Physiol Mol Biol Plants (March 2021) 27(3):605–617
https://doi.org/10.1007/s12298-021-00965-9

RESEARCH ARTICLE

The efficacy of machine learning algorithm for raw drug authentication in *Coscinium fenestratum* (Gaertn.) Colebr. employing a DNA barcode database

Remya Unnikrishnan[1,4] · M. Sumod[2] · R. Jayaraj[3] · P. Sujanapal[2] · Suma Arun Dev[1]

Received: 3 January 2021 / Revised: 19 February 2021 / Accepted: 2 March 2021 / Published online: 15 March 2021

Abstract Medicinal plants are a valuable resource for traditional as well as modern medicine. Consequently huge demand has exerted a heavy strain on the existing natural resources. Due to over exploitation and unscientific collection most of the commercially traded ayurvedic plants are in the phase of depletion. Adulteration of expensive raw drugs with inferior taxa has become a common practice to meet the annual demand of the ayurvedic industry. Although there are several recommended methods for proper identification varying from the traditional taxonomic to organoleptic and physiochemical, it is difficult to authenticate ayurvedic raw drugs available in extremely dried, powdered or shredded forms. In this regard, the study addresses proper authentication and illicit trade in *Coscinium fenestratum* (Gaertn.) Colebr. using CBOL recommended standard barcode regions viz. nuclear ribosomal–Internally Transcribed Spacer (nrDNA- *ITS*), maturase K (*matK),* ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*), and *psbA-trnH* spacer regions. Further, an integrated analytical approach employing Maximum Likelihood phylogenetic tree and Machine Learning Approach, Waikato Environment for Knowledge Analysis was employed to prove efficacy of the method. The automated species identification technique, Artificial Intelligence uses the ability of computers to build models that can receive the input data and then conduct statistical analyses which significantly reduces the human labour. Concurrently, scientific management, restoration, cultivation and conservation measures should be given utmost priority to reduce the depletion of wild resources as well as to meet the rapidly increasing demand of the herbal industries.

Keywords Machine learning algorithm · Artificial intelligence · DNA barcoding · Threatened species · Medicinal plants

Introduction

Coscinium fenestratum (Gaertn.) Colebr. a dioecious woody climber of Menispermaceae family is one of the commercially traded medicinal plant species in tropical forests of India, endemic to Kerala, Tamil Nadu and Karnataka (Ved et al. 2015). This liana has been listed as threatened by International Union for Conservation of Nature (IUCN) and Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Sarvalingam and Rajendran 2016). In India, distribution of the species is restricted to the Western Ghats, mostly in wet evergreen, moist evergreen, semi evergreen and semi deciduous forests within an altitudinal range of 500–750 mean sea level (msl) (Mohanan and Sivadasan 2002). The stems and roots of the plants are extensively used in diverse systems of medicine, such as Ayurveda, Folk, Tibetan, Siddha, and Thai because of the presence of major alkaloid, berberine (Tran et al. 2003; Thriveni et al. 2015). *C.*

✉ Suma Arun Dev
sumadev@kfri.res.in

1 Forest Genetics and Biotechnology Division, Kerala Forest Research Institute, Peechi, Thrissur, Kerala 680653, India

2 Sustainable Forest Management Division, Kerala Forest Research Institute, Peechi, Thrissur, Kerala 680653, India

3 Forest Ecology and Biodiversity Division, Kerala Forest Research Institute, Peechi, Thrissur, Kerala 680653, India

4 Cochin University of Science and Technology, Kochi, Kerala, India

fenestratum is the major ingredient of 62 Ayurvedic formulations and credited with anti-diabetic, anti-inflammation, anti-proliferative, anti-hepatotoxic, anti-plasmodial, CNS depressant and analgesic properties (Ueda et al. 2002; Tran et al. 2003; Punitha et al. 2005; Udayan et al. 2005; Rai et al. 2012). Recent studies have shown that the plant is also exploited as a source of berberine which acts effectively against chronic respiratory inflammatory diseases and multiple myeloma (Chunming et al. 2020; Tew et al. 2020). An average annual collection of 2000 tons of dried stem to meet industrial demand has negatively affected survival of the species (Thriveni et al. 2015). Further, removal of this liana from wild even before reaching its reproductive maturity of fifteen years is another setback affecting long term survival of the species. Unscientific collection along with intrinsic properties of the liana such as habitat specificity, dioecious nature, poor seed set and lack of regeneration had labelled the species as critically endangered (Ramasubbu et al. 2012; Thriveni et al. 2015). Consequently, an export ban of the species was introduced by the Ministry of Commerce, Government of India, accentuating the demand (vide notification No. 47 (PN)/92–97 dated 30 March 1994). This further indicates that, the current demand for *C. fenestratum* has been met probably by wide spread adulteration/substitution of the species with other plant materials (Tushar et al. 2008).

Recently, raw drug adulteration has become a burning problem in herbal industries wherein the quality of formulations is compromised with look-alike plants of inferior properties (Srirama et al. 2017). Consequences of herbal drug adulteration were reported from the countries like Australia, Japan, Taiwan and China, where chronic use of *Artistolochia fangchi* adulterated products led to death of patients due to renal failure (Michl et al. 2013; Jadot et al. 2017). Earlier, US Food and Drug Administration also banned *Piper methysticum* containing products in Germany, Switzerland, France, Canada and UK owing to health issues related to hepatitis, cirrhosis and liver failure (U.S. Food and Drug Administration 2002). Adulteration and contamination of herbal medicinal products resulted in agranulocytosis, meningitis, multi-organ failure, perinatal stroke, arsenic, lead or mercury poisoning, malignancies or carcinomas, hepatic encephalopathy, hepato-renal syndrome, nephrotoxicity, rhabdomyolysis, metabolic acidosis, renal or liver failure, cerebral edema, coma, intracerebral haemorrhage, and even death (Posadzki et al. 2012). In this regard, WHO pharmacopoeia highlights the importance of ensuring the quality of raw drugs through proper identification of recommended species for the purpose (WHO 2011). Accordingly, Indian Pharmacopoeia recommends methods such as traditional taxonomic to organoleptic and physiochemical to authenticate raw drugs in the ayurvedic formulations that are manufactured, distributed and sold by licensed manufacturers in India. Traditional plant identification such as microscopic and macroscopic methods are cost effective and capable of dealing with the multi-herbal formulations and impurities; however, it has low efficiency in highly processed samples (Upton et al. 2019; Ichim et al. 2020). Chemical methods also had wide applicability in the quality assurance of herbal medicines, nevertheless, studies have reported the discrepancy of chemical markers in delineating medicinal plants, due to variation with respect to age and environmental heterogeneity of plant species (Liu et al. 2011; Kaur et al. 2016; Moustafa et al. 2016; Cao et al. 2017). A stable system for checking the authenticity of herbal drugs and safety of consumers are thus envisaged for ensuring safe usage of herbal dugs (Mishra et al. 2016). Many researchers have diverted their focus towards the multi-tiered or integrated approach, by amalgamating already existing methods which are yet to be fine-tuned with molecular tools (Palhares et al. 2014,2015; Urumarudappa et al. 2016; Seethapathy et al. 2018).

Recently, DNA barcode based plant identification has been considered as a powerful and potential method in herbal pharmacovigilance research for quality assurance along with hyphenated methods (De Boer et al. 2015; Palhares et al. 2015; Urumarudappa et al. 2016; Seethapathy et al. 2018; Ichim et al. 2019). DNA barcode analysis has revealed wide-range of discrepancies in the claimed composition of various North American herbal products viz. in saw palmetto dietary supplements, ginkgo products, black cohosh, herbal teas and in ginseng (Stoeckle et al. 2011; Baker et al. 2012; Wallace 2012; Little and Jeanson et al. 2013; Newmaster et al. 2013; Ichim and de boer 2020). Though DNA barcoding can be used specifically for unprocessed plant materials, it has practical limitations to authenticate highly processed multi-herbal formulations (Raclariu et al. 2017b). The post harvesting process or manufacturing of herbal materials affects the quality of DNA which further affects DNA barcoding as well as meta barcoding (de Boer et al. 2015).

