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DNA Barcoding of the Market Samples of Single-Drug Herbal Powders Reveals Adulteration with Taxonomically Unrelated Plant Species

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Abstract: Herbal drugs are increasingly becoming a viable alternative to allopathic medicine. Since powdered herbal drugs are more prone to adulteration than intact plant parts, their authentication becomes essential to ensure the safety and efficacy of herbal drugs. This study authenticated 107 single-drug herbal powders, representing 65 species from 60 genera and 35 families, collected from the markets in Tamil Nadu, India. DNA barcoding using the *rbcL* marker revealed that 58 samples (54%) were authentic, and 49 (46%) were adulterant. About 41% of the adulterant samples were a mixture of more than one species, possibly due to unintentional cross-contamination during processing. In 59% of the adulterant samples, the authentic species was entirely substituted with taxonomically and medicinally unrelated species, 72% of which belonged to different orders and families, while 28% were from other genera. Despite the taxonomic diversity, 20% of adulterant spe, cies had a morphological resemblance to the authentic species. It is not known whether their use as adulterants is intentional. In a detailed study on DNA barcoding of 17 powder samples from *Ocimum tenuiflorum*, 88% of the samples were authentic. These results indicate that the extent of adulteration is not high in all the species. Approximately, 95% of the samples collected for this study were produced by companies with limited resources and expertise in the unorganized sector. Hence, training them on species identification and providing simple and cost-effective authentication tools will likely reduce adulteration in the market samples.

Keywords: single-drug herbal powder; *rbcL*; molecular authentication; Tulsi powder; adulteration

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1. Introduction

India is one of the 17 countries in the world with mega-biodiversity, and 954 species of medicinal plants are actively traded in the markets [1]. About 40% to 90% of the people in different countries use traditional medicine for their primary healthcare needs [2]. These plant-based medications are less expensive and more accessible in rural areas of developing countries [3]. The expected benefits of any medicine can be realized only if authentic materials are used. It is also essential from the safety aspect of the patients. Unlike allopathic medicine, herbal medicine remains largely unregulated. This paves the way for intentional and unintentional adulterations and admixtures raising concerns about the efficacy and safety of the herbal drugs [4,5]. Increasing demand for herbal medicine is expected to boost the trade of medicinal drugs from USD 120 billion to USD 7 trillion by 2050 [6]. Such vast business and employment opportunities will be lost if the consumers do not trust the authenticity of the herbal drugs traded in the markets. Therefore, it becomes essential for consumers and traders to be interested in only authentic plant materials being traded as herbal drugs.

Several techniques such as morpho-taxonomic keys, anatomy, pharmacognosy, chemical fingerprinting, and DNA barcoding are used to differentiate authentic and non-authentic

plant materials. Morpho-anatomical studies of the leaves and stems were used to identify the *Tinospora* species in dietary supplements, and it was further supported by HPTLC fingerprinting [7]. Chemical characterization was used to authenticate the presence of *Salvia* species in traditional herbal preparations in Spain [8]. DNA barcoding was used to authenticate the nut species in milk beverages [9]. Each method has its own merits and demerits [10]. While the morpho-taxonomic approach helps collect authentic plant material, it will be challenging to use the same for processed and powdered materials. Chemical fingerprinting suffers from the fact that it is difficult to establish species-specific chemical markers, and the markers are sensitive to the age, season, and place of collection of the plant material [11]. With technological advancements, drastic reduction in sequencing cost, and increasing richness of reference sequences, DNA barcoding has emerged as a more versatile and robust method for authenticating herbal products and raw drugs through molecular species identification [12,13]. DNA barcoding uses the markers that are conserved within species but divergent between species so that species-specific sequences can be retrieved using a single pair of universal primers. DNA barcoding can be used for species delimitation, identification of cryptic species, and understanding species composition in biodiversity hotspots, which are useful in taxonomy, biodiversity assessment, conservation, and environmental protection [14–16]. Several studies have employed DNA barcoding techniques to detect adulterations, product substitution, contamination, mislabeling, and admixture in herbal products and raw drugs [17–21]. In the market samples of Ashwagandha, we found that 88% of the adulterant samples were in the form of powders [22]. Therefore, we initiated a larger study to authenticate a diverse set of herbal drugs that are traded in the form of powders. A reference DNA herbal drug barcode library was assembled, and 117 single-drug herbal powders collected from the markets were authenticated. Additionally, a detailed study was conducted by analyzing 17 market samples of one herbal powder.

