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"DNA BARCODING FOR THE AUTHENTICATION OF AYURVEDIC RAW DRUGS: A REVIEW OF ADULTERATION AND SUBSTITUTION IN INDIAN TRADE, METHODOLOGICAL LIMITS, AND A PATH TO INTEGRATION WITH PHARMACOPOEIAL STANDARDS"

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ABSTRACT
Correct identification of a drug is the first requirement of Dravyaguna, and classical texts already recognise disputed (sandigdha) drugs and permitted substitutes (pratinidhi). In modern trade, raw drugs are sold as dried roots, barks, stems and powders that cannot easily be identified by appearance, and deliberate or accidental substitution is common. DNA barcoding identifies species from short standard DNA sequences and can work on fragmented or powdered material. This review summarises DNA-based evidence of adulteration in Indian herbal trade, the barcode regions in use, methodological limits, and integration with pharmacopoeial standards. A global analysis of 206 DNA-based studies covering 5,957 products found 27% adulterated, with 31% of 752 products from India. In raw drug markets in South India, 12% of 203 samples of 30 species were adulterated, with substitution ranging from 20% to 100% by species; all samples sold as *Sida cordifolia* were *Sida acuta*. In 93 finished products from Coimbatore, about 60% contained species not on the label. Barcoding has also resolved confusions between *Tinospora* species, between *Bacopa monnieri* and *Centella asiatica*, and among plants sold as Bala. Its limits are real: DNA is degraded or absent in extracts, oils and processed formulations, public sequence databases contain unvouchered errors, and barcoding detects identity rather than quality or potency. We recommend a vouchered national reference library for pharmacopoeial species, barcoding of raw drugs at procurement, and combined DNA and chemical methods for finished products.

KEYWORDS: DNA barcoding, adulteration, substitution, raw drug trade, authentication, sandigdha dravya, pratinidhi dravya, Ayurvedic Pharmacopoeia

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INTRODUCTION

Dravyaguna begins with identification. Charaka states that a physician should know a drug by its name and form, and that knowledge of its use depends on this.[1] Classical and later texts recognise both the problem and partial solutions: some drugs are disputed (sandigdha), with more than one plant used under the same name, and others may be replaced by approved substitutes (pratinidhi) when unavailable.[2] These are early responses to the same problem that modern authentication methods address.

In today's market the problem is larger. Most Ayurvedic raw drugs are traded as dried roots, barks, stems, seeds or powders by collectors and intermediaries with limited botanical training, and pharmacies and manufacturers receive material that cannot easily be identified by eye. Pharmacopoeial monographs rely on macroscopy, microscopy, physicochemical tests and thin-layer chromatography.[3] These methods work well for intact material and characteristic markers but struggle with powders, with closely related species and with mixtures. DNA barcoding offers a complementary approach: species are identified from short standard regions of DNA, which can be recovered from small, dried or powdered samples.[4,5]

This review summarises DNA-based evidence on adulteration and substitution of herbal raw drugs and products in India, describes the barcode regions and methods in use, examines methodological limits, and proposes how barcoding could be integrated with pharmacopoeial standards for Ayurvedic drugs.

METHODS

This is a critical review with a scoping approach. We searched PubMed, publisher websites, institutional repositories and the grey literature for studies that used DNA barcoding or related DNA-based methods to authenticate medicinal plant raw drugs or herbal products sold in India, together with methodological papers on plant barcodes and reviews of their use in herbal authentication.

DNA BARCODING IN BRIEF

A DNA barcode is a short, standard region of the genome that varies enough between species to identify them but little within a species. For land plants, the Consortium for the Barcode of Life recommended a two-locus combination of the chloroplast genes rbcL and matK as the core barcode.[4] For medicinal plants, a large validation study of more than 6,600 samples from about 4,800 species found that the nuclear ITS2 region identified 92.7% of samples to species, and proposed it as the preferred barcode for medicinal plants.[5] The chloroplast intergenic spacer psbA-trnH is also widely used. In practice, most Indian studies combine a nuclear and a chloroplast region (Table 1).

