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ORIGINAL ARTICLE

# Assessment of adulteration in raw herbal trade of important medicinal plants of India using DNA barcoding

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## Abstract

A number of studies have shown that there could be widespread substitution and/or adulteration (hereafter referred to as substitution) in raw herbal trade of medicinal plants. Substitution could potentially endanger the health and safety of the consumers. In this study, the extent of adulteration in raw herbal trade of 30 important medicinal plants in South India was analyzed. Biological reference material (BRM) consisting of taxonomically authenticated samples of each of the 30 species along with 14 other co-occurring and congeneric allied species that are likely to be used in adulteration was established. DNA barcode signatures of 124 BRM using two candidate regions, nr-*ITS* and *psb*A-*trn*H were identified. A total of 203 herbal trade samples representing the 30 medicinal plant species were collected from 34 locations in South India. Using the DNA barcode sequences of the BRM as reference, the analysis indicated that the substitution ranged from 20 to 100%. Overall, approximately 12% of the market samples were adulterated. Considering the potential health hazard that such adulteration can cause, the need for a national regulatory framework that can authenticate and regulate raw herbal trade in the country is discussed.

**Keywords** DNA barcoding · Biological reference material · Raw herbal drugs · Substitution

India is known for its rich diversity of medicinal plants and has a long history of traditional medicinal practices (Valithan 2006). The Codified Indian System of Medicine recognizes the use of about 2400 medicinal plants, though about 6000 higher plant species are used in several folk healthcare traditions (Ved and Goraya 2007). An estimated 9500 registered herbal industries along with a multitude of unregistered cottage-level industries depend on the supply of medicinal plants for manufacturing raw herbal products (Ved and Goraya 2007). However, barely 10% of the supply is met from cultivated sources, the remaining obtained are from collection of naturally occurring populations (Ved and Goraya 2007; Seethapathy et al. 2014). Collections of plants from the wild were often plagued by adulterations either intentionally or unintentionally (substitution) (Srirama et al. 2010; Seethapathy et al. 2014; Santhosh et al. 2015). For example, substitution of samples could arise due to more than one co-occurring species sharing the same vernacular name and hence leading to confusion for the collectors. Alternatively, it could occur due to the inability of the collector to distinguish two or more co-occuring species because of their close morphological similarity (Srirama et al. 2010; Santhosh et al. 2015).

Species substitution may adversely affect consumer health as it could cause severe allergies and will not have the intended effect (Seethapathy et al. 2014; Santhosh et al. 2015, 2016; Srirama et al. 2017). Visual detection of species adulteration in the raw herbal trade is often difficult, as the plants are usually in a dry state and do not retain the original features of the plant (Seethapathy et al. 2014; Santhosh et al.

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s13205-018-1169-3) contains supplementary material, which is available to authorized users.

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2015, 2016). In recent years, a number of techniques have been developed to identify medicinal plants in trade including the use of morpho-taxonomical keys, histological techniques, chemical fingerprinting and DNA-based approach, each having their own advantages and disadvantages (Smillie and Khan 2009). However, the major disadvantage of morphological technique is that they cannot identify the market samples if they are in powdered form. Similarly, the disadvantage of using chemical and histological approach is that they are sensitive to age and season of collection (Smillie and Khan 2009).

Among these techniques, DNA barcoding has been extensively used as an accurate, cost-effective, and reliable tool to identify medicinal plant material used in trade (Srirama et al. 2010; Wallace et al. 2012; Newmaster et al. 2013; Seethapathy et al. 2014; Santhosh et al. 2015). Santhosh et al. (2016) investigated the authenticity of *Saraca asoca* herbal products sold in India using DNA barcoding and NMR metabolomics and found that 80% of the products did not contain the species reported to be that of *Saraca asoca*. Several other studies have employed DNA barcoding to detect adulterations, including product substitution, contamination, use of endangered species for medicinal purposes and the use of unlabeled fillers that pose considerable health concerns (Srirama et al. 2010; Wallace et al. 2012; Kool et al. 2012; Newmaster et al. 2013; Jian et al. 2014; Parvathy et al. 2014; Swetha et al. 2014; Santhosh et al. 2015, 2016).

This study attempts to establish DNA barcodes for 30 important medicinal plants that are highly traded in South India. Using these barcodes as reference, the extent of species adulteration in the raw herbal trade samples pertaining to these species was assessed. Species adulteration occurred in nearly 12% of all the market samples examined. These results are discussed in the light of the increasing concern over safety of raw herbal drug. The need for a national regulatory mechanism that can authenticate medicinal plants used in raw trade is discussed that could offer quality assurance to customers (Srirama et al. 2017).

