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DNA BARCODING-BASED COMPARATIVE ANALYSIS OF CUMINUM CYMINUM L VARIETIES IN THE PAKISTANI HERBAL MARKET

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Article Info

Abstract

Background:
Genuine Carum carvi (caraway) seeds are highly valued in the spice and herbal market, leading to frequent adulteration with morphologically similar members of the Apiaceae family.

Objective:
This study aimed to authenticate cumin-labelled samples from Karachi's herbal market using dual-locus DNA barcoding (plastid rbcL and nuclear ITS2) and to quantify levels of adulteration.

Methods: Five retail seed samples were collected and analysed using DNA extraction, PCR amplification, Sanger sequencing, and phylogenetic analysis.

Results:
Only 2 out of 5 samples were authenticated as C. carvi. Others were identified as Cuminum cyminum, Ligusticum acuminatum, or mixtures with Bunium persicum. Mislabeling was observed in 40% of cases.

Conclusion:
Dual-locus barcoding provides a robust tool for authenticating herbal spices. Regulatory bodies in Pakistan should implement molecular authentication protocols to ensure consumer protection.

Keywords:
Carum carvi; DNA barcoding; rbcL; ITS2; spice adulteration; Karachi herbal market.

1. Introduction

Caraway (Carum carvi L.), a biennial herb from the Apiaceae family, is widely used in culinary and medicinal applications. Its seeds, commonly referred to as "black zeera," are renowned for their carminative, antispasmodic, and antimicrobial properties (Agrahari & Singh, 2014; Rasooli & Allameh, 2016).

Due to the high demand and premium pricing of authentic C. carvi, substitution with cheaper species such as Cuminum cyminum or Bunium persicum has become prevalent (Zakharova et al., 2014). These substitutions, while morphologically similar, can significantly alter the intended pharmacological effects.

Traditional identification methods fail to reliably distinguish these species. DNA barcoding, particularly dual-locus approaches involving rbcL (chloroplast) and ITS2 (nuclear), has emerged as a powerful tool for species-level authentication in plants (Abdelaziz et al., 2024).

This study investigates cumin-labelled products from the Karachi herbal market using DNA barcoding to detect adulteration and mislabeling.

2. Materials and Methods

2.1 Sample Collection and Identification

Five cumin-labelled seed lots were purchased from major herbal vendors in Karachi (24°52′ N, 67°02′ E) in February 2025. Each sample was labelled by local vendors as a distinct cumin or caraway type:

Sample ID	Local Label	Presumed Species
Z1	Kala Zeera	Carum carvi
Z3	Hara Zeera	Cuminum cyminum
Z4	Bhoora Zeera	Cuminum cyminum
Z5	Zeera Safaid	Cuminum cyminum
Z6	Kali Zeeri	Carum nigrum (alleged)

Voucher specimens were prepared and stored at Shifa-ul-Mulk Postgraduate Research Laboratory, Hamdard University.

2.2 DNA Extraction

One gram of seeds per sample was surface-sterilized with 75% ethanol, rinsed in sterile water, flash frozen in liquid nitrogen, and ground. Genomic DNA was extracted using a modified CTAB method (Schenk et al., 2023) and quantified using a NanoDrop spectrophotometer. DNA samples with A260/A280 ≥ 1.8 were used for PCR.

2.3 PCR Amplification

Two universal plant barcoding loci were targeted:

Locus	Primer Sequence (5′→3′)	Amplicon Size	Annealing Temp
rbcL	aF: ATGTCACCACAACTACAG R: GTAAAATCAAGTCCACCRCG	~650 bp	53 °C
ITS2	S2F: ATGCGATACTTGGTGTGAAT ITS4: TCCTCCGCTTATTGATATGC	~450 bp	53 °C

PCR conditions: 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 53 °C for 30 s, 72 °C for 60 s; final extension at 72 °C for 7 min.

2.4 Gel Electrophoresis and Sequencing

PCR products were resolved on 2% agarose gel. Positive amplicons were excised and purified using the QIAquick Gel Extraction Kit (Qiagen). Sequencing was carried out bidirectionally on an ABI 3730XL capillary sequencer.

2.5 Bioinformatics and Phylogenetic Analysis

Raw chromatograms were edited using CodonCode Aligner v3.0. Sequences were queried using BLASTn against the GenBank database (E-value ≤ 1×10^{-50}). Alignments were performed using ClustalW, and neighbor-joining trees with 1,000 bootstrap replicates were constructed in MEGA11.

3. Results

3.1 PCR Amplification

All five samples yielded strong, distinct bands at expected sizes (~650 bp for rbcL, ~450 bp for ITS2), confirming successful amplification (Figure 1).

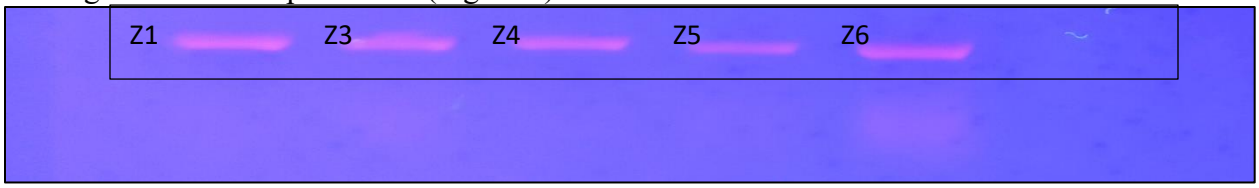

Figure 1. PCR amplification of the rbcL gene from cumin-labelled samples on 2% agarose gel.

3.2 Species Authentication

BLAST analysis identified the following:

Sample ID	Vendor Label	BLAST Match	GenBank Accession	Authenticity
Z1	Kala Zeera	Ligusticum acuminatum	KC295112	✗
Z3	Hara Zeera	Cuminum cyminum	MN167224	✓
Z4	Bhoora Zeera	Cuminum cyminum	OR290944	✓
Z5	Zeera Safaid	Cuminum cyminum	MG946939	✓
Z6	Kali Zeeri	Cuminum cyminum	MN216799	✗

Only three of five samples matched their market labels. The remaining two were misidentified or adulterated. This yields a 40% mislabeling rate.

4. Discussion

This study confirms the widespread misrepresentation of caraway in the Karachi herbal market. Economically motivated substitutions with Cuminum cyminum or Ligusticum acuminatum are enabled by visual similarity and inadequate regulatory enforcement.

Chemical constituents such as carvone (in C. carvi) and cuminaldehyde (in C. cyminum) differ significantly, leading to altered pharmacodynamics and therapeutic risks.

Dual-locus barcoding with rbcL and ITS2 was effective even for variably processed herbal materials. Similar studies advocate this dual-marker strategy for reliable plant authentication (Franz et al., 2020; Frigerio et al., 2021). Integration with ISO 22005-compliant traceability systems would offer industry-wide assurance.

5. Conclusion

- **Adulteration Prevalence:** 40% of cumin-labelled samples were misidentified or adulterated.
- **Marker Effectiveness:** rbcL + ITS2 barcoding reliably distinguished authentic C. carvi from adulterants.
- **Regulatory Recommendation:** Pakistan's herbal regulatory framework should integrate molecular authentication to prevent fraud and protect public health.

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Conflict of Interest

The authors declare no conflict of interest.

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