The present study addresses the extent of adulteration and illicit trade of *C. fenestratum* in south India using integrated approach of DNA barcoding and HPTLC (High Performance Thin Layer Chromatography) fingerprinting. The study also analyses the efficiency of approaches like Maximum Likelihood phylogenetic tree and Machine Learning Algorithm (WEKA) for molecular sequence data and hierarchical clustering for chemical fingerprints to ascertain the extent of adulteration. This is for the first time MLA is used in herbal drug authentication.

Materials and methods

Collection of authenticated biological reference material

The common market adulterants of *C. fenestratum* were identified through a preliminary market and industrial survey in south India as part of a previous research project. Accordingly, four species with similar features (woody stem with berberine) were selected. Mature stem, leaf and flower samples of *C. fenestratum* and its market adulterants viz. *Anamirta cocculus* (L.) Wight & Arn., *Diploclisia glaucescens* (Blume) Diels, *Morinda pubescens* Sm. were collected from different geographic locations of south India and *Berberis aristata* DC. from north India (Fig. 1). GPS coordinates of the collected locations were also recorded (Supplementary Table S1). Taxonomically confirmed samples at the Institute were then assigned a specific voucher number and deposited in the herbarium at KFRI, known by the acronym KFRI by Index Herbariorum (Taxon 37: 503. 1988). This taxonomically authenticated Biological Reference Materials (BRM) were used to develop species specific barcodes for four of the standard barcode gene regions (*rbc*L, *mat*K, ITS and *psb*A-*trn*H). The generated DNA barcode database was further analysed for validating the authenticity of market raw drugs.

Collection of commercially traded samples

Commercially traded samples were purchased from the selected authorised dealers of ayurvedic raw drugs and major ayurvedic industries in south India (Kerala and Tamil Nadu). Thirty raw drug samples of *C. fenestratum* sold under common vernacular name maramanjal/daruharidra were purchased from Kerala and Tamil Nadu. About 100 g of raw drug sample (available in extremely dried and shredded form) was collected from each shop to check adulteration. Each of the collected samples was given a Herbal Authentication Service Code (HAS) with details of location (Supplementary Table S2). To avoid the chances of mixing up, strict protocol was followed from collection to final data analysis. Most of collected samples had not retained any of the morphological features of the original plant.

Genomic DNA extraction, PCR amplification and Sequencing

Total genomic DNA was extracted from BRM leaf samples (n = 25 with 5 individuals for each species) using Qiagen Plant DNeasy Mini Kit (Qiagen Inc., USA). 100 mg tissue from silica gel dried leaf was used for genomic DNA extraction. DNA was quantified using Nanodrop photometer programmed through CFR 21 software (Implen, Germany), and quality was analyzed by agarose gel electrophoresis. Total genomic DNA was extracted from commercially traded samples using Qiagen Plant DNeasy Mini Kit (Qiagen Inc., USA) with slight modifications (samples were finely powdered using blender followed by 3-h incubation at 65° C). Working concentration of genomic DNA was prepared by diluting the stock solution at a concentration 25 ng/µL. PCR reaction mixture comprised of 2.5 µL PCR buffer at 1X (supplied with10X concentration), 1 µL each of forward and reverse primers (5 pmol), 2.5 µL of dNTPs from 10 mM stock, 2 U/25 µL of Taq-polymerase, 1 µL template DNA with the concentration of 25 ng/µL, and the final volume of the PCR reaction mixture was made up to 25 µL with sterile distilled water. Total genomic DNA of BRM was used for the amplification of CBOL recommended barcode regions viz. *ITS*, *rbcL, psbA-trnH* and *matK* (Table 1). The eluted PCR products were subjected to Sanger's dideoxy sequencing using Applied Biosystems DNA Sequencer (ABI) and ABI DNA Sequencing Analysis Software v5.1 (Perkin-Elmer, made in USA).

HPTLC

CAMAG Linomat 5 with twin plate chamber and CAMAG TLC scanner instrument programmed through Win CATS software (Merck, made in Germany) was used for HPTLC fingerprinting. Matured stem of BRM (n = 10, with 2 individuals in each species) was powdered coarsely. Ten gram powdered samples from each stem was accurately weighed (10 g) and extracted using 300 mL methanol. Extracts were filtered and concentrated under reduced pressure and made up to 10 mL in standard flasks separately. Stationary phase of aluminium TLC plates precoated with Silica gel 60 F_{254} of 0.2 mm thickness and mobile phase of toluene: ethylaceate: acetic acid (5:1:0.6 v/v) were used for the analysis. Samples were visualised in 366 nm and 254 nm (Supplementary Figs. 1 and 2). The plate was derivatized with anisaldehyde sulphuric acid reagent at 366 nm for band visualization of phenolic components in the plant parts used.

HPTLC banding profile of BRM was documented under 366 nm UV light. Chemical profile of each sample was analysed according to their RF values (Retention factor). Dendrogram was constructed by SPSS v.16.0 (SPSS Inc 2007) using nearest neighbour adopting euclidean distance, which revealed the relation between each species according to their phytochemical constituents. Owing to variations in HPTLC fingerprints between geographical accessions of a species, the technique cannot be further considered for market sample validation.

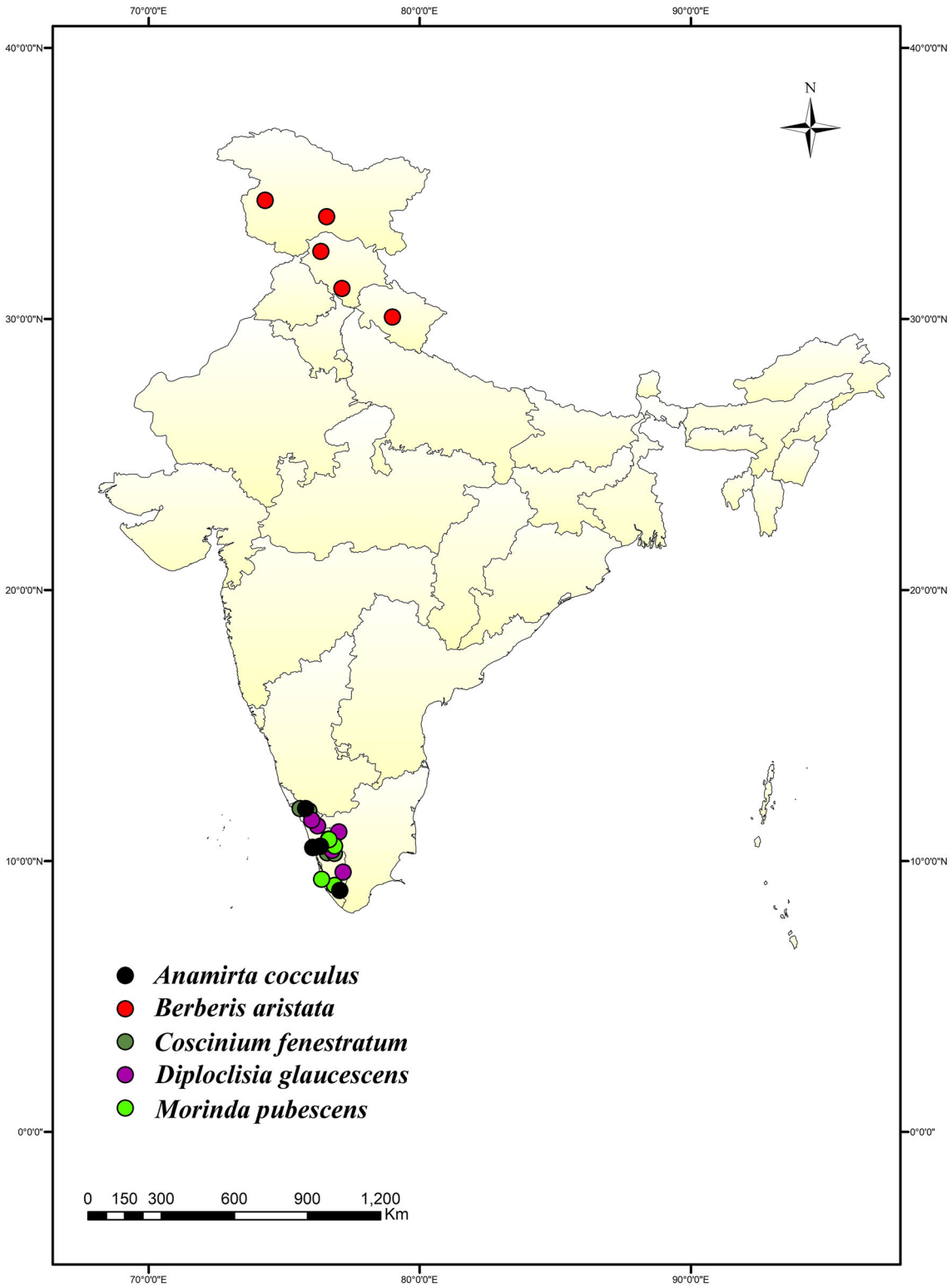

Fig. 1 Map showing the Biological Reference Material (BRM) collected from multiple locations

