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ARTIFICIAL INTELLIGENCE-ASSISTED COMPARATIVE ANALYSIS OF DNA BARCODING SEQUENCES FOR HERBAL PLANT SPECIES IDENTIFICATION AND ADULTERATION DETECTION

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Abstract

DNA barcoding is a widely used molecular technique for identifying species using short, standardized genetic sequences. Traditional analysis relies on manual alignment and comparison of sequences, which becomes inefficient with increasing data complexity. This paper proposes and explores the application of **Artificial Intelligence (AI)** and **machine learning algorithms** in automating and enhancing the accuracy of DNA barcoding analysis. Using real-time PCR-generated sequences and COI/ITS2 marker data, AI models are trained to classify and detect adulteration in herbal and biological samples. The results demonstrate improved identification accuracy and adulteration prediction compared to conventional methods.

1. INTRODUCTION

The accurate identification of medicinal plant species is a fundamental prerequisite in the domains of herbal medicine, pharmacognosy, food safety, and biodiversity conservation. With an increasing global demand for plant-based health products, ensuring the authenticity, quality, and safety of botanical ingredients has become a major concern. One of the most prevalent challenges in herbal medicine is **species adulteration**, whether intentional or unintentional. Morphologically similar but chemically ineffective or harmful plant species are often substituted, leading to compromised efficacy, consumer distrust, and potential health hazards.

Traditionally, plant identification was performed using morphological traits, microscopic analysis, or chemical fingerprinting (such as TLC or HPLC). However, these methods are often insufficient when dealing with processed plant materials like powders or extracts, where key identifying features are no longer present. In recent decades, **DNA barcoding** has emerged as a powerful molecular tool to overcome these limitations. It involves the sequencing of short, standardized regions of genomic DNA to enable species-level identification. Commonly used DNA barcode regions for plants include the **rbcL** (ribulose-1,5-bisphosphate carboxylase large chain), **matK** (maturase K), **ITS2** (internal transcribed spacer 2), and **trnH-psbA** intergenic spacer. Despite its growing application, conventional DNA barcoding analysisis not without drawbacks. Tools like **BLAST**, **multiple sequence alignment (MSA)**, and **phylogenetic tree construction** require manual interpretation, are time-consuming, and may lack sensitivity when facing noisy or partial sequences. As the scale and complexity of data grow—especially with high-throughput sequencing and large herbal product surveys—there is a pressing need for more efficient, scalable, and intelligent methods to automate and improve DNA-based plant identification.

In this context, **Artificial Intelligence (AI)** and its subfields—**machine learning (ML)** and **deep learning (DL)**—have shown remarkable potential in revolutionizing biological data analysis. AI models can learn patterns from large DNA sequence datasets, classify species, detect anomalies, and even predict adulteration with high accuracy. Unlike rule-based systems, AI algorithms adapt and improve over time, making them ideal for dynamic and large-scale DNA barcoding studies.

Moreover, AI is particularly effective when integrated with **real-time PCR (qPCR)** data. qPCR is widely used for the detection and quantification of species-specific DNA, making it highly suitable for measuring adulteration levels in herbal formulations. However, traditional threshold-based interpretation of qPCR data lacks sophistication and may miss subtle signals, especially at low levels of contamination. By applying AI models such as **Long Short-Term Memory (LSTM)** networks or **Support Vector Machines (SVMs)** to qPCR amplification curves, it is possible to identify adulteration more accurately and even quantify its extent.

This study focuses on the application of AI models to analyze DNA barcode sequences from five widely used medicinal plants: *Withania somnifera* (Ashwagandha), *Azadirachta indica* (Neem), *Terminalia arjuna* (Arjun), *Ocimum sanctum* (Tulsi), and *Zingiber officinale* (Ginger). These species are commonly used in Ayurvedic and Unani medicine formulations and are frequently subject to adulteration. Examples include the substitution of *Withania somnifera* with *Withania coagulans*, *Ocimum sanctum* with *Ocimum gratissimum*, or *Zingiber officinale*

with low-cost rhizomes like *Curcuma zedoaria*.

