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DNA BARCODING OF UNANI MEDICINAL PLANTS: A CRITICAL REVIEW OF MOLECULAR AUTHENTICATION AND EMERGING TECHNOLOGIES

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ABSTRACT

The authentication of medicinal plants is essential for ensuring the identity, quality, and safety of herbal drugs used in the Unani system of medicine. Conventional identification methods may be challenging when plant materials are fragmented, powdered, processed, or morphologically similar to other species. DNA barcoding offers a complementary molecular approach for identifying medicinal plants through standardized DNA sequences. This review discusses the principles of DNA barcoding, commonly used herbal drugs barcode markers, sequence-based identification, and emerging technologies relevant to Unani medicinal plant authentication. The applications of GenBank and the Barcode of Life Data System (BOLD) in taxonomic identification are examined, alongside the potential of next-generation sequencing, DNA metabarcoding, and mini-barcoding. The review also evaluates challenges associated with degraded DNA, incomplete reference databases, closely related species, and complex herbal formulations. DNA barcoding cannot independently establish phytochemical composition, drug potency, or therapeutic efficacy; therefore, its integration with Pharmacognostic examination and chromatographic fingerprinting is important. Developing a curated reference library of authenticated Unani medicinal plants could strengthen botanical identification and support the scientific standardization of Unani drugs.

KEYWORDS: DNA barcoding; Unani medicinal plants; molecular authentication; *Mufradāt*; GenBank; BOLD; DNA metabarcoding; herbal drug standardization.

1. INTRODUCTION

The Unani system of medicine relies substantially on medicinal plants for the preparation of single drugs (*Mufradāt*) and compound formulations (*Murakkabāt*). The correct botanical identity of medicinal raw materials is fundamental to maintaining the quality, consistency, and safety of these preparations. However, the presence of closely related species, vernacular naming variations, substitution, and adulteration can complicate the authentication of herbal drugs. Traditional identification approaches, including macroscopic and microscopic examination, organoleptic evaluation, and Pharmacognostic analysis, remain essential components of medicinal plant quality control. Nevertheless, these methods may have limitations when diagnostic structures are damaged or absent in powdered and processed materials. DNA barcoding provides an additional approach by identifying taxa through short, standardized DNA regions compared against reference sequences.[1]

Although DNA barcoding has been investigated extensively in medicinal plant research, its systematic application to Unani Materia medica requires further attention. Unani-focused approach could help establish molecular reference data for authenticated medicinal species and improve the reliability of botanical identification.[2]

2. Principles of DNA Barcoding and Marker Selection

DNA barcoding involves extracting DNA from a biological specimen, amplifying selected genetic regions, sequencing the amplified products, and comparing the resulting sequences with authenticated reference records. The reliability of identification depends on the quality of

the sequence, the discriminatory power of the selected marker, and the accuracy of the reference database.

The Consortium for the Barcode of Life Plant Working Group recommended the combination of two chloroplast regions, *rbcL* and *matK*, as core DNA barcodes for land plants.[1] Additional regions, including the nuclear internal transcribed spacer (ITS), ITS2, and the chloroplast intergenic spacer *psbA–trnH*, may be useful where greater discriminatory power is required.[1,3]

The principal markers have different strengths and limitations:

- ***rbcL*:** Generally, offers reliable amplification and sequence recovery but may not distinguish some closely related plant species.
- ***matK***: Can provide useful species-level discrimination, although amplification may be difficult in certain taxa.
- **ITS/ITS2:** Nuclear markers that may provide greater discriminatory power in some plant groups, but can present challenges involving amplification and sequence interpretation.
- ***psbA–trnH***: A variable chloroplast spacer that can help distinguish related taxa, although sequence alignment and interpretation may be complicated by insertions and deletions.

No single marker is universally effective for all medicinal plants. A combination of loci may therefore be required, depending on the plant group and the identification problem. Marker selection for Unani medicinal plants should be validated using authenticated specimens rather than assumed to perform equally well across all taxa.[4,6]

Table 1: Comparison of DNA barcode markers relevant to medicinal plant authentication.

