and phenolic compounds, is widely used for preliminary authentication. However, phytochemical composition can fluctuate due to environmental conditions, soil type, harvesting season, and post-harvest handling. Overlapping phytochemical signatures among congeners and unrelated adulterants further undermine the specificity of this approach [8].

These challenges collectively highlight the inadequacy of sole reliance on conventional methods for S. cordifolia authentication and underscore the need for integrating robust molecular tools into routine quality control protocols.

3.2 Drivers for Molecular Authentication

The incorporation of molecular tools into the authentication pipeline for S. cordifolia is driven by several interrelated scientific, regulatory, and conservation imperatives:

Therapeutic integrity – Accurate species identification ensures that the raw material retains its intended pharmacological profile, thereby guaranteeing consistency in therapeutic efficacy across batches [11]. Molecular authentication minimizes the risk of substituting pharmacologically inferior or inactive species, which could compromise formulation outcomes.

Consumer safety – Substitution with morphologically similar but chemically distinct species can introduce toxic compounds into the supply chain. DNA-based methods can detect even trace levels of adulterants, safeguarding against adverse health effects [8].

Regulatory compliance – National and international regulatory frameworks, including those of the Ministry of AYUSH, the World Health Organization (WHO), and various herbal pharmacopeias, emphasize species-level identification as a prerequisite for market approval [12, 13]. Molecular authentication provides legally defensible evidence of botanical identity.

Biodiversity conservation – Overharvesting of wild populations due to misidentification can threaten non-target species and destabilize ecosystems. Molecular traceability systems help monitor species sourcing, enabling sustainable cultivation programs and compliance with the Convention on Biological Diversity (CBD) protocols [6].

Research reproducibility – Linking pharmacological, phytochemical, and clinical outcomes to authenticated voucher specimens is critical for reproducibility and meta-analysis. Molecular methods allow retrospective verification of plant material used in experimental studies, enhancing the reliability of ethnopharmacological research [7].

4. Molecular Identification Approaches for S. cordifolia

4.1 DNA Barcoding Markers

DNA barcoding has emerged as a robust molecular tool for species-level authentication in medicinal plants, including S. cordifolia. It involves sequencing standardized genomic regions—short yet variable enough to differentiate closely related taxa—and comparing them against curated databases such as GenBank and BOLD. In the case of Sida, several nuclear and chloroplast loci have been evaluated for discriminatory power.

Based on current literature, the ITS2 + psbA–trnH combination provides the most validated and complementary approach for S. cordifolia authentication, balancing universality, discriminatory power, and applicability to degraded or processed material.

4.2 Species-Specific Diagnostic Markers

Species-specific diagnostic markers are indispensable tools for the unequivocal identification of S. cordifolia in complex herbal mixtures and processed formulations, where morphological traits are obscured. These markers target unique, reproducible genomic regions that distinguish S. cordifolia from closely related species within the Sida genus, thereby overcoming the limitations of universal DNA barcodes, which may exhibit high interspecific similarity.

4.2.1 Sequence Characterized Amplified Region (SCAR) Markers

SCAR markers are PCR-based tools developed from species-specific DNA fragments, often identified through ITS2, plastome intergenic spacers, or RAPD/AFLP fragments. The development process involves sequencing the polymorphic fragment, designing longer (\~18–24 bp) primers, and amplifying a single, reproducible diagnostic band only in the target species.

For S. cordifolia, SCAR markers derived from ITS2 polymorphisms have been reported to provide high specificity and robustness, even with degraded DNA from powdered herbal drugs.

Advantages:

High specificity for target species.

Compatible with processed material (e.g., dried powders, tablets).

Low-cost and requires standard PCR equipment.

Limitations:

Initial development requires sequencing and validation across a wide range of reference species.

May fail if adulterant species share highly similar target sequences (rare but possible in congeners).

Key Example:

In Withania somnifera, ITS2-derived SCAR markers have been used successfully for market authentication [14], demonstrating a transferable approach for S. cordifolia authentication.

