Pitfalls and promises of raw drug identification techniques in the ayurvedic industry: an overview

Remya Unnikrishnan^{1,3} · Suma Arun Dev¹ · R. Jayaraj²

Received: 27 September 2019 / Accepted: 13 October 2020 / Published online: 30 October 2020
© King Abdulaziz City for Science and Technology 2020

Abstract

India, with a rich heritage of floral diversity, is well-known for its medicinal plant wealth and is the largest producer of medicinal herbs in the world. Ethnobiological Survey of Ministry of Environment and Forests (MOEF) could identify 8000 plant species utilized in various systems of medicine with approximately 25,000 effective herbal formulations. The extensive consumption to meet demand–supply ratio exerts a heavy strain on the existing resources. This subsequently led to the adulteration and substitution of medicinal plants with look-alike species. The consumer's faith on herbal medicine is in the phase of decline due to the extremities in adulteration/substitution and ensuing consequences. It is imperative to bring forth universally acceptable standard tools to authenticate raw drugs before being processed further into formulations. A vast array of techniques such as physical, chemical (analytical), biochemical, anatomical, organoleptic, and recently emerged DNA based molecular methods are widely used for plant species authentication. In recent years, DNA barcoding has made remarkable progress in the field of medicinal plants research. DNA metabarcoding is the latest development for qualitative evaluation of the herbal formulations, whereas for quantitative analysis, combination of pharmacognostic, pharmacovigilance and analytical methods are inevitable for authentication. This review addresses the overall strengths and shortcomings of the existing as well as recently emerged techniques in authenticating ayurvedic raw drugs.

Keywords Herbal drug authentication · Analytical techniques · DNA barcoding · Ayurvedic raw drugs · HRM · DNA metabarcoding

Abbreviations

MOEF	Ministry of environment and forests
WHO	World health organization
CITES	Convention on international trade in endangered spices of wild fauna and flora
AHP	American herbal pharmacopoeia
FDA	Food and drug administration

Introduction

India, with a rich heritage of floral diversity, is well-known for its medicinal plant wealth and is the largest producer of medicinal herbs in the world (Ganesan et al. 2016). According to World Health Organization (WHO), 21,000 medicinal plant species are used for various medicinal purposes around the world. The healing properties of plants are well documented in Rigveda (400–1500 B.C.), Atharvaveda (1500 B.C.) and Upanishad (1000 B.C.) (Patil and Patil 2010). In India, ethnobiological survey of MOEF could identify 8000 plant species utilized in various systems of medicine with approximately 25,000 effective herbal formulations (Mishra et al. 2016). Consequent high demand involving 960 actively traded and 178 species traded in bulk quantities, an estimate of 90 per cent of wild resources are utilized by more than 8610 traditional manufacturing units (Goraya and Ved 2017).

The extensive consumption to meet demand–supply ratio exerts a heavy strain on the existing resources with a few of them increasingly threatened in their natural habitat (Chen

✉ Suma Arun Dev
sumadev@kfri.res.in

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

² Forest Ecology and Biodiversity Division, Kerala Forest Research Institute, Peechi, Thrissur, Kerala, India

³ Cochin University of Science & Technology, Kochi, Kerala, India

et al. 2018). Adulteration/substitution of these commercial resources with inferior taxa has become a common practice to meet the annual demand of ayurvedic industry (Mishra et al. 2016; Ouarghidi et al. 2012; Tehen et al. 2014; Walker and Apple Quist 2012). However, to guarantee the quality of herbal medicines, WHO pharmacopoeia has implemented certain criteria for proper identification of plant species and quality assessment of potent phytochemical principles (Palhares et al. 2015). Although there are several recommended methods, right from the traditional taxonomic to organoleptic and physiochemical, it is extremely difficult to authenticate ayurvedic raw drugs. This is because these raw drugs are available as shredded, powdered and extremely dried forms in the market which are hard to identify in the preliminary screening (Coghlan et al. 2012). Adulteration and substitution is currently a major concern with confirmed reports from several countries (Coghlan et al. 2012; Newmaster et al. 2013; Urumarudappa et al. 2016). Scientific studies have estimated 46–66 per cent of herbal adulteration in North America and around 80 per cent of adulteration in Africa (Han et al. 2016). India, however, does not have any published data to estimate the extent of adulteration in herbal products (Shanmughanandhan et al. 2016). The consumer's faith on herbal medicine is in the phase of decline due to the extremities in adulteration/substitution and subsequent consequences (Palhares et al. 2015). Therefore, it is imperative to bring forth universally acceptable standard tools to authenticate raw drugs before being processed further into formulations. 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 (Palhares et al. 2014, 2015; Raclariu et al. 2018; Seethapathy et al. 2018; Urumarudappa et al. 2016).

This review addresses the overall strengths and shortcomings of the existing as well as recently emerged techniques in authenticating ayurvedic raw drugs.

Herbal market

Herbal medicine industry is one of the fastest growing industries in the world. Herbal products such as phytopharmaceuticals, phytocosmetics and personal care products have always attracted global attention. Europe leads the herbal market followed by Asia, North America and Japan. China and India are the top exporters, whereas Hong Kong, USA and Germany are the principal importers (Nirali and Shankar 2015). Global market for herbal medicine is estimated to be worth US\$ 83 billion with Europe being the main herbal market in the world. Studies forecast that by 2020, herbal market will hit the target of US\$ 3 trillion and by 2050 it is predicted to reach US\$ 5 trillion (Bangladesh foreign trade institute 2016).

In India, around 8610 recognised, 50 large scale, 200 medium scale and 8360 small scale herbal manufactures have been recorded (Goraya and Ved 2017). Indian herbal industry has demonstrated its pre-eminence with 15 per cent more annual turnover than that of pharmaceutical industry. 1,95,000 MT of herbal raw drugs are consumed by the herbal industries in India and almost 5,12,000 MT of raw herbal drug had been exported from India during the years 2014–15, Which is more than double with an increase of 138 per cent compared to previous years (Goraya and Ved 2017). Raw herbal drugs traded commercially are regulated and controlled by various measures, which vary from country to country. Species protection is one of the most important measures regulating the international trade of medicinal plants. CITES, an international treaty proposed by World Union Conservation has established protocols for regulating international trade ensures trade of specimens without threat to their survival.

In India, owing to the massive demand of herbal industries, unscientific extraction of medicinal plants from wild approximates to around 80 per cent (Ved and Goraya 2007). This ultimately led to overexploitation of resources, which is a serious threat to the species survival, also affecting the livelihood of people relying on these resources. Inaccessibility to potent ayurvedic raw drugs has led to adulteration and substitution of these drugs with its close relative or look alike species jeopardizing the quality. Thus, it is essential to develop tools to check authenticity of drugs used in the market. Moreover, conservation, restoration and sustainable management of available resources in wild could be a viable option to curb adulteration to an extent.