Identification depends on comparing the unknown sequence with a reference library. Public databases such as GenBank contain many sequences from unvouchered specimens, some of which are misidentified. In an Indian study, a reference library built from authenticated voucher specimens matched all 187 species correctly, compared with 64% for GenBank, and the authors advised against relying on GenBank for herbal identification.[6]

Table 1. Barcode regions are commonly used for medicinal plant authentication.

Region	Genome	Notes
rbcL	Chloroplast	Easy to amplify and align; limited resolution among closely related species; part of the core plant barcode[4]
matK	Chloroplast	Higher variation than rbcL; amplification less universal; part of the core plant barcode[4]
ITS2	Nuclear ribosomal	92.7% species-level identification in a large medicinal plant dataset;[5] short, works on degraded DNA
ITS (full)	Nuclear ribosomal	High resolution; used in Indian raw drug studies[7,8]
psbA-trnH	Chloroplast	Short and variable; discriminated all *Sida* species tested[8]

EVIDENCE OF ADULTERATION IN INDIA

Table 2. DNA-based studies of adulteration in herbal raw drugs and products relevant to India.

Study	Material	Main finding	Markers
Ichim, 2019[9]	206 studies, 5,957 products, 37 countries	27% adulterated overall; Asia 23%; India 31% of 752 products	Various
Santhosh Kumar et al., 2018[7]	203 raw drug samples of 30 species from 34 locations, South India	12% of all samples adulterated; species-level substitution 20–100%	ITS, psbA-trnH
Santhosh Kumar et al., 2015[8]	Raw "Bala" (*Sida*) from 10 markets, South India	Widespread adulteration; all *S. cordifolia* samples were *S. acuta*	ITS, matK, psbA-trnH
Shanmughanandhan et al., 2016[6]	93 finished products from 10 companies, Coimbatore	About 40% authentic; 60% contained unlabelled species (contamination 50%, substitution 10%, fillers 6%)	ITS2, rbcL
Newmaster et al., 2013[10]	44 products from 12 companies, North America (comparator)	48% authentic; substitution 32%; fillers 21%	rbcL, ITS2

The global picture comes from a review of 206 DNA-based studies covering 5,957 commercial herbal products in 37 countries, which found that 27% were adulterated relative to their labelled species.[9] Of the 752 Indian products included, 31% were adulterated.[9]

Studies of the raw drug trade are most directly relevant to Ayurvedic manufacturing. In a survey of 30 important medicinal plants traded in South India, a vouchered reference set of 124 samples covering the target species and 14 likely substitutes was used to test 203 market samples from 34 locations; about 12% of samples were adulterated, but the rate varied from 20% to 100% across species, so that for some drugs authentic material was rare or absent.[7] A study of Bala, the root of *Sida cordifolia*, found that every market sample labelled as this species was *Sida acuta.*[8] For an important rasayana and vatahara drug, the material in trade was therefore entirely a different species.

Finished products are also affected. Of 93 herbal products from ten companies purchased in Coimbatore, about 40% were authentic, while about 60% contained species not on the label, mostly as contamination rather than outright substitution.[6] The authors noted that mixing with other herbs may dilute the effect of the main ingredient.[6] The North American study that drew wide attention to the method found broadly similar proportions.[10]

RESOLVING DISPUTED AND CONFUSED DRUGS

Barcoding is particularly useful for drugs where classical or trade names cover more than one species. For Guduchi, species-specific DNA markers developed from next-generation sequencing distinguished *Tinospora cordifolia* from its common substitute *T. sinensis* with full sensitivity and specificity in validation,[11] and a metabolomic approach found 20 of 25 commercial *Tinospora* products authentic.[12] This is directly relevant to the debate over Guduchi-associated liver injury, in which the species of the consumed material was never confirmed.[13] For Brahmi, *Bacopa monnieri* and *Centella asiatica* share a common name and are frequently mislabelled in trade,[14] while historical scholarship argues that classical Brahmi and Mandukaparni may both denote *C. asiatica;*[15] barcoding can at least establish which plant is in a given product. For Bala, barcoding showed that the market had effectively replaced one species with another.[8] In each case the method answers the question of what is present, while the question of what the classical text intended remains a matter for textual scholarship.