Table 1 Details on primers and PCR reaction conditions

Barcode loci	Primer name	Sequence 5′-3′	PCR profile (35 Cycles)
ITS	*ITS 1*	TCCGTAGGTGAACCTGCGG	95 °C 5 min, 94 °C 1 min, 60 °C 1 min, 72° 1 min, 72° C 10 min
	ITS 2	TCCTCCGCTTATTGATATGC	
psbA-trnH	*psbA*	GTWATGCAYGAACGTAATGCTC	95 °C 5 min, 94 °C 1 min, 58 °C 1 min, 72° 1 min, 72° C 10 min
	trnH	CGCGCATGGTGGATTCACAATCC	
matK	*matk* 427 F	CCCRTYCATCTGGAAATCTTGGTT	95 °C 5 min, 94 °C 1 min, 50 °C 1 min, 72° 1 min, 72° C 10 min
	matK 1248 R	GCTRTRATAATGAGAAAGATTTCTGC	
rbcL	*rbcL* 1 F	ATGTCACCACAAACAGAAAC	95 °C 5 min, 94 °C 1 min, 60 °C 1 min, 72° 1 min, 72° C 10 min
	rbcL 724 R	TCGCATGTACCTGCAGTAGC	

DNA barcode development from BRM samples

Raw chromatograms were edited and trimmed using BioEdit software (Hall 1999). Edited sequences were aligned using Clustal W (Thompson et al. 1994) and submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank/) (Supplementary Table S3). Homology searches of all the sequences obtained from BRM barcode library were performed using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), to confirm the identity of sequences. For pair-wise genetic distance (PWG) method, genetic distances of BRM samples (interspecific as well as intraspecific distances) were determined by MEGA v.7.0 using Kimura two-parameter distance model (K2P) adopting complete deletion (Tamura et al. 2013). The interspecific divergence among species was calculated using three parameters; (1) average interspecific distance, (2) average theta prime and (3) minimum interspecific distances. Intraspecific parameters; (4) average intraspecific distance, (5) theta (θ) and (6) coalescent depth were also calculated to characterize intraspecific divergences (Meyer and Paulay 2005). DNA barcoding gaps were calculated by comparing intra and interspecific genetic distances (Kress and Erickson 2007). An ideal barcode region with higher interspecific but low intraspecific divergence was further used to differentiate the market samples.

Data analysis and validation of commercial sample

Total genomic DNA from 30 commercially traded samples was initially amplified with CBOL recommended barcode regions viz. *ITS*, *rbcL*, *psbA-trnH* and *matK*. The genomic DNA extracted from extremely dried samples was slightly degraded and three barcode regions viz. *ITS*, *psbA-trnH* and *matK* failed to give amplification consistently. *rbcL* barcode region successfully amplified even from degraded specimens, was thus considered for the validation analysis. The eluted PCR products were subjected to Sanger's dideoxy sequencing and sequences obtained from traded samples were analysed along with the BRM barcode reference database. The BLAST analysis was performed with *rbcL* sequences as queries to identify similar nucleotide reference sequences in the GenBank database with highest homology, maximum query coverage, and maximum score to assign the identity of the species. Maximum Likelihood tree was constructed using developed barcode sequences of commercially traded samples and BRM, with 1000 bootstrap using MEGA v.7. 0 adopting Kimura 2 model with partial deletion (Kumar et al. 2016).

Machine learning approach (MLA)

In MLA, DNA barcoding analysis can be performed with a reference data set composed of DNA sequence of known species (BRM) and query data set with the sequence of unknown species (market samples). In the adopted algorithm namely, Waikato Environment for Knowledge Analysis (WEKA), the function-based method Support Vector Machines (SMO) (Suykens and Vandewalle 1999), the rule-based RIPPER (Jrip) (Shahzad et al. 2013), the decision tree C4.5 (J48) (Quinlan 1996) and the Bayesian-based method Naive Bayes (Lewis 1998) were tested on DNA barcodes with tenfold cross validation. The ".fasta" files of barcode sequences were converted to ".arff" format using "Fasta2Weka" programme for analysis in WEKA (Weitschek et al. 2014). All four classification methods in WEKA were run with four barcode primer sequences of BRM. Best classifier was selected according to their efficiency in species discrimination. Using the best classifier, *rbc*L sequences from commercially traded market samples were further analysed along with BRM sequence database.

Result

DNA barcode analysis

All the analysed barcode regions (*ITS, psb*A-*trn*H, *mat*K and *rbc*L) amplified successfully with 100 percent PCR efficiency. Among these barcode regions, *psbA-trnH* spacer

Table 2 Sequence characteristics of four barcode regions and their combinations

Comparison	*rbcL*	*matK*	*psbA-trnH*	*ITS*
Sequence length	590	780	648	423
Conserved sites	378	580	188	257
Variable site	212	200	460	166
Informative site	375	287	455	166
Singleton site	3	0	15	0

region showed highest nucleotide variation (460/648), followed by *rbcL* (212/590), *matK* (200/780) and *ITS* (166/423) regions, respectively (Table 3). Intra and Inter specific genetic divergences of these sequences analysed from the four barcode regions showed only interspecific divergences and none of them displayed any intraspecific divergences (Supplementary Fig. 3). Among these four barcode regions, *psbA-trnH* and *rbcL* showed highest interspecific divergence in the Biological Reference Materials (Table 4). Based on intra and inter specific distances, barcode gap was also estimated. The barcode regions viz. *ITS, psb*A-*trn*H, *mat*K and *rbc*L showed distinct barcode gap of 0.0276, 0.175, 0.0234 and 0.0632 respectively. Wilcoxon's signed rank test performed to test the significance of interspecific divergences in barcode regions (*psb*A-*trn*H, *mat*K, *rbc*L, and *ITS*) showed significant values. Based on barcode gap analysis, *psbA-trnH* and *rbcL* gene regions can be considered as effective barcodes (Supplementary Fig. 4). Further, *rbc*L barcode region alone was adopted for authentication of commercially traded samples successfully owing to difficulty in amplification of other barcode regions from degraded DNA. The barcode sequences developed from these samples were clustered into separate clades corresponding to the respective sequences of BRM samples. The Maximum Likelihood tree with 1000 bootstraps using MEGA v.7. 0 adopting Kimura 2 model with partial deletion generated showed a clear clustering of thirty commercially traded samples with those of BRM (Fig. 2). Thus, among the thirty samples, twenty samples could be identified as *B. aristata* and remaining ten samples were authenticated as *C. fenestratum*.

Table 4 Species identification rates as percentage (correctly identified/misidentified/not identified) for the BRM using Machine Learning Algorithm (WEKA)

Barcode loci	Naïve Bayes	J48	JRip	SMO
WEKA classifiers				
rbcL	50/50/0	100/0/0	91/9/0	100/0/0
matK	77/23/0	100/0/0	90/10/0	100/0/0
ITS	44/56/0	100/0/0	72/28/0	100/0/0
psbA-trnH	77/23/0	94/6/0	55/45/0	100/0/0

Similar results were also generated in the MLA analysis. All four classification methods were run in WEKA with ten-fold cross validation (Table 5). Among four machine learning algorithms, Naive bayes and JRip failed to identify the sequences of reference data set as well as the test sequence database. SMO and J48 showed species identification in BRM samples with 100 percent discriminatory power (Supplementary Figs. 5–8). These two machine learning algorithms were subsequently employed for authentication of sequences of unknown samples. When the test data of unknown commercially traded samples with large variations were analysed, the J48 classifier could identify only 35 percent of the species. Best performance was shown by SMO with 100 % accuracy in authenticating the test data of thirty market samples (Fig. 3). Test data set of thirty market samples showed similarity with the reference data set (BRM sequence database). Twenty commercially traded samples showed similarity with *B. aristata*, and ten with *C. fenestratum* provided in the reference data set, which again substantiated the dendrogram based sequence analysis in MEGA.