2. Materials and Methods

2.1. Collection of Single-Drug Herbal Powders

Single-drug herbal powders were selected based on their therapeutic value and trade volume [1,23–28]. The binomial and vernacular names of the herbal powders were obtained from the Traded Medicinal Plants Database (http://envis.frlht.org/botanical_search.php, accessed on 2 February 2022). This database maintains the data on the 960 medicinal plants that are traded from India. It provides vernacular names used in different parts of India and the botanical names for medicinal plants. It also provides the details regarding plant parts traded. The same vernacular names were used, and 117 single-drug herbal powders were collected from the markets in Tamil Nadu, India. Of these, 106 powders were from known plant parts (whole plant, root, rhizome, stem, bark, leaf, flower, and seed), and 11 were from an unknown origin. The samples collected for this study were traded by three registered companies and seven unregistered companies from the unorganized sector. Details of the samples collected for this study are given in Table S1. For detailed analysis, 17 powder samples of Tulsi (*Ocimum tenuiflorum*) were obtained from 17 manufacturers (Table S2).

2.2. Genomic DNA Extraction, PCR Amplification, and DNA Sequencing

Genomic DNA was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) method, with minor modifications [29,30]. About 100 mg herbal powder samples were thoroughly suspended in 0.5 mL DNA extraction buffer, and the suspension was incubated for 16 h at room temperature. The suspension was mixed briefly by vortexing and incubated at 55 °C for 30 min. DNA was isolated as described before [31] and dissolved in TE buffer. Universal primers for *rbcL* (*rbcL*a-F and *rbcL*ajf634-R) [32,33], *trnH-psbA* [34], and *ITS2* [35] were used for PCR amplification of the DNA barcode markers. The PCR reaction mixture contained 1X buffer with 1.5 mM MgCl2, 0.2 mM dNTPs, 5.0 pmol primers, 1 unit Taq DNA polymerase (GenetBio Inc., Nonsan-si, Korea), and 20–50 ng genomic DNA. PCR

amplification was started with an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min, final extension at 72 °C for 5 min, and held at 16 °C. The PCR amplified products were purified using the EZ-10 Spin Column PCR Purification Kit (Bio Basic Inc., Markham, ON, Canada). The sequencing of PCR products was carried out with BigDye Terminator v3.1 chemistry in SeqStudio, following the standard manufacturer's protocol. The quality of sequences was analyzed in Sequence Scanner Software v1.0 (Applied Biosystems, Waltham, MA, USA).

2.3. Reference DNA Barcode Library

We assembled a reference DNA barcode library consisting of 1325 accessions from 656 species (Table S3) from our previous DNA barcoding projects [12,18,30,31,36,37]. It included all species of the herbal powders collected for this study.

2.4. Data Analysis and Species Authentication

DNA barcode sequences from the single-drug herbal powders were compared with the sequences in the reference DNA barcode library. Authentic samples were identified based on the clustering pattern in the phylogenetic tree constructed using the neighbor-joining (NJ) method in MEGA version 7 [38] with Kimura 2 parameter distance model [39,40] and bootstrap analysis, with 1000 replications. The sequences from the non-authentic samples were searched against the non-redundant nucleotide database of NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 12 February 2022) and the BOLD database (https://www.boldsystems.org/index.php/IDS_OpenIdEngine, accessed on 12 February 2022) using the BLAST algorithm for species identification.