DNA marker	Main advantage	Important limitation	Potential application
rbcL	Relatively easy to amplify and align	May have limited resolution among closely related species	Initial plant identification
matK	Often provides useful species discrimination	Amplification may be difficult in some taxa	Species-level authentication
ITS2	Useful discriminatory variation in many plant groups	May show intragenomic variation or amplification problems	Differentiating related medicinal species
psbA–trnH	Variable region useful for distinguishing some taxa	Insertions, deletions and alignment issues may complicate interpretation	Supplementary species identification

Source: Adapted conceptually from the CBOL Plant Working Group (2009) and 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. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. PLoS One. 2010 Jan 7;5(1): e8613.

3. Molecular Authentication of Unani Medicinal Plants

DNA barcoding may be particularly useful for authenticating medicinal plant raw materials whose diagnostic morphological features are unavailable or difficult to interpret. In Unani pharmacognosy, potential applications include confirming the botanical identity of crude drugs, investigating suspected substitution, and differentiating closely related medicinal species.

A reliable authentication workflow begins with the collection of correctly identified plant specimens, preferably supported by voucher specimens deposited in a recognized herbarium. Plant material is then subjected to DNA extraction, polymerase chain reaction (PCR) amplification, sequencing, and quality assessment. The resulting sequence is compared with reference records to determine its closest taxonomic matches. Sequence similarity alone should not be treated as definitive proof of identity. Identification should consider sequence coverage, alignment quality, the presence of ambiguous bases, the distribution of matches among related taxa, and the reliability of the reference records. Where several species have highly similar sequences, additional barcode regions and independent taxonomic evidence may be necessary.

For Unani medicinal plants, an important research priority is to establish species-specific reference data linked to accepted botanical names, synonyms, plant parts used, Unani drug names, and authenticated voucher specimens. Such a resource could facilitate the comparison of traditional drug identities with accepted botanical taxonomy.[3,5,8]

4. Reference Databases and Sequence Identification

The National Center for Biotechnology Information (NCBI) GenBank database and the Barcode of Life Data System (BOLD) are important resources for molecular identification. GenBank enables researchers to compare nucleotide sequences using the Basic Local Alignment Search Tool (BLAST). BOLD provides a specialized platform for DNA barcode data management and taxonomic identification using its identification tools.

The principal steps in sequence-based identification are:

1. Obtain a high-quality DNA sequence and inspect the chromatogram or sequence-quality data.

2. Compare the sequence against GenBank using BLAST and against suitable reference records in BOLD.
3. Examine sequence identity, alignment coverage, the number and taxonomic distribution of matching records, and the quality of reference annotations.
4. Compare the proposed identification with botanical descriptions, voucher specimens, and other taxonomic evidence.
5. Record the marker used, database searched, accession numbers, identification criteria, and any unresolved taxonomic uncertainty.

Neither database should be treated as an infallible authority. Public sequence repositories may contain incomplete annotations, misidentified specimens, or records lacking sufficient voucher information. Therefore, the highest percentage sequence match does not necessarily establish the correct species. Concordant results from independently authenticated reference specimens, multiple markers where appropriate, and conventional botanical identification provide stronger evidence. For Unani drug standardization, transparent documentation of the complete identification process would improve reproducibility and facilitate the development of validated molecular authentication protocols.[9-11]

5. Emerging Technologies and Their Applications

5.1 Next-generation sequencing

Next-generation sequencing (NGS) enables high-throughput analysis of DNA and can support the investigation of multiple taxa in a sample. Compared with conventional single-sequence approaches, sequencing technologies may facilitate the analysis of complex biological materials and the development of larger reference libraries. Their utility depends on the sample, marker selection, sequencing accuracy, and analytical workflow.[12]

5.2 DNA metabarcoding

DNA metabarcoding combines barcode markers with high-throughput sequencing to investigate multiple biological taxa within a mixed sample. It offers potential for studying the botanical composition of polyherbal preparations, where conventional single-species barcoding may be insufficient.