HPTLC analysis

Dendrogram generated using RF values was used to analyse the banding pattern of BRM samples (Fig. 4). Each species showed specific banding pattern with intra species variation (Supplementary Fig. 1) belonging to different geographical locations were grouped in different clades. *C. fenestratum* from Aralam, south India and *B. aristata* from Himachal Pradesh, north India were grouped together,

Table 3 Evaluation of the four DNA barcode regions used in this study

Parameters	*rbcL*	*matK*	*PsbA-trnH*	*ITS*
Average intraspecific distance	0	0	0	0
Average interspecific divergence	0.0632 ± 0.0015	0.0234 ± 0.0064	0.175 ± 0.0880	0.0276 ± 0.0064

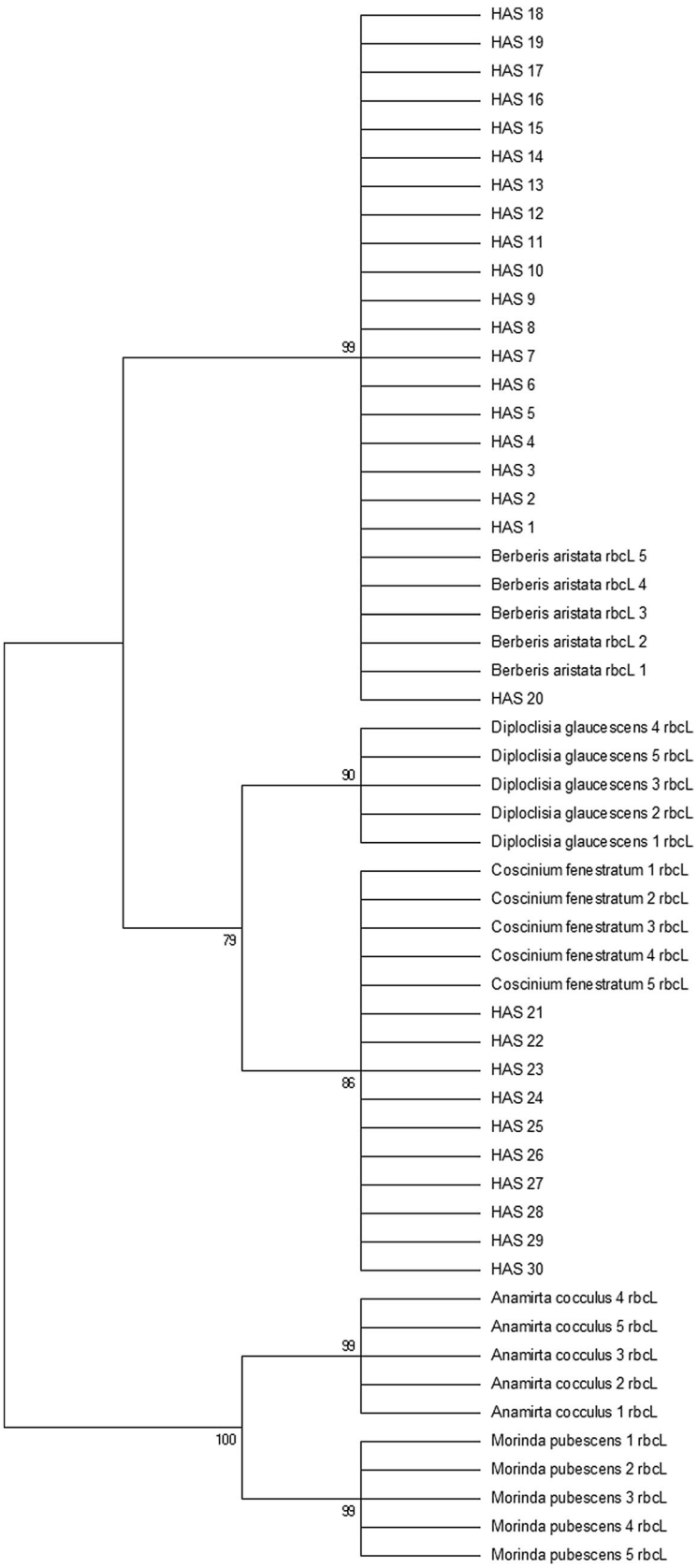

Fig. 2 Maximum Likelihood tree (ML) of market samples along with BRM using *rbcL* barcode

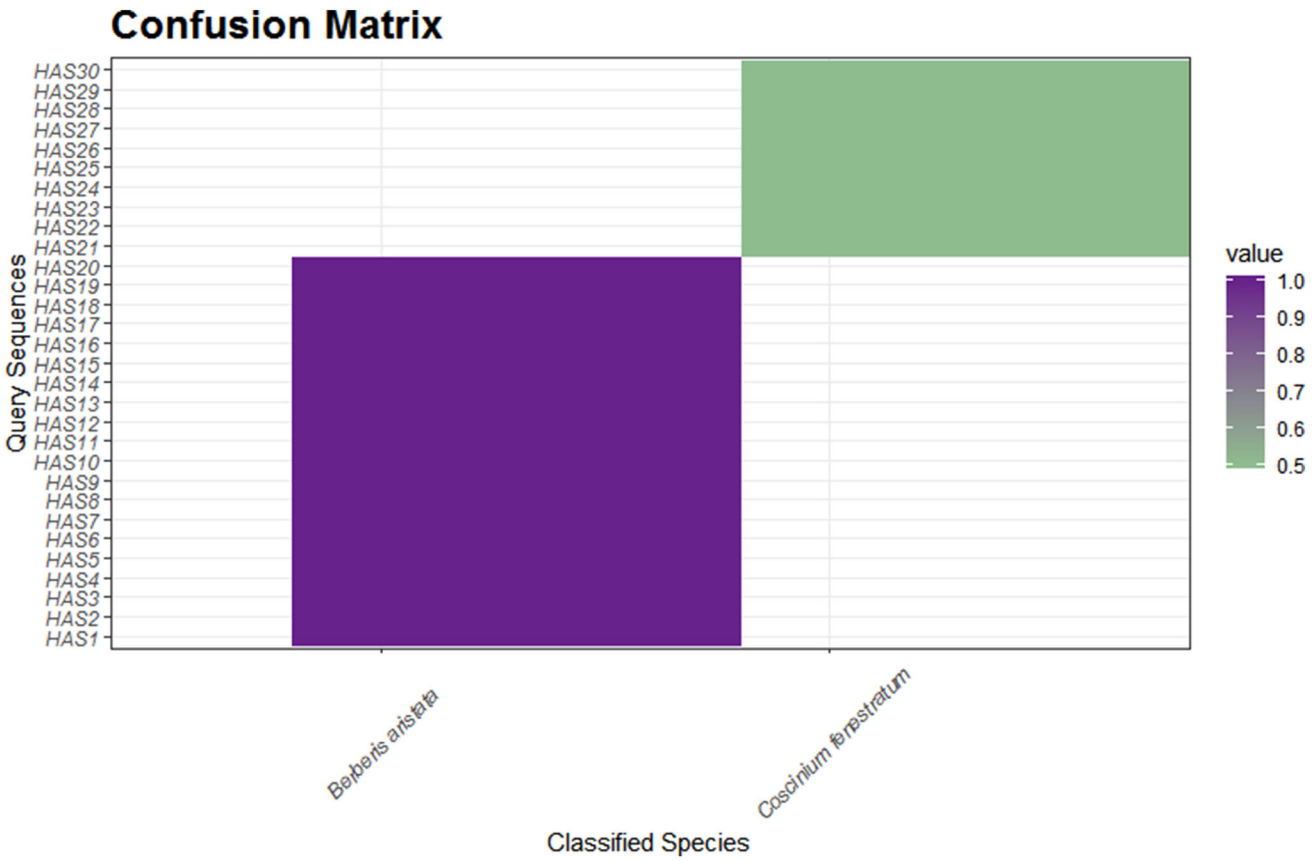

Fig. 3 The confusion matrix showing identification rate of market samples along with BRM based on *rbcL* using J48 and SMO

while *C. fenestratum* from Sholayar, south India showed similarity with *Morinda pubescens*. Likewise, *B. aristata* showed similarity with *Anamirta cocculus* and *Diploclisia glaucescens*. Therefore, HPTLC fingerprinting could not be further used as a species authentication tool because of the inconsistencies in the fingerprints generated.

