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## Identification of species adulteration in traded medicinal plant raw drugs using DNA barcoding

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### Abstract

Plants are the major source of therapeutic ingredients in Complementary and Alternative Medicine (CAM). However, species adulteration in traded medicinal plant raw drugs threatens the reliability and safety of the CAM. Since morphological features of the medicinal plants are often not intact in the raw drugs, DNA barcoding was employed for species identification. Adulteration in 112 traded raw drugs was tested after creating a reference DNA barcode library consisting of 1,452 *rbcL* and *matK* barcodes from 521 medicinal plant species. Species resolution of this library was 74.4%, 90.2%, and 93.0% for *rbcL*, *matK* and *rbcL+matK*, respectively. DNA barcoding revealed adulteration in about 20% of the raw drugs, and at least 6% of them were derived from plants with completely different medicinal or toxic properties. Raw drugs in the form of dried roots, powders and whole plants were found to be more prone to adulteration than rhizomes, fruits, and seeds. Morphological resemblance, co-occurrence, mislabeling, confusing vernacular names, and unauthorized or fraudulent substitutions might have contributed to species adulteration in the raw drugs. Therefore, this library can be routinely used to authenticate traded raw drugs for the benefit of all stakeholders; traders, consumers, and regulatory agencies.

**Keywords:** Medicinal plants; Species adulteration; Raw drug trade; DNA barcoding; Complementary and alternative medicine.

**Abbreviations:** *rbcL*, ribulose-bisphosphate carboxylase; *matK*, maturase K; CTAB, cetyl trimethyl ammonium bromide; EDTA, ethylene diamine tetra acetic acid; PCR, polymerase chain reaction; NCBI, national center for biotechnology information; BOLD, barcode of life data systems; CAM, complementary and alternative medicine; IUCN, international union for conservation of nature; ENVIS, environmental information system.

## Introduction

Complementary and alternative medicine (CAM) is widely used in developing countries where it contributes up to 80% of the healthcare needs of the people. It is also becoming more popular in developed countries due to the side effects and high cost of allopathic drugs. World Health Organization has reported that about 38 to 75% of the people in Australia, Belgium, Canada, France, and the USA have used CAM at least once in their lifetime (WHO 2002). Increasing global demand for CAM is expected to boost the medicinal plant raw drug trade from approximately 120 billion USD to 7 trillion USD by the year 2050 (Marichamy et al. 2014). Yet, frequent reports on species adulteration in traded raw drugs are major threat to the efficacy and safety of CAM (Stoeckle et al. 2011; Revathy et al. 2012; Ouarghidi et al. 2013; Seethapathy et al. 2014; Mishra et al. 2015), which could ultimately affect the trade industry. Therefore, identification of species adulteration in traded raw drugs becomes essential to consumers as well as traders.

Species authentication is a challenging task as most of the raw drugs are traded in the form of dried, fragmented or powdered leaves, flowers, seeds, stem bark, roots, and other plant parts, which do not possess intact diagnostic characters required for morphological

identification by Linnaean taxonomy. Alternative methods such as chemotaxonomy, chromatography, and microscopy, which were adopted for this purpose showed only limited success in species identification. Major limitations of these methods includes involvement of complex chemistries, lack of unique compounds, influence of environmental factors, plant's age, and geographical variations (Techen et al. 2014). All these challenges can be largely overcome by using DNA barcoding, which does not rely on the morphology of plants, and therefore, is not affected by the above mentioned factors. Moreover, DNA barcoding can be done using live or dead tissue from any part of the plant at any stage of its life cycle. In fact, identification of species adulteration in traded raw drugs is one of the most useful applications of plant DNA barcoding. Though several chloroplast markers have been used for DNA barcoding of plants (Sucher and Carles 2008; Techen et al. 2014) the Consortium for the Barcode of Life Plant Working Group has recommended *rbcL* and *matK* as core DNA barcode markers for plants (CBOL Plant Working Group 2009).

The power of DNA barcoding to identify species has been demonstrated in several studies. Bruni et al. (2010) have demonstrated that DNA barcoding can be used for rapid and accurate identification of poisonous plant materials. Mattia et al. (2011) have employed a DNA barcoding approach for species identification in processed plant materials of commercial kitchen spices. Cornara et al. (2013) have successfully used DNA barcoding in a multidisciplinary approach for the identification of ingredients in commercial plant mixtures. However, there are only a few DNA barcoding studies in which a large number of medicinal plant raw drugs and natural health products were tested for species adulteration (Kool et al. 2012; Wallace et al. 2012; Newmaster et al. 2013). Such studies are very limited in India, which is endowed with about 7,500 recognized medicinal plants in various codified and non-codified systems of Indian medicine (Ved and Goraya 2008). The current study, reports a large scale study on species adulteration in diverse traded medicinal plant raw drugs. A

medicinal plant reference DNA barcode library was assembled for 521 medicinal plants using *rbcL* and *matK* markers, and subsequently used to identify species adulteration in 112 traded raw drugs collected from the markets.

## **Material and methods**

### **Reference DNA barcode library**

Plants for this study were selected from the medicinal plant list provided by National Medicinal Plants Board (NMPB), Government of India, available at <http://nmpb.nic.in/>. Leaf samples were collected from different localities in India, which includes botanical gardens, herbal gardens, cultivated, and natural fields. Collected samples were identified by plant taxonomists and their corresponding vouchers were deposited at the SRM University herbarium (SRMUH). DNA barcodes were generated for 400 medicinal plant species, and combined with DNA barcodes of 121 species from our previous studies (Purushothaman et al. 2014; Nithaniyal et al. 2014; Vassou et al. 2015) to create a larger reference DNA barcode library for medicinal plant species.

