

Review article

DNA fingerprinting in industrially important medicinal trees

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Abstract

Herbal medicine has been considered as a promising future medicine for the management of healthcare. The shift "Return back to Nature" has enhanced the consumption of plant-based phytomedicines and other botanicals in recent years. Medicinal plants are considered as rich sources of phytochemical ingredients. With a big gap in the demand and supply, there is heavy exploitation of these resources. The fast depleting are the medicinal trees, which, due to their long gestation, fails to find a place in cultivation. There is a need to encourage cultivation of these species. Traditional breeding and biotechnology can be used in their genetic improvement. Environmental conditions affect the active constituents of medicinal plants and, therefore their activity profiles. Hence, there is a need to understand the geographical variation at the genetic level. DNA-based techniques have been widely used for assessing diversity and authentication of plant species of medicinal importance. This paper describes the demand of some medicinal trees which are in high demand in the Ayurvedic industry, and the application of DNA techniques to understand the geographic distribution, authentication and confirmation of identity of the species.

Keywords : Molecular markers, authentication, genetic variation, medicinal trees

1. Introduction

India prides in practicing some of the very old systems of medicine that continue to be the mainstay of healthcare delivery to a very large segment of its population even today (Manoharachary and Nagaraju, 2016). These Indian Systems of Medicine are finding increasing acceptance globally also. Their continuance is largely dependent upon assured supply of quality herbal raw material. Indian herbal medicine industry recognizes the importance of conservation of these valuable bioresources and encourages their cultivation (Narayana and Johnson, 2017). Nearly, 1200 medicinal plant species are in commercial use by the domestic herbal industry and traditional practitioners (Goraya and Ved, 2017).

The National Medicinal Plants Board in India has been making efforts to strengthen the medicinal plants resource base in the country through conservation and cultivation. Some of the species have been successfully domesticated and brought under cultivation. The Indian Council of Forestry Research and Education (ICFRE) has been making significant contribution to the strengthening of medicinal plants resource base through development of domestication and cultivation protocols in respect of various medicinal plant species of conservation concern.

1.1 Indian medicinal industry

Healthcare market and medical devices market in India is expected to reach US\$ 372 billion and US\$ 11 billion by 2022. The usage of herbal products in Indian households has increased from 69% in

2015 to 77% in 2017 (The Economic Times, 2018). It is predicted that global Ayurvedic market would increase by three folds in 2022 (TOI, 2018) with a contribution of US\$ 9.7 billion compared to that of US\$ 3.4 billion in 2015.

Among various Indian System of Medicine practitioners, the largest share is Ayurveda system contributing 55.4% share (IBEF, 2019). During 2014-15, it was estimated that the domestic and international demand for medicinal plants was 1,95,000 MT and 1,34,500 MT, respectively. Considering the herbal drug market, it is estimated that the total consumption in India for the year 2014-15 was 5,12,000 MT which amounted to trade value of 5,500 crore rupees. There has been approximately nine fold increase in export value from Rs. 345.80 crore to Rs. 3211 crore in 2005-06 to 2014-15 (NMPB, 2019). With this sort of continuous increase in demand for crude drugs and natural products, a big challenge that emerges in this scenario is protecting the highly valued medicinal plant resources from over exploitation and habitat destruction (Srivastava *et al.*, 1996).

1.1.1 Demand for species

Medicinal plants are not only a major resource base for the traditional medicine and herbal industry but also provide livelihood and health security to a large segment of Indian population. The domestic trade of the AYUSH industry is of the order of Rs. 80 to 90 billion (Biradar, 2015). In India, out of 17 to 18 thousand species of flowering plants, considering the folk and documented systems of medicine, more than 7000 species have one or the other medicinal properties. Out of these, 1178 species are traded in the market and 242 species are known to have consumption demand of more than 100 metric tons per year. The number of botanicals traded is estimated to vary from 400 as per the report on medicinal plants commissioned by Planning Commission of India (Anon, 2002) to 1500 as per Jain (1996). However, Bode (2004) mentioned that for

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production of Unani medicines, Hamdard Pharmaceutical Company uses more than 2000 fruits, shrubs, leaves, metals, minerals as well as animals. Due to enormous demand for Ayurveda products, industries have resorted to huge production of herbal products (Nayanabhirama, 2016). Large quantities of plant raw material is needed which is mostly harvested from the wild (Valiathan, 2006; Goraya and Ved, 2017; Joshi *et al.*, 2017).

Though, cultivation of medicinal plants is gaining momentum in the recent years, yet consumption from the wild sources continues to be the first choice. The NMPB report (Ved and Goraya, 2008) reveals that of the 1178 species used, nearly 53% of these species are subjected to destructive harvest while in 2018, the authors report that 582 species (66%) are reported to be harvested destructively (Figure 1).

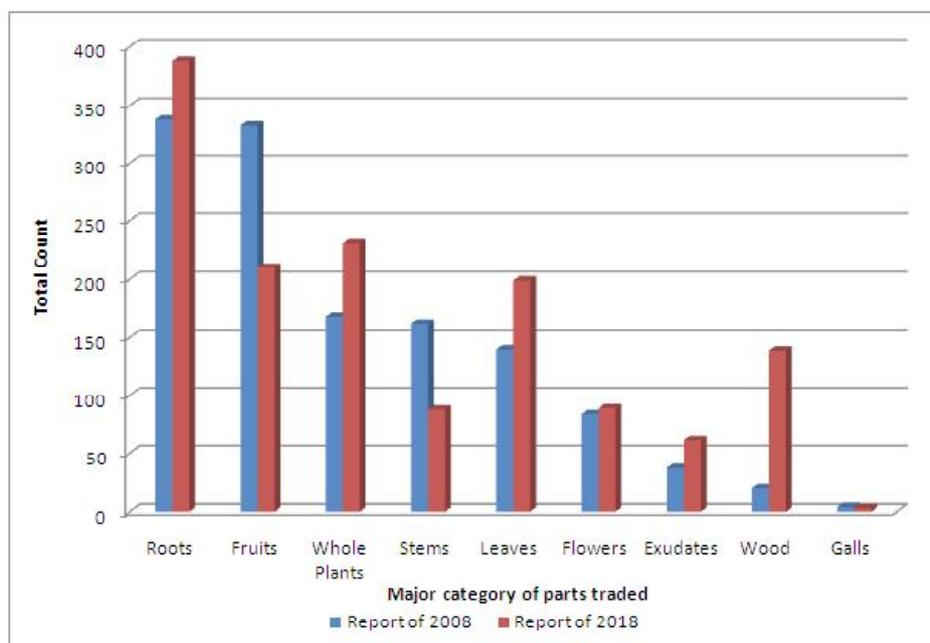


Figure 1: Part-wise distribution of herbal raw drugs in commercial demand in 2008 and 2018

India exports a large number of botanical raw drugs to many countries across the globe. United States of America has been the largest importer of botanical raw drugs from India. An average

annual increase of 11% in export volumes of botanical raw drugs, recorded from 2005 to 2015, reveals significant increase (Figure 2).