Discussion

Adulteration/substitution is recently a critical issue in ayurvedic industries and hence authentication constitutes a pertinent role in assuring quality, safety and efficacy of ayurvedic formulations. A vast array of techniques such as physical, chemical (analytical), anatomical, organoleptic, and recently emerged DNA based molecular methods are widely used for plant species authentication (Mishra et al. 2016; Raclariu et al. 2017a). Conventional taxonomic method generally emphasize on morphology and anatomy of the species. However, recent studies have shown the impact of ecological and climatic variables in morphology and anatomy of species (Li et al. 2019, 2020), which makes species discrimination, a tedious job. The lack of expertise, phenotypic plasticity and species delimitation make the conventional methods more complicated. Since raw drugs are available in extremely dried, shredded or powdered form, species identification using microscopic and macroscopic methods may not always be feasible. In many cases, chemical fingerprinting/chemical methods are utilized for the qualitative and quantitative analysis of herbal drugs. However, chemical fingerprints are controlled by external environmental factors such as plant age, storage conditions, post-harvest technology, type of plant parts used (Kaur et al. 2016; Liu et al. 2011) and show discrepancy in the results. But DNA barcode based plant identification method has been comparatively considered as most powerful and potential method in herbal pharmacovigilance research (De Boer et al. 2015; Raclariu et al. 2017b) owing to its consistency. As compared to other traditional methods, DNA barcode database once developed can be made available in a public domain for species identification without involving much technical expertise. In India, species adulteration in commercially traded samples of *Aswagandha, Cinnamomum verum, Cassia, Myristica fragrans, Phyllanthus, Sida, Santalum album, Saraca asoca, Senna* and *Garcina* was analysed using DNA barcodes (Srirama et al. 2010; Dev et al. 2013; Seethapathy et al. 2015,2018; Urumarudappa et al. 2016).

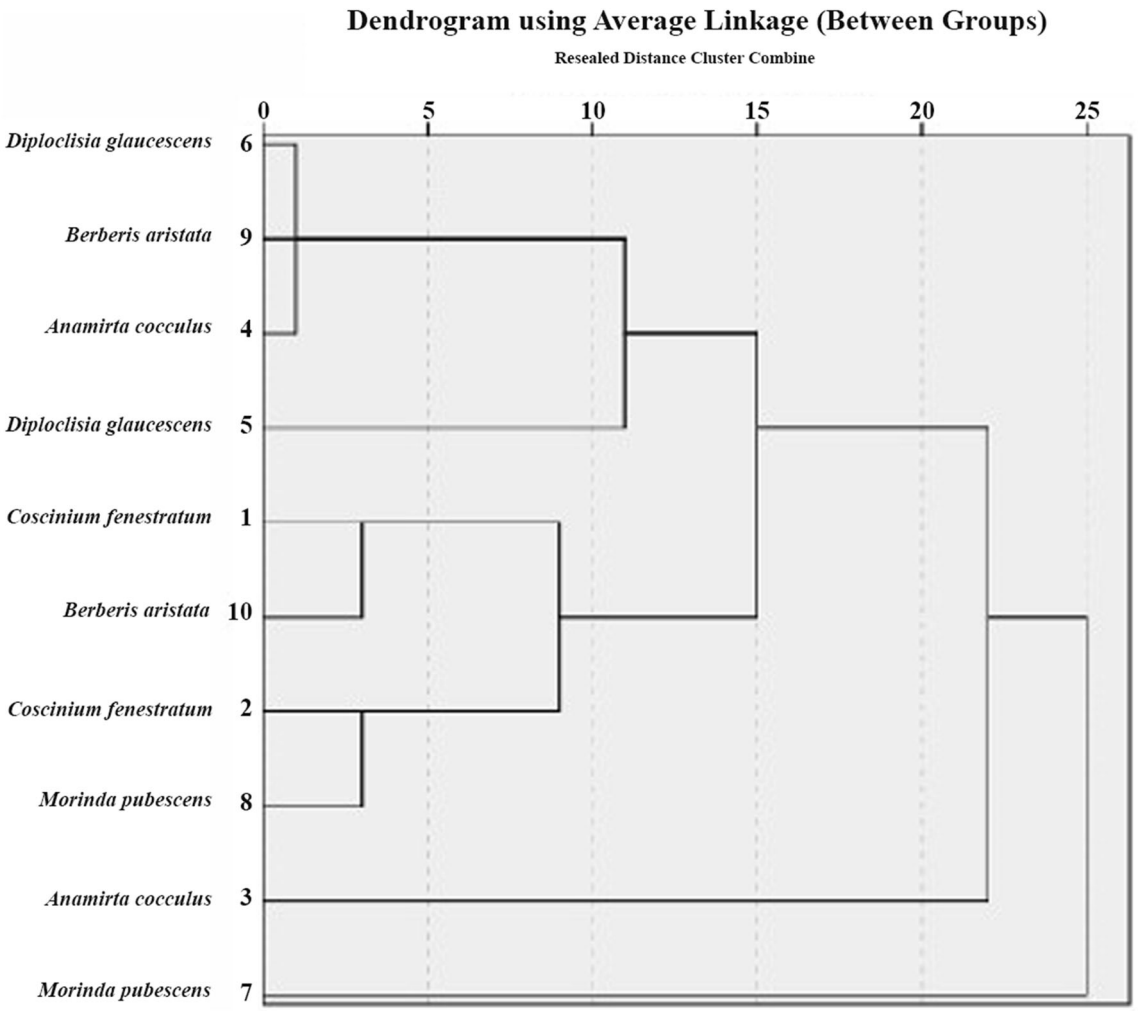

Fig. 4 Dendrogram constructed using HPTLC banding pattern (RF value)

In our study, CBOL recommended barcode primers *rbcL, matK, psbA-trnH* and *ITS* were used to create BRM library in which *psbA-trnH* and *rbcL* barcode regions showed higher interspecific divergence. High level of interspecific variation, difficulty of sequence alignments due to high number of indels and lack of high quality bidirectional sequences are major limitations of *psbA-trnH* (Chase et al. 2007; Hollingsworth et al. 2009; CBOL 2009) and hence was not further utilised in this study. Consequently, *rbc*L database was used to authenticate the market samples. PCR amplification was inconsistent and difficult to achieve for other barcode regions due to degradation of DNA in extremely dried market samples. The poor storage conditions (results in fungal and humus contamination) and inappropriate post-harvest methods along with degraded DNA might have further hindered the primer annealing and subsequent amplification in the traded samples (Newmaster et al. 2013; Santhosh Kumar et al. 2018). Prior studies also showed potentiality of *rbcL* barcode gene region to authenticate the congeneric plants as well as distantly related species (Newmaster et al. 2006; Chase et al. 2007).