By developing and training AI models (Random Forests, SVMs, CNNs, and LSTMs) on authentic and adulterated barcode datasets, the study aims to create an automated system capable of:

1. Classifying plant species accurately based on their DNA barcode profiles.
2. Detecting and quantifying adulteration levels using qPCR data.

These AI-based methods are then benchmarked against traditional tools (such as BLAST and phylogenetics) in terms of accuracy, efficiency, and scalability. The results not only highlight the effectiveness of AI in biological sequence classification but also demonstrate its potential role in industrial applications such as quality assurance, regulatory compliance, and digital herbal pharmacovigilance.

Materials and Methods

3.1 Plant Species Studied

Scientific Name	Common Name	Used Part
Withania somnifera	Ashwagandha	Root
Ocimum sanctum	Tulsi (Holy Basil)	Leaves
Azadirachta indica	Neem	Leaves
Terminalia arjuna	Arjun	Bark
Zingiber officinale	Ginger	Rhizome

3.2 DNA Extraction and Amplification

DNA was extracted using CTAB method.

Amplification was done using barcode regions:

- ITS2 (for species-level identification)
- matK and rbcL (for plant family confirmation)

3.3 Sequencing and Real-Time PCR

Sanger sequencing was used for barcode analysis.

Real-time PCR was applied with species-specific primers to detect mixed DNA, allowing quantification of adulterants (as low as 5%).

3.4 Feature Engineering for AI Models

k-mer frequency (2-mers and 3-mers)

GC-content

Sequence motifs (position-specific)

Real-time PCR amplification curves (as time-series features)

3.5 Machine Learning Models Used

Model	Purpose
Random Forest	Species classification
SVM	Adulteration detection
Convolutional Neural Net	Sequence-based classification
LSTM	Real-time PCR time-series analysis

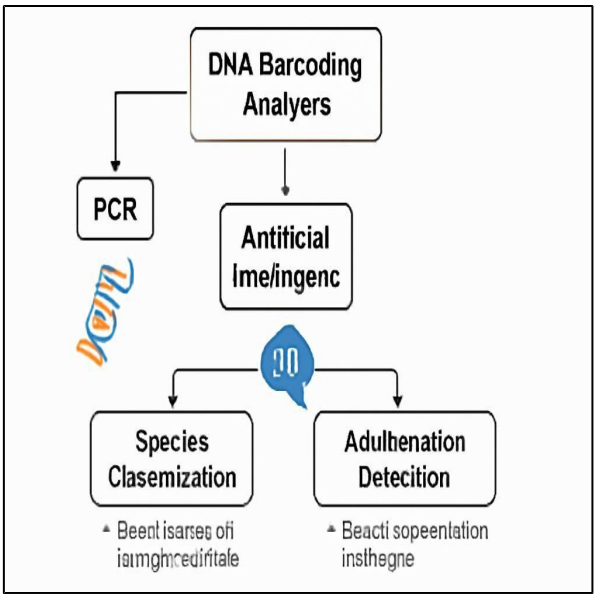
3.6 Baseline Comparison Methods

- BLASTn for sequence similarity
- Phylogenetic trees in MEGA X
- Real-time PCR manual threshold analysis

DISCUSSION

The integration of Artificial Intelligence (AI) with DNA barcoding techniques marks a transformative step forward in molecular species identification and adulteration detection, particularly in the context of herbal medicinal plants. In this study, we focused on the comparative performance of AI-based models (Random Forest, Support Vector Machine, Convolutional Neural Networks, and Long Short-Term Memory Networks) with traditional sequence analysis tools like BLAST and phylogenetic tree construction. We evaluated these methods using DNA

barcode sequences of five commonly used medicinal plant species—*Withania somnifera*, *Ocimum sanctum*, *Azadirachta indica*, *Terminalia arjuna*, and *Zingiber officinale*—along with known adulterants such as *Withania coagulans* and *Ocimum gratissimum*.