*Authenticated biological reference material (BRM):* Multiple samples of each of the 30 medicinal plant species that are highly traded were collected from different geographic locations in Southern India (Supplementary Table 1S). Fourteen other congeneric plant species that co-occured with some of the 30 medicinal plant species and had a higher likelihood of being used as an adulterant, were also collected. All these plant species ($n = 44$) were authenticated using the morphological characters described in a monographic study (Kaplan 2001) as well as by two taxonomists (Dr. Senthilkumar U, SRM University, Chennai, India and Dr. Srikanth Gunaga, Forestry College, Sirsi, India) independently and each sample was assigned a specific voucher identification number. This constituted the "Biological Reference Material (BRM)" of the medicinal plants. For each species, multiple herbarium sheets were prepared and deposited in the Herbarium of the Ashoka Trust for Research in Ecology and the Environment (ATREE), Bangalore, India.

*Collection of raw herbal trade material:* For each of the 30 species, region-specific vernacular names and trade names were obtained from the literature or from "Ayurvedic Pharmacopoeia of India" and ENVIS FRLHT, Bangalore (http://envis.frlht.org/). Using the identified trade names and vernacular names, raw drug samples of the different species were collected from four Southern states of India viz., Karnataka, Andhra Pradesh, Tamil Nadu and Kerala (Fig. 1). Markets in 34 locations in South India were visited and 203 samples of raw drug trade were purchased (Table 3S).

The trade samples included different plant parts such as fruits, leaf, stem, bark and roots. All the market samples were collected for a single species, and multiple individuals (at least three) were sampled for DNA analysis. Most of the market samples were difficult to identify morphologically as they were in dry form and had not retained the characteristic features of the plant. Each market sample was given a unique sample number so as to ensure a chain of custody protocol. Each market sample also contained details about the date and location of collection, shop name, and collection number. The market samples were also deposited in the herbarium of ATREE, Bangalore.

*DNA barcode of biological reference material (BRM):* Genomic DNA was extracted from leaves of 124 BRM samples representing the 30 medicinal plant species and their 14 allied species using the CTAB protocol (Doyle and Doyle 1987). PCR amplification was conducted using the universal primers, namely complete nr-*ITS* region, ITS1-TCCGTAGGTGAACCTGCGG; ITS4- TCCTCCGCTTATTGATATGC (White et al. 1990) and *psb*A-*trn*H region, *psb*A-GTTATGCATGAACGTAATGCTC; *trn*H-CGCGCATGGTGGATTCACAAATC (Sang et al. 1997) spacer region. PCR amplification was carried out in 25 µL reaction volume which consisted of template concentration of 80 ng/µL, 2.5 µL of 10X Taq buffer(Genie), 1 µL of 2 mM $MgCl_2$, 2.5 µL of 1 mM dNTP's mixture, 5 pM of 1.5 µL each primer, 1.5 U Taq DNA polymerase(Genie) and sterile distilled water. PCR was performed in an Eppendorf Mastercycler Gradient (Hamburg, Germany). The amplification profile was 94 °C for 4 min followed by 30 cycles of 94 °C for 60 s, 55 °C for 45 s, 72 °C for 90 s with a final extension step at 72 °C for 10 min. The amplified products were sequenced in unidirectional at Amnion Biosciences Pvt. Ltd, Bangalore, India. Direct sequencing of the gel-purified amplicon yielded a sequence length between 640 and 750 bp and 380 and 450 bp for nr-*ITS* and *psb*A-*trn*H spacer region, respectively. The obtained sequence results were edited manually using the BioEdit software (Version 5.0.6). The sequences of the nr-*ITS* region and *psb*A-*trn*H spacer region have been deposited at GenBank. The GenBank accession