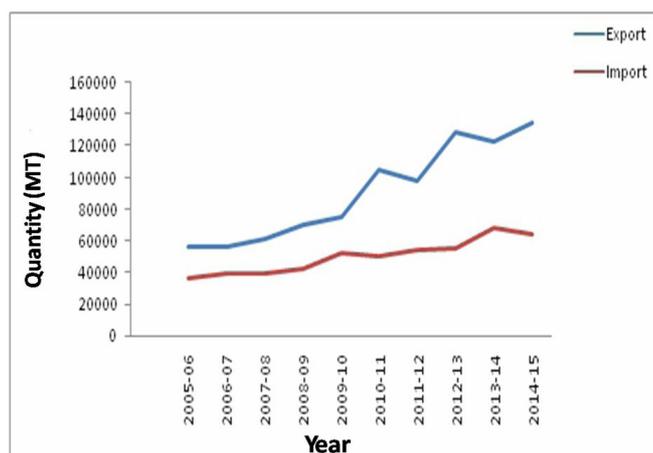


Figure 2: Gross volume of foreign trade of botanical raw drugs (Goraya and Ved, 2017).

1.1.2 Markers

Authenticity of the raw drugs used in various preparations is presently the major concern of the growing herbal sector. Though, the use of acceptable substitutes, in case of non-availability of the original entity has been suggested in classical texts, inexperience of wild gatherers results in collection of look alike species, leading to

adulteration. The other is deliberate mixing of foreign or inferior material in the accepted herbal raw drug with an intent to enhance the returns.

For safety and efficacy of herbal medicine, one of the most essential aspect is the correct identification of the species, proper extraction of constituents and its subsequent quality assurance so that,

reproducible quality of herbal medicine is achieved (Joshi *et al.*, 2004). To attain this, most of pharmacopoeias and regulatory guidelines suggest that the botanical material has to undergo suitable pharmacognostic techniques in the form of micro and macroscopic evaluation along with proper chemical profiling for quality control and standardization (WHO, 1996).

Some of the innovative technologies such as high throughput screening (HTS) and recent advances in extraction, chromatographic techniques like TLC and HPLC, electrophoresis and spectroscopy generally use those chemical markers that may not be active therapeutically. Apart from that, secondary metabolites which are considered as markers may not be stable as they are liable to changes due to environmental factors may also create hindrance in correct identification of active principles or chemical constituents.

To overcome these difficulties, use of ubiquitous molecular markers is gaining prominence. Molecular markers identify plants at genomic level and establish new standards in for quality control of botanicals (Joshi *et al.*, 2004). With the advancement in the field of genetics and molecular biotechnology, for authentication of herbal materials at DNA level, genetic tools are considered to be more reliable (Kumar *et al.*, 2009). For species characterization in medicinal plants, various DNA-based markers are being extensively used now (Shaw *et al.*, 2002; Zhang *et al.*, 2007; Sucher and Carles, 2008). This technology is also useful specially in cases of those species which are frequently adulterated, substituted or in cases where it is difficult to distinguish samples having morphological or chemical similarity.

One of the main issues of chemical fingerprinting is that the signature is strongly influenced by some of the intrinsic as well as external factors such as age of the sample, physiological, environmental factors, cultivation area, harvesting period and process, drying and storage conditions, *etc.* However, DNA is extremely stable and not influenced by external factors, easily recovered from either fresh or dried material and sometimes even from processed biological material. Moreover, the molecules are not tissue specific and are required in very small quantity.

1.1.2.1 Authentication of herbal material on the DNA level

DNA based approaches are useful analytical tools for assessing and assuring the quality of herbs / herbal products (Parveen *et al.*, 2016). Using short and standardized gene regions, known as 'barcodes', DNA barcoding technique is cost effective and suitable for species level identification (Hebert *et al.*, 2003). In India, the herbal drug markets were surveyed, and the authenticity of the herbal drugs has been tested using DNA barcoding.

It was found that raw drugs in Indian herbal markets were not genuine to the tune of 21% (Vassou *et al.*, 2016). Out of 93 herbal products sold in the form of plant powders, capsules and in local stores in India, 60 per cent were adulterated (Shanmuganandhan *et al.*, 2016). Raw drug samples obtained from *Phyllanthus amarus* showed 24 per cent substitution with phenotypically similar species of *Phyllanthus* (Srirama *et al.*, 2010).

Substitution has also been reported in *Cinnamomum verum* by 70% (Swetha *et al.*, 2014); *Myristica fragrans* by 60% (Swetha *et al.*, 2017); *Senna auriculata* by 50%, *Senna tora* by 37% and *Senna alexandrina* (8%) (Seethapathy *et al.*, 2015) and *Sida cordifolia* by 76% (Vassou *et al.*, 2015, 2016).

Studies to authenticate herbal products obtained from various species by combining spectroscopic methods such as NMR along with DNA bar coding or microscopy reported 80% in *Berberis aristata* (Srivastava and Rawat, 2013), 22% in *Piper nigrum* (Parvathy *et al.*, 2014) and 80% adulteration in *Saraca asoca* (Urumarudappa *et al.*, 2016). Various such studies on herbal products have shown concern about the quality and good labeling practices (Veldman *et al.*, 2017; Seethapathy *et al.*, 2019).

1.1.2.2 Diversity analysis

Medicinal plants are the primary raw material for pharmaceutical drugs. They differ in quality depending on the populations. Knowledge about inherent variability is required for conservation, and/or protection of genetic diversity. For optimum genetic improvement and resource management, developing conservation strategies and sustainable utilisation of medicinal plants resources, documenting and understanding the genetic variation within and among the population is a basic step.

It is an accepted fact that due to increased demand for medicinal plants, natural populations are extensively exploited. For its long term survival as well as extensive semi-domestication success, the essential factor needs immediate attention is to maintain sufficient genetic variability. This would also provide options for developing superior genotypes by human interference as well as accommodating natural selection pressures as a result of continuous environmental changes (Barrett and Kohn, 1991).

In India, medicinal plants are gaining popularity and cultivated extensively by farmers, however, it is to be noted that many of the species are still semi-domesticated in nature. Numerous studies have been carried out to understand the impact of cultivation on genetic diversity of crop plant as well as tree species (Bahulikar *et al.*, 2004; Shaanker *et al.*, 2004; Bodare *et al.*, 2013; Harish *et al.*, 2014). However, studies are still to be carried out to understand the changes in genetic structure of various species due to impact of environmental factors like variation in latitude, longitude along with meteorological variables (Britto *et al.*, 2009; Haji *et al.*, 2014; Panda *et al.*, 2015).