The present study demonstrated the efficiency of different approaches for DNA barcode sequence data analysis to validate the occurrence of adulterants in commercially traded samples of *C. fenestratum*. The competence of *rbcL* barcode sequence data to authenticate the market samples was analysed employing Maximum Likelihood (ML) based phylogenetic tree as well as Machine learning algorithm, WEKA. ML tree was the most efficient and statistical suitable method to understand the process of sequence evolution compared to Maximum Parsimony (MP) and Neighbour Joining (NJ). ML tree also has a rich repertoire of sophisticated evolutionary models and computer stimulations (Kuhner and Felsenstein 1994; Guindon and Olivier 2003; Ziheng et al. 2012). Moreover, previous studies also reported the use of ML tree to detect raw drug adulteration (Seethapathy et al. 2018). Compared to classical barcode sequence data analysis methods such as Maximum

Likelihood (ML), BLAST and nearest-neighbour, recent studies have shown the higher efficacy of Machine Learning Algorithm (MLA) for species authentication (Hartvig et al. 2015). Present study could demonstrate the effectiveness of both sequence based phylogenetic tree as well as WEKA analyses to authenticate the identity of unknown samples based on the BRM reference databases created. Machine Learning Approaches were earlier performed for authentication of timber species like *Pterocarpus* and *Dalbergia* (He et al. 2018, 2019). Large data analysis ability being one of the major advantages of Artificial Intelligence (AI), provides quite a large platform for rapid analysis of raw drug samples, which significantly reduces the human labour (He et al. 2018). AI utilises the ability of computers to build models that can receive input data and then conduct statistical analyses. Even though, MLA recommends minimum of four specimens per species for the BRM library, it provides higher precision, greater cost-effectiveness, time efficiency, and a lower threshold than other analytical methods.

Most of the market samples showed similarity with *B. aristata* when compared to the authentic species *C. fenestartum*. Similar instances of adulteration were earlier reported wherein *Saraca asoca* was adulterated with *Polyalthia longifolia*, *Piper nigrum* with *Capsicum annuum* and *Hypericum perforatum* with *Senna alexandrina,* among others (Urumarudappa et al. 2016; Parvathy et al. 2014). This adulteration of *C. fenestratum* with *B. aristata* may be due to the fact that *B. aristata*, a common species of north Indian states, are easily available in huge quantities in the wild and that they share the same vernacular name and also has the potent alkaloid berberine. However, *C. fenestratum* and *B. aristata* belong to two different families with dissimilar anatomical, morphological and sexual features. Unavailability of *C. fenestratum* in south Indian markets point to the overexploitation and consequent loss of species in the wild. A recent survey conducted by KFRI indicates that the total number of mature individuals in Kerala is around 110 and the representation of female plants was only 45. Moreover, the unscientific collection and extensive consumption to meet the huge industrial demand exerts a heavy strain on existing resources of the endangered populations of *C. fenestratum*. Adulteration with inferior taxa is reported to significantly alter the therapeutic properties of ayurvedic formulations (Srirama et al. 2010). Most herbal drugs available in the market are sourced from the wild through supply chains in the formal or informal sectors (Larsen et al. 2006; Goraya and Ved 2017). In India, major herbal trade occurs through conventional collection centres and wholesale markets. Along with government agencies like Forest Department, tribal cooperative society and Vana Samrakshana samiti, there are number of stakeholders ranging from herb gatherers, local middlemen, urban traders, wholesalers, manufacturers, exporters and herbal healers in the medicinal plants trade sector (Goraya and Ved 2017). In India, there is no proper guidelines to coordinate and maintain the information related to collection, supply, trade and consumption of botanicals (Kala et al. 2006; Goraya and Ved 2017). Recently, National Medicinal Plant Board (NMPB), Government of India, has launched an online platform e-charak, to create transparent trade linkage among primary collectors to end users of medicinal plant sector (http://www.nmpb.nic.in).

B. aristata is a commonly available species in north India and according to current market price list of National Medicinal Plant Board (NMPB), *B. aristata* belongs to low price category of medicinal plants compared to *C. fenestratum* (http://www.nmpb.nic.in). However, there is no codified market price for the raw drugs and the price of collected raw drugs often tend to vary from shop to shop. In India, there is neither a centralised agency to monitor the proper collection, processing, production and sale of herbal medicine nor an effective regulatory framework to evaluate the quality, authenticity and safety of raw drugs or drug formulations available in the market (Sahoo et al. 2013). Prior studies have also suggested *B. aristata* as substitute of *C. fenestratum,* mainly due to the presence of potent alkaloid berberine (Tamilselvi et al. 2014). However, it is not recommended as a substitute by Pharmacopoeia Commission for Indian Medicine & Homoeopathy (PCIM&H) and there is no scientific study available comparing the phytochemical constituents in *B. aristata* and *C. fenestratum*.

Recently, the combination of two or more diverse techniques were employed to authenticate the species with high precision. The efficiency of multitier approach comprising DNA barcoding in combination with HPTLC for the identification of popular species such as *Hamamelis virginiana*, *Matricaria recutita*, *Maytenus ilicifolia*, *Mikania glomerata*, *Panax ginseng*, *Passiflora incarnata*, *Peumus boldus* and *Valeriana officinalis* (Palhares et al. 2014), with NMR for finding out adulteration in *Garcinia* species and *Sarca asoca* (Kumar et al. 2016; Seethapathy et al. 2018), with TLC and HPLC for authentication of *Marsdenia* (Yu et al. 2018) were reported to authenticate species with high precision. However, studies have also reported the inconsistency of chemical markers in delineating medicinal plants, owing to variation with age of plant and environmental heterogeneity (Liu et al. 2011; Kaur et al. 2016; Moustafa et al. 2016; Cao et al. 2017). In the present analysis, for developing HPTLC fingerprint, *C. fenestratum* accessions were collected from environmentally heterogeneous areas and of unknown age and sex, which must have contributed to the discrepancies in HPTLC profiles. In

Tinosporia cordifolia, sex specific disparity in chemical constituents was earlier reported (Bajpai et al. 2017).

C. fenestratum is hermaphrodite in nature with only very few female individuals at reproductive maturity. An imbalance in the sex ratio has affected the population viability and regeneration efficiency, which negatively affected the long term survival of the available scattered populations of this endangered species in the Western Ghats. This warrants an immediate action to be taken to sustain the available resources augmented through recovery, assisted regeneration, restoration and sustainable management of the available resources of wild *C. fenestratum* (Sumod et al 2016). DNA barcode tool can monitor and regulate the illicit trade in their raw drug sector and subsequently can protect and conserve the available germplasm in situ. Recently, KFRI has initiated the in situ conservation of *C. fenestratum* in Kerala, south India through aided natural regeneration (ANR) in its natural microhabitats. As part of this programme, 14,500 hardened seedlings were transplanted through National Afforestation and Eco-Development Board (NAEB), Ministry of Environment & Climate Change (MOEF &CC), Government of India.

Conclusion

Adulteration of ayurvedic drugs is quite rampant to meet the industrial demand and strict regulation is therefore warranted to monitor the quality of herbal products by authenticating right from the time of collection to the formulation of herbal drugs. A national level certification agency for authentication and chain of custody validation is vital in India to ensure safety and quality of ayurvedic formulations. Our study could demonstrate the effectiveness of DNA barcode tool together with artificial intelligence (AI) based automated species identification technique to detect the extent of adulteration in commercially traded raw drugs. Therefore, including DNA barcoding as one among the standard techniques in Indian Ayurvedic Pharmacopoeia to validate the authenticity of raw drugs is highly recommended. Simultaneously, conservation and restoration measures through in situ and ex situ means of the available resources in commercially traded medicinal plants is recommended to replenish and enrich the rapidly declining resources.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12298-021-00965-9.

Acknowledgements We are thankful to the State Forest Department of Kerala, for their permission to collect wild samples. The financial support received from National Medicinal Plant Board (NMPB), Ministry of AYUSH, Government of India (No. F.No. Z.18017/187/CSS/R&D/KE-01/2016-17-NMPB-IV A) is gratefully acknowledged.

Author contribution Suma Arun Dev, Jayaraj R: Fund acquisition, conceptualization, methodology & designing the experiments, writing, editing and finalization of manuscript. Remya Unnikrishnan: Conducted wet lab experiments as part of doctoral research, data analysis & manuscript writing. Sujanapal P, Sumod M: Sample collection and manuscript writing.