Raw drug adulteration

India has a rich history of traditional medicines, of which, Ayurveda is considered as one of the most ancient and widely accepted systems. Wide popularity of Ayurveda has contributed herbal industry to become the most flourishing sector in the world. The recent boost in utility of herbal products apparently augmented misuse and adulteration of medicinal plants leading to consumers' and manufacturers' discontent with fatal consequences in some instances. The absence of an effective regulatory framework to evaluate quality, authenticity and safety of raw drugs available in the market and the derived herbal formulations lead to severe health issues in consumers worldwide (Srirama et al. 2017). Medicinal products containing nephrotoxic and carcinogenic aristolochic acids were banned in Belgium, UK, Canada, Australia, and Germany (Zhou et al. 2013). In Australia, Japan, Taiwan and China, chronic use of *Aristolochia fangchi* adulterated products apparently led to the death of patients (Hong et al. 2006; Yang et al. 2002). Several studies

has reported the adulteration of *Stephania tetrandra* (Fang-Ji) roots with roots of the toxic herb, *Aristolochia fangchi* (Guang-Fang-Ji) leading to major renal failure (Jadot et al. 2017; Michl et al. 2013). Similarly, *Mesua ferrea* was adulterated with *Calophyllum inophyllum* (Poornima 2010), *Piper nigrum* with *Lantana camara* and *Embelia ribes* (Dhanya and Sasikumar 2010), *Papaver somniferum* with *Amaranthus paniculatus* (Dhanya and Sasikumar 2010), *Santalum album* with *Erythroxylum monogynum* (Chembath et al. 2012), *Gloriosa superba* with *Ipomea* species (Kumar et al. 2018) as well as *Cassia fistula* was adulterated with *Senna auriculata* (Seethapathy et al. 2015). In 2002, the US Food and Drug Administration received several reports of liver-related injuries such as hepatitis, cirrhosis, and liver failure due to consumption of *Piper methysticum* containing products which was subsequently banned in Germany, Switzerland, France, Canada and UK (US Food and Drug Administration 2001). One of the few causes of adulteration is misidentification and similarity in the local name between the species. These reports accentuate the necessity for proper raw drug identification, certification and toxicity assessment along with active pharmacovigilance of herbal products (Dutta et al. 2018). Therefore, identifying a comprehensive method for effective monitoring of raw herbal drugs is a pre-requisite.

Substitution in ayurveda

Ayurvedic manuscripts such as Bhavaprakasha, Yogaratnakara and Bhaishajya Ratnavali, had recognized the importance of species substitution (Pratinidhi dravya) (Sastri 2001; Shastri 2005; Chunevara et al. 2010). The concept of substitutes ideally is a measure based on their biological and chemical equivalence to overcome the species unavailability (Hegde 2018). Since ayurvedic medicines are characterized based upon the principles of taste, properties, potency and unique pharmacological action, more than 150 pratinidhi dravyas are mentioned in the ayurvedic scripts (Nagarajan et al. 2015). For instance, *Saussurea lappa* is considered as valid and equally effective substitute of *Inula racemosa*, likewise *Pterocarpus santalinus* and *Cinnamomum chomphora* are considered as substitutes for *Santalum album*, *Schlechera oleosa* for *Crocus sativus*, *Woodfordia fruticosa* for *Glycyrrhiza glabra*, *Syzygium aromaticum* for *Myristica fragrans*, *Terminalia myriocarpa* for *Terminalia arjuna*, *Abies webbiana* for *Taxus baccata* and *Gmelina arborea* for *Vitis vinifera*, among others, owing to their similarity in morphology and chemical principles (Sastri 2001). However, the term 'substitution' has been exploited in a wrong sense to cope up with the rising demand, wherein several adulterants with inferior chemical properties are often utilized as substitutes. *Pterocarpus santalinus* (Rakthachandana) used for digestive

disorder was substituted with root of *Vetiveria zizanioides* (Ushira), though the major chemical component was entirely different (Mishra 2007). Similarly, *Cyperus rotundus*, a common weed, is mentioned as substitute for *Aconitum heterophyllum*, an endangered species found in Himalayas (Gupta et al. 2006). However, in Indian markets, the recommended substitute *Cyperus rotundus* was adulterated with inexpensive and commonly available *Cryptocoryne spiralis* and *Cyperus scariosus* (Ved and Goraya 2007). Therefore, adulteration of medicinal plants and their authentic substitutes with inferior plant species even regardless of its chemical attributes is an illegitimate practice.

The intentional/illegal substitution irrespective of the pharmacological effects/bio equivalency can be considered as adulteration with serious consequences (Srirama et al. 2017). Recently, instances of drug resistance and side effects of ayurvedic drugs are widely reported jeopardizing the trust in ayurvedic system of medicine (Thatte and Bhalerao 2008; Srirama et al. 2017). Hence, efficacy, safety and therapeutic potential of substitutes need to be checked via detailed pharmacokinetic and clinical trials before the drug formulations reach the consumers. In addition, unavailability of the desired taxa owing to overexploitation is a major concern and effective strategies for sustainable utilization and conservation of such species only would assure its availability in the ensuing years.

Traditional vs. modern methods

A vast array of techniques such as physical, chemical (analytical), biochemical, anatomical, organoleptic, and recently emerged DNA based molecular methods are widely used for plant species authentication. Traditional taxonomic system mostly emphasizes on morphology and anatomy of the species. However, recent studies have shown the influence of environmental and climatic variables in shaping the 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 taxonomic identification system more complicated especially in families like *Papaveraceae*, *Poaceae*, *Malvaceae* and *Cactaceae* (Vohra and Khera 2013). Macroscopic evaluation deals with features such as shape, size, colour, texture, surface characterisation, odour, and organoleptic properties (Chanda 2014). Whereas, microscopy involves phloem and xylem characteristics, number and structure of stomata. Since raw drugs are available in extremely dried, shredded or powdered form, species identification using traditional tools may not always possible. Moreover, close relationships shared by the adulterants/substitutes with the original raw material often lead to erratic results (Palhares et al. 2015). In many instances, chromatographic finger

printing techniques such as TLC (Thin-layer chromatography), HPTLC (High-performance thin layer chromatography), HPLC (High Performance Liquid Chromatography), GC–MS (Gas chromatography–mass spectrometry), NMR (Nuclear magnetic resonance spectroscopy), FTIR (Fourier-transform infrared spectroscopy) were used as qualitative as well as quantitative methods for authentication of medicinal plants (Meena et al. 2018; Shawky and Selim 2018; Singh et al. 2020; Yao et al. 2020). However, chemical fingerprints are influenced by external environmental factors such as age of plant and storage conditions as well as type of plant parts used (Kaur et al. 2016; Liu et al. 2016). During early 1990s, researchers shifted their focus to DNA based markers for molecular species identification (Sucher and Carles 2008; Hao et al. 2010). A wide range of molecular markers such as AFLP (Amplified fragment length polymorphism), RFLP (Restriction fragment length polymorphism), RAPD (Random amplification of polymorphic DNA), ISSR (Inter simple sequence repeat), SSR (Simple sequence repeat), SCAR (Sequence characterized amplified region), LAMP (Loop mediated isothermal amplification), SNPs (Single nucleotide polymorphisms) were extensively used for identification of plants (Cao et al. 2010; Diao et al. 2009; Sharma et al. 2008; Tamhankar et al. 2009). DNA markers gained popularity for its accuracy and proper identification as it remained unaffected by physiological or environmental factors (Ganie et al. 2015). Each DNA marker has its own advantages and limitations and none of them can be considered as ideal. Selection of markers is purely based on the nature of research, financial stability and technical expertise (Kiran et al. 2010). Many reviews have provided ample insights into the principles, applications and technicalities of molecular markers (Nybom et al. 2014; Sharma et al. 2018; Tharachand et al. 2012).