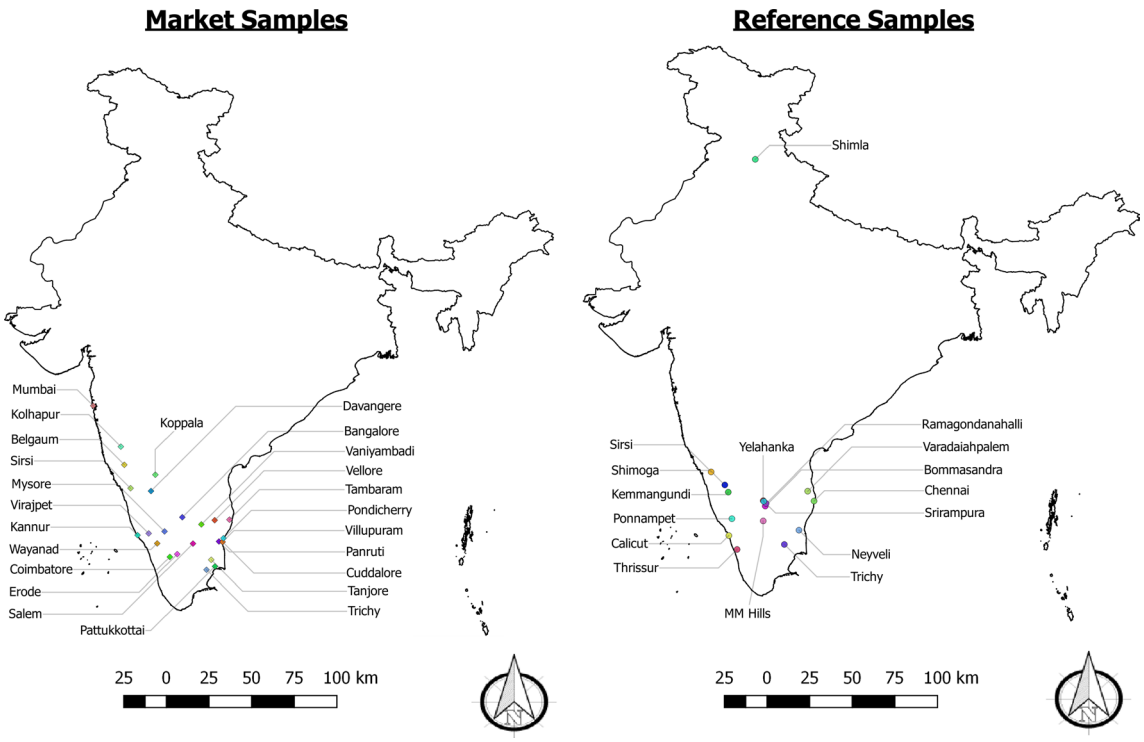


**Fig. 1** Map showing the collection sites of raw drug trade samples in South India

numbers for all the BRM samples are given in Supplementary Table 1S. The obtained BRM sequences were used as a query sequence in BLASTn in GenBank to identify the best matching sequences. Those sequences with the best match in blast search were downloaded as FASTA format from the GenBank and were included in the analysis (Supplementary Table 2S).

*Assessment of adulteration in raw herbal trade market:* To assess the extent of adulteration, if any, the raw herbal drug samples obtained from 34 locations in the South India were processed for determining the identity of the species using the BRM barcode as the reference. The plant material obtained from the shop was either in the form of stem, leaf, root or bark. This material was randomly separated into three parts representing three replicates with each of the part containing roughly one-third of the original material.

The selected samples were ground, using liquid nitrogen and the genomic DNA was extracted from each of these three replicate samples using the Qiagen kit following the manufacturer's instructions (Cat No./ID 69104). Extracted DNA was purified using commercially available kits (Qiagen, Cat No./ID 28604). Genomic DNA was amplified using nr-*ITS* region and *psb*A-*trn*H spacer and sequenced as described above. The sequences obtained from the herbal drug samples and the sequences downloaded from GenBank were analyzed along with the reference barcode library (BRM) as single query sequence (Table 2, Supplementary Table 2S).

Both the barcode regions (nr-*ITS* and *psb*A-*trn*H*)* successfully amplified all the 124 BRM samples comprising the 44 species in the BRM library. All the BRM sequences matched either with the same species or the same genera (Table 2, Supplementary Table 2S). The BRM sequences have been deposited in the GenBank and their accession numbers obtained (Supplementary Table 1S). The amplification and sequencing of the barcode regions for the trade samples were relatively difficult compared to the BRM samples and required multiple attempts to obtain good sequences. The sequences obtained were compared with those obtained from the BRM DNA barcode library.

Of the 203 market samples, 24 pertaining to eight species, namely *Coscinium fenestratum* (Goetgh.) Colebr*, Embelia ribes* Burm.f.*, Boerhavia diffusa* L.*, Mesua ferrea* L.*, Tinospora cordifolia* (Willd.) Miers*, Gloriosa superba* L.*, Morinda citrifolia* L., and *Plumbago zeylanica* L., were found to be adulterated. Over 90% of the market samples of *Coscinium fenestratum,* commonly referred as Daruharidra or Mara manjal were adulterated by *Berberis* spp. (Berberidaceae) (Table 1). Similarly, 75% of the samples claimed to be *Embelia ribes* was actually that of *Embelia tsjeriam-cottam* (Roem. & Schult.) A.DC and *Maesa indica* (Roxb.) A. DC (Primulaceae) (Tables 1, 2).

About 20% of raw drug samples of *Tinospora cordifolia* were adulterated with a closely related species, *Tinospora sinensis* (Lour.) Merr. Both *T. sinensis* and *T. cordifolia*