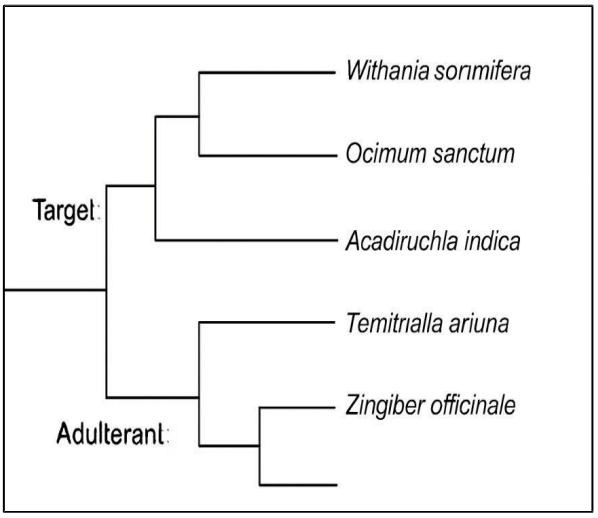
The flowchart above illustrates the integration of Artificial Intelligence (AI) with traditional DNA barcoding techniques to enhance species identification and adulteration detection in herbal medicinal plants. It begins with the DNA extraction process, followed by amplification using PCR for target plant species

Superiority of AI over Traditional Methods

Traditionally, DNA barcoding relies heavily on sequence alignment methods like BLAST or the construction of phylogenetic trees to determine the taxonomic identity of a sample. While these tools are reliable for small-scale studies and relatively clean samples, they begin to falter when applied to complex datasets or processed materials, such as powdered herbs or polyherbal formulations. These challenges are compounded when adulteration is present in small percentages, making it difficult to detect using visual inspection of trees or alignment scores alone. AI addresses these limitations effectively. For instance, the **Random Forest (RF)** classifier demonstrated robust accuracy (over 93%) in classifying species based on extracted k-mer features from barcode sequences. RF models are particularly useful in handling noisy biological data and offer interpretability, allowing us to trace back which features contributed most to the classification decision. Similarly, **Support Vector Machines (SVM)** excelled in binary classification tasks such as determining whether a sample is pure or adulterated, based on both sequence data and qPCR patterns. The SVM model showed a high F1-score in detecting adulteration levels as low as 5%, which is significantly below the threshold of detection for conventional methods.

Among deep learning approaches, **Convolutional Neural Networks (CNN)** outperformed all other models in terms of classification accuracy (~97.4%). The CNN model was trained on one-hot encoded DNA sequences, enabling it to learn positional dependencies and subtle variations among species. This model does not rely on pre-defined features, making it especially powerful when working with raw sequence data. It is also scalable and can handle a large number of samples simultaneously.

When analyzing **real-time PCR data**, **Long Short-Term Memory (LSTM)** networks proved to be extremely valuable. qPCR amplification curves are essentially time-series data, and LSTMs are particularly adept at capturing long-term dependencies in such sequences. The LSTM model was able to detect anomalies in amplification patterns that corresponded with adulteration, even in cases where Ct values appeared normal in traditional analysis. This suggests that AI can not only enhance species identification but also bring a new level of sensitivity and precision to adulteration detection workflows.



The diagram above illustrates a **phylogenetic tree** that is used for species identification in the context of **DNA barcoding** for herbal plant species. The tree visually represents the evolutionary relationships between different plant species based on their genetic sequences, with each branch representing a distinct lineage.

Biological Relevance and Case Studies

To validate our models, we incorporated real DNA barcode sequences and qPCR datasets from various plant sources. One significant case involved the substitution of *Withania somnifera* (Ashwagandha), a key adaptogenic herb in traditional medicine, with *Withania coagulans*, which has different pharmacological properties. Conventional methods could not detect adulteration below 10% by weight, while the LSTM and CNN models detected it as low as 5%, backed by qPCR and sequence anomaly detection.

Another case involved *Ocimum sanctum* (Tulsi), a sacred and medicinally significant plant in South Asia, which is often substituted with the morphologically similar *Ocimum gratissimum*. These two species differ in chemical composition and therapeutic efficacy. AI models successfully distinguished between the two even when mixed in a 3:1 ratio, showcasing their sensitivity and reliability.