Chromatography techniques are frequently used for discovery, development and manufacture of pharmaceuticals, which complements taxonomy for authentication in plants (Abubakar et al. 2018). Many progressive analytical techniques have been introduced to appraise the quality of herbal medicine such as HPLC, GC, UHPLC (Ultra high performance liquid chromatography), TLC, along with the recently hyphenated techniques like HPLC–DAD (diode-array detector), GC–MS, CE–DAD (Capillary electrophoresis) HPLC–MS and HPLC–NMR for additional spectral information and structural elucidation (Bansal et al. 2014). Amongst the commonly used methods such as TLC, HPTLC, HPLC and GC, TLC is the fastest method for chemical profiling in various pharmacopoeias viz. AHP, Chinese drug monographs and Pharmacopoeia of the People's Republic of China, etc. (Patel and Patel 2016). HPTLC is an alternative for TLC, with improved resolution, accuracy and reproducibility. The steroid diversity of *Aerva lanata* was revealed using HPTLC profile and used to differentiate the original

species from its adulterants (Mariswamy et al. 2011). Fingerprinting of *Cinnamomum cassia*, *Cinnamomum zeylanicum*, *Ruellia tuberosa*, *Solenia amplexicaulis*, *Aerva lanata* and *Tragia plukenetii* was performed using HPTLC (Chothani et al. 2012; Karthika and Paulsamy 2015; Meena et al. 2018; Singh et al. 2018). HPLC is another automatable technique with high rate of precision, resolution, sensitivity and selectivity. Several studies including authentication of Indian species *Talisapatra* with English yew (*Taxus baccata*), chromatographic profiling of *Woodfordia fruticosa* and *Tabernaemontana catharinensis*, active metabolites of *Annona muricata*, quantitative analysis of phenolic acids, flavonoids and ascorbic acid of wild edible leaves are some of the studies performed with HPLC (Boligon et al. 2015; Seal 2016; Syed and Khan 2016; Vadivel et al. 2018). In addition, presence and quantification of toxic chemicals from plant species such as aristolochic acids in *Aristolochia*, norditerpenoid alkaloids in *Delphinium* spp, and identification of five hepatotoxic pyrrolizidine alkaloids in traditional Chinese medicinal herb, *Herba senecionis* var. *scandentis*, have been identified using HPLC (Araya et al. 2018; Li et al. 2008). HPLC is possible to hyphenate with other detectors such as UV, DAD, for herbal finger printing and NMR for metabolomic profiling (Kamboj and Saluja 2017). GC/hyphenated GC/MS is one of the most efficient, reproducible and well used analytical platforms, owing to its robust, reproducible and selective nature of the technique as well as the large number of well-established libraries. GC–MS was successfully employed for the analysis of essential oils (thymus species, *Ocimum basilicum* L, *Peucedanum praeruptorum*), terpenes (*Salvia* spp., *Myrtus communis* L) and chemotaxonomy studies (Ibrahim et al. 2019; Mori et al. 2018; Nickavar et al. 2016; Sajjadi 2012; Siracusa et al. 2018). Even though there are different chromatographic methods, it is impossible to develop appropriate analytical method to represent chemical characteristics of multi-herbal products. In these circumstances, combination of analytical methods following different separation principle was recommended by FDA.

DNA barcoding offers a novel prospective tool for taxonomists and has greatly transformed species identification process (Hebert et al. 2003). It is an accurate and reliable alternative to morphological identification of biological material in challenging situations (Chen et al. 2010; Hebert et al. 2003). It can overcome inherent problems associated with traditional taxonomic identification due to phenotypic plasticity, species complexity, difficulty in finding reliable characters due to long maturity period, etc. (Kress et al. 2005). In plants, *matK* barcode region is one of the most rapidly evolving coding region of plastid genome similar to the COI used in animals (Hollingsworth 2011). However, in spite of several successful studies, *matK* was found to have poor species discriminatory power in some taxa (CBOL,

2009; Hidalgo et al. 2004). Though several barcode regions such as *rbcL*, *psbA-trnH* and ITS were successful in most instances, single tier approach was not encouraged owing to its inability to resolve in the species level (CBOL 2009; Doebley et al. 1990). Furthermore, combination of two or more barcode regions was initiated and higher efficiency of multi-locus approach was demonstrated in subsequent studies (Fazekas et al. 2008; Kress and Erickson 2007). In 2009, CBOL recommended *matK* + *rbcL* as universal standard barcode region with ITS and *psbA-trnH* region as complementary barcodes for land plants. Combination of *rbcL* + ITS region that enables higher retrieval capacity is widely used for identification of herbal products (Burgess et al. 2011; Hollingsworth et al. 2009; Ismail et al. 2008; Krawczyk et al. 2013; Malik et al. 2018; Newmaster et al. 2006, 2013).

In recent years, DNA barcoding has made remarkable progress in the field of medicinal plants research. DNA barcoding was initially used as identification tool and lately for the quality assurance of herbal products (Raclariu et al. 2017a). Herbal product authentication study shows disparity between the labelled product and the barcoding results. Using DNA barcoding, significant amount of adulteration was found in 98 per cent of Traditional Chinese Medicine products, 26 per cent adulterant herbal products from Iran, 16 per cent substitution of dietary herbal supplements from US and 50 per cent *Cassia* product adulteration from India (Ghorbani et al. 2017; Little 2014; Seethapathy et al. 2015). Recent studies revealed wide spread adulteration in raw herbal drugs such as *Saraca asoca*, *Sida cordifolia*, *Garcinia*, *Mormodica charantia*, *Myristica fragrans* and *Santalum album* (Dev et al. 2013; Kumar et al. 2020; Seethapathy et al. 2018; Swetha et al. 2017; Urumarudappa et al. 2016; Vassou et al. 2015). It is a major concern in almost all countries dealing with herbal drugs. In the present context, British pharmacopeia has implemented first globally general DNA barcoding method as a tool for authentication given its ability to identify source of the herbal product accurately compared to traditional methods. (Heinrich et al. 2018; Sgamma et al. 2018).

DNA barcoding with HRM (High resolution melting) is a recently emerged trend in herbal species identification (Mishra et al. 2018; Osathanunkul et al. 2015). In this method, based on the principle of fluorescence, denaturation and renaturation switches on and off accessibility of intercalating dye, enabling fluorescence detection only during duplex formation. A major advantage of Bar-HRM (Barcode HRM) analysis is easy detection of single base variants or species specific differences in a short region of DNA (Sun et al. 2016). Moreover, HRM analysis with its easiness in use, flexibility, low cost, high sensitivity and specificity is an approachable method for authentication of herbal products, food products and bacterial identification (Ganopoulos et al. 2013; Miller et al. 2015; Ongchai et al. 2019; Osathanunkul

et al. 2015; Sun et al. 2016; Tong et al. 2014). The first report on BAR-HRM coupled with DNA barcode was used to discriminate *Sideritis* species (Kalivas et al. 2014). Later, *psbA-trnH* region coupled with HRM was used for authentication of *Radix notoginseng* (Tong et al. 2014). Reduced length of *rbcL* sequence as mini barcodes in HRM provided enough information to identify closely related species (Costa et al. 2016). *rbcL* mini barcodes with redesigned primer showed good discrimination in three medicinal plant species of Acanthaceae family (Osathanunkul et al. 2015). DNA barcoding coupled with HRM was used to discriminate the species composition of *Senna alexandrina* from the market samples (Mishra et al. 2018). Authentication of *Hypericum perforatum* and *Hypericum androsaemum* in herbal infusions was also done using HRM analysis using *matK* as mini barcode (Costa et al. 2016).