Not many medicinal plants have been evaluated for genetic diversity and gene pool wealth. There is no data to support how much of any species can be harvested on a sustainable yield basis-without reducing existing populations. Basic research on reproductive biology, population dynamics and demographics of medicinal species is yet to be conducted (Rao, 2016).

In the last three decades, three classes (Gupta *et al.*, 2001) of markers have been developed. The first generation molecular markers include restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPDs) and their modifications. This was followed by inter simple sequence repeats (ISSR), simple sequence repeats SSRs, amplified fragment length polymorphism (AFLP) and their modified forms which formed the second generation. The current day markers in vogue are ESTs and SNPs - the third generation markers.

First generation markers

RAPD-based molecular markers helped to differentiate different accessions of *Andrographis paniculata* (Fu *et al.*, 2003), *Atractylodes* (Kohjyouma *et al.*, 1997), *Glycirrhiza* (Yamasaki *et al.*, 1994) and

Piper nigrum (Khan *et al.*, 2010). The markers differentiated related species as in the case of *Lycium barabarum* (Zhang *et al.*, 2001), *Scutellaria* (Hosokawa *et al.*, 2000), *Melissa officinalis* (Wolf *et al.*, 1999). The markers were also efficient in identifying adulterants of species like *Annonum villosum* (Wang *et al.*, 2000), *Panax* sp. (Shim *et al.*, 2003), *Cuscuta* (Khan *et al.*, 2010) and *Echinacea* (Nieri *et al.*, 2003). Modifications of the RAPD has been applied to authenticate *Herba* species (Cao *et al.*, 1996; 1997) and *Magnolia officinalis* (Wang *et al.*, 2001). Similarly, RFLP was also used to identify *Fritillaria* (Tsoi *et al.*, 2003; Wang *et al.*, 2005), *Rheum* (Yang *et al.*, 2004) and *Dendrobium* (Li *et al.*, 2005).

Second generation markers

The advent of AFLP not only helped in distinguishing closely related individuals at the sub-species level but also in mapping genes. Species-specific markers were developed to identify different *Swertia* species (Misra *et al.*, 2010). An AFLP map was generated for *Cannabis* (Datwyler *et al.*, 2006). ISSR analysis represented a compromise between AFLP and RAPD. It was used to identify *Cistanche* (Shi *et al.*, 2009), *Fritillaria* (Li *et al.*, 2009), *Salvia* (Song *et al.*, 2010), *Vitex* (Hu *et al.*, 2007), *Cannabis* (Kojoma *et al.*, 2002) and the confirmation of *Dendrobium officinale* (Shen *et al.*, 2006). SSRs were used to identify *Panax ginseng* (Kim *et al.*, 2007), *Acanthopanax senticosus* (Kim *et al.*, 2007), *Dendrobium fimbriatum* (Fan *et al.*, 2009), *Cymbopogon* species (Kumar *et al.*, 2007), *Bupleurum* (Chun *et al.*, 2009) and *Schisandra* (Boqian *et al.*, 2009).

Third generation markers

Sequence characterized amplified region (SCAR) is a PCR based marker, generated from polymorphic regions, differing in size between species. It also permits sample authentication on the basis of SCAR size shifts. SCAR markers are highly efficient and robust. SCAR markers have high detection efficiency as the SCAR primers are able to retrieve a single clear band from the genuine sample, differentiating it from the adulterants. A 320 bp SCAR marker was developed for distinguishing *P. tuberosa* from *Adenia hondala*, *Cycas circinalis* and *Ipomea mauritiana* (Devaiah *et al.*, 2010). SCAR primers were designed to get unique bands for *Embelia ribes*, to identify it from the adulterants (Devaiah *et al.*, 2008).

Phyllanthus amarus was distinguished using SCAR primers from *P. debilis* and *P. urinaria*, which are morphologically similar (Theerakulpisut *et al.*, 2008). Development of SCAR markers have been done for *Zingiber* also (Chavan *et al.*, 2008). Seethapathy *et al.* (2014) authenticated *Aconitum heterophyllum* and *Cyperus rotundus* using nrDNA ITS sequence based SCAR markers.

Cultivar-specific SNP markers and established real-time allele-specific PCR system have been applied for molecular authentication, seed purity testing, and the marker assisted selection of the narrow genetic base of *P. ginseng* cultivars (Wang *et al.*, 2016). To evaluate genetic structure of cultivated varieties and populations in Chamomile, Genotyping-by-sequencing (GBS) was used. The study focussed on genes having larger effect on flowering time and alpha-bisabolol content (Otto *et al.*, 2017). SNP markers in *rbcL* and *matK* genes from the chloroplast genome helped to recognize *C. denticulatum* from other related taxa (Do *et al.*, 2019).

1.1.3 DNA barcoding

DNA barcoding can be an accurate and reliable alternative to morphological identification for biological material (Hebert *et al.*, 2003). It is now regularly used when identification using macroscopic or microscopic methods is difficult. Parvathy *et al.* (2014) used DNA barcodes to detect adulteration in traded black pepper powder while Rai *et al.* (2012) developed DNA barcodes from ITS2 region for Asparagaceae and Asclepiadaceae members.

DNA barcodes have been used for authentication of *Ginkgo biloba* dietary supplements (Little, 2014) and molecular authentication of *Peucedanum praeruptorum* (Zhou *et al.*, 2014). Enan and Ahamed (2014) identified DNA barcodes from plastid *matK* and RNA polymerase for assessing the genetic identity of date cultivars while Chandramohan *et al.* (2013) used the same for molecular characterization of *Croton bonplandianum*. Vassou *et al.* (2015) reported the efficiency of DNA barcoding for species identification from powdered plant parts of *Sida cordifolia* from the raw drug market. Plant barcodes *rbcL*, *matK*, *trnH-psbA*, and ITS2 have been tested in a wide variety of plant species

Table 1: Limitations of genetic markers

| RAPD | RFLPs | SSR | AFLP | ISSR | SCAR | Barcodes |
|--------------------------|-------------------------------------|----------------------------|--|--------------------------|--|---|
| Less reproducible marker | Limited sensitivity of detection | Time consuming | Involves radioactive materials (overcome using fluorescent tags) | Less reproducible marker | Prior knowledge on sequence data essential | Fails to distinguish recently diverged species |
| Dominant marker | Laborious and difficult to automate | Costly | Purified and high molecular weight DNA | Dominant marker | | No single barcode available to distinguish all plants |
| | Time consuming | Occurrence of null alleles | | | | |
| | Involves radioactivity | | | | | |

Source : *Adapted from Ganie *et al.* (2015)

Limitations in the use of molecular markers: Molecular marker analysis involves cost and time. In many cases, significant polymorphism is not detected. Thus, it is not possible to apply them in all plants. Some of the limitations of different markers are listed below (Table1).