**Table 1** Percentage of species adulteration in the raw herbal trade of medicinal plants in South India

| Sl. no. | Trade name | Corresponding scientific name | Major source supply | Parts used | Percentage of adulteration |
|---|---|---|---|---|---|
| 1. | Bilva/bael | *Aegle marmelos* (L.) Corrêa | W | Leaf, root, fruit | 0 |
| 2. | Kalmegh/nilavembu | *Andrographis paniculata* (Burm.f.) Nees | C/W | Leaf | 0 |
| 3. | Shatavari | *Asparagus racemosus* Willd. | C/W | Root | 0 |
| 4. | Brahmi/neer-brahmi/vallarai | *Bacopa monnieri* (L.) Wettst./*Centella asiatica* (L.) Urb. | C/W | Leaf, whole plant | 0 |
| 5. | Punarnava/raktapunarva | *Boerhavia diffusa* L. | W | Root, whole plant | 40 |
| 6. | Akhaphool | *Calotropis procera* (Aiton) Dryand. | W | Flowers | 0 |
| 7. | Malkangani/bavanthibeeja/valuzhuvai | *Celastrus paniculatus* Willd. | W | Fruit (Seed) | 0 |
| 8. | Daruharidra/mara manjal | *Coscinium fenestratum* (Goetgh.) Colebr. | W | Stem | 90 |
| 9. | Kali musli/talamuli | *Curculigo orchioides* Gaertn. | W | Roots | 0 |
| 10. | Aaldi, karimanjal/haridra | *Curcuma longa* L. | W/C | Rhizome | 0 |
| 11. | Musta/nagarmotha | *Cyperus rotundus* L. | W | Rhizome | 0 |
| 12. | Bhringraj/bhiranraja | *Eclipta prostrata* (L.) L. | W | Whole pla nt | 0 |
| 13. | Vidanga | *Embelia ribes* Burm.f. | W | Fruit | 75 |
| 14. | Langali/kalihari/kalappakilangu | *Gloriosa superb* L. | W/C | Rhizome | 50 |
| 15. | Gudmaar/sirukurinjan | *Gymnema sylvestre* (Retz.) R.Br. ex Sm. | W | Leaf | 0 |
| 16. | Anatmool/sariwa/sarasaparilla/svetasariva | *Hemidesmus indicus* (L.) R. Br. ex Schult. | W | Root | 0 |
| 17. | Vasa/adusa/adhatoda | *Justicia adhatoda* L. | W/C | Leaf | 0 |
| 18. | Champaka | *Magnolia champaca* (L.) Baill. ex Pierre | W/C | Flower | 0 |
| 19. | Nagakesar/nagakesari/nagkeshar | *Mesua ferrea* L. | W | Flower | 33 |
| 20. | Isapgul | *Plantago ovate* Forssk. | W/C | Seeds | 0 |
| 21. | Citraka/chitrak | *Plumbago zeylanica* L. | W | Bark/stem | 25 |
| 22. | Manjantthi | *Morinda citrifolia* L. | W | Fruit | 100 |
| 23. | Arjuna/arjun/maruthapattai | *Terminalia arjuna* (Roxb. ex DC.) Wight &Arn. | W/C | Bark | 0 |
| 24. | Behda/Bibhitaki/thandrekai | *Terminalia bellirica* (Gaertn.) Roxb. | W/C | Fruit | 0 |
| 25. | Harda/himaj/kadukkai | *Terminalia chebula* Retz. | W/C | Fruit | 0 |
| 26. | Amrithaballi/guduci | *Tinospora cordifolia* (Willd.) Miers | W | Stem | 20 |
| 27. | Vettiver/lavancha | *Chrysopogon zizanioides* (L.) Roberty | W/C | Roots | 0 |
| 28. | Neergundi/nocchi/renuka | *Vitex negundo* L. | W/C | Whole plant | 0 |
| 29. | Asvagandha/ammukira | *Withania somnifera* (L.) Dunal | C/W | Root, whole plant | 0 |
| 30. | Shunti/ardaka/chukka | *Zingiber officinale* Roscoe | C/W | Rhizome | 0 |

*W* WILD, *C* cultivation

share a common vernacular name. Similarly, *Plumbago auriculata* Lam was adulterated with another closely related species, *Plumbago zeylanica*. 33% of the market samples of *Mesua ferrea* contained entirely different species, which did not match any of the BRM sequences (Table 1, Table 2).

*Boerhavia diffusa* commonly traded as Punarnava or Raktapunarva for alleviating disorders related to urinary tract was adulterated with a co-occurring species *Boerhavia erecta* L. *Morinda citrifolia* is one of the highly traded plants widely used in the preparation of health drinks. Analysis of the market samples of *M. citrifolia* revealed that a different species, namely *Moringa oleifera* was traded in its name (Supplementary Table 2S). Similarly, 50% of the tubers of *Gloriosa superba* were adulterated with rhizomes of *Ipomea* spp. (Table 1). The raw drug sales of Brahmi, a product sold for increasing memory, contained both *Bacopa monnieri* (L.) Wettst. and *Centella asiatica* (L.) Urb. Ayurvedic literature acknowledges (Sharma 1987; Kareem 1997) both these species as legitimate substitutes of each other, probably because of their similar properties.

Medicinal plants used in raw herbal trade are often marketed as dry twigs, powder or billets and thus are usually difficult to identify morphologically. Identification of these medicinal plants at the species level is traditionally achieved by careful examination of the specimen's macroscopic and microscopic morphology. However, morphological identification is often not possible when the original plant material has been processed or converted into morphologically