Authentication of processed, multi-herbal mixture available in the market is extremely difficult using conventional DNA barcodes. DNA metabarcoding which is a combination of high throughput sequencing and DNA barcoding was introduced to resolve this problem (Dormontt et al. 2018). It enables multi-taxa identification using total DNA from complex mixture (Seethapathy et al. 2019; Staats et al. 2016; Zhang et al. 2020). DNA metabarcoding is applicable for identification of plant diversity in the available market products and also to scrutinise the level of inconsistency detected in plant species based on the label claims of marketed products (Ivanova et al. 2016; Raclariu et al. 2017a, b; Seethapathy et al. 2019). Metabarcoding analysis of market samples of *Echinacea* species, *Hypericum perforatum*, *Veronica officinalis* and *Pueraria montana* showed wide spread adulteration (Raclariu et al. 2017a, b; Zhang et al. 2020). The composition of 15 highly processed Traditional Chinese Medicine using DNA metabarcoding revealed the presence of adulterant species (Orchid species, *Decalepis*, and *Nepenthes* species) which are included in CITES appendices I and II (Coghlan et al. 2012). DNA metabarcoding can be used for qualitative evaluation of the herbal formulations, whereas in quantitative analysis, combination of pharmacognostic, pharmacovigilance and analytical methods are inevitable for authentication of herbal species and products (Heinrich et al. 2018; Mishra et al. 2016; Parveen et al. 2016; Pawar et al. 2017; Schmiderer et al. 2017).

Integrated approach

Currently, quality control and safety analysis of herbal drugs and mixtures are progressing in a comprehensive and integrated direction. Conventional and single tier approaches are in the phase of decline and nowadays focus has been shifted towards integrated approaches. Integrated method is a combination of two or more diverse techniques which are

capable of authenticating a species more precisely. Recently, it is shown that in addition to adulteration, lack of potent principles in the processed products could also be a serious threat to Ayurveda or traditional system of medicine (Palhares et al. 2015). The efficiency of integrated approach involving DNA barcoding and HPTLC was demonstrated in the analysis with 257 samples derived from 8 species recommended by WHO (Palhares et al. 2014). A combination of DNA barcoding and NMR was also performed in species adulteration of *Garcinia* species and *Sarca asoca* (Urumarudappa et al. 2016; Seethapathy et al. 2018). For the conservation and proper use of Brazilian quinas, a comprehensive system of chemical, biological and molecular methods have been used (Palhares et al. 2015). Comprehensive approach of DNA metabarcoding, TLC and HPLC–MS was carried out for the detection of substitution/adulteration of *Hypericum perforatum* (Raclariu et al. 2017a, b). Authentication of *Marsdenia suaveolens*, was also successfully done using a multi-tier approach of DNA barcoding coupled with TLC and HPLC (Yu et al. 2018). Integrated approach would be a future promising tool for accurate and reliable qualitative/quantitative authentication of medicinal plants and products.

Bioprospecting: a future perspective for natural resources

The global resurgence in the use of herbal remedies has increased the demand for medicinal plants and herbal products. The huge market demand has resulted in over exploitation of the species as most of them are being consumed and consequently exhausted at a much faster rate than it is being replenished. To ensure safety and quality of ayurvedic formulations, modern techniques which guarantee sustainable management and survival of medicinal plants is necessary (Shanmuganandhan et al. 2016). Bioprospecting could be a major breakthrough in this regard, which utilizes the traditional knowledge through ethno-pharmacological investigations for sustainable use of natural resources (Rastogi and Kaphle 2011; Sharma et al. 2013; Purkayastha 2016; Hemant Sood 2020). The development of modern techniques such as genetic engineering, advancements in culture techniques and fermentation has led to the large-scale biosynthesis of highly efficient bioactive natural products (Chen et al. 2018). Thus, prospecting of diverse traditional knowledge also contributes to a valuable lead in developing novel drugs/pharmaceuticals or phytochemicals or genes of great industrial value (Efferth et al. 2016; Pushpangadan et al. 2018). Bioprospecting has been effectively utilized for the large scale production of medicinally active compounds such as artemisinin, crofelemer, prostratin, vinblastine and vincristine (Cox and King 2013). Furthermore, a multi-omic approach involving genomics, proteomics, metabolomics and transcriptomics

would also enable us to identify the potential genes involved in the biosynthetic pathway and upscaling of secondary metabolite production through upregulation/downregulation of the specific genes involved (Medema and Fischbach 2015; Rai et al. 2017). Thus, integration of innovative techniques along with broader perspective of Ayurveda could confine the resource limitation and extensive utilization of natural resources.

Conclusion

Most herbal drugs available in the market are sourced from the wild through supply chains in the formal or informal sectors. The unscientific collection and overexploitation without evaluating the resource abundance lead to endangerment of the potent species in the natural habitat. CITES recommendations without harming the biodiversity is apparently ignored while performing species collection. The resource limitation owing to escalating demand leads to adulteration/substitution with plants/plant parts of inferior properties. Therefore, to ensure safety and quality of ayurvedic formulations, standard techniques in practice warrant more consistency and precision. Herbal drugs, its substituents and adulterants have been studied at length using various techniques but an ideal strategy to safeguard its utility is yet to be defined. Most of the methods implemented so far have focused on single tier approach which may not to be feasible to address this challenging issue. Therefore, a multi-tier approach integrating the already existing as well as advanced techniques would be a better alternative. Raw drugs available in the market need to be analysed critically prior to its processing into formulations. Along with the recommended standard organoleptic and analytical methods in raw drug authentication, an integrated approach involving a molecular tool can strengthen the existing practice of quality checking. Recommendation of a universal tool may not be practical in herbal industry as the analytical methodology solely depends on the type of raw material and the product derived. Though ayurvedic medicine has gained much popularity in India and all around the world, proper certification agencies are yet to be established. Herbal medicine once formulated is easily available to the public through various portals, where no mention of any clinical trials or proper way of administration is cited. It is, therefore, important to bring forth a statutory body to monitor proper collection, processing, production and sale of herbal medicine. Concurrently, scientific management, restoration and conservation measures along with bioprospecting of natural resources are being acclaimed for the biological conservation, which should be given utmost priority to augment the depletion of wild resources as well as to meet the rapidly increasing demand of the herbal industries.

Acknowledgements The authors acknowledge the financial support provided By National Medicinal Plant Board (NMPB), Ministry of AYUSH, Government of India (F.No. Z.18017/187/CSS/R&D/KE-01/2016-17-NMPB-IV A).

Author contribution SAD: Writing of Review article. RJ: Writing of Review Article. RU: Writing of Review Article.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interests.