### **Collection of raw drug samples**

Medicinal plant raw drugs are traded across India through various outlets such as wholesale markets, food stores, and pharmacies. We collected single plant raw drugs that contained only one ingredient as per the label. Vernacular names for the raw drugs were obtained from traded-medicinal plants database ([http://envis.frlht.org/botanical\\_search.php](http://envis.frlht.org/botanical_search.php)) using botanical name search. Vernacular names in Tamil were used to collect the plant raw drugs from markets. Specifically, we collected 112 raw drug samples from eight markets in Tamil Nadu, India (Fig. 1, Table S1) of which 68 plant raw drugs were in the form of dried plant parts such as leaves, carved roots, seeds, rhizomes, fruits, and whole plants, and the remaining 44 were in the form of powders of known (leaf, bark, seed, fruit, and whole plant) or unknown origin. All voucher samples of raw drugs were deposited in the SRM University

herbarium. Details of the plant raw drugs collected are provided in Table S1. (supplementary data, Table S1.).

### **Genomic DNA Isolation, PCR Amplification and DNA Sequencing**

Total genomic DNA was isolated by following the protocol of Saghai-Marroof et al. with minor modifications (Saghai-Marroof et al. 1984). About 100 mg of sample was taken and ground using mortar and pestle by adding 500  $\mu$ l of CTAB buffer (100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% beta-mercaptoethanol, and 2% polyvinylpyrrolidone). Homogenized samples were transferred to 1.5 ml centrifuge tubes, incubated in water bath at 60°C for 30 minutes. Samples were then extracted with equal volume of chloroform and centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was transferred to 1.5 ml centrifuge tubes, precipitated by adding equal volume of ice-cold isopropanol, and centrifuged at 10,000 rpm for 10 minutes. The DNA pellet was washed twice with 70% ethanol, air-dried at room temperature, and dissolved in 100  $\mu$ l TE buffer.

Genomic DNA suitable for PCR amplification was successfully isolated from all samples. Universal primer pairs were used for PCR amplification of *rbcL* (*rbcLa*-F and *rbcLajf634*-R) (Levin et al. 2003; Fazekas et al. 2008) and *matK* (*matK-1RKIM*-F and *matK-3FKIM*-R, Ki-Joong Kim, School of Life Sciences and Biotechnology, Korea University, Seoul, unpublished). PCR reaction mixture contained 1X buffer with 1.5 mM MgCl<sub>2</sub>, 0.2  $\mu$ M dNTPs, 5 pmol primers, and 1 unit *Taq* DNA polymerase. PCR amplification was done in a thermal cycler (Eppendorf, Germany) using the following protocol: Initial denaturation at 95°C for 5 minutes, 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, final extension at 72°C for 5 minutes, and hold at 16°C. The PCR amplified products were purified using EZ-10 Spin Column PCR Purification Kit (Bio Basic Inc. Ontario, Canada). Sequencing of the PCR products was performed using Big-dye terminator chemistry in 3130xl Genetic Analyzer (Life

Technologies, California, USA) by following the standard protocol as recommended by the manufacturer.

### **Data analyses and identification of traded raw drugs**

Sequence quality was analyzed with Sequence Scanner Software v1.0 (Applied Biosystems, CA, USA) and full-length sequences were assembled using Codon Code Aligner, version 4.2.4 (CodonCode Corporation, MA, USA). Identification of traded raw drugs was done by comparing DNA barcodes obtained from the raw drug with the medicinal plant reference DNA barcode library. Species identification of the raw drugs was performed by best match method using TaxonDNA/Species identifier version 1.6.2 (Meier et al. 2006). This method assigns a query sequence to its closest match based on the genetic divergence. Genetic divergence was calculated as the percentage of mismatched nucleotides over the total number of aligned nucleotides. Unmatched samples were searched against the non-redundant nucleotide database of NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the Barcode of Life Data Systems (BOLD; [www.boldsystems.org](http://www.boldsystems.org); Ratnasingham and Hebert 2007) using BLAST algorithm.

## **Results**

### **Assembly of reference DNA barcode library**

We generated a reference DNA barcode library consisting of 1,452 barcodes from 521 medicinal plant species (1.64±1.01 and 1.13±1.24 barcodes per species for *rbcL* and *matK*, respectively), which was subsequently used for the purpose of identifying species adulteration in the medicinal plant raw drugs that were collected from the markets. The 521 medicinal plant species were taxonomically diverse representing 374 genera, 119 families and 44 orders. DNA isolation was successful for all the samples and purity of the genomic DNA was sufficient for the purpose of DNA barcoding as indicated by 100% PCR and sequencing success with *rbcL* marker. However, PCR and sequencing success rate was only

60% for *matK* marker. Bidirectional sequencing yielded 94% full-length sequences for *rbcL* (607bp). In the case of *matK*, full-length sequences were obtained from 47% of the samples, and the size varied between 828bp and 858bp. Currently, the reference DNA barcode library contains 1,452 DNA barcodes from *rbcL* and *matK* markers. The size of the barcodes varies from 504bp to 607bp and 624bp to 858bp for *rbcL* and *matK*, respectively. All the DNA barcode data were submitted to BOLD under the accessions numbers SRM000170A to SRM000296A and SRM000670A to SRM000943C.

### **Species resolution of reference DNA barcode library**

We calculated pairwise genetic divergence between species to evaluate the ability of *rbcL* and *matK* markers to differentiate medicinal plant species in the reference DNA barcode library. The *rbcL* marker differentiated 388 out of 521 species (74.4%) with the pairwise genetic divergence ranging between 0.2% and 19.0%. The *matK* marker differentiated 287 out of 318 species (90.2%) with the pairwise genetic divergence ranging between 0.2 and 21.0%. When these two markers were combined, 296 out of 318 species (93.0%) could be differentiated. However, in the tiered approach wherein the *rbcL* sequence acts as scaffold on which the *matK* sequence is placed for species differentiation, 450 out of 521 species (86.3%) were differentiated.