4.2.2 Quantitative PCR (qPCR) and Digital Droplet PCR (ddPCR) Assays

Real-time quantitative PCR (qPCR) and droplet digital PCR (ddPCR) enable species-specific detection with quantification, making them powerful tools for adulteration monitoring. Species-specific primers or hydrolysis probes (TaqMan®) are designed from unique SNPs or indels in S. cordifolia chloroplast or nuclear genomes.

qPCR allows rapid amplification and detection in real time, with Ct values correlating to template concentration. It is well-suited for routine quality control in manufacturing units.

ddPCR partitions the PCR reaction into thousands of nanoliter-sized droplets, allowing absolute quantification without reference standards and improved tolerance to PCR inhibitors.

Advantages:

Sensitive detection of low-level adulteration (as low as 1%).

Quantification capability—essential for regulatory compliance with pharmacopeial purity standards.

Applicable to highly processed herbal matrices (capsules, syrups, polyherbal blends).

Limitations:
Requires advanced instrumentation and technical expertise.
Higher operational costs compared to SCAR or endpoint PCR.

Recent Developments:
Chen et al. [15] applied ddPCR to detect adulteration in Panax ginseng products, showing that even trace contamination could be quantified accurately. For S. cordifolia, a similar assay could be developed by targeting plastid-encoded genes such as matK, rbcL, or intergenic spacers with unique SNP signatures.
Species-specific diagnostic markers, especially SCAR markers for cost-effective specificity and qPCR/ddPCR for quantitative precision, represent the next-generation toolkit for the regulatory authentication of S. cordifolia. Their adoption can significantly reduce adulteration incidents, safeguard consumer health, and enhance the credibility of herbal supply chains. Integrating these molecular assays into routine pharmacognostic testing is a crucial step towards Good Agricultural and Collection Practices (GACP) and Good Manufacturing Practices (GMP) compliance.

4.3 Advanced Genomics Approaches and Necessity for Molecular Identification
The accurate identification of plant species is essential for ensuring authenticity, safety, and efficacy in herbal products. Traditional morphological methods often fail, especially in cases involving processed, powdered, or closely related species where diagnostic features are lost. This limitation is particularly critical for medicinal plants prone to adulteration, substitution, or mislabeling, which can lead to compromised therapeutic outcomes or potential health risks. Molecular identification thus becomes indispensable, providing species-specific precision that surpasses conventional approaches.
Integrating these advanced genomic approaches with robust bioinformatics pipelines ensures a forensically defensible framework for species authentication. As herbal supply chains globalize, such molecular precision is no longer optional—it is a regulatory and ethical necessity to safeguard consumer health, maintain product integrity, and preserve biodiversity.

4.4 Rapid Field-Deployable Methods and Their Necessity in Molecular Identification
The urgent need for rapid, reliable, and field-deployable molecular identification tools arises from several constraints in conventional plant authentication workflows. Morphological identification, while cost-effective, is often hindered by phenotypic plasticity, seasonal variations, and post-harvest morphological degradation, especially when dealing with powdered or processed botanical materials. Traditional laboratory-

based molecular assays, such as PCR and sequencing, though highly accurate, require sophisticated infrastructure, skilled personnel, and longer turnaround times—factors that limit their practical applicability in field surveillance, border inspections, herbal supply chain monitoring, and on-site biodiversity assessments.

To address these challenges, recent advancements have led to the development of point-of-care (POC) nucleic acid-based diagnostic platforms that combine molecular specificity with portability and operational simplicity.

(a) Loop-Mediated Isothermal Amplification (LAMP)

LAMP is an equipment-light, isothermal DNA amplification method that operates typically at 60–65°C, eliminating the need for thermal cycling. It employs a set of four to six primers recognizing distinct regions of the target DNA, enabling exceptionally high specificity. Reaction results can be visualized in real time through turbidity, fluorescence, or colorimetric dyes, allowing non-specialists to perform assays in resource-limited settings. In plant authentication, LAMP assays have been successfully developed for species-specific detection of medicinal plants (e.g., Panax ginseng, Curcuma longa), enabling on-site discrimination from adulterants in under an hour. Its robustness against inhibitors and compatibility with crude DNA extracts make LAMP highly suited for quality control in herbal product supply chains and field biodiversity studies.