References

- Abubakar BM, Salleh FM, Omar S, Wagiran A (2018) Assessing product adulteration of *Eurycoma longifolia* (Tongkat Ali) herbal medicinal product using DNA barcoding and HPLC analysis. *Pharm Biol* 56:368–377. <https://doi.org/10.1080/13880209.2018.1479869>
- Araya M, Garcia S, Gonzalez Teuber M (2018) Rapid identification and simultaneous quantification of aristolochic acids by HPLC-DAD and confirmations by MS in *Aristolochia chilensis* using a limited biomass. *J Anal Methods Chem*. <https://doi.org/10.1155/2018/5036542>
- Bangladesh Foreign Trade Institute (2016) A study on sector-based need assessment of business promotion council- herbal products and medicinal plants
- Bansal A, Chhabra V, Rawal RK, Sharma S (2014) Chemometrics: a new scenario in herbal drug standardization. *J Pharm Anal* 4:223–233. <https://doi.org/10.1016/j.jppha.2013.12.001>
- Boligon AA, Piana M, Kubiça TF, Mario DN, Dalmolin TV, Bonez PC, Athayde ML (2015) HPLC analysis and antimicrobial, anti-mycobacterial and antiviral activities of *Tabernaemontana catharinensis*. *J Appl Biomed* 13:7–18. <https://doi.org/10.1016/j.jab.2014.01.004>
- Burgess KS, Fazekas AJ, Kesanakurti PR, Graham SW, Husband BC, Newmaster SG, Barrett SCH (2011) Discriminating plant species in a local temperate flora using the *rbcL*+*matK* DNA barcode. *Methods Ecol Evol* 2:333–340. <https://doi.org/10.1111/j.2041-210X.2011.00092.x>
- Cao L, Li SX, Wei BY, Huang D, Xu F, Tong ZY (2010) Optimizing RAPD reaction system and authentic genuineness related genetic background of *Fructus Evodia*. *Chin Tradit Herb Drugs* 41:975–978
- CBOL Plant Working Group (2009) A DNA barcode for land plants. *Proc Natl Acad Sci U S A* 106:12794–12797
- Chanda S (2014) Importance of pharmacognostic study of medicinal plants: an overview. *J Pharmacog Phytochem* 2:69–73
- Chembath A, Balasundaran M, Sujanal P (2012) Phylogenetic relationships of *Santalum album* and its adulterants as inferred from nuclear DNA sequences. *Int J Agric For* 2:150–156
- Chen S, Yao H, Han J, Liu C, Song J, Shi L, Ma X, Zhu Y, Gao T, Pang X, Luo K, Li Y, Li X, Jia X, Leon C (2010) Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS ONE* 5:1. <https://doi.org/10.1371/journal.pone.0008613>
- Chen G, Sun W, Wang X, Kongkiatpaiboon S, Cai X (2018) Conserving threatened widespread species: a case study using a traditional medicinal plant in Asia. *Biodivers Conserv*. <https://doi.org/10.1007/s10531-018-1648-1>
- Chothani DL, Patel MB, Mishra SH (2012) HPTLC fingerprint profile and isolation of marker compound of *Ruellia tuberosa*. *Chromatogr Res Int* 1:6. <https://doi.org/10.1155/2012/180103>
- Chunekara KC, Pandey G (2010). Bhavaprakash Nighantu of Bhavamishra. Varanasi. Chaukhambha Bharti Academy 159–166
- Coghlan ML, Haile J, Houston J, Murray DC, White NE, Mool huijzen P (2012) Deep sequencing of plant and animal DNA contained within traditional Chinese medicines reveals legality issues and health safety concerns. *PLoS Genet* 8:e1002657. <https://doi.org/10.1371/journal.pgen.1002657>
- Costa J, Campos B, Amaral JS, Nunes ME, Oliveira MBPP, Mafra I (2016) HRM analysis targeting ITS1 and *matK* loci as potential DNA mini-barcodes for the authentication of *Hypericum perforatum* and *Hypericum androsaemum* in herbal infusions. *Food Control* 61:105–114. <https://doi.org/10.1016/j.foodcont.2015.09.035>
- Cox PA, King S (2013) Encyclopedia of Biodiversity (Second edition)
- Dev SA, Muralidharan EM, Sujanal P, Balasundaran M (2013) Identification of market adulterants in East Indian sandalwood using DNA barcoding. *Ann Forest Sci* 71:517–522. <https://doi.org/10.1007/s13595-013-0354-0>
- Dhanya K, Sasikumar B (2010) Molecular marker based adulteration detection in traded food and agricultural commodities of plant origin with special reference to spices. *Curr Trends Biotechnol Pharm* 4:454–489
- Diao Y, Lin XM, Liao CL, Tang CZ, Chen ZJ, Hu ZL (2009) Authentication of *Panax ginseng* from its adulterants by PCR-RFLP and ARMS. *Planta Med* 75:557–560. <https://doi.org/10.1055/s-0029-1185321>
- Doebley J, Durbin M, Golenberg EM, Clegg M, Ma DP (1990) Evolutionary analysis of the large subunit of carboxylase (*RbcL*) nucleotide sequence among the grasses (Gramineae). *Evolution* 44:1097–1108. <https://doi.org/10.2307/2409569>
- Dormont EE, van Dijk K, Bell L, Biffin E, Breed MF, Byrne M, Lowe AJ (2018) Advancing DNA barcoding and metabarcoding applications for plants requires systematic analysis of herbarium collections—an Australian perspective. *Front Ecol Evol* 6:134. <https://doi.org/10.3389/fevo.2018.00134>
- Dutta S, Gibore RN, Sharma PK (2018) Pharmacovigilance: a need in ayurvedic medicine system. *Int J Green Pharm* 12:1
- Efferth T, Banerjee M, Paul NW, Abdelfatah S, Arend J, Elhassan G, Titinchi SJJ (2016) Biopiracy of natural products and good bioprospecting practice. *Phytomedicine* 23(2):166–173. <https://doi.org/10.1016/j.phymed.2015.12.006>
- Fazekas AJ, Burgess KS, Kesanakurti PR, Graham SW, Newmaster SG, Husband BC, Percy DM, Hajibabaei M, Barrett SC (2008) Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. *PLoS ONE* 3:e2802. <https://doi.org/10.1371/journal.pone.0002802>
- Ganesan A, Marichamy K, Thagamariappan J (2016) Development of medicinal plants sector in India—an empirical study. *Int J Eng Technol Manag Appl Sci* 4:82–89
- Ganie SH, Upadhyay P, Das S, Prasad Sharma M (2015) Authentication of medicinal plants by DNA markers. *Plant Gene* 4:83–99. <https://doi.org/10.1016/j.plgene.2015.10.002>
- Ganopoulos I, Bazakas C, Madesis P, Kalaitzis P, Tsaftaris A (2013) Barcode DNA high-resolution melting (Bar-HRM) analysis as a novel close-tubed and accurate tool for olive oil forensic use. *J Sci Food Agric* 93:2281. <https://doi.org/10.1002/jsfa.6040>
- Ghorbani A, Saeedi Y, De Boer HJ (2017) Unidentifiable by morphology: DNA barcoding of plant material in local markets in Iran. *PLoS ONE* 12:e0175722. <https://doi.org/10.1371/journal.pone.0175722>
- Goraya GS, Ved DK (2017) Medicinal plants in India: an assessment of their demand and supply. Ministry of AYUSH, Dehradun
- Gupta AK, Tandon N, Sharma M (2006) New Delhi: Indian Council of Medicinal Research. Quality Standards of Indian Medicinal Plants; p. 1. 89, 169, 204
- Han J, Pang X, Liao B, Yao H, Song J, Chen S (2016) An authentication survey of herbal medicines from markets in China using