### **DNA barcoding of the traded raw drugs**

Genomic DNA was successfully isolated from all 112 raw drug samples using a standard protocol. PCR amplification and sequencing of *rbcL* DNA barcodes were successful for all samples and chromatogram of the *rbcL* DNA barcodes did not show mixed peaks in any of the raw drug sample analyzed. Therefore, the recovered barcodes could be directly compared with those present in the reference DNA barcode library for species identification. The DNA barcodes from 90 traded raw drugs (approx. 80%) showed the highest identity with expected species in the medicinal plant reference DNA barcode library. However, ten of them

also showed the highest identity with other species in the same genus or closely related genus in the same family.

### **Identification of the species adulteration in traded raw drugs**

DNA barcodes from the remaining 22 raw drugs (approx. 20%) showed 1.5 to 10.2% genetic divergence with expected species in the medicinal plant reference DNA barcode library, which strongly indicated species adulteration. DNA barcodes were searched against the non-redundant nucleotide database of NCBI and BOLD to establish their species identity. The DNA barcodes from 15 raw drugs showed 100% identity with single species, and that from seven species showed 100% identity with multiple species from the same genus or different genera of the same family. Therefore, botanical identity could be established at species, genus, and family level for 15, 4, and 3 adulterated plants, respectively (Table 1). All 22 adulterant species belonged to different genera when compared with the species mentioned on the label, and 14 of them even belonged to a different family. The majority of traded raw drugs were in the form of powders, seeds, roots, and whole plants, and among them, adulteration was found to be the least within seeds (4.0%). Adulteration with roots, whole plants, and powders was 42.9%, 33.3%, and 22.7%, respectively. We then compared medicinal properties of the expected species and the adulterant species for the 15 raw drugs in which the adulterated plant materials were identified to species level. Eight adulterant species shared some but not all the medicinal properties of the expected species. The remaining seven adulterant species had no resemblance with the expected species in terms of the medicinal values, and some of them were even reported to be toxic (supplementary data, Table S2.).

### **Discussion**

Species adulteration, presence of one species in place of the other, in traded medicinal plant raw drugs is a growing concern in the context of increasing popularity and demand for CAM throughout the World (WHO 2004). Different terminologies such as adulteration, substitution, fraudulent substitution, admixture, mislabeling, contamination, filler, etc., are used to refer to species adulteration in raw drugs (Srirama et al. 2010; Wallace et al. 2012; Newmaster et al. 2013; Swetha et al. 2014; Kumar et al. 2015). In the present study, a traded medicinal plant raw drug sample was considered to have species adulteration if DNA barcoding did not identify the species that was mentioned on the label, and the actual species found was referred to as an adulterant species. Analysis of 112 traded medicinal plant raw drugs revealed the presence of adulterant species in about 20% of the samples. Adulteration was frequent in dried roots, powders, and whole plants, which are more difficult to identify based on morphology. All adulterations were presumably in the form of complete replacement of one species for the other as indicated by the absence of characteristic mixed peaks. Interestingly, all adulterant species were from a different genus, and in some cases, even from a different family. Taxonomic analysis of controversial drug plants (Nair 2004) that are often found as adulterant species show that 78% of them belong to other genera and families. The conserved *rbcL* marker, which is capable of mostly genus level taxonomic discrimination but credited with good PCR amplification and sequencing success (Bafeel et al. 2012; Parmentier et al. 2013; Chen et al. 2014) would be sufficient to establish species adulteration in most of the traded raw drugs. However, ITS2 is evolving as a successful core or supplementary marker to increase species resolution and raw drug authentication (Newmaster et al. 2013; Seethapathy et al. 2014).

The efficiency of identifying species adulteration by DNA barcoding primarily depends on the taxonomic coverage of the reference DNA barcode library. Our reference DNA barcode library of 521 medicinal plant species contains authentic drug plants, closely related

species, and their possible adulterants. The library covers 347 actively traded medicinal plants including 115 species of high trade value (>100 metric tonnes per year) (Ved and Goraya 2008) and 42 controversial drug plants, which are frequently adulterated with non-authentic species (Nair 2004). Therefore, this reference DNA barcode library could be utilized by the regulatory authorities to monitor the indiscriminate collection of medicinal plants from wild source, which becomes a major threat to biodiversity and sustainable utilization. Medicinally useful but threatened plants may be traded under the label of other plants to evade legal action. The reference DNA barcode library currently comprises 31 threatened plant species (IUCN, <http://www.iucnredlist.org>; ENVIS, <http://envis.frlht.org/junclist.php>) and can provide scientific evidence for species identity to support legal action against fraudulent or illicit trade.

The species adulterations found in the present study may be due to mislabeling, morphological resemblance, co-occurrence, confusion in vernacular names, substitution of one species for another as result of non-availability or high cost of the authentic species, etc. For example, initially we recovered *Eclipta prostrata* (L.) L. in place of *Mukia maderaspatana* (L.) M. Roem. However, DNA barcoding of seven more samples from the same market recovered *M. maderaspatana* in all the cases, which indicated the possibility of accidental mislabeling during packaging. Adulteration of *Zaleya pentandra* (L.) C. Jeffrey with *Boerhavia diffusa* L. may be due to their morphological resemblance (Suthari et al. 2011), and adulteration of *Tephrosia purpurea* (L.) Pers. with *Sida cordifolia* L. may be because they co-occur as weeds along the roadsides and barren lands. We recovered *Aerva lanata* (L.) Juss. and *Andrographis paniculata* (Burm.f.) Nees from the raw drugs labeled as *Bergenia ciliata* (Haw.) Sternb. and *Swertia chirata* Buch.-Ham. ex Wall., respectively. This may be because “Sirupilai” is the vernacular name for both *A. lanata* and *B. ciliata* and “Nilavembu” is the vernacular name for both *S. chirata* and *A. paniculata*. *Bergenia ciliata*

and *S. chirata* are highly traded raw drugs but are distributed only in the Himalayan regions of India (Rai et al. 2000). Their non-availability in other regions might have encouraged substitution due to identical vernacular names. We recovered *Abrus precatorius* L. in place of *Adenantha pavonina* L. which have very similar vernacular names, Kundumani and Anai Kundumani, respectively. This is also true for *Azadirachta indica* A. Juss. and *Toona ciliata* M. Roem. with vernacular names, Vembu, and Santhana Vembu, respectively. Species adulteration due to confusion in vernacular names was also reported in traded raw drugs of *S. cordifolia* (Vassou et al. 2015).