However, the detection of DNA does not necessarily establish the quantity of an ingredient or confirm that it contributes a pharmacologically relevant concentration. Differential DNA degradation, primer bias, extraction efficiency, and manufacturing processes can influence which taxa are detected. A negative result also does not conclusively demonstrate the absence of a botanical ingredient.[3,12,13]

5.3 Mini-barcoding and nanopore sequencing

Mini-barcoding uses shorter DNA regions and may improve amplification when DNA is degraded, as can occur in processed herbal materials. Nanopore sequencing provides a portable sequencing option with potential for rapid molecular analysis. Nevertheless, the suitability of these approaches depends on marker performance, sequence accuracy, contamination control, and validated taxonomic reference data.

For Unani medicine, these technologies should be evaluated through controlled studies involving authenticated raw drugs, powdered samples, and representative formulations before routine implementation is recommended.[5,12-15]

Table 2: Comparison of molecular and conventional authentication methods.

Method	Main purpose	Key limitation
DNA barcoding	Identifies a plant taxon from a selected DNA region	Requires recoverable DNA and suitable reference sequences
DNA metabarcoding	Detects multiple taxa in mixed samples	Detection can be affected by DNA degradation and amplification bias
Microscopy	Examines diagnostic plant structures	Less informative when characteristic structures are destroyed
HPTLC/HPLC	Compares chemical fingerprints and relevant constituents	Chemical profiles can vary with source, processing and storage

Source: Adapted from Raclariu AC, Heinrich M, Ichim MC, de Boer H. Benefits and Limitations of DNA Barcoding and Metabarcoding in Herbal Product Authentication. Phytochemical Analysis., 2018; 29(2): 123–128.

6. Limitations and Integration with Quality Control

Despite its potential, DNA barcoding has important limitations. Closely related species may share similar barcode sequences, while hybridization, incomplete lineage sorting, and variation within species can complicate identification. DNA may also be degraded or absent after extraction, heating, or other manufacturing processes. Most importantly, DNA barcoding identifies biological origin; it does not independently determine the concentration of active constituents, chemical purity, pharmacological potency, toxicity, or therapeutic efficacy. These characteristics require complementary analytical and biological assessments.

A comprehensive quality-control strategy for Unani medicinal plants should therefore integrate molecular identification with macroscopic and microscopic examination, physicochemical testing, and chromatographic fingerprinting, such as high-performance thin-layer chromatography (HPTLC) or

high-performance liquid chromatography (HPLC). This complementary approach is consistent with published evaluations of DNA-based herbal product authentication.[3,5,7,8]

7. Future Perspectives

Future research should prioritize the development of a curated DNA barcode reference library for medicinal plants documented in authoritative Unani Materia medica and Pharmacopoeial sources. The library should include authenticated voucher specimens, accepted botanical names, relevant synonyms, plant parts, geographical provenance, barcode sequences, and accession identifiers.

Collaborative work involving Unani pharmacologists, pharmacognosy's, taxonomists, molecular biologists, and bioinformaticians could establish validated protocols for high-priority medicinal species. Research should also compare the performance of individual and combined barcode markers, assess the effects of processing on DNA recovery, and evaluate metabarcoding approaches for selected compound formulations. The resulting evidence could support improved traceability of medicinal plant raw materials and provide a foundation for incorporating molecular authentication into existing quality-assurance systems.

8. CONCLUSION

DNA barcoding is a promising complementary tool for authenticating medicinal plants used in the Unani system of medicine. The use of validated barcode markers, reliable reference databases, and appropriate sequence interpretation can strengthen botanical identification and help investigate species substitution. Emerging technologies, particularly DNA metabarcoding and mini-barcoding, offer additional opportunities for research on complex and processed herbal materials. However, molecular identification must be interpreted cautiously and integrated with Pharmacognostic and phytochemical methods. Establishing a curated reference library of authenticated Unani medicinal plants is an important step towards strengthening scientific standardization and quality assurance.

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