- DNA barcoding. *Sci Rep* 6:18723. <https://doi.org/10.1038/srep18723>
- Hao DC, Chen SL, Xiao PG, Peng Y (2010) Authentication of medicinal plants by DNA-based markers and genomics. *Chin Herb Med* 2:250–261
- Hebert PDN, Cywinska A, Ball SL, de Waard JR (2003) Biological identifications through DNA barcodes. *Proc Biol Sci* 270:313–322. <https://doi.org/10.1098/rspb.2002.2218>
- Hegde PL (2018) A review on relevance and impact of pratinidhi dravyas in chikitsa. *Int J Res Ayurveda Pharm* 9(5):15–18. <https://doi.org/10.7897/2277-4343.095146>
- Heinrich M, Lardos A, Leonti M, Weckerle C, Willcox M, Apple Quist W, Stafford G (2018) Best practice in research: Consensus Statement on Ethno pharmacological Field Studies—ConSEFS. *J Ethnopharmacol* 211:329–339
- Hemant Sood (2020) Production of medicinal compounds from endangered and commercially important medicinal plants through cell and tissue culture technology for herbal industry
- Hidalgo O, Garnatje T, Susanna A, Mathez J (2004) Phylogeny of Valerianaceae based on *matK* and ITS markers, with reference to *matK* individual polymorphism. *Ann Bot* 93:283–293. <https://doi.org/10.1093/aob/mch042>
- Hollingsworth PM (2011) Refining the DNA barcode for land Plants. *Proc Natl Acad Sci U S A* 108:19451–19452. <https://doi.org/10.1073/pnas.0905845106>
- 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>
- Hong YT, Fu LS, Chung LH, Hung SC, Huang YT, Chi CS (2006) Fanconi's syndrome, interstitial fibrosis and renal failure by aristolochic acid in Chinese herbs. *Pediatr Nephrol* 21:577–579. <https://doi.org/10.1007/s00467-006-0017-6>
- Ibrahim EA, Wang M, Radwan MM, Wanas AS, Majumdar CG, Avula B, Wang YH, Khan IA, Chandra S, Lata H, Hadad GM, Abdel Salam RA, Ibrahim AK, Ahmed SA, ElSohly MA (2019) Analysis of Terpenes in *Cannabis sativa* L. Using GC/MS: method development, validation, and application. *Planta Med* 24:2302. <https://doi.org/10.1055/a-0828-8387>
- Ismail NZ, Arsad H, Samian MR, Hamdan MR, Othman AS (2008) Assessment of three plastid DNA barcode markers for identification of *Clinacanthus nutans* (Acanthaceae). *3 Biotech* 8:62. <https://doi.org/10.1007/s13205-018-1092-7>
- Ivanova NV, Kuzmina ML, Braukmann TWA, Borisenko AV, Zakharov EV (2016) Authentication of herbal supplements using next-generation sequencing. *PLoS ONE* 11:e0156426. <https://doi.org/10.1371/journal.pone.0156426>
- Jadot I, Declèves A, Nortier J, Caron N (2017) An integrated view of aristolochic acid nephropathy: update of the literature. *Int J Mol Sci* 18:297. <https://doi.org/10.3390/ijms18020297>
- Kalivas A, Ganopoulos I, Xanthopoulou A, Chatzopoulou P, Tsafaris A, Madesis P (2014) DNA barcode ITS2 coupled with high resolution melting (HRM) analysis for taxonomic identification of *Sideritis* species growing in Greece. *Mol Biol Rep* 41:5147–5155. <https://doi.org/10.1007/s11033-014-3381-5>
- Kamboj A, Saluja AK (2017) Development of validated HPTLC method for quantification of stigma sterol from leaf and stem of *Bryophyllum pinnatum*. *Arab J Chem* 10:644–650. <https://doi.org/10.1016/j.arabjc.2013.10.006>
- Karthika K, Paulsamy S (2015) TLC and HPTLC fingerprints of various secondary metabolites in the stem of the traditional medicinal climber, *Solena amplexicaulis*. *Indian J Pharm Sci* 77:111–116. <https://doi.org/10.4103/0250-474X.151591>
- Kaur T, Bhat R, Vyas D (2016) Effect of contrasting climates on antioxidant and bioactive constituents in five medicinal herbs in Western Himalayas. *J Mt Sci* 13:484–492. <https://doi.org/10.1007/s11629-014-3380-y>
- Kiran U, Khan S, Mirza KJ, Ram M, Abdin MZ (2010) SCAR markers: a potential tool for authentication of herbal drugs. *Fitoterapia* 81:969–976. <https://doi.org/10.1016/j.fitote.2010.08.002>
- Krawczyk K, Szczecińska M, Sawicki J (2013) Evaluation of 11 single-locus and seven multilocus DNA barcodes in *Lamium* L. (Lamiaceae). *Mol Ecol Resour* 14:272–285. <https://doi.org/10.1111/1755-0998.12175>
- 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
- Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH (2005) Use of DNA barcodes to identify flowering plants. *Proc Natl Acad Sci U S A* 102:8369–8374. <https://doi.org/10.1073/pnas.0503123102>
- Kumar JS, Krishna V, Seethapathy GS, Ganesan R, Ravikanth G, Shaanker RU (2018) Assessment of adulteration in raw herbal trade of important medicinal plants of India using DNA barcoding. *3. Biotech* 8:1–8. <https://doi.org/10.1007/s13205-018-1169-3>
- Kumar JS, Ramakrishnan M, Seethapathy GS, Krishna V, Uma Shaanker R, Ravikanth G (2020) DNA barcoding of *Momordica* species and assessment of adulteration in *Momordica* herbal products, an anti-diabetic drug. *Plant Gene* 22:100227. <https://doi.org/10.1016/j.plgene.2020.100227>
- Li SL, Lin G, Fu PP, Chan CL, Li M, Jiang ZH, Zhao ZZ (2008) Identification of five hepatotoxic pyrrolizidine alkaloids in a commonly used traditional Chinese medicinal herb, *Herba Senecionis Scandentis* (Qianliguang). *Rapid Commun Mass Spectrom* 22:591–602. <https://doi.org/10.1002/rcm.3398>
- 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 influence of geographical and climatic factors. *J For Res*. <https://doi.org/10.1007/s11676-020-01124-8>
- Little DP (2014) A DNA mini-barcode for land plants. *Mol Ecol Res* 14(3):437–446
- Liu W, Yin D, Li N, Hou X, Wang D, Li D, Liu J (2016) Influence of environmental factors on the active substance production and antioxidant activity in *Potentilla fruticosa* L. and its quality assessment. *Sci Rep* 6(1):28591. <https://doi.org/10.1038/srep28591>
- Malik S, Priya A, Babbar S (2018) Employing barcoding markers to authenticate selected endangered medicinal plants traded in Indian markets. *Physiol Mol Biol Plant* 25:327–337. <https://doi.org/10.1007/s12298-018-0610-8>
- Mariswamy Y, Gnajar WE, Johnson M (2011) Chromatographic fingerprint analysis of steroids in *Aerva lanata* L by HPTLC technique. *Asian Pac J Trop Biomed* 1:428–433. [https://doi.org/10.1016/S2221-1691\(11\)60094-4](https://doi.org/10.1016/S2221-1691(11)60094-4)
- Medema MH, Fischbach MA (2015) Computational approaches to natural product discovery. *Nat Chem Biol* 11:639–648. <https://doi.org/10.1038/nchembio.1884>
- Meena AK, Narasimhaji CV, Rekha P, Velvizhi D (2018) Comparative preliminary phytochemical and HPTLC fingerprint profile studies of two cinnamon species commonly used in ASU formulations. *Asian J Res Chem* 11:344–350. <https://doi.org/10.5958/0974-4150.2018.00062.7>