3. Results and Discussion

3.1. DNA Isolation, PCR, and Sequencing

Total genomic DNA was isolated from 107 out of the 117 powder samples. The ten samples in which the DNA isolation failed were from Kottai karanthai (HRD023), Lavangappattai (HRD062), Koraikizhangu (HRD065), Boomi sakkarai kilangu (HRD066), Maathulai (HRD074), Kadukkai (HRD083), Aduthinnaipalai (HRD089), Thanrikkai (HRD117), Naval (HRD139), and Poonnankanni (HRD142). Incubation of the powders in the DNA extraction buffer overnight at room temperature was essential for better extraction of genomic DNA. In general, compared with the powders from leaves and flowers, the powders from root, rhizome, stem, and bark yielded much less DNA. However, the quantity of DNA obtained was more than sufficient to PCR amplify the DNA barcode markers. Often 10 to 30 times dilution of the DNA gives better results in terms of PCR amplification, likely due to dilution of the co-precipitated PCR inhibitors [31]. We obtained a 100% success rate for PCR amplification and sequencing of the *rbcL* marker. Therefore, we subjected all of the 107 samples for authentication. As per the label, these samples were derived from 65 species, which belong to 60 genera and 35 families. Agarose gel electrophoresis of DNA and *rbcL* marker amplified from ten samples are given in Figure 1. The chromatograms of the *rbcL* sequences were manually edited before using the data for further analysis.

3.2. Non-Authentic Mixed Samples

Chromatograms of 20 samples (19%) were completely not readable or contained several overlapping peaks originating from more than one DNA fragment in the same sample (Figure 2). These samples included tissues from more than one divergent species and, therefore, are called mixed samples. Since we collected single-drug herbal powders, all of the mixed samples were considered non-authentic (Table S4). The significant number of mixed samples found among the single-drug herbal powders samples is a concern. This may be due to unintentional cross-contamination during sample processing, though economically motivated intentional adulteration cannot be ruled out. As PCR amplification is very sensitive, DNA barcoding can detect even a minute quantity of contamination and

identify it as a mixed sample. Although PCR was used to detect adulteration of up to 0.5% Chili in pepper [41], its sensitivity could be much higher considering the extraordinarily high copy number of the chloroplast genome, which can reach as high as 10,000 copies per cell [42]. Therefore, the proportion of the adulterant species needs to be determined to evaluate the clinical consequences of using the mixed samples for treatment purposes. Additionally, it would require determining the species composition of the mixed samples. The DNA sequence from mixed samples cannot be used for any DNA-sequence-based analysis, and therefore, the adulterant species present in them cannot be determined by DNA barcoding. The species composition of the mixed samples can be determined by meta-DNA barcoding [43,44] or DNA barcoding after cloning [45].

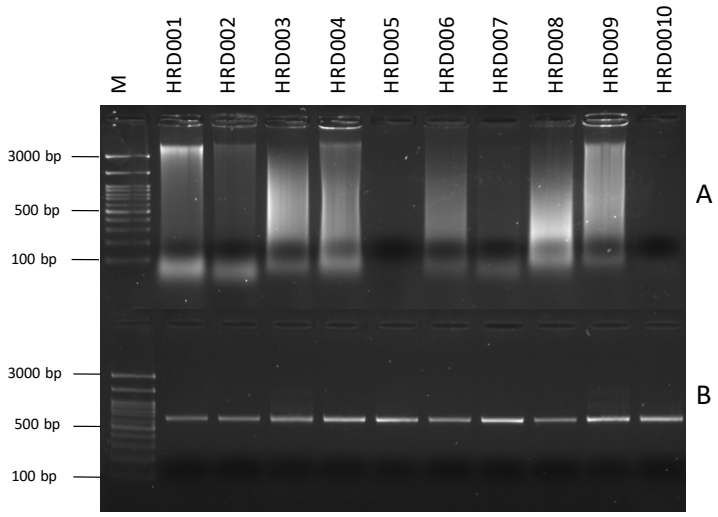

Figure 1. Agarose gel electrophoresis of the genomic DNA from ten single-drug herbal powder samples (**A**) and the PCR amplified *rbcL* DNA barcode markers (**B**) from the respective samples along with 100 bp DNA ladder (M).