(b) CRISPR-based Detection Systems

The integration of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology into field diagnostics marks a next-generation leap in molecular identification. CRISPR-associated (Cas) proteins, such as Cas12 and Cas13, exhibit collateral cleavage activity upon recognizing a specific nucleic acid sequence. When coupled with isothermal pre-amplification methods (e.g., LAMP or Recombinase Polymerase Amplification), CRISPR-based systems can detect plant species with single-nucleotide resolution. Readouts can be visualized via lateral flow strips, portable fluorescence readers, or even smartphone-based platforms, making them highly adaptable for border biosecurity checkpoints, wildlife trade monitoring, and rapid verification of herbal raw materials.

These rapid molecular diagnostic platforms not only accelerate species authentication but also reduce dependence on centralized laboratories, enabling real-time decision-making in conservation management, regulatory enforcement, and commercial transactions. In the context of herbal product authentication, where misidentification can lead to safety hazards, economic losses, and erosion of consumer trust, the deployment of LAMP and CRISPR-based assays represents a paradigm shift towards decentralized, accessible, and precise identification systems.

5. Importance of Molecular Identification

Molecular identification has emerged as a cornerstone in ensuring authenticity, safety, and sustainability within herbal medicine value chains. Traditional morphological identification is often inadequate—particularly for dried, powdered, or processed botanical materials—leading to misidentification and adulteration. Advances in DNA-based technologies provide an accurate, reproducible, and globally recognized approach to species authentication.

5.1 Quality Assurance in Herbal Supply Chains

Accurate molecular authentication ensures that the botanical raw materials conform to the species specified on product labels. In complex herbal supply chains, where sourcing often involves multiple intermediaries and cross-border transactions, DNA barcoding and related methods act as a quality checkpoint. For instance, a study by Newmaster et al. [8] revealed that over 30% of commercial herbal products were contaminated or substituted with non-listed species, underscoring the need for routine molecular verification. Such authentication protects brand integrity, reduces the risk of regulatory penalties, and ensures consumer trust.

5.2 Pharmacovigilance and Safety

Adulteration with toxic or pharmacologically active substitutes can lead to adverse health outcomes. For example, substitution of Stephania tetrandra with Aristolochia fangchi resulted in cases of aristolochic acid nephropathy in Europe. Molecular identification enables proactive detection of such substitutions before products reach consumers, thereby supporting post-market surveillance and pharmacovigilance frameworks [7]. This not only safeguards public health but also assists in tracing the source of contamination or adulteration.

5.3 Global Trade Compliance

The herbal trade, valued at over USD 120 billion annually, is increasingly governed by stringent quality and authenticity requirements across major markets. DNA-based species authentication aligns with pharmacopoeial monographs, including the Indian Pharmacopoeia, European Pharmacopoeia, and Chinese Pharmacopoeia, which are progressively integrating molecular markers into official guidelines. Implementing molecular identification reduces the risk of export rejections, ensures compliance with good manufacturing practices (GMP), and facilitates smoother customs clearance for botanical products.

5.4 Conservation Biology and Biodiversity Protection

Unsustainable harvesting and trade in endangered species, such as those listed under the Convention on International Trade in Endangered Species of Wild Fauna and

Flora (CITES), threaten global biodiversity. Molecular identification plays a dual role in conservation:

1. Trade regulation – enabling enforcement agencies to monitor and regulate illegal trade by verifying the species identity of seized plant materials.
2. Species conservation – supporting population genetics studies that guide sustainable harvesting, habitat protection, and restoration strategies.

By integrating molecular diagnostics into conservation programs, both biodiversity preservation and livelihood protection for communities dependent on medicinal plant resources can be achieved.

6. Scientific Reliability

Ensuring the reproducibility of research outcomes is a fundamental principle of scientific investigation, particularly in pharmacological and clinical evaluations of herbal medicines. In the case of S. cordifolia, authenticated botanical material forms the basis for correlating phytochemical profiles with pharmacological efficacy. Without rigorous molecular identification, experimental results risk being confounded by the inclusion of adulterants or misidentified taxa, thereby undermining the reliability of therapeutic claims and compromising meta-analyses or systematic reviews. By integrating DNA-based authentication into research pipelines, studies achieve higher reproducibility, enabling meaningful comparisons across laboratories and facilitating the translation of preclinical findings into clinical practice.