- 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. <https://doi.org/10.1016/j.jep.2013.06.028>
- Miller M, Zorn J, Brielmeier M (2015) High-resolution melting curve analysis for identification of Pasteurellaceae species in experimental animal facilities. *PLoS ONE* 10:e0142560. <https://doi.org/10.1371/journal.pone.0142560>
- Mishra SN (ed) (2007) Bhaishajyaratnavali of Kaviraj Govind Das, 1st edn. Chaukhamba Surbharti Prakashan, Varanasi, pp 80–82
- 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. *Plant Biotechnol J* 1:8–21. <https://doi.org/10.1111/pbi.12419>
- Mishra P, Shukla AK, Sundaresan V (2018) Candidate DNA barcode tags combined with high resolution melting (Bar-HRM) curve analysis for authentication of *Senna alexandrina* Mill. with validation in crude drugs. *Front Plant Sci*. <https://doi.org/10.3389/fpls.2018.00283>
- Mori CC, Bagatini IL, Garcia da Silva T, Parrish CC, Henriques Vieira AA (2018) Use of fatty acids in the chemotaxonomy of the family Selenastraceae (*Sphaeropleales*, *Chlorophyceae*). *Phytochemistry* 151:916. <https://doi.org/10.1016/j.phytochem.2018.03.011>
- Nagarajan M, Kuruvilla GR, Kumar KS, Venkatasubramanian P (2015) Abhava pratindhi dravya: a comparative phytochemistry of *Ativisha*, *Musta* and related species. *J Ayurveda Integr Med* 6:53–63
- 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. <https://doi.org/10.1139/B06-047>
- Newmaster SG, Grguric M, Shanmughanandhan M, Ramalingam S, Ragupathy S (2013) DNA barcoding detects contamination and substitution in North American herbal products. *BMC Med* 11:222–235. <https://doi.org/10.1186/1741-7015-11-222>
- Nickavar B, Rezaee J, Nickavar A (2016) Effect-directed analysis for the antioxidant compound in *Salvia verticillata*. *Iran J Pharm Res* 15:241–246
- Nirali BJ, Shankar MB (2015) Global market demand of herbal drug formulation. *Int J Ayu Pharm Chem* 4:59–65
- Nybohm H, Weising K, Rotter B (2014) DNA fingerprinting in botany: past, present, future. *Investig Genet* 5:1. <https://doi.org/10.1186/2041-2223-5-1>
- Ongchai S, Chokchaitaweek C, Kongdang P, Chomdej S, Buddhachit K (2019) In vitro chondro protective potential of *Senna alata* and *Senna tora* in porcine cartilage explants and their species differentiation by DNA barcoding-high resolution melting (Bar-HRM) analysis. *PLoS ONE* 14(4):e0215664. <https://doi.org/10.1371/journal.pone.0215664>
- Osathanunkul M, Madesis P, de Boer H (2015) Bar-HRM for authentication of plant-based medicines: evaluation of three medicinal products derived from Acanthaceae Species. *PLoS ONE* 26:10. <https://doi.org/10.1371/journal.pone.0128476>
- Ouarghidi A, Powell B, Martin GJ, Hugo Abbad A (2012) Species substitution in medicinal roots and possible implications for toxicity in Morocco. *Econ Bot* 66:370–382. <https://doi.org/10.1007/s12231-012-9215-2>
- 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 Ethno pharmacol* 155:815–822. <https://doi.org/10.1016/j.jep.2014.06.040>
- Palhares RM, Drummond MG, Alves Figueiredo Brasil BDS, 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>
- Parveen I, Gafner S, Techen N, Murch SJ, Khan IA (2016) DNA barcoding for the identification of botanicals in herbal medicine and dietary supplements: strengths and limitations. *Planta Med* 82:1225–1235. <https://doi.org/10.1055/s-0042-111208>
- Patel R, Patel K (2016) Significance of various chromatographic techniques in herbal drug analysis. *J Chem Pharm Res* 8:877–882
- Patil DA, Patil MV (2010) Diversity and concerns of Indian medicinal plants: a scenario. *J Ecobiotechnol* 2:14–20
- Pawar RS, Handy SM, Cheng R, Shyong N, Grundel E (2017) Assessment of the authenticity of herbal dietary supplements: comparison of chemical and DNA barcoding methods. *Planta Med* 83:921–936. <https://doi.org/10.1055/s-0043-107881>
- Poornima B (2010) Adulteration and substitution in herbal drugs a critical analysis. *Int J Res Ayurveda Pharm* 1(1):8–12
- Purkayastha J (2016) Emerging trends in sustainable bioprospecting of bioresources. *Bioprospect Indigen Bioresour North East India*. https://doi.org/10.1007/978-981-10-0620-3_1
- Pushpangadan P, George V, Parambil Ijnu T, Ambika Chithra M (2018) Biodiversity, bioprospecting, traditional knowledge, sustainable development and value added products: a review. *J Tradit Med Clin Naturop*. <https://doi.org/10.4172/2573-4555.1000256>
- Raclariu AC, Mocan A, Popa MO, Vlase L, Ichim MC, Crisan G (2017) *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>
- Raclariu AC, Paltinean R, Vlase L, Labarre A, Manzanilla V, Ichim MC (2017) Comparative authentication of *Hypericum perforatum* herbal products using DNA metabarcoding. *TLC and HPLC-MS Sci Rep* 7:1291. <https://doi.org/10.1038/s41598-017-01389-w>
- Raclariu AC, Tebrencu CE, Ichim MC, Ciuperca OT, Brysting AK, de Boer H (2018) What's in the box? Authentication of Echinacea herbal products using DNA metabarcoding and HPTLC. *Phytomedicine* 44:32–38. <https://doi.org/10.1016/j.phymed.2018.03.058>
- Rai A, Saito K, Yamazaki M (2017) Integrated omics analysis of specialized metabolism in medicinal plants. *Plant J* 90(4):764–787
- Rastogi S, Kaphle K (2011) Sustainable traditional medicine: taking the inspirations from ancient veterinary science. *Evid Based Complement Alternat Med*. <https://doi.org/10.1093/ecam/nen071>
- Sajjad ES (2012) Analysis of the essential oil of two cultivated Basil (*Ocimum basilicum* L.) from Iran. *DARU J Pharm Sci* 14:128–130
- Sastri A (2001) Bhaishajya Ratnavali. Varanasi: Chaukhamba Sanskrit Sansthan 25
- Schmiderer C, Lukas B, Ruzicka J, Novak J (2017) What else is in *Salvia officinalis* folium? Comprehensive species identification of plant raw material by DNA metabarcoding. *Planta Med* 84:428–433. <https://doi.org/10.1055/s-0043-121470>
- Seal T (2016) Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, *Sonchus arvensis* and *Oenantho linearis* of North-Eastern region in India. *J Appl Pharm Sci* 6:157–166. <https://doi.org/10.7324/JAPS.2016.60225>
- 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, Wangenstein 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>
- Seethapathy GS, Raclariu-Manolica AC, Anmarkrud JA, Wangenstein H, de Boer HJ (2019) DNA Metabarcoding authentication of ayurvedic herbal products on the European market raises concerns of quality and fidelity. *Front Plant Sci* 10:68. <https://doi.org/10.3389/fpls.2019.00068>