2. DNA marker studies in medicinal trees

The present paper reviews the application of different molecular markers in 11 commercially important medicinal trees of India (Table 2). These species are mainly sources from the forests, leading to a depletion of their genetic stocks in the natural conditions. Some of these species are destructively harvested as root bark or stem bark are the major parts used in medicinal preparations. Large scale cultivation of these species is also not very encouraging.

2.1 *Aegle marmelos* L. Correa

2.1.1 Species description

Aegle marmelos, a deciduous tree, is a small to moderate size growing to a height of 20-25 feet and girth of 3-4 feet. It is naturally

distributed in India, Myanmar and Sri Lanka, invariably in dry deciduous forests and widely cultivated in Southeast Asia and Tropical Africa (Warrier *et al.*, 2010). The bark is shallowly furrowed and corky, leaves are trifoliate and aromatic which the branches has long straight spines.

The sweet scented greenish white flowers are bisexual, nearly 2 cm wide and are borne in clusters. The calyx is shallow with five short sepals and is pubescent on the outside. The five petals are pale greenish white, oblong ovoid, blunt, thick and dotted with oil glands. It has numerous stamens which sometimes are coherent in bundles. It has oblong ovoid ovary which is slightly tapering, with wide axis, cells numerous (8-20), small circularly arranged with numerous ovules in each cell. The brown coloured fruits are 5-7.5 cm in diameter, having globose, oblong pyriform shape, the rind is gray or yellow coloured while the pulp is thick yellow, orange to brown in color having sweet taste. The numerous seeds have wooly hairs and are arranged in the cells that are enclosed by slimy transparent mucilage, (Morton, 1987; Mishra, 1999).

Table 2: Medicinal tree species in commercial demand sourced from tropical forests

| S. No. | Species | Trade name | Parts used | Estimated Demand (MT/ Year) |
|--------|----------------------------------|---|---------------------------------|-----------------------------|
| 1. | <i>Aegle marmelos</i> | Bael, Bel, Bael, Belgiri, Bilva, Vilvam | Leaf, Fruits, Bark (Root, Stem) | 2000-5000 |
| 2. | <i>Commiphora wightii</i> | Guggulu, Gugal | Gum resin | 1000-2000 |
| 3. | <i>Emblica officinalis</i> | Amla, Nelli Amalaki | Fruits | >10000 |
| 4. | <i>Gmelina arborea</i> | Ghambar chal, Gambhari | Bark (Root) | 500-1000 |
| 5. | <i>Oroxylum indicum</i> | Tetuchaal, Syonaka | Bark (Stem, Root) | 500-1000 |
| 6. | <i>Santalum album</i> | Chandan, Sveta candana | Wood (Heartwood) | 500-1000 |
| 7. | <i>Saraca asoca</i> | Ashoka | Bark (Stem) | 1000-2000 |
| 8. | <i>Stereospermum chelonoides</i> | Patala, Padal fali, Patalai | Root | 500-1000 |
| 9. | <i>Strychnos nux-vomica</i> | Kuchla, Nirmali, Visamus | Fruits, Stem or Bark | 500-1000 |
| 10. | <i>Terminalia arjuna</i> | Arjun, Arjuna | Fruits, Bark (Stem) | 2000-5000 |
| 11. | <i>Terminalia bellirica</i> | Behdea, Bibhitaka | Fruits | 2000-5000 |
| 12. | <i>Terminalia chebula</i> | Harda, Haritaki | Fruits | 5000-10000 |

Source: NMPB, 2018. https://www.nmpb.nic.in/medicinal_list

2.1.2 Parts used and properties

Roots, leaves and fruits are mainly used. The roots are sweet, astringent, bitter and febrifuge. They are useful in diarrhea, dyspepsia, dysentery, stomachalgia, cardiopalmus, uropathy, vitiated conditions of vata, seminal weakness, and swellings. The leaves are useful in diabetes and asthmatic complaints, deafness, catarrh, ophthalmia and inflammations. The unripe bael fruits are sour, acrid, bitter having astringent, digestive and stomachic properties. It is also used in diarrhea and dysentery. The ripe fruits

are sweet, astringent, cooling, aromatic, laxative, febrifuge and tonic. It is good for heart, brain and dyspepsia (Warrier *et al.*, 2002).

2.1.3 Diversity

PCR-based RAPD markers were first reported by Britto *et al.* (2009) assessing the genetic variability in ten genotypes of the species from the Western Ghats. Twenty nine polymorphic loci were obtained using five primers, suggesting the variability existing in the selections. These markers generated 476 polymorphic loci with 64% polymorphism in *A. marmelos* collected from the Andaman

Islands (Nayak *et al.* 2013). Recently, a study conducted by Parvesh and Vijaya (2018) revealed that these markers are effective in distinguishing genotypes in the Chhattisgarh region also. This implies that RAPD markers could be used to efficiently manage germplasm collections of the species for future improvement programmes. RAPD markers could also detect the genetic uniformity in *in vitro* grown plants (Pati *et al.*, 2008 a, b; Mishra *et al.*, 2008). Four inter-simple sequence repeats (ISSR) used to assess genetic variability among 18 *A. marmelos* genotypes, revealed 12 monomorphic bands (Mujeeb *et al.*, 2017; Princy *et al.*, 2015). Mishra *et al.* (2008) tested the genetic uniformity of regenerated plantlets and mother plants using directly amplified minisatellite DNA (DAMD) and ISSR markers. Microsatellite markers revealed 100% polymorphism in genotypes of *A. marmelos* (Sharma and Sharma, 2015). Chloroplast matK barcode has been identified as a unique barcode to distinguish the species from its adulterants (Sivalingam *et al.*, 2016). The transcriptome analysis of the species revealed 1298 complete genes with an average of 2.03 orthologs/core gene (Kaushik and Kumar 2018). The gene QNS is identified as responsible for quinolone biosynthesis and conferring salt and drought tolerance (Resmi *et al.*, 2013; 2015).

2.2 *Commiphora wightii* (Arnott) Bhandari

2.2.1 Species description

Commiphora wightii (Family Burseraceae) is a shrub or small tree, attaining maximum height of 4 m. The bark is thin and papery and the branches are thorny. The leaves are simple or trifoliate, having ovate leaflets which are 1-5 cm long, 0.5-2.5 cm broad and is irregularly toothed. The small red to pink flowers are sessile, unisexual, occurring solitary or in groups of 2-3, 8-10, having lobed disc, oblong-ovoid ovary with 8-10 stamens and four small petals. Some plants bear bisexual and male flowers and others with female flowers only. The red drupe fruits are ovate, acuminate and separating into two fleshy leaves and the nut is enveloped by a 2-3, 8-10 lobed disc and an oblong-ovoid ovary. The nuts are ovoid acute in shape, splitting into two each with one cell (Chaudhary *et al.*, 2002)

2.2.2 Parts used and properties

The roots and leaves are used. Roots are astringent, sweet, cooling, aphrodisiac and diuretic. They are used in diabetes, strangury, fever and vitiated conditions of vata and pitta. The leaves are anodyne and used in rheumatism (Warrier *et al.*, 2002).