When adulterant species in raw drugs are used directly or in formulations, it is unlikely that the consumer will derive the same health benefits as expected from authentic species. In some cases, adulterant species is also claimed to possess the same medicinal properties as that of the authentic species. If so, fair practice would be to mention the correct name of that species on the label so that the consumer or the practitioner of the alternative medicine can take informed decision on using a particular raw drug. Alarming, some of the adulterant species are known to contain compounds that are toxic if consumed. For example, *Z. pentandra* was found in place of *B. diffusa*, which was reported to cause abortion (Quattrocchi 2012). Similarly, *A. pavonina* was found in place of *A. precatorius* which is known for the presence of toxins such as abrin and isolectins. Abrin is a toxin with the potential to pose a severe threat to both human and animal health, and its possession is regulated by law in several countries including the USA ([www.selectagents.gov/Select Agents and Toxins List](http://www.selectagents.gov/SelectAgentsandToxinsList)). The raw drugs from *Albizia lebbek* (L.) Benth., *Flacourtia indica* (Burm.f.) Merr., and *Gmelina asiatica* L. were found to be replaced with species of *Senna*, which is reported to also have several species with toxic compounds (Nelson et al. 2007; Vashishtha et al. 2009; Quattrocchi 2012).

Sale of raw drugs and herbal medicines as supplements remains largely unregulated in most countries. Trading of adulterant species with unrelated medicinal properties or toxic properties not only questions the ethical practices followed in the trade but also undermines the credibility and safety of CAM, which will ultimately affect the trade industry itself. Therefore, ensuring that only authentic plant materials are traded in the markets is important for both the consumers as well as the traders. The corrective measures to prevent species adulteration in raw drugs would include i) prohibiting the use of multiple vernacular names for single species or making it mandatory to provide botanical name along with vernacular name(s) on the label, ii) including DNA barcoding as a routine quality control measure, and iii) authentication of marketed plant materials using DNA barcoding by an independent agency.

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### **Author Contributions Statement**

P.M. and S.N. conceived and designed the research. S.N., S.L.V., P.S. and B.R. performed the experiments. P.M. and S.N. analyzed the data and wrote the manuscript. All authors reviewed and approved the final manuscript.

**Conflict of interest:** The authors declare that there is no conflict of interest associated with this publication.

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**Table 1.** Details of the adulterant species found among the 112 traded medicinal plant raw drugs that were tested by DNA barcoding.

Sample ID	Form	Expected plant species	Species ID by DNA barcoding	Family
RD066	Dried leaf	<i>Adenanthera pavonina</i> L.	<i>Abrus precatorius</i> L.	Fabaceae
RD044	Powder	<i>Albizia lebbeck</i> (L.) Benth.	<i>Senna</i> sp.	Fabaceae
RD026	Dried root	<i>Anisomeles malabarica</i> (L.) R.Br. ex Sims	Araliaceae	Araliaceae
RD067	Dried whole plant	<i>Barleria prionitis</i> L.	<i>Andrographis paniculata</i> (Burm.f.) Nees	Acanthaceae
RD068	Powder	<i>Bauhinia purpurea</i> L.	<i>Peltophorum</i> sp.	Fabaceae
RD140	Powder	<i>Bergenia ciliata</i> (Haw.) Sternb.	<i>Aerva lanata</i> (L.) Juss. <i>Zaleya pentandra</i> (L.) C.Jeffrey	Amaranthaceae
RD045	Dried root	<i>Boerhavia diffusa</i> L.	C.Jeffrey	Aizoaceae
RD061	Dried rhizome	<i>Calamus rotang</i> L.	Araceae	Araceae
RD193	Powder	<i>Calotropis procera</i> (Aiton) Dryand.	<i>Enicostema axillare</i> (Poir. ex Lam.) A.Raynal	Gentianaceae
RD084	Dried whole plant	<i>Calycopteris floribunda</i> (Roxb.) Lam. ex Poir. <i>Catharanthus roseus</i> (L.) G.Don	<i>Iphigenia indica</i> (L.) A.Gray ex Kunth	Colchicaceae
RD071	Powder	G.Don	<i>Ocimum tenuiflorum</i> L. <i>Tephrosia purpurea</i> (L.) Pers.	Lamiaceae
RD004	Dried root	<i>Clitoria ternatea</i> L.	Pers.	Fabaceae
RD073	Dried root	<i>Cordia obliqua</i> Willd. <i>Flacourtia indica</i> (Burm.f.) Merr.	<i>Azima tetraacantha</i> Lam.	Salvadoraceae
RD074	Powder	Merr.	<i>Senna</i> sp.	Fabaceae
RD075	Powder	<i>Gmelina asiatica</i> L.	<i>Senna</i> sp.	Fabaceae
RD100	Powder	<i>Melia azedarach</i> L. <i>Mukia maderaspatana</i> (L.) M.Roem.	<i>Andrographis paniculata</i> (Burm.f.) Nees	Acanthaceae
RD011	Powder	M.Roem.	<i>Eclipta prostrata</i> (L.) L.	Asteraceae
RD080	Dried root	<i>Polyalthia longifolia</i> (Sonn.) Thwaites	Asclepiadiaceae <i>Tephrosia purpurea</i> (L.) Pers.	Asclepiadiaceae
RD054	Dried root	<i>Sida cordifolia</i> L.	Pers.	Fabaceae
RD056	Dried whole plant	<i>Swertia chirata</i> Buch.-Ham. ex Wall. <i>Teramnus labialis</i> (L.f.) Spreng.	<i>Andrographis paniculata</i> (Burm.f.) Nees <i>Vigna radiata</i> (L.) R.Wilczek	Acanthaceae
RD188	Dried seed	Spreng.	R.Wilczek	Fabaceae
RD147	Powder	<i>Toona ciliata</i> M.Roem.	<i>Azadirachta indica</i> A.Juss.	Meliaceae