Declaration

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

References

Bajpai V, Kumar S, Singh A, Singh J, Negi MPS, Bag SK, Kumar B (2017) Chemometric based identification and validation of specific chemical Markers for Geographical, Seasonal and Gender Variations in *Tinospora cordifolia* stem using HPLC-ESI-QTOF-MS Analysis. Phytochem Anal 28:277–288. https://doi.org/10.1155/2012/18010310.1002/pca.2673

Baker DA, Stevenson DW, Little DP (2012) DNA barcode identification of black cohosh herbal dietary supplements. J AOAC Int 95:1023–1034. https://doi.org/10.1155/2012/1801310.5740/jaoacint.11-261

Cao Y, Fang S, Yin Z, Fu X, Shang X, Yang W, Yang H (2017) Chemical fingerprint and multicomponent quantitative analysis for the quality evaluation of *Cyclocarya paliurus* leaves by HPLC–Q–TOF–MS. Molecules 22:1927. https://doi.org/10.1155/2012/18010310.3390/molecules22111927

CBOL Plant Working Group (2009) A DNA barcode for land plants. Proc Natl Acad Sci USA 106:12794–12797. https://doi.org/10.1073/pnas.0905845106

CFSAN March 25 (2002) Centre for Food Safety and Applied Nutrition(CFSAN): Kava-containing dietary supplements may be associated with severe liver injury. U.S. Department of Health and Human Services, Food and Drug Administration; Rockville, Maryland

Chase MW, Cowan RS, Hollingsworth PM, Van den Berg C, Madrinan S, Petersen G, Seberg O, Jorgsensen T, Cameron KM, Carine M, Pedersen N, Hedderson TAJ, Conrad F, Salazar GA, Richardson JE, Hollingsworth ML, Barraclough TG, Kelly L, Wilkinson M (2007) A proposal for a standardised protocol to barcode all land plants. Taxon 56:295–299

Chunming G, Zhao Y, Hong N, Yanjun L, Juhua Y et al (2020) Identification of berberine as a novel drug for the treatment of multiple myeloma via targeting UHRF1. BMC Biol 18(1):33. https://doi.org/10.1186/s12915-020-00766-8

De Boer HJ, Ichim MC, Newmaster SG (2015) DNA barcoding and pharmacovigilance of herbal medicines. Drug Saf 38:611–620. https://doi.org/10.1007/s40264-015-0306-8

Dev SA, Muralidharan EM, Sujanapal Balasundaran P (2013) Identification of market adulterants in East Indian sandalwood using DNA barcoding. Ann For Sci 71:517–522. https://doi.org/10.1007/s13595-013-0354-0

Goraya GS, Ved DK (2017) Medicinal plants in India: an assessment of their demand and supply, national medicinal plants board, ministry of AYUSH, Government of India. New Delhi and Indian Council of Forestry Research & Education, Dehradun

Guindon C, Olivier G (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by Maximum Likelihood. Syst Biol 52(5):696–704. https://doi.org/10.1080/10635150390235520

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis. Department of Microbiology, North Carolina State University

Hartvig I, Czako M, Kjær ED, Nielsen LR, Theilade I (2015) The Use of DNA barcoding in identification and conservation of rosewood (Dalbergia spp). PLoS ONE. https://doi.org/10.1371/journal.pone.0138231

He T, Jiao L, Wiedenhoeft AC, Yin Y (2019) Machine learning approaches outperform distance- and tree-based methods for DNA barcoding of *Pterocarpus* wood. Planta. https://doi.org/10.1007/s00425-019-03116-3

He T, Jiao L, Yu M, Guo J, Jiang X, Yin Y (2018) DNA barcoding authentication for the wood of eight endangered *Dalbergia* timber species using machine learning approaches. Holzforschung. https://doi.org/10.1515/hf-2018-0076

Hollingsworth ML, Andra clark A, Forrest LL, Richardson J, Pennington RT, Long DG, Hollingsworth PM (2009) Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. Mol Ecol Resour 9:439–457. https://doi.org/10.1111/j.1755-0998.2008.02439.x

Ichim MC (2019) The DNA-based authentication of commercial herbal products reveals their globally widespread adulteration. Front Pharmacol. https://doi.org/10.3389/fphar.2019.01227

Ichim MC, Haser A, Nick P (2020) Microscopic authentication of commercial herbal products in the globalized market: potential and limitations Front. Pharmacol 11:876. https://doi.org/10.3389/fphar.2020.00876

Ichim MC, de Boer H (2020) A review of authenticity and authentication of commercial ginseng herbal medicines and food supplements Front. Pharmacol. https://doi.org/10.3389/fphar.2020.612071

Jadot I, Decleves A, Nortier J, Caron N (2017) An integrated view of aristolochc acid nephropathy: update of the literature. Int J Mol Sci 18:297

Kala C, Dhyani P, Sajwan B (2006) Developing the medicinal plants sector in northern India: challenges and opportunities. J Ethnobiol Ethnomed 2:32. https://doi.org/10.1186/1746-4269-2-32

Kaur T, Bhat R, Vyas D (2016) Effect of contrasting climates on antioxidant and bioactive constituents in five medicinal herbs in Western Himalayas. JMS 13:484–492. https://doi.org/10.1007/s11629-014-3380-y

Kress WJ, Erickson DL (2007) A two-locus global DNA barcode for land plants: the coding *rbcL* gene complements the non-coding *trnH-psbA* spacer region. PLoS ONE 2:e508

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Kuhner MK, Felsenstein J (1994) A simulation comparison of phylogeny algorithms under equal and unequal evolutionary rates. Biol. Evol, Mol. https://doi.org/10.1093/oxfordjournals.molbev.a040126

Larsen HO, Olsen CS (2006) Unsustainable collection and unfair trade? Uncovering and assessing assumptions regarding Central Himalayan medicinal plant conservation. Biodivers Conserv 16:1679–1697. https://doi.org/10.1007/s10531-006-9039-4

Lewis DD (1998) Naïve (Bayes) at forty: the independence assumption in information retrieval. Mach Learn 98:4–15

Li J, Wu J, Peng K, Fan G, Yu H, Wang W, He Y (2019) Simulating the effects of climate change across the geographical distribution of two medicinal plants in the genus. Nardostachys PeerJ 7:e6370. https://doi.org/10.7717/peerj.6730

Li Y, Li S, Lu X, Wang Q, Han H, Zhang X, Gan X (2020) Leaf phenotypic variation of endangered plant *Tetracentron sinense* Oliv. and infuence of geographical and climatic factors. J For Res. https://doi.org/10.1007/s11676-020-01124-8

Little DP, Jeanson ML (2013) DNA barcode authentication of saw palmetto herbal dietary supplements. Sci Rep. https://doi.org/10.1038/srep03518

Liu Y, Reich PB, Li G, Sun S (2011) Shifting phenology and abundance under experimental warming alters trophic relationships and plant reproductive capacity. Ecology 92:1201–1207. https://doi.org/10.1890/10-2060.1

Meyer CP, Paulay G (2005) DNA barcoding: error rates based on comprehensive sampling. PLoS ONE 3:e422

Michl J, Jennings HM, Kite GC, Ingrouille MJ, Simmonds MSJ, Heinrich M (2013) Is aristolochic acid nephropathy a widespread problem in developing countries? A case study of *Aristolochia indica* L. in Bangladesh using an ethnobotanical-phytochemical approach. J Ethnopharmacol 149:235–244

Mishra P, Kumar A, Nagireddy A, Mani DN, Shukla AK, Tiwari R, Sundaresan V (2016) DNA barcoding: an efficient tool to overcome authentication challenges in the herbal market. J Plant Biotechnol 1:8–21. https://doi.org/10.1111/pbi.12419

Mohanan N, Sivadasan M (2002) Flora of Agathymala. Bishen singh Mahendra Pal Singh, Dehra Dun India, p 65

Moustafa MF, Hesham AEL, Quraishi MS, Alrumman SA (2016) Variations in genetic and chemical constituents of *Ziziphus spina-christi* L. populations grown at various altitudinal zonation up to 2227m height. J Genet Eng Biotechnol 14:349–362

Newmaster SG, Grguric M, Shanmughanandhan D (2013) DNA barcoding detects contamination and substitution in North American herbal products. BMC Med 11:222. https://doi.org/10.1186/1741-7015-11-222

Newmaster SG, Fazekas AJ, Ragupathy S (2006) DNA barcoding in land plants: evaluation of *rbcL* in a multigene tiered approach. Can J Bot 84:335–341

Palhares RM, Gonçalves Drummond M, Brasil AF, Cosenza GP, Lins Brandao MDG, Oliveira G (2015) Medicinal plants recommended by the World Health Organization: DNA barcode identification associated with chemical analyses guarantees their quality. PLoS ONE 10:e0127866. https://doi.org/10.1371/journal.pone.0127866