**Table 2** List of sample code, vernacular name, simple BLAST results and final identifications based on the DNA barcoding

| Sl. no. | Trade name | Market samples code | ITS_blast Vs BRM | ITS_blast Vs NCBI | PsbA_blast Vs BRM | PsbA_blast Vs NCBI | Identification by DNA barcoding approach |
|---|---|---|---|---|---|---|---|
| 1 | Bilva/bael | HAS 8, 52, 66, 76, 91, 113, 147, 232, 242, 252, 269 | *Aegle marmelos* | *Aegle marmelos* | *Aegle marmelos* | *Aegle marmelos* | *Aegle marmelos* |
| 2 | Kalmegh/nila-vembu | HAS 41, 157, 201, 494, 520, 571 | *Andrographis paniculata* | *Andrographis paniculata* | *Andrographis paniculata* | *Andrographis paniculata* | *Andrographis paniculata* |
| 3 | Shatavari | HAS 45, 110, 156, 186, 210, 278, 287 | *Asparagus racemosus* | *Asparagus racemosus* | *Asparagus racemosus* | *Asparagus racemosus* | *Asparagus racemosus* |
| 4 | Brahmi/neer-brahmi/vallarai | HAS 36,117, 499, 528, 569 | *Bacopa monnieri* | *Bacopa monnieri* | *Bacopa monnieri* | *Bacopa monnieri* | *Bacopa monnieri* |
| | | HAS 85 | Not matching | *Centella asiatica* | Not matching | *Centella asiatica* | *Centella asiatica* |
| | | HAS 146 | Not matching | *Centella asiatica* | Not matching | *Centella asiatica* | *Centella asiatica* |
| | | HAS 174 | Not matching | *Centella asiatica* | Not matching | *Centella asiatica* | *Centella asiatica* |
| 5 | Punarnava/rakta-punarva | HAS 162 | *Boerhavia diffusa* | *Boerhavia diffusa* | *Boerhavia diffusa* | *Boerhavia diffusa* | *Boerhavia diffusa* |
| | | HAS 205 | Not matching | *Boerhavia erecta* | Not matching | *Boerhavia erecta* | *Boerhavia erecta* |
| | | HAS 501 | *Boerhavia diffusa* | *Boerhavia diffusa* | *Boerhavia diffusa* | *Boerhavia diffusa* | *Boerhavia diffusa* |
| | | HAS 537 | *Boerhavia diffusa* | *Boerhavia diffusa* | *Boerhavia diffusa* | *Boerhavia diffusa* | *Boerhavia diffusa* |
| | | HAS 563 | Not matching | *Boerhavia erecta* | Not matching | *Boerhavia erecta* | *Boerhavia erecta* |
| 6 | Akhaphool | HAS 512, 572 | *Calotropis procera* | *Calotropis procera* | *Calotropis procera* | *Calotropis procera* | *Calotropis procera* |
| 7 | Malkangani/ bavanthibeeja/ valuzhuvai | HAS 517, 522, 556, 587 | *Celastrus paniculatus* | *Celastrus paniculatus* | *Celastrus paniculatus* | *Celastrus paniculatus* | *Celastrus paniculatus* |
| 8 | Daruharidra/mara manjal | HAS 31 | *Coscinium fenestratum* | *Coscinium fenestratum* | *Coscinium fenestratum* | *Coscinium fenestratum* | *Coscinium fenestratum* |
| | | HAS 75 | Not matching | *Berberis asiatica* | Not matching | *Berberis asiatica* | *Berberis asiatica* |
| | | HAS 93 | Not matching | *Berberis gyalaica* | Not matching | *Berberis gyalaica* | *Berberis gyalaica* |
| | | HAS 151 | Not matching | *Berberis asiatica* | Not matching | *Berberis asiatica* | *Berberis asiatica* |
| | | HAS 180 | Not matching | *Berberis gyalaica* | Not matching | *Berberis gyalaica* | *Berberis gyalaica* |
| | | HAS 217 | Not matching | *Berberis asiatica* | Not matching | *Berberis asiatica* | *Berberis asiatica* |
| | | HAS 507 | Not matching | *Berberis asiatica* | Not matching | *Berberis asiatica* | *Berberis asiatica* |
| | | HAS 540 | Not matching | *Berberis minutiflora* | Not matching | *Berberis minutiflora* | *Berberis minutiflora* |
| 9 | Kali musli/tala-muli | HAS 79, 96, 136, 171, 177, 206, 283, 431 | *Curculigo orchioides* | *Curculigo orchioides* | *Curculigo orchioides* | *Curculigo spp* | *Curculigo orchioides* |
| 10 | Aaldi, karimanjal/haridra | HAS 513, 531, 553 | *Curcuma longa* | *Curcuma longa* | *Curcuma longa* | *Curcuma longa* | *Curcuma longa* |
| 11 | Musta/nagarmotha | HAS 33, 84, 94, 114, 150, 183, 202 | *Cyperus rotundus* | *Cyperus rotundus* | *Cyperus rotundus* | *Cyperus rotundus* | *Cyperus rotundus* |
| 12 | Bhringraj/bhiranraja | HAS 122, 160, 503, 524 | *Eclipta prostrata* | *Eclipta prostrata* | *Eclipta prostrata* | *Eclipta prostrata* | *Eclipta prostrata* |
| 13 | Vidanga | HAS 34, 555, 580 | *Embelia tsjeriam-cottam* | *Embelia tsjeriam-cottam* | *Embelia tsjeriam-cottam* | *Embelia tsjeriam-cottam* | *Embelia tsjeriam-cottam* |
| | | HAS 45, 542 | *Embelia ribes* | *Embelia ribes* | *Embelia ribes* | *Embelia ribes* | *Embelia ribes* |
| | | HAS 111, 224, 543 | *Maesa indica* | *Maesa indica* | *Maesa indica* | *Maesa indica* | *Maesa indica* |