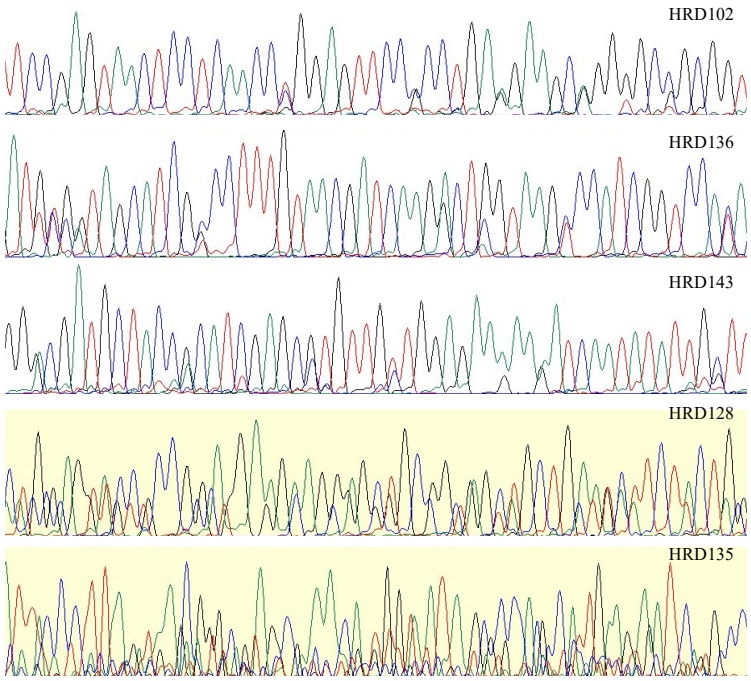

Figure 2. Chromatograms of the mixed samples showing a few overlapping peaks (HRD102, HRD136, and HRD143) to a completely unreadable DNA sequence (HRD128 and HRD135).

3.3. Non-Authentic Samples with Complete Substitution

All of the 87 samples that yielded readable DNA sequences were subjected to further analysis to identify authentic samples. In the phylogenetic tree based on the sequences from the reference DNA barcode library and market samples, 58 samples (~54%) clustered with the expected species. These samples were considered as authentic (Table S5, Figure S1). The remaining 29 samples (~27%) did not cluster with the expected species. In these samples, authentic species were completely substituted with a different species and, therefore, non-authentic samples (Table S6, Figure S1). About 72% of them clustered with species from other orders and families. The remaining 28% of the non-authentic samples clustered with species from different genera, and none of them clustered with congeneric species. These results demonstrate that the adulterant species are not closely related to the authentic species. In our earlier study on authentication of traded medicinal plants not specific to powder samples, about 13% and 7% of samples were adulterated with species from different families and genera [12]. This observation has two implications. First, the adulterant species is not likely to have the same medicinal property as the authentic species. Second, the identification of authentic samples may not need species-specific markers.

3.4. Identification of the Adulterant Species in Non-Authentic Samples

The DNA barcode sequences of the 29 non-authentic samples in which the expected species was completely substituted with a different species were subjected to BLAST analysis for species identification. The species that showed the highest identity (99.65% to 100%) are given in Table 1. The presence of adulterant species in market samples may be due to the same or similar vernacular names, morphological resemblance, mishandling, mislabeling, and species admixture [12,43,46,47]. In this study, only in one sample in which *Pavonia zeylanica* was substituted with *Sida acuta,* the adulteration may be due to a similar vernacular name ("Kurunthotti" is the vernacular name in the Tamil language for both species). Though their vernacular names are the same, the medicinal properties of these two species are different. While the *P. zeylanica* roots are used as a laxative and expectorant [48], *S. acuta* is used as an aphrodisiac and liver tonic [49]. In contrast, 35% of the completely substituted samples (10 samples from six species) likely contain a different species due to morphological resemblance (Figure 3).