7. Research Gaps and Future Directions

Despite significant progress in molecular authentication of medicinal plants, several critical gaps remain in the context of S. cordifolia. Addressing these will not only strengthen species authentication but also enhance pharmacological research, regulatory compliance, and conservation strategies.

8. Proposed Workflow for S. cordifolia Authentication

Ensuring the accurate identification of S. cordifolia is critical for quality control, pharmacovigilance, and research reproducibility. Based on recent advances in molecular systematics and herbal product authentication methods, we propose a multi-tiered workflow integrating standard and advanced DNA-based techniques.

Step 1: DNA extraction

Employ the CTAB method supplemented with polyvinylpolypyrrolidone (PVPP) to remove polyphenolic compounds common in Malvaceae tissues [16, 17]. This approach yields high-quality DNA even from dried or processed herbal material.

Step 2: PCR amplification of primary barcode markers

Target the Internal Transcribed Spacer 2 (ITS2) and the plastid intergenic spacer psbA–trnH, both known for high species-level resolution in medicinal plants [9, 15].

Step 3: Sequencing and database comparison

Perform bidirectional sequencing and match the consensus sequences against a curated reference database (e.g., BOLD, GenBank with validated sequences). BLAST or local alignment tools can be used to assess similarity scores and identity thresholds.

Step 4: Secondary markers for inconclusive cases

If ITS2 and psbA–trnH yield ambiguous results (e.g., due to closely related Sida species), incorporate additional plastid markers such as matK, rbcL, ycf1, or even plastome hotspot regions, which have shown phylogenetic utility in Malvaceae [18].

Step 5: Authentication of processed herbal drugs

For highly degraded DNA in processed products, employ mini-barcodes (<200 bp) or quantitative PCR-based methods (qPCR or ddPCR) targeting species-specific regions to enable both identification and quantification of S. cordifolia versus adulterants [19].

Step 6: Reporting

Provide a species identification report with:

1. Confidence level (e.g., >98% match to reference sequence).
2. A note on potential adulteration if detected.
3. Sequence accession numbers for transparency.

9. Future Research Directions

The authentication of Sida cordifolia remains a critical priority in ensuring the safety, efficacy, and integrity of herbal drug supply chains. Future research should focus on advancing molecular tools to overcome current limitations in species identification and adulteration detection.

1. Expansion and Curation of Reference Databases

Comprehensive and high-quality reference sequences—particularly from diverse geographic origins—are essential to improve molecular diagnostic accuracy. Collaborative initiatives should prioritize sequencing authenticated S. cordifolia specimens using standard barcodes (ITS2, psbA–trnH) and whole plastome data, with rigorous voucher documentation.

2. Development of Mini-Barcodes for Processed Samples

Given the degraded DNA in powdered or heat-treated herbal products, designing shorter, highly discriminatory DNA fragments (mini-barcodes) will improve detection in processed samples while maintaining taxonomic resolution.

3. Integration of Multi-Locus and Genomic Approaches

Future authentication should integrate multi-locus barcoding with next-generation sequencing (NGS) and genome skimming approaches to capture comprehensive genetic signals, especially in closely related Sida species.

4. Portable and Field-Deployable Molecular Diagnostics

Advancements in portable PCR, isothermal amplification (LAMP, RPA), and nanopore sequencing platforms present opportunities for rapid, on-site verification of herbal raw materials, enabling real-time supply chain monitoring.

5. Quantitative Adulteration Assessment

Digital PCR and quantitative real-time PCR should be explored for estimating the proportion of adulterants in mixed herbal formulations, enhancing both regulatory enforcement and consumer transparency.

6. Bioinformatics Tools for Rapid Authentication

Developing user-friendly, cloud-based analytical platforms will facilitate molecular identification by non-specialists, accelerating adoption in industry and regulatory laboratories.