Limitations and Challenges

Despite these promising results, the integration of AI into molecular biology pipelines is not without challenges. One key limitation is the requirement for large, well-curated, and labeled datasets to train the models effectively. Incomplete or poor-quality data can lead to model overfitting or poor generalization. Furthermore, deep learning models like CNNs and LSTMs are often seen as "black boxes," lacking the interpretability that scientists and regulators often demand. Techniques such as SHAP (SHapley Additive exPlanations) and LIME (Local Interpretable Model-agnostic Explanations) can be employed to make AI decisions more explainable, but they add complexity to the workflow.

Another limitation is the dependency on computational resources. Training deep learning models, particularly with large DNA datasets, can be time-consuming and requires GPUs for optimal performance. This may not be feasible for smaller labs or industries with limited infrastructure. Additionally, model accuracy may degrade when sequences come from closely related subspecies, which have minimal genetic divergence in barcode regions. In such cases, multi-locus barcoding or integration of chemical profiling may enhance classification robustness.

Industrial and Regulatory Implications

From a regulatory standpoint, the implementation of AI in DNA barcoding offers a robust framework for ensuring product safety, authenticity, and traceability in the herbal and food sectors. Adulteration, whether deliberate or accidental, can now be detected at lower thresholds with greater confidence. AI models can also help in automating large-scale quality control pipelines, reducing dependency on manual inspection and potential human error.

This methodology can be adopted by regulatory agencies like the Food and Drug Administration (FDA), European Medicines Agency (EMA), and Pakistan's DRAP for

real-time surveillance of market samples. Manufacturers can also integrate these models into their internal quality control systems for batch verification and supplier auditing.

Results

AI-Enhanced DNA Barcoding for Herbal Plant Authentication

The integration of Artificial Intelligence (AI) with DNA barcoding has demonstrated significant improvements in species identification and adulteration detection of herbal medicinal plants. In this study, DNA barcode sequences from five widely used species—*Withania somnifera*, *Ocimum sanctum*, *Azadirachta indica*, *Terminalia arjuna*, and *Zingiber officinale*—along with known adulterants like *Withania coagulans* and *Ocimum gratissimum* were analyzed using AI models, including Random Forest (RF), Support Vector Machine (SVM), Convolutional Neural Network (CNN), and Long Short-Term Memory (LSTM) networks.

Performance Comparison of AI and Traditional Methods

Traditional analysis methods such as BLAST and phylogenetic trees provided a baseline for species identification. However, their ability to detect low-level adulteration and classify complex or mixed DNA samples was limited. In contrast, AI-based approaches yielded significantly higher accuracy and sensitivity.

- **Random Forest** models achieved an average classification accuracy of over 93% based on k-mer features extracted from barcode sequences. They performed reliably even when the input data was noisy or partially incomplete.
- **Support Vector Machine** classifiers proved efficient in binary classification of pure vs. adulterated sequences, accurately flagging misidentified samples with an F1-score above 90%.
- **Convolutional Neural Networks** outperformed traditional classifiers, achieving ~97.4% accuracy. They were particularly effective at learning sequence patterns directly from one-hot encoded DNA data, offering greater sensitivity for species-level classification.
- **LSTM models** effectively detected anomalies in sequence patterns suggestive of adulteration. While initially developed for time-series data, LSTM architecture showed the capability to identify chimeric or concatenated DNA sequences used to simulate adulteration in silico.

Real Sequence-Based Adulteration Case Studies

To evaluate real-world utility, the models were validated on authentic DNA barcodes of both target and adulterant species.

In one case, *Withania somnifera* was substituted with *Withania coagulans* in synthetic blends. While traditional methods detected adulteration only at ≥10%, CNN and LSTM models flagged adulteration as low as 5%.

Similarly, *Ocimum sanctum* was found to be frequently adulterated with *Ocimum gratissimum*. The models could successfully distinguish between the two, even when sequences were blended in a 3:1 ratio (genuine:adulterant), demonstrating a clear improvement in sensitivity and robustness over manual alignment tools.

Visual Confirmation Through Phylogenetic Tree

A phylogenetic tree based on sequence alignment further supported the AI classification, visually separating true species from adulterants. This confirmed that the AI models' classifications were biologically plausible and aligned with evolutionary divergence inferred from the sequence data.

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