We collected two powder samples of *Abutilon indicum*—one was a mixed sample, and the other was substituted with *Sida cordifolia*. Earlier, we found *A. indicum* as an adulterant in *S. cordifolia* [31]. While the aerial parts of *A. indicum* are used for treating asthma [50], the roots of *S. cordifolia* are used to prepare nervine tonic [51]. It appears that these two species are often mixed up during collection due to highly similar leaves and fruits (Figure 3A,B). It is also possible that, after harvesting the roots of *S. cordifolia,* the leftover aerial parts are used for adulteration in *A. indicum*. Flowers of *H. rosa-sinensis* are used for treating hair loss and extracting natural dyes [52]. We collected three powder samples of *H. rosa-sinensis* flowers, and all were adulterated with *Rhododendron*. *H. rosa-sinensis* is commonly available in the areas from where we collected the market samples. However, due to high demand, this species seems to be adulterated with *Rhododendron*, which grows in the Himalayan regions. The red-colored flowers of the *Rhododendron* highly resemble that of *H. rosa-sinensis* (Figure 3C,D). Based on morphological and powder microscopy studies, it was reported that the dried flowers of *R. arboreum* are adulterated with *H. rosa-sinensis* [53]. *Cynodon dactylon* is used as a laxative, expectorant, analgesic, and in the treatment of dropsy and diabetes [54,55]. We collected three powder samples of *C. dactylon* leaves, and all of them were adulterated with *Sporobolus helvolus*. Both are grass species with phenotypic resemblance (Figure 3E,F), and they co-occur in the same habitat. We collected three powder samples of *Senna auriculata* flowers, which are used for hair wash, as well as for treating diabetes and fever [56]. We recovered *Indigofera tinctoria* in place of *S. auriculata*, and both have similar leaf morphology (Figure 3G,H). Similarly, the morphological resemblance of the leaves could be associated with the adulteration of *Mukia maderaspatana* with *Cucumis melo* (Figure 3I,J).

Tribulus terrestris (Devil's thorn) is a highly traded medicinal plant (~3000 metric tonnes per year), and its dried fruits are used for treating urinary stones, impotence, and venereal diseases in the Indian Ayurvedic and the Chinese traditional medicine [57]. We collected four powder samples of *T. terrestris* fruits from four different manufacturers. Three samples were authentic, but one was adulterated with *Harpagophytum* (Devil's claw). Dried fruits of both *T. terrestris* and *Harpagophytum* are brown with thorns. Species of *Harpagophytum* are distributed only in southern parts of Africa [58]. It needs to be further investigated how it is found to be an adulterant in *T. terrestris* samples collected from India. The rhizomes of *H. procumbens* are used for treating arthritis, rheumatism, and labor pain [59] and are exported from Africa to Europe [60]. There is no reported medicinal use for the fruits. After harvesting the rhizomes, it is surmised that the fruits may be exported to countries such as India for adulteration with *T. terrestris*. (Figure 3K,L).

Table 1. Non-authentic single-drug powder samples in which authentic species was entirely substituted with adulterant species. Taxonomic affiliations of the authentic and substituted species are provided for comparison.