By addressing these areas, molecular authentication of S. cordifolia can evolve into a more robust, rapid, and widely applicable system, ensuring not only the integrity of herbal medicines but also supporting biodiversity conservation and sustainable trade practices.

10. Conclusion

The accurate authentication of Sida cordifolia is no longer a supplementary step. But a cornerstone of herbal drug safety, efficacy, and regulatory compliance. Traditional identification methods are inadequate once plant materials are powdered or processed, making DNA-based tools indispensable. A hierarchical strategy-starting with primary barcodes (ITS2, psbA–trnH), supported by SCAR markers for cost-effective specificity, and extending to qPCR/ddPCR for quantitative adulteration analysis—offers a robust workflow for industry and regulatory adoption. Emerging technologies such as LAMP, CRISPR-based diagnostics, and portable sequencing platforms further enhance field-level surveillance, addressing real-time challenges in supply chain monitoring. Future research should prioritize curated reference databases, mini-barcodes for degraded DNA, and accessible point-of-care diagnostic kits. Integrating these molecular tools into pharmacopoeial and regulatory frameworks will safeguard therapeutic integrity, restore consumer confidence, and support biodiversity conservation—securing the enduring legacy of Sida cordifolia in traditional and modern medicine.

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Authorcontributions

Conceptualization: RP, GC; Methodology: RP; Data curation and analysis: RP, GC, PG; Writing—original draft: RP, GC, PG; Writing—review & editing: GC, PG, KR, BUV; Supervision: GC. All authors have read and approved the final manuscript.

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References

1. Dinda, B., Das, N., Dinda, S., Dinda, M., & SilSarma, I. (2015). The genus Sida L. – A traditional medicine: Its ethnopharmacological, phytochemical and pharmacological data for commercial exploitation in herbal drugs industry. Journal of Ethnopharmacology, 176, 135–176.
2. Srinivasan, N., Murali, R., & Sivakrishnan, S. (2022). Sida cordifolia – An update on its traditional use, phytochemistry, and pharmacological importance. International Journal of Pharmaceutical Research and Allied Sciences, 11(1), 74–86.
3. Sulaiman, C., Saji, A., Anandan, E. M., & others. (2024). Phytochemical comparison of selected Sida species using spectrophotometric and multiple chromatographic analyses. Future Journal of Pharmaceutical Sciences, 10, 118.
4. Vassou, S. L., Kusuma, G., & Parani, M. (2015). DNA barcoding for species identification from dried and powdered plant parts: A case study with authentication of the raw drug market samples of Bala (Sida cordifolia). Journal of Ethnopharmacology, 171, 264–272.
5. Srirama, R., Senthilkumar, U., Sreejayan, N., Ravikanth, G., Gurumurthy, B. R., Shivanna, M. B., & Shaanker, R. U. (2017). Assessing species admixtures in raw drug trade of Bala (Sida cordifolia–Malvaceae) using molecular tools. Journal of Ethnopharmacology, 197, 165–175.
6. Sucher, N. J., & Carles, M. C. (2008). Genome-based approaches to the authentication of medicinal plants. Planta Medica, 74(6), 603–623.
7. Newmaster, S. G., Grguric, M., Shanmughanandhan, D., Ramalingam, S., & Ragupathy, S. (2013). DNA barcoding detects contamination and substitution in North American herbal products. BMC Medicine, 11, 222.
8. Kress, W. J., Wurdack, K. J., Zimmer, E. A., Weigt, L. A., & Janzen, D. H. (2005). Use of DNA barcodes to identify flowering plants. Proceedings of the National Academy of Sciences, 102(23), 8369–8374.