**Table 2** (continued)

| Sl. no. | Trade name | Market samples code | ITS_blast Vs BRM | ITS_blast Vs NCBI | PsbA_blast Vs BRM | PsbA_blast Vs NCBI | Identification by DNA barcoding approach |
|---|---|---|---|---|---|---|---|
| 14 | Langali/kalihari/kalappakilangu | HAS 144 | *Gloriosa superba* | *Gloriosa superba* | *Gloriosa superba* | *Gloriosa superba* | *Gloriosa superba* |
| | | HAS 547 | *Not matching* | *Ipomoea spp* | *Not matching* | *Ipomoea spp* | *Ipomoea spp* |
| 15 | Gudmaar/sirukurinjan | HAS 504, 541, 567, 591 | *Gymnema sylvestre* | *Gymnema sylvestre* | *Gymnema sylvestre* | *Gymnema sylvestre* | *Gymnema sylvestre* |
| 16 | Anatmool/sariwa/sarasaparilla/svetasariva | HAS 19, 46, 97, 143, 169, 190, 208, 581 | *Hemidesmus indicus* | *Hemidesmus indicus* | *Hemidesmus indicus* | *Hemidesmus indicus* | *Hemidesmus indicus* |
| 17 | Vasa/adusa/adhatoda | HAS 22, 71, 159, 502, 527, 554 | *Justicia adhatoda* | *Justicia adhatoda* | *Justicia adhatoda* | *Justicia adhatoda* | *Justicia adhatoda* |
| 18 | Champaka | HAS 534, 565 | *Magnolia champaca* | *Magnolia champaca* | *Magnolia champaca* | *Magnolia champaca* | *Magnolia champaca* |
| 19 | Nagakesar/nagakesari/nagkeshar | HAS 104, 165, 270, 526 | *Mesua ferrea* | *Mesua ferrea* | *Mesua ferrea* | *Mesua ferrea* | *Mesua ferrea* |
| | | HAS 185 | *Not matching* | *Calophyllum inophyllum* | *Not matching* | *Calophyllum inophyllum* | *Calophyllum inophyllum* |
| | | HAS 284 | *Not matching* | *Calophyllum inophyllum* | *Not matching* | *Calophyllum inophyllum* | *Calophyllum inophyllum* |
| 20 | Isapgul | HAS 78, 101, 106, 267, 507, 592, 559 | * | *Plantago ovata* | * | *Plantago ovata* | *Plantago ovata* |
| 21 | Citraka/chitrak | HAS 35, 109, 176, 495, 538, 562 | *Plumbago zeylanica* | *Plumbago zeylanica* | *Plumbago zeylanica* | *Plumbago zeylanica* | *Plumbago zeylanica* |
| | | HAS 218, 590 | *Plumbago auriculata* | *Plumbago auriculata* | *Plumbago auriculata* | *Plumbago auriculata* | *Plumbago auriculata* |
| 22 | Manjantthi | HAS 529 | *Not matching* | *Moringa oleifera* | *Not matching* | *Moringa oleifera* | *Moringa oleifera* |
| 23 | Arjuna/arjun/maruthapattai | HAS 25, 83, 148, 164, 178, 213, 263, 294, 309, 320, 327 | *Terminalia arjuna* | *Terminalia arjuna* | *Terminalia arjuna* | *Terminalia arjuna* | *Terminalia arjuna* |
| 24 | Behda/bibhitaki/thandrekai | HAS 98, 141, 167, 197, 218, 285, 296, 312, 322, 325 | *Terminalia bellirica* | *Terminalia bellirica* | *Terminalia bellirica* | *Terminalia bellirica* | *Terminalia bellirica* |
| 25 | Harda/himaj/kadukkai | HAS 27, 81, 103, 138, 166, 193, 214, 266, 282, 297, 307, 321, 324 | *Terminalia chebula* | *Terminalia chebula* | *Terminalia chebula* | *Terminalia chebula* | *Terminalia chebula* |

indistinguishable form (Sucher and Carles 2008; Smillie and Khan 2009). Each identification method uses different techniques and requires different levels of prior information, infrastructure, and skill sets to achieve proper authentication of a botanical product. Under such circumstances, other, more in-depth techniques can be applied to assist in the identification of botanical samples (Smillie and Khan 2009).