2.2.3 Diversity

Samantaray *et al.* (2009) developed an efficient DNA extraction protocol and standardised the PCR reactions for the species. Hague *et al.*, (2009 a, b) used ITS sequences to differentiate in *C. wightii* while three RAPD primers were observed to be associated with sex expression (Samantaray *et al.*, 2010). Suthar *et al.* (2010) grouped different accessions of *C. wightii* genotypes collected from Rajasthan and Gujarat into three subgroups using RAPD markers. Harish *et al.* (2014) estimated genetic diversity within and among different 45 populations of *C. wightii*. Krishnamurthy *et al.* (2015) used 20 RAPD primers; Vyas and Joshi (2015) used 11 markers for diversity assessment of *C. wightii* populations in Rajasthan while Kulhari *et al.* (2015) used ISSR and RAPD to analyze genetic variability among *C. wightii* germplasm of Rajasthan and Haryana. Sairkar *et al.* (2016) developed four SCAR markers for rapid identification of closely

related *Commiphora* species. Chaudhary *et al.* (2017) and Mohan *et al.* (2017) used fluorescent-labeled RAPD primers used for genetic analyses. Genetic diversity analysis conducted in 61 *C. wightii* accessions using 20 decamer primers revealed 121 out of 124 loci to be polymorphic. Bishoyi *et al.* (2018) has consolidated the application of different molecular markers in the discrimination *Commiphora* species distributed in India.

2.3 *Emblica officinalis* Gaertn. (*Phyllanthus emblica* L.)

2.3.1 Species description

Emblica officinalis (Family: Euphorbiaceae) is a moderate sized deciduous tree known as Amla or Emblic myrobalan. As it sheds branchlets as well as leaves, it is seldom entirely bare and, therefore sometimes mentioned as evergreen. The tree is found throughout tropical and sub-tropical India. The species is native to India and also grows in Pakistan, Uzbekistan, Sri Lanka, Bangladesh, Southeast Asia, China, and Malaysia (Krishnamurthy, 1993; Warrier *et al.*, 2003). Being a graceful ornamental tree, it normally reaches height up to 18 m and in rare occasions reaching upto 30 m. The pale grayish brown bark is fairly smooth and peels off in thin flakes. The small oblong leaves distichously disposed on very slender branchlets are 3 mm wide and 1.25-2.0 cm long. The small greenish yellow flowers found in the axils of the lower leaves are, inconspicuous and are borne in compact clusters. The female flowers are found above the male flowers that occur at the lower end of a growing branchlet. There are trees that are occasionally deciduous. Fruits are 1.5 to 2.5 cm in diameter, fleshy round rather indistinctly marked into 6 lobes. The skin is thin, translucent and adherent to the very crisp, juicy, concolorous flesh. There is a hexagonal stone which is tightly embedded in the center of the flesh containing 6 small seeds (Morton, 1987).

2.3.2 Parts used and properties

Amla is useful in treating anaemia, diarrhoea, dyspepsia, inflammation of eyes, and menorrhagia. It also finds use in medicinal preparations like chyawanprash and brahma rasayan, *etc.* The fruits are diuretic and laxative and are useful in digestive system disorders. They are also prescribed for treating jaundice and coughs. It is also one of the three important ingredients of Triphala, a famous ayurvedic preparation which is extensively used in treating biliousness, chronic dysentery, and other disorders. It is also known for its antiseptic properties and, therefore used for cleaning wounds and as a palliative for snake bites and scorpion stinging (Warrier *et al.*, 1997).

2.3.3 Diversity

Isolation of genomic DNA from young leaf tissues of different commercial varieties of *P. emblica* was standardized by employing modified DNA extraction protocols (Singh, 2003). RAPD markers were used to distinguish varieties of amla of same origin or even selection from same parents, suggesting its use in identification of varieties in crop improvement programme (Chaurasia, 2009). A 1.1 kb SCAR marker specific to *P. emblica* was developed from RAPD amplicons, useful for differentiating *P. emblica* from its adulterants (Dnyaneshwar *et al.*, 2006). Similarly, eighty RAPD primers were screened to develop DNA based marker for identification of *P. emblica* and a SCAR marker of 1.1 kb specific to *P. emblica* was identified (Warude *et al.*, 2006). Six microsatellite loci developed by Pandey and Changtragoon (2012) showed

polymorphism in five primers with the number of alleles ranging from four to seven while 20 microsatellite markers generated by Geethika *et al.* (2018) revealed 2-9 alleles per locus. Four polymorphic SSR markers and one SCAR marker was used to understand diversity in 66 genotypes of *P. emblica* from different locations of Sri Lanka (Mawalagedera *et al.*, 2015). Single nucleotide polymorphisms in trnI (UAA) intron sequences of chloroplast DNA was used as a potential marker to differentiate *P. emblica* from its allied species (Sangeetha *et al.*, 2010). For understanding the genetic structure and gene flow, to identify novel genes of interest and for developing markers for assisted breeding, 20 EST-SSR primer pairs showing polymorphisms in 90 individuals from three populations were developed from the transcriptome of *P. emblica* (Liu *et al.*, 2018).

2.4 *Gmelina arborea* Roxb.ex Smith

2.4.1 Species description

Gmelina arborea Roxb.ex Smith belongs to the family Verbenaceae, and is naturally distributed throughout India. It has been found and grown extensively in temperatures ranging from 1 to 48°C with annual rainfall varying from 760 to 4500 mm (Anandalakshmi *et al.*, 2016). The pubescent or glabrous leaves are opposite-decussate, petioles are cylindrical with 50 to 150 mm long.

Leaf blades are broadly ovate, 10-25 cm × 7-20 cm wide, and are apically long acuminate or caudate and is entire on mature plants. The reddish brown or yellow scented flowers are terminal or axillary having 1 to 3 cymes on the panicle branches which are about 8-40 cm long. Flowers vary from 2.5 to 5 cm in diameter, bracts 8 mm long which is linear lanceolate. Calyx is broad, campanulate and 5 mm long densely fulvous tomentose externally having rim with five small, triangular acute teeth. The yellow to orange or brilliant orange to reddish or brownish-yellow, dull yellow-brown corolla is large, tubular below, obliquely funnel-form at the throat, the limb is two lipped and the upper lip often orange-pink in colour is deeply divided into two oblong, obtuse backwardly curled lobules. The lower lip is three lobed often lemon yellow in colour and is twice longer than the upper lip. The fruit is a drupe, long obovoid shaped and 20 to 35 mm long. It is seated on the enlarged calyx and the ripened fruit is glossy yellow in colour. The exocarp is succulent and aromatic while the two celled endocarp is bony having 1 to 3 seeds (Mayavel and Nicodemus, 2017).