## Figure legend

**Fig. 1** Different forms of the traded medicinal plant raw drugs that were used for present study. Flower buds: *Michelia champaca* (a). Rhizomes: *Acorus calamus* (b), *Zingiber officinale* (c). Tubers: *Cyperus rotundus* (d). Whole plants: *Evolvulus alsinoides* (e), *Mollugo cerviana* (f). Dried stems: *Cissus quadrangularis* (g), *Tinospora cordifolia* (h). Roots: *Holarrhena antidysenterica* (i), *Hemidesmus indicus* (j), *Morinda pubescens* (k), *Ficus benghalensis* (l). Seeds: *Moringa oleifera* (m), *Cleome viscosa* (n), *Semecarpus anacardium* (o), *Strychnos nuxvomica* (p), *Mucuna pruriens* (q), *Eleusine coracana* (r), *Abrus precatorius* (s), *Papaver somniferum* (t). Powders: *Murraya koenigii* (u), *Erythrina variegata* (v), *Centella asiatica* (w), *Cardiospermum halicacabum* (x).

## Supplementary data

### Table legends

**Table S1.** Details of the 112 traded medicinal plant raw drugs that were collected for the current study.

**Table S2.** Comparison of the medicinal properties of plants used in complementary and alternative medicine versus the adulterant species



Fig. 1 Different forms of the traded medicinal plant raw drugs that were used for present study

101x143mm (300 x 300 DPI)

**Identification of species adulteration in traded medicinal plant raw drugs using DNA  
barcoding**

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**Table S1.** Details of the 112 traded medicinal plant raw drugs that were collected for the current study.

Sample ID	Place of collection	Form of the sample	Botanical name	Family	Vernacular Name in Tamil	Trade name
RD021	Vellore	Dried seed	<i>Abrus precatorius</i> L.	Fabaceae	Kundumani	Gunja
RD001	Madurai	Leaf powder	<i>Abutilon indicum</i> (L.) Sweet	Malvaceae	Thuthi	Tutti
RD022	Theni	Bark powder	<i>Acacia nilotica</i> (L.) Delile	Fabaceae	Karuvellam	Babul
RD023	Theni	Dried rhizome	<i>Acorus calamus</i> L.	Acoraceae	Vasambu	Vach
RD066	Madurai	Dried leaf	<i>Adenanthera pavonina</i> L.	Fabaceae	Anai Kundumani	Kamboji
RD024	Theni	Leaf powder	<i>Aegle marmelos</i> (L.) Corrêa	Rutaceae	Vilvam	Bael
RD043	Trichy	Powder	<i>Aerva lanata</i> (L.) Juss.	Amaranthaceae	Sirupilai	Cheroola
RD044	Trichy	Powder	<i>Albizia lebbek</i> (L.) Benth.	Fabaceae	Vagai	Vaakaveru
RD197	Tanjore	Dried seed	<i>Amaranthus tricolor</i> L.	Amaranthaceae	Thandukkeerai	Alpamarisa
RD161	Virudhunagar	Dried seed	<i>Anethum graveolens</i> L.	Apiaceae	Sadakuppai	Sowa
RD026	Vellore	Dried root	<i>Anisomeles malabarica</i> (L.) R.Br. ex Sims	Lamiaceae	Peymiratti	Karimthumpa
RD096	Virudhunagar	Dried seed	<i>Argemone mexicana</i> L.	Papaveraceae	Brahmathandi	Kusme Beeja
RD154	Kancheepuram	Dried root	<i>Aristolochia indica</i> L.	Aristolochiaceae	Easwaramuli	Ishwar mool
RD027	Theni	Powder	<i>Azadirachta indica</i> A.Juss.	Meliaceae	Vembu	Neem
RD060	Trichy	Powder	<i>Bambusa bambos</i> (L.) Voss	Poaceae	Moongil	Bansalochan
RD067	Madurai	Dried whole plant	<i>Barleria prionitis</i> L.	Acanthaceae	Semmulli	Vajradanti
RD068	Madurai	Powder	<i>Bauhinia purpurea</i> L.	Fabaceae	Mandharai	Sonachal
RD140	Virudhunagar	Powder	<i>Bergenia ciliata</i> (Haw.) Sternb.	Saxifragaceae	Sirupilai	Pashnabheda
RD045	Trichy	Dried root	<i>Boerhavia diffusa</i> L.	Nyctaginaceae	Mukkarattai	Punarnava
RD002	Chennai	Dried seed	<i>Brassica juncea</i> (L.) Czern.	Brassicaceae	Kadugu	Sasuve Bili
RD061	Trichy	Dried rhizome	<i>Calamus rotang</i> L.	Arecaceae	Pirapan kizhangu	Bet
RD193	Tanjore	Powder	<i>Calotropis procera</i> (Aiton) Dryand.	Fabaceae	Erukku	Akada Phool
RD084	Madurai	Dried whole plant	<i>Calycopteris floribunda</i> (Roxb.) Lam. ex Poir.	Combretaceae	Therulankodi	Pullaaniyila
RD159	Vellore	Dried seed	<i>Capsicum annum</i> L.	Solanaceae	Milagai	Marich