S. No	Collection ID	Species Expected as per the Label	Family	Order	Species Identified by DNA Barcoding	Family	Order
1	HRD031	*Abutilon indicum*	Malvaceae	Malvales	*Sida cordifolia*	Malvaceae	Malvales
2	HRD050	*Alpinia galanga*	Zingiberaceae	Zingiberales	*Indigofera stachyodes*	Fabaceae	Fabales
3	HRD017	*Cardiospermum halicacabum*	Sapindaceae	Sapindales	*Trigonella foenum-graecum*	Fabaceae	Fabales
4	HRD004	*Centella asiatica*	Apiaceae	Apiales	*Ipomea imperati*	Convolvulaceae	Solanales
5	HRD130	*Centella asiatica*	Apiaceae	Apiales	*Trigonella foenum-graecum*	Fabaceae	Fabales
6	HRD103	*Coscinium fenestratum*	Menispermaceae	Ranunculales	*Vigna mungo*	Fabaceae	Fabales
7	HRD084	*Curcuma aromatica*	Zingiberaceae	Zingiberales	*Cullen corylifolium*	Fabaceae	Fabales
8	HRD038	*Cynodon dactylon*	Poaceae	Poales	*Sporobolus helvolus*	Poaceae	Poales
9	HRD054	*Cynodon dactylon*	Poaceae	Poales	*Sporobolus helvolus*	Poaceae	Poales
10	HRD107	*Cynodon dactylon*	Poaceae	Poales	*Sporobolus helvolus*	Poaceae	Poales
11	HRD093	*Ficus benghalensis*	Moraceae	Rosales	*Thespesia populnea*	Malvaceae	Malvales
12	HRD085	*Ficus racemosa*	Moraceae	Rosales	*Abutilon indicum*	Malvaceae	Malvales
13	HRD138	*Ficus racemosa*	Moraceae	Rosales	*Abutilon grandiflorum*	Malvaceae	Malvales
14	HRD078	*Ficus religiosa*	Moraceae	Rosales	*Indigofera tinctoria*	Fabaceae	Fabales
15	HRD052	*Glycyrrhiza glabra*	Fabaceae	Fabales	*Canavalia* sp.	Fabaceae	Fabales
16	HRD039	*Gymnema sylvestre*	Apocynaceae	Gentianales	*Trigonella foenum-graecum*	Fabaceae	Fabales
17	HRD068	*Hibiscus rosa-sinensis*	Malvaceae	Malvales	*Rhododendron* sp.	Ericaceae	Ericales
18	HRD108	*Hibiscus rosa-sinensis*	Malvaceae	Malvales	*Rhododendron* sp.	Ericaceae	Ericales
19	HRD110	*Hibiscus rosa-sinensis*	Malvaceae	Malvales	*Rhododendron* sp.	Ericaceae	Ericales
20	HRD127	*Hybanthus enneaspermus*	Violaceae	Malpighiales	*Cardiospermum halicacabum*	Sapindaceae	Sapindales
21	HRD040	*Mangifera indica*	Anacardiaceae	Sapindales	*Mollugo cerviana*	Molluginaceae	Caryophyllales
22	HRD016	*Melia azedarach*	Meliaceae	Sapindales	*Justicia adhatoda*	Acanthaceae	Lamiales
23	HRD081	*Moringa oleifera*	Moringaceae	Brassicales	*Cassia senna*	Fabaceae	Fabales
24	HRD095	*Mukia maderaspatana*	Cucurbitaceae	Cucurbitales	*Cucumis melo*	Cucurbitaceae	Cucurbitales
25	HRD079	*Pavonia zeylanica*	Malvaceae	Malvales	*Sida acuta*	Malvaceae	Malvales
26	HRD019	*Senna auriculata*	Fabaceae	Fabales	*Indigofera tinctoria*	Fabaceae	Fabales
27	HRD099	*Terminalia arjuna*	Combretaceae	Myrtales	*Mucuna pruriens*	Fabaceae	Fabales
28	HRD098	*Tribulus terrestris*	Zygophyllaceae	Zygophyllales	*Harpagophytum* sp.	Ericaceae	Ericales
29	HRD126	*Zingiber officinale*	Zingiberaceae	Zingiberales	*Cajanus cajan*	Fabaceae	Fabales