9. Parveen, I., Gafner, S., Techen, N., Murch, S. J., & Khan, I. A. (2016). DNA barcoding for the identification of botanicals in herbal medicine and dietary supplements: Strengths and limitations. Planta Medica, 82(14), 1225–1235.
10. Rastogi, S., Pandey, M. M., & Rawat, A. K. S. (2016). DNA barcoding technology for authentication of medicinal plants from the Indian subcontinent. Pharmacognosy Reviews, 10(20), 164–170.
11. Chaudhary, A., et al. (2020). Development of ITS2-derived SCAR markers for authentication of Withania somnifera. Industrial Crops and Products, 152, 112474.
12. Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y., & Leon, C. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. PLoS ONE, 5(1), e8613.
13. Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19, 11–15.
14. Santhosh Kumar, J. U., Krishna, V., Seethapathy, G. S., Senthilkumar, U., Ragupathy, S., Ganeshaiah, K. N., Ganesan, R., Newmaster, S. G., Ravikanth, G., & Uma Shaanker, R. (2015). DNA barcoding to assess species adulteration in raw drug trade of "Bala" (Sida L.) herbal products in South India. Biochemical Systematics and Ecology, 61, 501–509.
15. Hollingsworth, P. M., et al. (2011). A DNA barcode for land plants. Proceedings of the National Academy of Sciences, 108(49), 12794–12797.
16. Xin, T., et al. (2015). Barcoding the kingdom Plantae: New PCR primers for ITS regions of plants with improved universality and specificity. Molecular Ecology Resources, 15(5), 999–1007.

Table 1. Comparative profile of Sida cordifolia and its common adulterants in herbal trade

Species	Common Names	Major Traditional Uses	Key Phytochemicals Reported	Risk of Substitution
Sida cordifolia L. (Malvales: Malvaceae)	Bala, Flannel weed, Country mallow	Rasayana (rejuvenative); treatment of arthritis, paralysis, fatigue, bronchial asthma, musculoskeletal and nervous system disorders	Ephedrine-like alkaloids (ephedrine, pseudoephedrine), flavonoids, sterols, fatty acids, withanolides	High demand leads to frequent adulteration
Sida acuta Burm.f.	Wireweed, Broomweed	Used in folk medicine for malaria, fever, diarrhea, liver disorders	Alkaloids (cryptolepine-type), tannins, saponins, flavonoids	Morphologically similar; often mixed with S. cordifolia
Sida rhombifolia L.	Arrowleaf sida, Jelly leaf	Traditional use in respiratory disorders, ulcers, inflammation, wound healing	Alkaloids (vasicine), sterols, phenolic acids, flavonoids	Substitution reduces efficacy of Bala formulations
Abutilon indicum (L.) Sweet (Malvales: Malvaceae)	Atibala, Indian mallow	Emollient, expectorant, demulcent, diuretic; used in piles, gonorrhea, cough, urinary infections	Flavonoids, glycosides, mucilage, terpenoids, steroids	Common adulterant due to morphological resemblance; lacks Bala's alkaloid profile

Table 2. Comparative Microscopic Features of Sida cordifolia and Its Common Adulterants

Species	Trichomes	Stomata	Epidermal Features	Remarks	References
Sida cordifolia L. (Malvales: Malvaceae)	Unicellular trichomes; stellate hairs	Paracytic stomata	Polygonal epidermal cells with straight walls	Some diagnostic features but overlap with congeners	Dinda et al., 2015; Kumar et al., 2018
Sida acuta Burm.f.	Long simple trichomes; sparse stellate hairs	Anisocytic stomata	Elongated epidermal cells	May be confused with S. cordifolia in powders	Sasidharan et al., 2011; Warrier et al., 2014
Sida rhombifolia L.	Stellate and glandular hairs	Paracytic stomata	Smaller epidermal cells with wavy walls	Morphology partly overlaps; difficult in ground samples	Nirmal et al., 2012; Srinivasan Nagaraj et al., 2022
Abutilon indicum (L.) Sweet (Malvales: Malvaceae)	Dense stellate trichomes	Paracytic stomata	Mucilage cells; lobed venation visible in peel	Often substituted due to gross similarity but lacks Bala's alkaloids	Jain et al., 2010; Joshi & Kaushik, 2020

Table 3. DNA barcoding markers evaluated for authentication of Sida cordifolia, their genomic origin, advantages, limitations, and reported applications.