In recent years, DNA barcoding techniques have emerged as a quick and alternative tool to identify species adulteration in the raw herbal trade (Newmaster et al. 2013; Seethapathy et al. 2014; Santhosh et al. 2015; Han et al. 2016). This technique is independent of the type of tissue collected and also the geographical source of the material. Unlike animal systems, where the mitochondrial *COI* is regarded as the universal bar code, in plants there is as yet no consensus on a universal barcode. A number of authors have shown that the chloroplastic gene regions such as *psb*A-*trn*H and nuclear region such as nr-*ITS* have been widely used in raw herbal drug authentication (Palhares et al. 2015; Seethapathy et al. 2014). Chen et al. (2010) has suggested *ITS*2 region as an important region for barcoding the medicinal plants. Similarly, *psb*A-*trn*H has also been one of the most preferred candidate region for species identification (Shaw et al. 2005; Kress et al. 2005, Srirama et al. 2010). In fact, the

**Table 2** (continued)

| Sl. no. | Trade name | Market samples code | ITS_blast Vs BRM | ITS_blast Vs NCBI | PsbA_blast Vs BRM | PsbA_blast Vs NCBI | Identification by DNA barcoding approach |
|---|---|---|---|---|---|---|---|
| 26 | Amrithaballi/guduci | HAS 24 | *Not matching* | *Tinospora sinensis* | *Not matching* | *Tinospora sinensis* | *Tinospora sinensis* |
| | | HAS 115, 279, 308, 319, 360, | *Tinospora cordifolia* | *Tinospora cordifolia* | *Tinospora cordifolia* | *Tinospora cordifolia* | *Tinospora cordifolia* |
| 27 | Vettiver/lavancha | HAS 17, 46, 69, 124,129, 155 | *Chrysopogon zizanioides* | *Chrysopogon zizanioides* | *Chrysopogon zizanioides* | *Chrysopogon zizanioides* | *Chrysopogon zizanioides* |
| 28 | Neergundi/nocchi/renuka | HAS 18, 72, 99, 505, 523 | *Vitex negundo* | *Vitex negundo* | *Vitex negundo* | *Vitex negundo* | *Vitex negundo* |
| 29 | Asvagandha/ammukira | HAS 39, 42, 82, 118, 265, 496, 530, 557 | *Withania somnifera* | *Withania somnifera* | *Withania somnifera* | *Withania somnifera* | *Withania somnifera* |
| 30 | Shunti/ardaka/chukka | HAS 38, 514, 546, 561 | *Zingiber officinale* | *Zingiber officinale* | *Zingiber officinale* | *Zingiber officinale* | *Zingiber officinale* |

*BRM* biological reference material

*BRM Vouchers not prepared

discriminatory power of *psb*A-*trn*H has been shown to be the highest (69%) among many other chloroplastic regions (Pennisi 2007).

In this study, a multi-locus approach of two barcode regions, namely nr-*ITS* and *psb*A-*trn*H was used to establish the DNA barcode signatures of 30 highly traded medicinal plants in South India. A common problem encountered while barcoding using nr-*ITS* was contamination with fungal flora, that were probably present in the raw drug samples. However, this was overcome by either scraping the outer tissues using a fine razor blade and or cleansing the outer surface using ethanol and/or by repeated extraction and amplification.

Using the DNA barcodes of the 30 species as a reference, the study showed that approximately 12% of the 203 raw herbal market samples were substituted. For example, the climber, *Embelia ribes* regarded for its medicinally active compound embelin, was highly prone to adulteration (over 75%) with *Embelia tsjeriam-cottam* and *Maesa indica*. Due to same vernacular name, Guduci (Ved and Goraya 2007), *Tinospora cordifolia*, an important immuno-modulatory plant was found to be adulterated by *Tinospora sinensis.* Overt morphological similarity of the rhizome and tubers, may have similarly lead to adulteration of *Gloriosa superba* tubers by rhizomes of *Ipomea* spp.

In summary, the BRM of the 30 medicinal plant species established in the study along with their respective DNA barcodes could be used to effectively identify the raw herbal trade material pertaining to these species. With decreasing cost of sequencing, DNA barcoding is rapidly becoming an important tool for medicinal plant identification. It could be used to rapidly evaluate samples from leaves, seeds, flowers, dry materials, museum specimens, powders or other products from which DNA can be obtained. The study reaffirms the belief that raw herbal trade in countries such as India may be plagued with issues of species adulteration. While the consequences of such adulteration on health and safety of consumers is only now beginning to be understood (Seethapathy et al. 2014; Santhosh et al. 2015), it is important to regulate the quality of raw herbal medicines. Efforts to integrate the use of such DNA barcoding tools to identify species adulterants can lead to ensuring quality of raw herbal products. A robust national herbal trade authentication system should be put in place such that both domestic and export markets are ensured of quality and safety of raw herbal trade material.

Adulteration in herbal products could have serious health implications, which could lead to lowering consumer confidence and ultimately to reducing the trade value of the herbal products. In this regard, there is an urgent need to develop an Herbal Authentication System (HAS), which could serve as a regulator for ensuring quality of herbal trade. A reliable discrimination and identification of species is critical especially for highly traded medicinal plant species. DNA barcoding enables easy species identification, even from small amounts of plant tissue. It's cost effectiveness and simplicity could be potentially used for authenticating raw herbal drugs and thus restoring consumer confidence in herbal products.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interests.