Palhares RM, Drummond MG, Brasil BS, Krettli AU, Oliveira GC, Brandao MG (2014) The use of an integrated molecular-chemical- and biological-based approach for promoting the better use and conservation of medicinal species: a case study of Brazilian quinas. J Ethnopharmacol 155:815–822

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. https://doi.org/10.1080/08905436.2013.870078

Posadzki P, Watson L, Ernst E (2012) Contamination and adulteration of herbal medicinal products (HMPs): an overview of systematic reviews. Europ J Clin Pharmacol 69(3):295–307. https://doi.org/10.1007/s00228-012-1353-z

Punitha ISR, Rajendran K, Shirwaikar A, Shirwaikar A (2005) Alcoholic stem extract of *Coscinium fenestratum* regulates carbohydrate metabolism and improves antioxidant status in streptozotocin-nicotinamide induced diabetic rats. Evid Based Complement Altern Med 2:375–381. https://doi.org/10.1093/ecam/neh099

Quinlan JR (1996) Improved use of continuous attributes in C4.5. JAIR 4:77–90

Raclariu AC, Heinrich M, Ichim MC, de Boer H (2017a) Benefits and limitations of DNA barcoding and metabarcoding in herbal product authentication. Phytochem Anal 29(2):123–128. https://doi.org/10.1002/pca.2732

Raclariu AC, Mocan A, Popa MO, Vlase L, Ichim MC, Crisan G (2017b) *Veronica officinalis* product authentication using DNA metabarcoding and HPLC-MS reveals widespread adulteration with *Veronica chamaedrys*. Front Pharmacol 8:378. https://doi.org/10.3389/fphar.2017.00378

Rai RV, Rajesh PS, Kim HM (2012) Medicinal use of *Coscinium fenestratum* (Gaertn.) Colebr.: an short review. Oriental Pharmacy Exp Med 13:1–9. https://doi.org/10.1007/s13596-012-0094-y

Ramasubbu R, Chandra Prabha A, Kumuthakalavalli R (2012) Seed biology of *Coscinium fenestratum* (Ga ertn.) Colebr.—a critically endangered medicinal plant of Western Ghats. J Med Plant Res 6:1094–1096

Sahoo N, Manchikanti P (2013) Herbal drug regulation and commercialization: an Indian industry perspective. J Altern Complement Med 19:957–963. https://doi.org/10.1089/acm.2012.0275

Santhosh Kumar JU, Krishna V, Seethapathy GS, Ganesan R, Ravikanth G, Uma Shaanker R (2018) Assessment of adulteration in raw herbal trade of important medicinal plants of India using DNA barcoding. 3 Biotech 8(3):135. https://doi.org/10.1007/s13205-018-1169-3

Sarvalingam A, Rajendran A (2016) Rare, endangered and threatened (RET) climbers of aouthern Western Ghats, India. Rev Chil de Hist Nat 89:2–5. https://doi.org/10.1186/s40693-016-0058-6

Seethapathy GS, Ganesh D, Kumar JUS, Senthilkumar U, Newmaster SG, Ragupathy S (2015) 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. https://doi.org/10.1007/s00414-014-1120-z

Seethapathy GS, Tadesse M, Urumarudappa SKJV, Gunaga S, Vasudeva R, Malterud KE, Wangensteen H (2018) Authentication of *Garcinia* fruits and food supplements using DNA barcoding and NMR spectroscopy. Sci Rep 8:10561. https://doi.org/10.1038/s41598-018-28635

Shahzad W, Asad S, Khan MA (2013) Feature subset selection using association rule mining and JRip classifier. Int J Phys Sci 8:885–896

SPSS Inc (2007) Released. SPSS for Windows, Version 16.0. Chicago, SPSS Inc

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. https://doi.org/10.1016/j.jep.2010.04.042

Srirama R, Santhosh Kumar JU, Newmaster SGS, Ragupathy S, Ganeshaiah KN, Ravikanth G (2017) Species adulteration in the herbal trade: causes, consequences and mitigation. Drug Saf 40:651–661. https://doi.org/10.1007/s40264-017-0527-0

Stoeckle MY, Gamble CC, Kirpekar R, Young G, Ahmed S, Little DP (2011) Commercial teas highlight plant DNA barcode identification; successes and obstacles. Sci Rep. https://doi.org/10.1038/srep00042

Sumod M, Sujanapal P, Robi AJ, Dantus KJ, Arun KR, Sathiyakumar K (2016) Population status and distribution of *Coscinium fenestratum* Colebr. (Menispermaceae) in northern Kerala. In: Proceedings of 26th Kerala Science Congress 2499–2507, Govt. of Kerala

Suykens JAK, Vandewalle J (1999) Least squares support vector machine classifiers. Neural Process Lett 9:293–300

Tamilselvi S, Venkatasubramanian P, Vasanthi NS (2014) Physico-Chemical characterization and anti-inflammatory activity of stem extracts of *Berberis aristata* DC and *Cosinium fenestratum* Linn in Carrageenan induced wistar rats. Pharmacogn J 6:72–77. https://doi.org/10.5530/pj.2014.4.11

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Tewa XN, Laua NJX, Chellappanb DK, Madheswaranc T, Zeeshanc TMM et al (2020) Immunological axis of berberine in managing inflammation underlying chronic respiratory inflammatory diseases. Chem Biol Interact 1:1. https://doi.org/10.1016/j.cbi.2020.108947

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Thriveni HN, Srikanth V, Gunaga RHN, Vasudeva R (2015) Ecological niche modeling, population status and regeneration of *Coscinium fenestratum* colebr. (Menispermaceae): a medicinally important liana of the central Western Ghats. Trop Ecol 56:101–110

Tran QL, Tezuka Y, Ueda JY, Nguyen NT, Maruyama Y, Begum K, Kadota S (2003) *In vitro* antiplasmodial activity of antimalarial medicinal plants used in Vietnamese traditional medicine. J Ethnopharmacol 86:249–252. https://doi.org/10.1016/s0378-8741(03)00045-x

Tushar KV, George S, Remashree AB, Balachandran I (2008) *Coscinium fenestratum* (Gaertn.) Colebr.—a review on this rare, critically endangered and highly traded medicinal species. J Plant Sci 3:133–145

Udayan PS, Satheesh George KV, Thushar IB (2005) Ethanomedicinal plant used by the Oorali tribe of Idukki district, Kerala state, India. J Econ Taxon Bot 29:219–220

Ueda J, Tezuka Y, Banskota AH, Le Tran Q, Tran QK, Harimaya Y, Kadota S (2002) Antiproliferative activity of Vietnamese medicinal plants. Biol Pharm Bull 25:753–760

Upton R, David B, Gafner S et al (2019) Botanical ingredient identification and quality assessment: strengths and limitations of analytical techniques. Phytochem Rev 19:1157–1177. https://doi.org/10.1007/s11101-019-09625-z

Urumarudappa SK, Gogna N, Newmaster SG, Venkatarangaiah K, Subramanyam R, Saroja SG, Gudasalamani R, Dorai K, Ramanan US (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

Ved D, Saha D, Ravikumar K, Haridasan K (2015) *Coscinium fenestratum*. The IUCN Red List of Threatened Species. e.T50126585A50131325

Wallace LJ, Boilard SMAL, Eagle SHC, Spall JL, Shokralla S, Hajibabaei M (2012) DNA barcodes for everyday life: routine authentication of natural health products. Food Res Int 49:446–452. https://doi.org/10.1016/j.foodres.2012.07.048

Weitschek E, Fiscon G, Felici G (2014) Supervised DNA Barcodes species classification: analysis, comparisons and results. Biodata Minining 7:4

WHO (2011) The World medicines situation—traditional medicines: global situation, issues and challenges. WHO Press, Geneva

Yu N, Wei Y, Zhu Y, Zhu N, Wang Y, Zhang H, Sun A (2018) Integrated approach for identifying and evaluating the quality of *Marsdenia tenacissima* in the medicine market. PLoS ONE 13:e0195240

Ziheng Y, Bruce R (2012) Molecular phylogenetics: principles and practice. Nat Rev Genet 13:303–314. https://doi.org/10.1038/nrg3186