Marker	Genome	Advantages	Limitations	Reported Applications
ITS2	Nuclear ribosomal	High interspecific variability, short length for degraded DNA, strong phylogenetic signal	intra-genomic variation	Separates S. cordifolia from S. acuta, S. rhombifolia, A. indicum (Rastogi et al., 2016; Chen et al., 2010)
psbA–trnH	Chloroplast spacer	High variability, diagnostic indels	Homopolymer issues in sequencing	Effective complement to ITS2 in two-locus barcoding (Kress et al., 2005; Mishra et al., 2021)
matK	Chloroplast gene	Standard plant barcode, large database coverage	Lower resolution within Sida	Supplementary for genus-level ID (CBOL Plant Working Group, 2009)
rbcL	Chloroplast gene	High universality, stable amplification	Low discrimination at species level	Baseline confirmation of genus (CBOL Plant Working Group, 2009)
ycf1	Chloroplast gene	High variability, emerging as powerful barcode	Fewer references	Distinguishes closely related Sida spp. (Dong et al., 2015)

Table 4. Molecular marker-based assays for precise identification and quality assessment of Sida cordifolia.

Marker Type	Description	Applications
SCAR markers	Derived from unique ITS2 or plastome sequences; yield a single diagnostic band for species identification.	Used for unambiguous identification of Sida cordifolia and differentiation from closely related species.
qPCR/ddPCR assays	Enable species-specific detection and quantification of adulteration levels with high sensitivity.	Applied in quality control of herbal raw materials, processed powders, and commercial formulations.

Table 5. Advanced plant genomics approaches for molecular authentication of medicinal plants, with applications to S. cordifolia and related taxa.

Approach	Purpose / Utility	Advantages	Example Applications
Whole Plastome Sequencing	Comprehensive sequencing of the chloroplast genome to identify novel barcode regions (e.g., intergenic spacers, simple sequence repeats \[SSRs], insertions/deletions \[indels])	High resolution; detects minute interspecific variations; useful in degraded samples	Development of species-specific chloroplast markers for S. cordifolia authentication |
Genotyping-by-Sequencing (GBS) & SNP Panels	High-throughput detection of thousands of single nucleotide polymorphisms (SNPs) for genetic differentiation	Enables fine-scale population structure analysis and discrimination among closely related species	Identification of geographically distinct populations of Phyllanthus amarus
Mitochondrial Genome Studies	Complement plastome data to resolve	Additional genomic resource when plastid sequences	Resolution of taxonomic confusion in

	phylogenetic ambiguities in complex taxa	are insufficient	Zingiber spp. |
Target Capture / Hybrid Enrichment	Selective sequencing of hundreds of nuclear loci for deep phylogenetic and diagnostic studies	Highly informative even with fragmented DNA from herbal powders	Authenticity testing in multi-herb formulations

Table 6. Key research gaps and recommended actions for molecular authentication of Sida cordifolia

Gap	Description	Recommended Action
Reference sequence quality	Many Sida sequences in public databases lack voucher specimen verification	Develop curated, voucher-linked ITS2 and plastome reference datasets
Intraspecific diversity	Limited data on genetic variation across S. cordifolia's natural range	Conduct population genomics and chemotype–genotype mapping
Mini-barcodes for degraded DNA	DNA from processed herbal products is often highly fragmented	Design <200 bp ITS2, psbA–trnH, and ycf1 mini-barcodes
Quantitative detection	Most molecular studies confirm only presence/absence of species	Develop ddPCR and qPCR assays to quantify adulterant levels
Field-ready kits	No widely available portable, low-cost authentication tools	Translate diagnostic SNPs into LAMP or CRISPR-based rapid kits
Chloroplast–nuclear integration	Overreliance on plastid markers may mislead in cases of hybridization	Integrate nuclear and plastid datasets for more robust species assignment