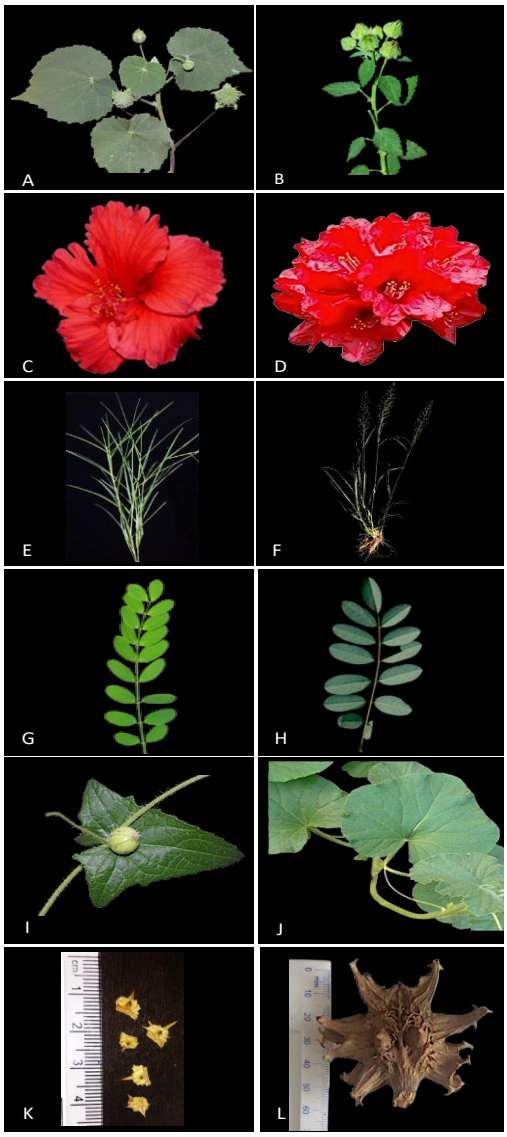

Figure 3. Morphological resemblance between the expected authentic species and non-authentic species identified by DNA barcoding. *Abutilon indicum* versus *Sida cordifolia* (**A**,**B**); *Cynadon dactylon* versus *Sporobolus helvolus* (**C**,**D**); *Hibiscus rosa-sinensis* versus *Rhododenron delavayi* (**E**,**F**); *Senna auriculata* versus *Indigofera tinctoria* (**G**,**H**); *Mukia maderaspatana* versus *Cucumis melo* (**I**,**J**); and *Tribulus terrestris* versus *Harpaophytum procumbens* (**K**,**L**).

3.5. Authentication of Tulsi (Ocimum tenuiflorum)

To investigate a single-drug herbal powder in more detail, we collected 17 samples of Tulsi (*O. tenuiflorum*) from 17 different manufacturers. Tulsi is an important medicinal species, and its leaves are used for treating bronchitis, rheumatism, pyrexia, asthma, and tooth pain [61,62]. DNA barcoding of *O. americanum*, *O. basilicum*, *O. filamentosum*, *O. gratissimum*, *O. kilimandscharicum*, *O. tenuiflorum*, and *O.* x *citriodorum*, using *rbcL*, *matK*, and *trnH-psbA* markers, showed that *trnH-psbA* was the most suitable marker for species differentiation; however, it did not differentiate all of the species [63]. In a similar study, *O. filamentosum* was replaced with *O. carnosum*, and DNA barcoding was performed using the same markers. None of the markers was species-specific [64]. In both studies, *ITS2* was not included, probably due to the problems in PCR amplification. We found that the *ITS2* marker of *O. basilicum* has GC rich sequence, and it could be PCR amplified only in the presence of 5% DMSO as a PCR additive [65]. In the current study, we used DMSO and obtained perfect amplification of *ITS2* from *O. americanum*, *O. basilicum*, *O. gratissimum*,

O. kilimandscharicum, and *O. tenuiflorum* (Figure 4). However, a complete *ITS2* sequence (451 bp) could be obtained only for *O. tenuiflorum*, and its GC content was 67%. Supplementing the sequencing reaction with DMSO indeed improved the sequence quality. Higher GC content and specific stretches of G and C nucleotides may affect strand separation or form strong secondary structures that affect the binding and extension of the sequencing primer. However, we obtained a good-quality sequence of 115 bp in the 5′ ends and 132 bp in the 3′ ends of the *ITS2* marker. Diagnostic nucleotides in these regions were used for authentication. Out of the 17 samples tested, 15 were authentic, and 2 were mixed samples. Since the *trnH-psbA* marker often shows length variations, we PCR-amplified this marker from the two mixed samples. Two differentially sized *trnH-psbA* markers were amplified from both samples (Figure 5). Sequencing and BLAST analyses of those two markers revealed that both samples contained *O. tenuiflorum* but were mixed with *Indigofera tinctoria* or *Trigonella foenum-graecum.* Compared with the overall adulteration in herbal powders, adulteration in Tulsi was relatively much less (~12%). This may be because Tulsi is abundantly available in the study area, and being a sacred species used in temples, people are familiar with this species.