RD109	Trichy	Powder	<i>Cardiospermum halicacabum</i> L.	Sapindaceae	Mudakkathan	Mudakkathan
RD160	Vellore	Dried seed	<i>Carica papaya</i> L.	Caricaceae	Pappali	Papaya
RD071	Madurai	Powder	<i>Catharanthus roseus</i> (L.) G.Don	Apocynaceae	Nithyakalyani	Sadabahar
RD003	Theni	Leaf powder	<i>Centella asiatica</i> (L.) Urb.	Apiaceae	Vallarai	Brahmi booti
RD047	Trichy	Dried stem	<i>Cissus quadrangularis</i> L.	Vitaceae	Pirandai	Asthisamhrta
RD030	Vellore	Dried seed	<i>Cleome viscosa</i> L.	Cleomaceae	Naikkadugu	Naikadugu
RD004	Chennai	Dried root	<i>Clitoria ternatea</i> L.	Fabaceae	Sankapushpam	Kajli
RD073	Madurai	Dried root	<i>Cordia obliqua</i> Willd.	Boraginaceae	Mookuchalipazham	Sapistan
RD005	Chennai	Seed powder	<i>Coriandrum sativum</i> L.	Apiaceae	Kothumalli	Dhaniya
RD104	Chennai	Flower powder	<i>Crocus sativus</i> L.	Iridaceae	Kungumappoo	Kesar
RD149	Vellore	Dried seed	<i>Cuminum cyminum</i> L.	Apiaceae	Seeragam	Shahjeera
RD195	Chennai	Powder	<i>Cynodon dactylon</i> (L.) Pers.	Poaceae	Arugampul	Durva
RD111	Kancheepuram	Dried tuber	<i>Cyperus rotundus</i> L.	Poaceae	Korai kizanghu	Nagarmotha
RD049	Trichy	Dried seed	<i>Datura metel</i> L.	Solanaceae	Umathai	Duttura
RD063	Trichy	Dried whole plant	<i>Desmostachya bipinnata</i> (L.) Stapf	Poaceae	Darbhaipul	Dharbha
RD050	Trichy	Dried Tuber	<i>Dioscorea bulbifera</i> L.	Dioscoreaceae	Kattuvalli	Varahi kand
RD131	Kancheepuram	Leaf powder	<i>Eclipta prostrata</i> (L.) L.	Asteraceae	Karisalangani	Bhringaraj
RD219	Kancheepuram	Dried seed	<i>Eleusine coracana</i> (L.) Gaertn.	Poaceae	Kezhvaragu	Nachani
RD031	Theni	Dried whole plant	<i>Enicostema axillare</i> (Poir. ex Lam.) A.Raynal	Gentianaceae	Vellarugu	Mamejava
RD006	Chennai	Leaf powder	<i>Erythrina variegata</i> L.	Fabaceae	Kalyana murungai	Murikkila
RD007	Chennai	Whole plant powder	<i>Euphorbia hirta</i> L.	Euphorbiaceae	Amman-pacharisi	Dudhika
RD051	Trichy	Dried whole plant	<i>Evolvulus alsinoides</i> (L.) L.	Convolvulaceae	Vishnukranthi	Shankhpush
RD064	Trichy	Dried root	<i>Ficus benghalensis</i> L.	Moraceae	Aal	Vada chhal
RD074	Madurai	Powder	<i>Flacourtia indica</i> (Burm.f.) Merr.	Fabaceae	Sothaikala	Kattar
RD210	Chennai	Dried fruit	<i>Foeniculum vulgare</i> Mill.	Apiaceae	Sombu	Badiyan Khatal
RD132	Chennai	Dried root	<i>Glycyrrhiza glabra</i> L.	Fabaceae	Athimathuram	Mulethi

RD075	Madurai	Powder	<i>Gmelina asiatica</i> L.	Lamiaceae	Nelakumizh	Kapas Beej
RD182	Kancheepuram	Dried seed	<i>Gossypium herbaceum</i> L.	Malvaceae	Paruthi	Kapas
RD122	Kancheepuram	Powder	<i>Gymnema sylvestre</i> (Retz.) R.Br. ex Sm.	Asclepiadaceae	Sirukurinjan	Gudmar
RD097	Virudhunagar	Dried fruit	<i>Helicteres isora</i> L.	Malvaceae	Valamburi	Marodphali
RD076	Madurai	Powder	<i>Heliotropium indicum</i> L.	Scrophulariaceae	Thekkodukku	Thekkada
RD098	Virudhunagar	Dried root	<i>Hemidesmus indicus</i> (L.) R. Br. ex Schult.	Apocynaceae	Nannari	Anatmool
RD032	Theni	Leaf powder	<i>Hibiscus rosa-sinensis</i> L.	Malvaceae	Semparuthi	Jashwanti
RD099	Virudhunagar	Dried seed	<i>Hibiscus sabdariffa</i> L.	Malvaceae	Pulichai-keerai	Patwa
RD105	Chennai	Dried seed	<i>Hippophae rhamnoides</i> L.	Elaeagnaceae	Ameel	Sea Buckthorn
RD144	Kancheepuram	Dried root	<i>Holarrhena antidysenterica</i> (Roth) Wall. ex A.DC.	Apocynaceae	Veppalai	Kutja
RD106	Kancheepuram	Whole plant powder	<i>Hybanthus enneaspermus</i> (L.) F.Muell.	Violaceae	Orithazh thamarai	Ratanpurus
RD123	Kancheepuram	Dried whole plant	<i>Hygrophila auriculata</i> (Schumach.) Heine	Acanthaceae	Neermulli	Tal makhana
RD087	Madurai	Dried stem	<i>Ichnocarpus frutescens</i> (L.) W.T.Aiton	Apocynaceae	Udarkodi	Dudhi, Sariva
RD008	Theni	Leaf powder	<i>Justicia adhatoda</i> L.	Acanthaceae	Adathodai	Adusa
RD009	Chennai	Leaf powder	<i>Lawsonia inermis</i> L.	Lythraceae	Marudhani	Mehndi
RD208	Chennai	Dried seed	<i>Luffa acutangula</i> (L.) Roxb.	Cucurbitaceae	Peerku	Tori
RD100	Virudhunagar	Powder	<i>Melia azedarach</i> L.	Meliaceae	Malaivembu	Bakayan phal
RD093	Vellore	Flower	<i>Mesua ferrea</i> L.	Calophyllaceae	Nagapoo	Nagkeshar
RD033	Vellore	Flower	<i>Michelia champaca</i> L.	Magnoliaceae	Shenbagam	Champaka
RD010	Chennai	Dried seed	<i>Mirabilis jalapa</i> L.	Nyctaginaceae	Andhimandhaarai	Gulabash
RD035	Chennai	Dried Whole plant	<i>Mollugo cerviana</i> (L.) Ser.	Molluginaceae	Parpadagam	Parpata
RD078	Madurai	Dried root	<i>Morinda pubescens</i> Sm.	Rubiaceae	Nuna	Manjanatthi
RD034	Chennai	Dried seed	<i>Moringa oleifera</i> Lam.	Moringaceae	Murungai	Sahenjana chaal
RD052	Trichy	Dried seed	<i>Mucuna pruriens</i> (L.) DC.	Fabaceae	Punaikkali	Kaunch beej
RD011	Theni	Leaf powder	<i>Mukia maderaspatana</i> (L.)	Cucurbitaceae	Musumusukkai	Mucukkai