2.4.2 Parts used and properties

The roots are acrid, bitter, sweet, stomachic, tonic, laxative, galactagogue and anthelmintic. It is useful in dyspepsia, hyperdipsia, hallucination, fever, haenonhoids, stomachalgia and burning sensation. Leaf paste is good for cephalalgia and the juice of the leaf is useful in washing foul ulcers. The flowers are sweet, refrigerant, bitter, astringent and acrid, and are used in treating skin diseases and leprosy. They are used for anaemia: leprosy, ulcers, constipation, strangury, leucorrhoea and colpitis (Warrier, 1993).

2.4.3 Diversity

A modified protocol for isolating DNA from dried leaves of *Gmelina arborea*, suitable for further molecular analysis was developed by Shankar and group (2009). Genetic diversity was analysed using Inter-simple sequence repeat (ISSR) primers. 95% polymorphic loci was obtained among eight populations (Naik *et al.*, 2009).

Genetic diversity of *G. arborea* (n=534) was evaluated using ten polymorphic microsatellite markers for 19 natural populations representing India, China, Thailand and Myanmar (Wee *et al.*, 2011).

2.5 *Oroxylum indicum* (L.) Kurz

2.5.1 Species description

It is a deciduous tree which is small or medium in size, growing to a height of 12 m. The bark is soft, light brown or grayish brown in colour having corky lenticels. The large glabrous leaflets are ovate or elliptic, acuminate in shape and are 90 to 180 cm long having 2-3 pinnate with five or more pairs of primary pinnae which are cylindrical and swollen at the junction of branches. The withered and fallen large leaf stalks get collected near the base of the trunk and appear as a pile of broken limb bones. The plant flowers in June-July and bears fruits in November. The numerous reddish purple flowers have pale pinkish-yellow colour inside and forms a large erect raceme. The nocturnal blooming flowers attracts bats as it emit a strong stinky odour. The long curved fruit pods hang down from the branches and resemble the wings of a large bird or appear like a dangling sickles or swords. These pods are 40 to 100 cm long and 5 to 10 cm broad. The dried pods burst open resulting in fluttering of numerous seeds on the ground. Sometimes, these thin papery winged seeds are carried by wind for some distance which resembles as though butterflies are flying (Kirtikar and Basu, 2001; Warriar *et al.*, 2016).

2.5.2 Parts used and properties

The root bark is bitter, pungent, acrid, astringent to the bowels, increases appetite, cooling, aphrodisiac, useful in "vata. It is also used in treating fevers, vomiting, intestinal worms, dysentery, leucoderma, bronchitis, asthma and anal troubles (Warrier *et al.*, 1995).

2.5.3 Diversity

In a study carried out in Andhra Pradesh to assess genetic diversity from eight locations, forty RAPD primers were used. It showed high levels of similarity (Jayaram and Prasad, 2008), revealing a very narrow genetic base while ISSR markers used for assessing the genetic diversity and population genetic structure of 39 accessions of belonging to South and North East India, showed high genetic diversity among populations but low within population variation (Rajasekaran *et al.*, 2017). In a similar study, Aparna and Srinivas (2018) reposted the same trend with accessions from Maharashtra and North East India. Both studies revealed clear differentiation between the Western Ghats and North East Indian populations. The only other marker study reported in the species is the assessment of clonal fidelity of *in vitro* regenerated plants (Gokhale and Bansal, 2015; Rami and Patel, 2017; Panchaksharaiah *et al.*, 2018).

2.6 *Santalum album* L.

2.6.1 Species description

This species is native to India, Sri Lanka and Indonesia. In India, the natural distribution of the species is Karnataka, Tamil Nadu, Andhra Pradesh and Kerala states; the species is introduced to other parts of India (Arunkumar *et al.*, 2012). In its natural habitat, subpopulations are not dense, devoid of larger girth classes and mature trees are entirely or nearly absent in the forest areas of

Karnataka and Tamil Nadu (Arunkumar *et al.*, 2019). It is a moderate sized evergreen tree attaining a girth of 1 to 2.4 m and height of 12 to 15 m (Sen Sarma, 1982).

The bark is found to be smooth when the tree is young and gradually as the tree matures, becomes rough by developing vertical cracks. The inner part of the bark is red in colour. The flowers are straw yellow coloured initially which turn to deep purplish brown as they mature. The flowers which occur axillary or terminal as cymose panicles are shorter than leaves and the floral organs develop in acropetal succession. The time required from initiation of bud stage to anthesis is around 30 to 35 days, while it takes 85 to 95 days for ripening of the fruit from the initial stage (Srinivasan *et al.* 1992). The fruit is a drupe, initially green in colour and gradually when fully matured becomes succulent and purplish black. The fruit shape varies from globose, ovate to elongate and sometimes show tapering ends (Arunkumar *et al.*, 2016).

2.6.2 Parts used and properties

The yellow coloured heartwood is bitter, sweet, acrid, cardiotoxic, diuretic, expectorant, antipyretic, haemostatic, diaphoretic, aphrodisiac, anodyne, aromatic, deodorant, refrigerant, restorative and intellect promoting tonic, useful when pitta condition is vitiated, foul odour due to hyperhidrosis, leprosy, forgetfulness, amentia, cardiac debility, jaundice, skin disease, inflammations, bronchitis and cough, gastric irritability, leucorrhoea, spermatorrhoea, general debility (Warrier *et al.*, 1996).

2.6.3 Diversity

Significant amount of genetic diversity among natural sandalwood populations has been reported in sandalwood (Arunkumar *et al.*, 2012). Using allozyme markers, genetic diversity of different sandalwood populations in peninsular India was studied and results indicated that populations found in Deccan plateau were genetically the most diverse and, thereby representing the 'hot-spot' of sandalwood genetic resources in peninsular India (Angadi *et al.*, 2003; Rao, 2004; Rao *et al.*, 2007). Clones selected based on growth and oil content, in the states of Karnataka and Tamil Nadu when subjected to isozyme studies, provided information about the relative amounts of genetic variation existing within sandalwood accessions (Arunkumar *et al.*, 2018).