LIMITATIONS OF DNA BARCODING

Barcoding has important limits, which have been emphasised by both proponents and critics.[16]

Table 3. Limitations of DNA barcoding for Ayurvedic drugs and how to address them.

Limitation	Implication and response
DNA degraded or absent in extracts, oils, asava–arishta and bhasma	Barcoding is suited to raw drugs and single-ingredient powders; use chemical methods for processed products[16]
Polyherbal formulations	Single-locus barcoding cannot resolve mixtures; metabarcoding can list species present but is semi-quantitative[16]
Errors in public databases	Use vouchered reference libraries rather than GenBank alone[6]
Fillers and contaminating DNA	Trace contamination may be detected and over-interpreted; report thresholds and controls
Closely related species	Some genera need additional or species-specific markers[11]
Identity is not quality	Barcoding does not measure active constituents, potency, plant part or contaminants such as heavy metals[17]
Lack of standard protocols	Methods need standardisation and validation before regulatory adoption[16]

The most important practical point is that barcoding and chemical methods answer different questions. Barcoding establishes species identity in material that still contains DNA; chemical fingerprinting establishes whether characteristic constituents are present and in what amounts. A review of the field concluded that barcoding suits single-ingredient products, that metabarcoding can assess species diversity in processed products, and that both should be combined with chemical methods for quality control.[16]

A DRAVYAGUNA PERSPECTIVE

The classical categories of sandigdha and pratinidhi dravya gain new meaning in light of these findings.[2] A sandigdha drug is one whose identity is disputed in the literature; barcoding cannot settle what a classical author intended, but it can document which plants are actually being sold under that name and thereby show where the dispute matters in practice. A pratinidhi drug is a sanctioned substitute; the evidence shows that unsanctioned substitution is widespread, often with species that have no classical status as substitutes. Distinguishing an approved pratinidhi from an unapproved adulterant requires knowing what is in the material. Barcoding therefore extends rather than replaces the classical requirement to know a drug by its name and form.[1]

RECOMMENDATIONS

- Build a national, vouchered DNA reference library for all species in the Ayurvedic Pharmacopoeia of India and their known substitutes, with herbarium specimens and open sequence data.[6]
- Add DNA identity tests to pharmacopoeial monographs for raw drugs that are frequently substituted, alongside existing macroscopic, microscopic and chromatographic tests.[3]
- Require barcode testing of raw drugs at procurement by manufacturers, particularly for high-risk species identified in market surveys.[7]
- Use combined DNA and chemical methods for finished products, and accept that barcoding is not suitable for extracts, oils, fermented products or bhasma.[16]
- Report species authentication, with the method used, in all clinical, pharmacological and pharmacovigilance studies of Ayurvedic drugs.[13,17]
- Develop rapid, low-cost species-specific assays for high-volume drugs so that testing is feasible for small manufacturers and traders.

LIMITATIONS OF THIS REVIEW

This review is not a full systematic scoping review, and several Indian market studies published in regional journals may not have been included. Adulteration rates depend on how samples were chosen, and surveys often target species suspected of adulteration, so their figures may not represent the whole market.

CONCLUSION

DNA-based studies show that adulteration and substitution of Ayurvedic raw drugs and herbal products in India are common, and for some species near-universal. DNA barcoding is a reliable tool for establishing species identity in raw drugs and single-ingredient powders, provided that it uses vouchered reference libraries, but it is not suitable for many

processed Ayurvedic formulations and does not measure quality. Incorporating barcoding into pharmacopoeial standards for raw drugs, together with chemical methods for finished products, would put into practice the classical principle that a drug must first be correctly known before it can be correctly used.

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