			M.Roem.			
RD036	Theni	Leaf powder	<i>Murraya koenigii</i> (L.) Spreng.	Rutaceae	Kariveppilai	Kariveppila
RD134	Virudhunagar	Powder	<i>Nelumbo nucifera</i> Gaertn.	Nymphaeaceae	Thamarai	Kamal phul
RD165	Kancheepuram	Dried seed	<i>Nigella sativa</i> L.	Ranunculaceae	Karunseeragam	Kalongi
RD012	Theni	Leaf powder	<i>Ocimum tenuiflorum</i> L.	Lamiaceae	Tulasi	Tulasi
RD101	Virudhunagar	Powder	<i>Oxalis corniculata</i> L.	Oxalidaceae	Puliyarai	Puliyarila
RD201	Tanjore	Dried seed	<i>Papaver somniferum</i> L.	Papaveraceae	Gasha-gasha	Khaskhas
RD108	Virudhunagar	Powder	<i>Phyla nodiflora</i> (L.) Greene	Verbenaceae	Poduthalai	Chota-okra
RD092	Theni	Fruit powder	<i>Phyllanthus emblica</i> L.	Phyllanthaceae	Nelli	Amla
RD037	Chennai	Dried inflorescence	<i>Piper longum</i> L.	Piperaceae	Thippili	Pippali
RD013	Chennai	Dried fruit	<i>Piper nigrum</i> L.	Piperaceae	Milagu	Kalimirch
RD080	Madurai	Dried root	<i>Polyalthia longifolia</i> (Sonn.) Thwaites	Annonaceae	Nettilingam	Ashok
RD014	Chennai	Dried fruit	<i>Pongamia pinnata</i> (L.) Pierre	Fabaceae	Pungam	Karanji
RD102	Virudhunagar	Dried whole plant	<i>Psoralea corylifolia</i> L.	Fabaceae	Karpokarishi	Bawachi
RD090	Tanjore	Dried root	<i>Rauvolfia serpentina</i> (L.) Benth. ex Kurz	Apocynaceae	Sarpagandhi	Sarpagandha
RD053	Trichy	Dried seed	<i>Semecarpus anacardium</i> L.f.	Anacardiaceae	Serankottai	Balave
RD028	Theni	Dried Flower	<i>Senna auriculata</i> (L.) Roxb.	Fabaceae	Avaram	Avarai
RD081	Madurai	Powder	<i>Senna tora</i> (L.) Roxb.	Fabaceae	Senavu	Chakoda Beeja
RD015	Chennai	Dried seed	<i>Sesamum indicum</i> L.	Pedaliaceae	Ellu	Til
RD054	Trichy	Dried root	<i>Sida cordifolia</i> L.	Malvaceae	Kurunthotti	Bala
RD017	Theni	Leaf powder	<i>Solanum trilobatum</i> L.	Solanaceae	Thoodhuvalai	Alarka
RD018	Chennai	Fruit powder	<i>Solanum xanthocarpum</i> Schrad. & H. Wendl.	Solanaceae	Kandanghathiri	Kateli
RD055	Trichy	Dried seed	<i>Strychnos nux-vomica</i> L.	Loganiaceae	Yetti-kottai	Kuchla
RD056	Trichy	Dried whole plant	<i>Swertia chirata</i> Buch.-Ham. ex Wall.	Gentianaceae	Nilavembu	Chiraiyata
RD038	Theni	Seed powder	<i>Syzygium cumini</i> (L.) Skeels	Myrtaceae	Naaval	Jamun
RD139	Chennai	Dried leaf	<i>Taxus baccata</i> L.	Taxaceae	Thalisa pathri	Talispatra

RD188	Kancheepuram	Dried seed	<i>Teramnus labialis</i> (L.f.) Spreng.	Fabaceae	Kattu uluntu	Masparni
RD039	Theni	Dried fruit	<i>Terminalia arjuna</i> (Roxb. ex DC.) Wight & Arn.	Combretaceae	Marudhu	Arjun
RD041	Theni	Dried fruit	<i>Terminalia chebula</i> Retz.	Combretaceae	Kadukkai	Harda
RD042	Chennai	Dried stem	<i>Tinospora cordifolia</i> (Willd.) Miers	Menispermaceae	Seendhal	Amrithaballi
RD147	Kancheepuram	Powder	<i>Toona ciliata</i> M. Roem.	Meliaceae	Santhanavembu	Thooniyanoikam
RD020	Theni	Fruit powder	<i>Tribulus terrestris</i> L.	Zygophyllaceae	Nerungil	Gokshura
RD103	Virudhunagar	Dried root	<i>Vetiveria zizanioides</i> (L.) Nash	Poaceae	Vetiver	Lavanha
RD107	Kancheepuram	Dried seed	<i>Vigna unguiculata</i> (L.) Walp.	Fabaceae	Karamani	Alasunda
RD065	Trichy	Leaf powder	<i>Vitex negundo</i> L.	Verbenaceae	Karunochi	Neergundi
RD211	Tanjore	Powder	<i>Withania somnifera</i> (L.) Dunal	Solanaceae	Aswaganthi	Ashwagandha
RD185	Chennai	Dried rhizome	<i>Zingiber officinale</i> Roscoe	Zingiberaceae	Sukku	Sonth
RD091	Madurai	Powder	<i>Ziziphus mauritiana</i> Lam.	Rhamnaceae	Elandhai	Ber