Genetic diversity of sandal was assessed using RAPD markers (Shashidhara *et al.*, 2003; Suma and Balasundaran, 2004; Azeez *et al.*, 2009; Dani *et al.*, 2011). Patel *et al.* (2016) characterized 20 sandalwood genotypes using RAPD, ISSR and SSR markers and found high level of genetic diversity and correlation among all markers. Genetic diversity of *S. album* using SSRs in 177 genotypes from 14 populations of three states (Karnataka, Telangana and Kerala) revealed that the populations were admixtures (Fatima *et al.*, 2019). Mohammed *et al.* (2012) used SSR markers to understand their applications through cross-transferability while and restriction fragment length polymorphism (RFLP) was used for regional differentiation (Byrne *et al.*, 2003). Chloroplast RFLPs were employed to identify ancestral lineages and determine whether Indonesian *S. album* is genetically distinct from Indian material (Jones *et al.*, 2009). Due to the range of the species, it is anticipated that there is high genetic variability but more studies are needed to confirm the extent of variation (Thomson *et al.*, 2018).

2.7 *Saraca asoca* (Roxb.) de Wilde.

2.7.1 Species description

Saraca asoca (Roxb.) de Wilde. belongs to the family Fabaceae, sub family Caesalpinioideae. The species is native of South India, Bangladesh and Western Myanmar; distributed in the Central and Eastern Himalayas, Khasi, Garo and Lushi hills, North eastern states of India, West Bengal, Orissa and in Western Ghats (Singh *et al.*, 2005a). It is a small, under storey evergreen tree growing up to a height of 10 m and has an average diameter of 20-30 cm. It has a blackish bark with reddish wood, found mostly along streams or under the shade of large evergreen patches of forests (Singh *et al.*, 2005b). The canopy is dense and the branches are somewhat droopy bear nearly sessile, large, abruptly pinnate leaves, 20-40 cm long, having 3-6 pairs of large oblong lanceolate leaflets, 8-16 cm long. The tender leaves are copper coloured and they resemble ponytail. Flowers are fragrant, large, snowy and yellow to orange in colour changes to red gradually and are borne in dense corymbose inflorescence. The pods are flat, leathery 15-30 cm long 2-5 cm wide, green when immature and turning to brown when the seeds are mature. Each pod contains 4-8 seeds, which are ellipsoid oblong and compressed with brown smooth seed coat and white cotyledons (Warrier *et al.*, 2007).

2.7.2 Parts used and properties

Bark is the main component used. The bark extract has been reported to possess a variety of therapeutic effects (Singh *et al.*, 2015). The bark is bitter, sweet, astringent, refrigerant, stomachic, anthelmintic, demulcent, constipating and demulcent. It is used in fever, dipisa, dyspepsia, burning sensation, colic ulcers, menorrhagia, leucorrhoea, metropathy and pimples. The flowers are used in vitiated conditions of pitta, considered to be uterine tonic, treated in syphilis, burning sensation, dysentery, inflammation and scabies in children, haemorrhoids, cervical adenitis and hyperdipsia. The dried flowers are used in treating haemorrhagic dysentery and diabetes. Seeds are used for stangury and vesical calculi and treating bone fractures (Warrier *et al.*, 1996).

2.7.3 Diversity studies

Numerous authors have worked on molecular markers in *Saraca asoca*. They include RAPDs, ISSRs, AFLPs, SCARs and DNA barcodes. The major focus of these studies were analyzing genetic variability and diversity, species authentication and detecting adulteration. DNA isolation and PCR protocol for RAPD for *Saraca asoca* was developed by Padmalatha and Prasad (2006). RAPD markers were used to study genetic variations in five different populations in Orissa (Senapati *et al.*, 2012); while Shrivastava *et al.* (2015) used 10 ISSRs, 9 AFLP primer and 5 SSRs to assess the genetic diversity in four natural populations in Orissa. All the SSR and AFLP markers revealed the existence of very narrow genetic base among the studied populations while RAPDs and ISSRs revealed high polymorphism, which could be attributable to the dominant nature of the markers. Recently, Saini *et al.* (2018) studied genetic diversity in *S. asoca* trees in seven different forest areas and three cultivated sites located in central and north-central Western Ghats using RAPD markers. The within population variation was higher (89%) than among population diversity (11%), suggesting good natural cross-pollination. As a modification, fluorescent-labelled RAPD primers were used to assess genetic diversity in individual trees from different

locations in Andhra Pradesh (Mohan *et al.*, 2017). RAPD markers produced distinct banding patterns to distinguish *S. asoca* from its common adulterant *Polyalthia longifolia* (Gahlaut *et al.*, 2013). Seventeen populations from Karnataka were assessed using 8 ISSR markers (Rosario, 2015). Populations growing in suitable habitats possessed high polymorphism. The within population variation was higher (82%) than among population diversity (18%). Microsatellite markers have also been developed for diversity analysis in this species (Sumangala *et al.*, 2013). *Saraca* specific SCAR markers with an amplicon length of 193 bp has been developed for identification and discrimination of *Saraca* from its adulterants (Kumar, 2016). AFLP analysis coupled with HPTLC fingerprinting revealed that chemical fingerprinting has proved as the better classifier than genetic markers in distinguishing species (Sharma *et al.*, 2018). Chloroplast matK barcode has been used to understand the evolutionary and phylogenetic relationship of *S. asoca* with other members present in the tribe Detariae (Saha *et al.*, 2013). DNA barcoding coupled with RP-HPLC (Hegde *et al.*, 2017) and NMR spectroscopy (Urumarudappa *et al.*, 2016) was successful in differentiating crude bark samples of *S. asoca* from its adulterants. The barcodes were generated from rbcL and psbA-trnH.

Hegde (2018) coupled ISSR fingerprints and rbcL-based DNA barcodes to differentiate common adulterants and substituents of *S. asoca*. Samples from six different states that consisted of ten localities revealed 47.06% polymorphism.

2.8 *Stereospermum chelonoides* (L. fil.) DC.

2.8.1 Species description

S. chelonoides (L. fil.) DC. is a large deciduous trees with large straight stem reaching a height of about 18 to 30 m and 2.8 m girth. It is found extensively in the moist regions of deciduous forests of India, reaching up to an altitude of about 1200 m (Parrota, 2001). It is commonly found in India, Myanmar, Sri Lanka; in the Western Ghats South, Central and South Maharashtra Sahyadris, commonly known as Yellow snake tree. The imparipinnate compound leaves are opposite, decussate and the rachis which is canaliculated and glabrous are 6 to 16.5 cm long. The leaflets are 3-5 pairs, opposite with odd terminal one. The petiole which is 0.8 to 1.5 cm long, the elliptical lamina 5 to 15 × 2.5-7.5 cm, having caudate apex and cuneate base with entire margin, glabrous, characeous, mid rib being flat above, and the secondary nerves which are 8 to 10 pairs gradually curved whereas the tertiary nerves are weakly precurrent.

2.8.2 Parts used and properties

Every part of the tree is used in treating many disorders. The leaves are used to treat wounds, malarial fever, otalgia, rehumatalgia and odantalgia. The decoction of the leaves is used to treat chronic dyspepsia and as antipyretic. The root which forms one of the ten ingredients in 'Dasamula' an Ayurvedic formulation is bitter astringent and acrid.