## References


Chen S, Yao H, Han J, Liu C, Song J, Shi L, Zhu Y, Ma X, Gao T, Pang X, Luo K (2010) Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. PLoS One 5:e8613

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Han J, Pang X, Liao B, Yao H, Song J, Chen S (2016) An authenticity survey of herbal medicines from markets in China using DNA barcoding. Sci Rep 6:18723

Jian C, Deyi Q, Qiaoyun Y, Jia H, Dexing L, Xiaoya W, Leiqing Z (2014) A successful case of DNA barcoding used in an international trade dispute. DNA Barcodes 2:21–28

Kaplan DR (2001) The science of plant morphology: definition, history, and role in modern biology. Am J Bot 88(10):1711–1741

Kareem AM (1997) Plants in ayurveda (A compendium of botanical and Sanskrit names). Foundation for Revitalisation of Local Health Tradition, Bangalore

Kool A, de Boer HJ, Krüger Å, Rydberg A, Abbad A, Björk L, Martin G (2012) Molecular identification of commercialized medicinal plants in Southern Morocco. PLoS One 7:e39459

Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH (2005) Use of DNA barcodes to identify flowering plants. PNAS USA 102:8369–8374

Newmaster SG, Grguric M, Shanmughanandhan D, Ramalingam S, Ragupathy S (2013) DNA barcoding detects contamination and substitution in North American herbal products. BMC Med 11:222

Palhares RM, Drummond MG, Brasil BD, Cosenza GP, Brandão MD, Oliveira G (2015) Medicinal plants recommended by the world health organization: DNA barcode identification associated with chemical analyses guarantees their quality. PLoS ONE 10(5):e0127866

Parvathy VA, Swetha VP, Sheeja TE, Leela NK, Chempakam B, Sasikumar B (2014) DNA barcoding to detect chilli adulteration in traded black pepper powder. Food Biotechnol 28:25–40

Pennisi E (2007) Wanted: a barcode for plants. Science 318:190–191

Sang T, Crawford DJ, Stuessy TF (1997) Chloroplast DNA phylogeny, reticulate evolution and biogeography of *Paeonia* (Paeoniaceae). Am J Bot 84:1120–1136

Santhosh Kumar JU, Krishna V, Seethapathy GS, Senthilkumar U, Ragupathy S, Ganeshaiah KN, Ravikanth G, Uma Shaanker R (2015) DNA barcoding to assess species adulteration in raw drug trade of "Bala" (genus: *Sida* L.) herbal products in South India. Biochem Sys Ecol 61:501–509

Santhosh Kumar JU, Gogna Navdeep, Newmaster Steven G, Krishna V, Ragupathy S, Seethapathy GS, Ravikanth G, Dorai Kavita, Uma Shaanker R (2016) DNA barcoding and NMR spectroscopy based assessment of species adulteration in the raw herbal trade of *Saraca asoca* (Roxb.) Willd, an important medicinal plant. Int J Legal Med 130:1457–1470

Seethapathy GS, Ganesh D, Santhosh Kumar JU, Senthilkumar U, Newmaster SG, Ragupathy S, Uma Shaanker R, Ravikanth G (2014) Assessing product adulteration in natural health products for laxative yielding plants, *Cassia, Senna,* and *Chamaecrista,* in Southern India using DNA barcoding. Int J Legal Med 129:693–700

Sharma PV (1987) Dravyaguna-vijnana, vol 2. Chaukhambha Bharati Academy, Varanasi **(in Hindi)**

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu WS, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL (2005) The tortoise and the hare. II. Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Am J Bot 92:142–166

Smillie TJ, Khan IA (2009) A comprehensive approach to identifying and authenticating botanical products. Clin Pharmacol Ther 87:175–186

Srirama R, Senthilkumar U, Sreejayan N, Ravikanth G, Gurumurthy BR, Shivanna MB, Uma Shaanker R (2010) Assessing species admixtures in raw drug trade of Phyllanthus, a hepato-protective plant using molecular tools. J Ethnopharmacol 130:208–215

Srirama R, Santhosh Kumar JU, Seethapathy GS, Newmaster SG, Ragupathy S, Ganeshaiah KN, Shaanker RU, Ravikanth G (2017) Species adulteration in the herbal trade: causes, consequences and mitigation. Drug Saf 40:1–11

Sucher NJ, Carles MC (2008) Genome-based approaches to the authentication of medicinal plants. Planta Med 74:603–623

Swetha VP, Parvathy VA, Sheeja TE, Sasikumar B (2014) DNA barcoding for discriminating the economically important *Cinnamomum verum* from its adulterants. Food Biotechnol 28:183–194

Valiathan MS (2006) Ayurveda: putting the house in order. Curr Sci 90:1

Ved DK, Goraya GS (2007) Demand and supply of medicinal plants in India. NMPB, New Delhi, p 18

Wallace LJ, Boilard SM, Eagle SH, Spall JL, Shokralla S, Hajibabaei M (2012) DNA barcodes for everyday life: routine authentication of natural health products. Food Res Int 49:446–452

White TJ, Bruns T, Lee SJWT, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc 18:315–322