**Table S2. Comparison of the medicinal properties of plants used in complementary and alternative medicine versus the adulterant species**

S.No	Expected Plants Name	Adulterant Plants Name
1.	<i>Adenanthera pavonina</i> L. Pulmonary Affection, Chronic Ophthalmia, Chronic Rheumatism, Gout, Haematuria, and Haematemesis.	<i>Abrus precatorius</i> L. Diuretic, Skin Diseases, Neurological disorders, Purgative, Emetic, Antiphlogistic, and Aphrodisiac.
2.	<i>Barleria prionitis</i> L. Toothache, Catarrhal Affections, Cough, and Anasarca.	<i>Andrographis paniculata</i> (Burm.f.) Nees Anthelmintic, Diuretic, Lung Disorders, Febrifuge, and Diabetes.
3.	<i>Bergenia ciliata</i> (Haw.) Sternb.* Demulcent, Astringent, Dysuria, Calculus, Laxative, Pulmonary Affection, Dysentery, Ulcers, Spleen enlargement, Cough, and Fever.	<i>Aerva lanata</i> (L.) Juss. Dysuria, Calculus, Cough, and Sore throat
4.	<i>Boerhavia diffusa</i> L. Purgative, Anthelmintic, Febrifuge, Diuretic, Asthma, Anemia, and Oedema.	<i>Zaleya pentandra</i> (L.) C.Jeffrey Abortifacient, Used in Scorpion-Sting.
5.	<i>Calotropis procera</i> (Aiton) Dryand. Skin Diseases, Pruritus, Gulma, Ascites, Wound Healing, Respiratory Disorders, and Piles.	<i>Enicostema axillare</i> (Poir. ex Lam.) A.Raynal Laxative, Oedema, Diabetes Mellitus, Obesity, and Intermittent fever.
6.	<i>Calycopteris floribunda</i> (Roxb.) Lam. ex Poir.* Anthelmintic, Colic, Headache, Dyspepsia, Diarrhea Dysentery, Laxative, Astringent, and Carminative.	<i>Iphigenia indica</i> (L.) A.Gray ex Kunth Anthelmintic, Colic, and Headache.
7.	<i>Catharanthus roseus</i> (L.) G.Don Diabetes, Menorrhagia, Hypotensive, Sedative, Tranquilizers and Used in wasp-sting	<i>Ocimum tenuiflorum</i> L. Respiratory Disorders, Anthelmintic, Skin diseases, Calculus, and Eye Diseases.
8.	<i>Clitoria ternatea</i> L.* Diarrhea, Asthma, Urinary Disorders, Laxative, and Demulcent.	<i>Tephrosia purpurea</i> (L.) Pers. Diarrhea, Asthma and Urinary disorders, Scabies, and Eczema.
9.	<i>Cordia obliqua</i> Willd.* Demulcent, Expectorant, Astringent, Relieve Cough, Asthma, and Used in snake-bite.	<i>Azima tetracantha</i> Lam. Expectorant, Cough, Asthma, and Rheumatism.

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| 10. | <i>Melia azedarach</i> L.*<br>Anthelmintic, Diuretic, Lung Disorders, Febrifuge, Cathartic, Emetic, Emmenagogue, Stomachic, Skin diseases and Diabetes.                           | <i>Andrographis paniculata</i> (Burm.f.) Nees<br>Anthelmintic, Diuretic, Lung Disorders, Febrifuge, and Diabetes. |
| 11. | <i>Mukia maderaspatana</i> (L.) M.Roem.<br>Toothache, Flatulence, Diuretic, Aperient Stomachic, and Vertigo.  | <i>Eclipta prostrata</i> (L.) L.<br>Anemia, Spleen enlargement, Skin troubles, and Respiratory Disorders.         |
| 12. | <i>Sida cordifolia</i> L.<br>Hemiplegia, Sciatica, Facial Paralysis, and Leucorrhoea.   | <i>Tephrosia purpurea</i> (L.) Pers.<br>Diarrhea, Asthma, Urinary Disorders, Scabies, and Eczema.                 |
| 13. | <i>Swertia chirata</i> Buch.-Ham. ex Wall.*<br>Anthelmintic, Diuretic, Lung Disorders, Febrifuge, Diabetes, Skin disease, Stomachic, Anti- Diarrheic, and Used in Scorpion sting. | <i>Andrographis paniculata</i> (Burm.f.) Nees<br>Anthelmintic, Diuretic, Lung Disorders, Febrifuge, and Diabetes. |
| 14. | <i>Teramnus labialis</i> (L.f.) Spreng.*<br>Nerve diseases, Paralysis, Diarrhea, Febrifuge, Vertigo, Stomachic, and Rheumatism.   | <i>Vigna radiata</i> (L.) R.Wilczek<br>Neuropathy, Diarrhea, Febrifuge, Vertigo, and Stomachic.                   |
| 15. | <i>Toona ciliata</i> M.Roem.*<br>Astringent, Dysentery, Menstrual Disorder, Ulcer, Skin diseases, and Diabetes.   | <i>Azadirachta indica</i> A.Juss.<br>Astringent, Anthelmintic, Ulcer, Skin diseases, and Diabetes.                |

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(\*) denotes the species sharing medicinal properties of expected and adulterant species.

## Sources

1. Anonymous. The Ayurvedic Pharmacopoeia of India, 1<sup>st</sup> Edition, Vol. I–VI. Published by Govt. of India, Ministry of Health & Family Welfare, Dept. of Indian system of Medicine & Homoeopathy, New Delhi (2008).
2. Yoganarasimhan, S. N. Medicinal plants of India vol.2 Tamil Nadu, Regional Research Institute (Ay.), Bangalore: Cybermedia (2000).
3. Nadkarni, A. K. Indian Materia Medica, Popular Prakashan, Bombay (1976).