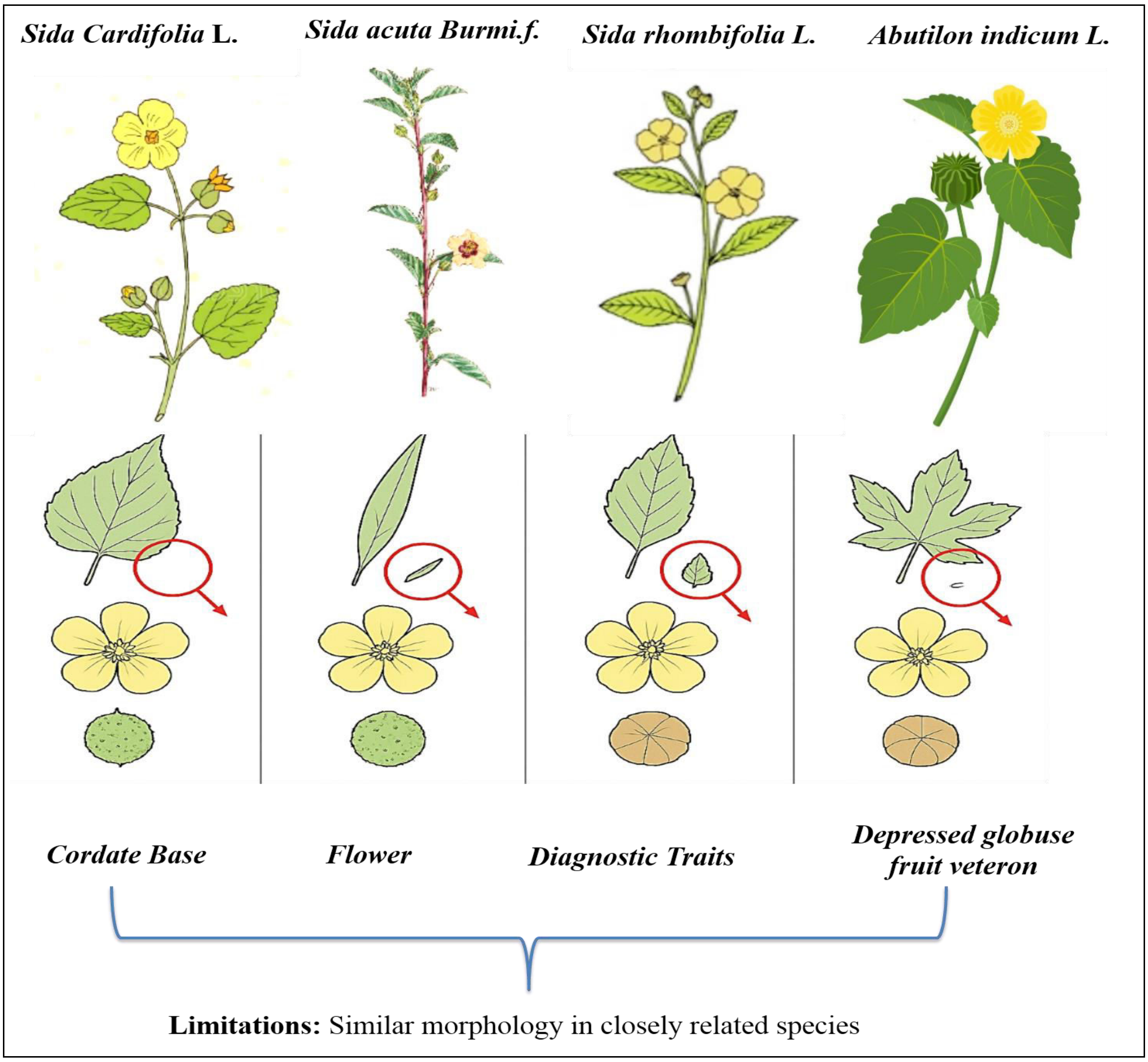

Figure 1. Comparative morphological traits distinguishing Sida cordifolia from allied congeners. Diagnostic traits include variation in leaf lamina shape and venation, petiole length, indumentum density, floral morphology (calyx lobation, corolla symmetry, androecium–gynoecium arrangement), and fruiting structures (schizocarp segmentation, mericarp size and ornamentation, seed surface features). These morphological markers provide reliable taxonomic delineation, particularly when dried or powdered herbal material complicates species-level identification (Bhandari, 1990; Cooke, 1967; Singh et al., 2001; Murthy et al., 2015).