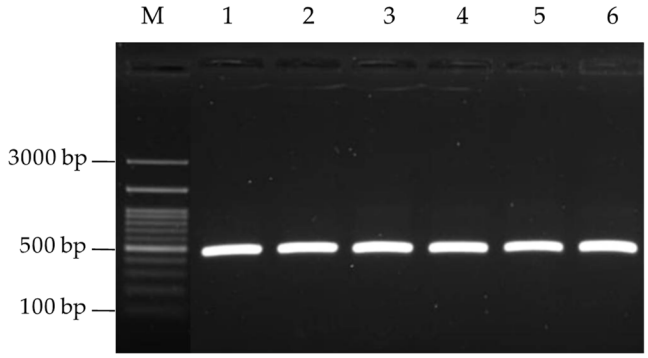

Figure 4. Agarose gel electrophoresis of PCR amplified *ITS2* DNA barcode markers from *O. tenuiflorum*—Rama type (1), *O. tenuiflorum*—Krishna type (2), *O. americanum* (3), *O. basilicum* (4), *O. gratissimum* (5), and *O. kilimandscharicum* (6). Lane M was loaded with 100 bp DNA.

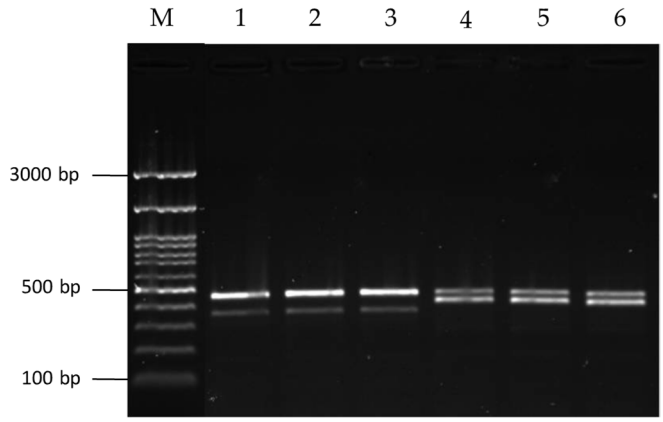

Figure 5. Agarose gel electrophoresis of PCR amplified *trnH-psbA* DNA barcode markers from the two mixed samples of Tulsi, TUL008 (lanes 1–3) and TUL015 (lanes 4–6), along with 100 bp DNA ladder (M).

4. Conclusions

The present study supports the applicability of DNA barcoding to authenticate the market samples of single-drug herbal powders by successfully identifying adulterated samples. It was not necessary to use species-specific markers because the adulterant species were not taxonomically closely related to the authentic species. Mixed samples constituted a

significant percentage of adulterated samples; however, information on species composition and the proportion of the adulterant species is needed to assess the clinical significance of such adulterations. Though the authentic and adulterant species were morphologically similar in quite a few cases, it is unknown if adulteration was due to a lack of knowledge or intentionally carried out by taking advantage of the morphological resemblance. The adulteration range varies, and the authentic and adulterated species often have unrelated medicinal properties. It is worth noting that 95% of the market samples authenticated in this study belonged to unregistered companies from unorganized sectors. These companies typically sell their products through local herbal shops. Large numbers of rural and urban populations from low-income groups depend on these herbal shops for their herbal drug requirements. However, their products may reach a wider population directly through online sales or indirectly through large herbal manufacturers. Since adulteration is not high in all cases, proper training on species identification for people who collect samples and the development of simple and cost-effective technologies to verify the collected samples' taxonomic identity are likely to decrease adulteration. Therefore, it is necessary to develop methods to authenticate the market samples so that only the correct species appropriate for the particular treatment or formulation are used.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14060495/s1, Figure S1: Phylogenetic tree based on the sequences from the reference DNA barcode library and the market samples. Authentic and non-authentic market samples are highlighted in green and red, respectively, File S1: DNA barcode sequences from the market samples collected for this study, Table S1: Details of the single-drug herbal powder samples collected for the current study, Table S2: Details of the single-drug herbal powder samples of Tulsi collected for the current study, Table S3: Details of the species included in the reference DNA barcode library, Table S4: Details of the single-drug herbal powders identified as non-authentic mixed samples, Table S5: Details of the single-drug herbal powders identified as authentic samples, Table S6: Details of the single-drug herbal powders identified as non-authentic samples with complete substitution.

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