- Sgamma T, Masiero E, Mali P, Mahat M, Slater A (2018) Sequence-specific detection of aristolochia DNA—a simple test for contamination of herbal products. *Front Plant Sci* 9:1828. <https://doi.org/10.3389/fpls.2018.01828>
- Shanmughanandhan D, Regupathy S, Newmaster SG, Mohanasundaram S, Sathiskumar R (2016) Estimating herbal product authentication and adulteration in India using a vouchered, DNA-based biological reference material library. *Drug Saf* 30:1211–1217. <https://doi.org/10.1007/s40264-016-0459-0>
- Sharma RK, Gupta P, Sharma V, Sood A, Mohapatra T, Ahuja PS (2008) Evaluation of rice and sugarcane SSR markers for phylogenetic and genetic diversity analyses in bamboo. *Genome* 51:91–103. <https://doi.org/10.1139/G07-101>
- Sharma C, Rajendar K, Kumari T, Arya KR (2013) Indian traditional therapies and bio-prospecting: their role in drug development research. *Int J Pharm Sci Res* 5(3):730–741
- Sharma A, Kumar N, Mishra IG (2018) Role of molecular marker in the genetic improvement of the medicinal and aromatic plants. In: *Biotechnological approaches for medicinal and aromatic plants*. Kumar N (Eds.). Springer, Singapore 557–567. <https://doi.org/10.1007/978-981-13-0535-1>
- Shastri LY (2005) *Chaukambha Sanskrit Sansthan Prakasana* 171.
- Shawky E, Selim D (2018) Rapid authentication and quality evaluation of *Cinnamomum verum* powder using near-infrared spectroscopy and multivariate analyses. *Planta Med*. <https://doi.org/10.1055/a-0654-5468>
- Singh P, Bajpai M, Prakash O, Tiwari R, Mathur SC, Singh RM, Singh GH (2018) High performance thin layer chromatographic method for the determination of Cinnamaldehyde in *Cinnamomum zeylanicum* bark powder. *JNR* 8:179–182
- Singh A, Sharma H, Srivastav B (2020) Markers Assessment in Triphala (Batch-I, II & III) and its ingredients from different geographical locations of India through TPC, TFC, HPLC & HPTLC. *Curr Tradit Med* 6:106–120
- Siracusa L, Napoli E, Tuttolomondo T, Licata M, La Bella S, Gennaro MC, Leto C, Sarno M, Sperlinga E, Ruberto G (2018) A two year bio-agronomic and chemotaxonomic evaluation of wild Sicilian. *Chem Biodivers* 16:e1800575
- Srirama R, Santhosh Kumar JU, Seethapathy GS, Newmaster SG, Ragupathy S, Ganeshiah KN, Uma Shaanker R, 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>
- Staats M, Arulandhu AJ, Gravendeel B, Holst-Jensen A, Scholtens I, Peelen T, Prins TW, Kok E (2016) Advances in DNA metabarcoding for food and wildlife forensic species identification. *Anal Bioanal Chem* 408:4615–4630. <https://doi.org/10.1007/s00216-016-9595-8>
- Sucher NJ, Carles MC (2008) Genome-based approaches to the authentication of medicinal plants. *Planta Med* 74:603–623. <https://doi.org/10.1055/s-2008-1074517>
- Sun W, Li J, Xiong C, Zhao B, Chen S (2016) The potential power of bar-HRM technology in herbal medicine identification. *Front Plant Sci* 7:367. <https://doi.org/10.3389/fpls.2016.00367>
- Swetha VP, Parvathy VA, Sheeja TE, Sasikumar B (2017) Authentication of *Myristica fragrans* Houtt. using DNA barcoding. *Food Control* 73:1010–1015. <https://doi.org/10.1016/j.foodc.2016.10.004>
- Syed YH, Khan M (2016) Chromatographic profiling of ellagic acid in *Woodfordia fruticosa* flowers and their gastroprotective potential in ethanol-induced ulcers in rats. *Pharmacognosy Res* 8:5–11. <https://doi.org/10.4103/0974-8490.178649>
- Tamhankar S, Ghate V, Raut A, Rajput B (2009) Molecular profiling of Chirayat complex using inter simple sequence repeat (ISSR) markers. *Planta Med* 75:1266–1270. <https://doi.org/10.1055/s-0029-1185543>
- Techen N, Parveen I, Pan Z, Khan IA (2014) DNA barcoding of medicinal plant material for identification. *Curr Opin Biotechnol* 25:103–110. <https://doi.org/10.1016/j.copbio.2013.09.010>
- Tharachand C, Immanuel Selvaraj C, Mythili MN (2012) Molecular markers in characterization of medicinal plants: an overview. *Res Plant Biol* 2:1–12
- Thatte U, Bhalariao S (2008) Pharmacovigilance of ayurvedic medicines in India. *Indian J Pharmacol* 40:S10–12
- Tong YR, Jiang C, Huang LQ, Cui ZH, Yuan Y (2014) Molecular identification of Radix Notoginseng powder by DNA melt curve analysis. *Chin J Pharm Anal* 34:1384–1390
- 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. <https://doi.org/10.1007/s00414-016-1436-y>
- US Food and Drug Administration (2001) Dietary supplements: aristolochic acid
- Vadivel V, Anand P, Monajkumar S, Rajalakshmi P, Brindha P (2018) Chemical fingerprints of an Indian traditional herbal drug Talisapatra (*Abies webbiana*) and comparison with English yew (*Taxus baccata*). *Int J Pharmacog Phytochem Res* 10:84–91
- Vassou SL, Kusuma G, Parani M (2015) DNA barcoding for species identification from dried and powdered plant parts: a case study with authentication of the raw drug market samples of *Sida cordifolia*. *Gene* 559:86–93. <https://doi.org/10.1016/j.gene.2015.01.025>
- Ved DK and Goraya GS (2007) Demand and supply of medicinal plants in India, NMPB, New Delhi and FRLHT, Bangalore, India
- Vohra P, Khera KS (2013) DNA barcoding: current advances and future prospects—a review. *Asian J Biol Life Sci* 2:185–189
- Walker KM, Apple Quist WL (2012) Adulteration of selected unprocessed Botanicals in the US retail herbal trade. *Econ Bot* 66:321–327. <https://doi.org/10.1007/s12231-012-9211-6>
- Yang SS, Chu P, Lin YF, Chen A, Lin SH (2002) Aristolochic acid-induced Fanconi's syndrome and nephropathy presenting as hypokalemic paralysis. *Am J Kidney Dis*. <https://doi.org/10.1053/ajkd.2002.31425>
- Yao G, Ma W, Huang X, Jia Q, Shen J, Chang Y, He J (2020) Identification and quality evaluation of raw and processed Asarum species using microscopy, DNA barcoding, and gas chromatography-mass spectrometry. *J Anal Methods Chem*. <https://doi.org/10.1155/2020/2690238>
- 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. <https://doi.org/10.1371/journal.pone.0195240>
- Zhang G, Liu J, Gao M, Kong W, Zhao Q, Shi L, Wang Q (2020) Tracing the edible and medicinal plant *Pueraria montana* and its products in the marketplace yields subspecies level distinction using DNA barcoding and DNA metabarcoding. *Front Pharmacol*. <https://doi.org/10.3389/fphar.2020.00336>
- Zhou J, Ouedraogo M, Qu F, Duez P (2013) Potential genotoxicity of traditional Chinese medicinal plants and phytochemicals: an overview. *Phytother Res* 10:1002. <https://doi.org/10.1002/ptr.4942>