It is used such as cardio tonic, appetizer, diuretic, anodyne, aphrodisiac, lithotropic, antiinflammatory, antibacterial, anticancer, febrifuge tonic, antiemetic, antipyretic. The root decoction is used to treat cough and asthma (Warrier, 2002).

2.8.3 Diversity

No reports on DNA marker studies are available.

2.9 *Strychnos nux-vomica* Linn.

2.9.1 Species description

Strychnos nux-vomica Linn. (Loganiaceae) is an important medicinal plant, native to Southeast Asia, especially India and Myanmar (American Cancer Society, 2000). It is a medium sized deciduous tree which is found naturally on wastelands and is found in degraded forests of West Coast and in Western and Eastern Ghats of India (Sivakumar *et al.*, 2006). Branches are irregular and covered with smooth ash colored bark and shiny dark green young shoots. The greenish white coloured flower occurs as terminal cymes, appearing with young leaves on axillary shoots or at the end of branchlets. The hermaphrodite funnel shaped flowers are small in size, nectariferous and emits unpleasant odour. The pentamerous flowers have ovate calyx lobes having dense hair outside. Corolla is slender tube of 1 cm long, widening at the throat abruptly, glabrous outside having pubescence at base, narrowly ovate greenish white to white lobes are 3 mm long, having thickened margin and minute hairs. The stamens are inserted at the mouth of the corolla and the tubes alter with corolla lobes. Pale cream coloured anthers are ditheous, introrse and dehiscence longitudinally. The bicarpellary ovoid ovary is superior, glabrous and with axile placentation. The style is filiform and similar in length to that of corolla tube with head shaped stigma. The ellipsoid or orbicular lens shaped seeds are covered with dense silky hairs that radiate from the centre which results in providing a characteristic shine to the seed. The seed is concave on one side and the other side, it is convex, having a small depression in the centre of either side. The hard endosperm is dark grey in colour, bitter in taste, odourless and contain small embryo (Schmelzer and Gurib-Fakim, 2013; Patel *et al.*, 2017; Behera *et al.*, 2017).

2.9.2 Parts used and properties

As recommended by the Ayurvedic Pharmacopoeia of India, the detoxified seeds are used in treating facial paralysis, sciatic and impotency (Kirtikar and Basu, 1991). The bark of the root is useful in intermittent fever, cholera and snake bite. The poultice made out of the leaves, is used in treatment of chronic wound and ulcers. The fruit pulp is used in treating paralytic affections of palms and foot. The bitter seeds are used as digestive, appetizer, antiperiodic, aphrodisiac and stimulant. They are applied to treat anemia, skin disease, bronchitis, asthma, malarial fever, diabetes, muscle weakness, colic, chronic constipation, insomnia, mental emotions, hysteria, paralysis, emphysema, nervous debility, gout, chronic rheumatism, hydrophobia and spermatorrhoea (Warrier *et al.*, 1993). Apart from medicinal use, it is also a rodenticide, avicide, insecticide, mematicide and piscicide (Behera *et al.*, 2017).

2.9.3 Diversity

No reports on DNA marker studies are available.

2.10 *Terminalia arjuna* (Roxb.) Wight & Arn., *T. bellirica* (Gaertn.) Roxb., *T. chebula* (Gaertn.) Roxb.

2.10.1 Species description

Terminalia arjuna: A native of the Indian subcontinent, *T. arjuna* is found eastwards in Myanmar and southwards in Sri Lanka also. Being a riparian species, it occurs along the river banks and streams in South and North India. A large deciduous trees which reaches a height of 30 m and a girth of 2 to 2.5 m having a buttressed trunk. The crown spread is large with drooping branches. The grey or

pinkish-green bark is smooth, thick and exfoliating in thin irregular sheets. The oblong or elliptically oblong shaped simple leaves are opposite to sub-opposite, 5-25 × 4-9 cm in length and width, is glabrous, hard and often inequilateral. The leaf apex is obtuse or sub-acute having round base or sometimes cordate. The short petiole is 2-4 cm long is sericeous and has two prominent glands at the apex of the petiole. The inflorescence is short axillary spikes or small terminal panicles, 9-13 cm long with 2.5 to 6 cm branches. The short rachis is pubescent and white in colour. The white, creamy or greenish-white coloured cup shaped flowers are small, regular, polygamous, sessile and strongly honey scented. Fruits are indehiscent drupe, dark brown to reddish brown coloured, obovoid-oblong shaped 2.5-6 × 1.8-2.8 cm long, glabrous with 5-7 equal thick narrow stiff-wings which are striated having numerous upwards-curved veins (Orwa *et al.*, 2009).

Terminalia bellirica occurs most frequently in the moist valleys and not in arid regions. The tree populations are distributed throughout the forests of India, below elevations of about 3000 ft. It mostly represents scattered populations. The large leaves are alternate, glabrous, broadly elliptic to obovate-elliptical, 4-24 cm × 2-11 cm. The base is rounded to cuneate, rufous-sericeous but soon glabrescent, with 6-9 pairs of secondary veins. The secondary and tertiary venation is prominently found on both the leaf surfaces. The petiole is 2.5 to 9 cm long. The young leaves are copper red in colour which soon changes into parrot green and then to dark green. The solitary greenish white, simple flowers are small 3-15 cm long, having axillary spikes. The calyx tube is densely sericeous or tomentulose. The flowers are found along with new leaves and have a strong honey like smell. The sub-globular to broadly ellipsoid fruits is light yellow in colour, densely velutinous or sericeous, 2-4 × 1.8-2.2 cm. It is obscurely five angled and minutely brown tomentose.

Terminalia chebula: It is found in the sub-Himalayan tracts from the river Ravi eastwards upto West Bengal and Assam, ascending upto an altitude of 1,500 m in the Himalayas, in deciduous and moist forests, West Coasts and hills of Deccan and South India. The deciduous tree reaches 25 m tall. The dark brown to black coloured bark is 5 to 6 mm thick having shallow vertical fissures which is exfoliating in thick scales. When blazed, has yellowish brown colour. The young shoots are densely pubescent and the glabrous branchlets are brownish or grayish in colour. The simple leaves are opposite to alternate, exstipulate. The stout and pubescent petiole is 12-25 mm long, grooved above, having two sessile glands at the top. The leaf lamina is 9.5-28 × 4-13 cm long and wide, and is ovate, elliptic, obovate or elliptic-obovate in shape with base being round, obtuse, oblique or subtruncate, the leaf apex is acuminate, obtuse or apiculate, margin is entire, coriaceous, glabrous above tawny villous beneath, lateral nerves 6-12 pairs, pinnate, ascending, prominent, arched towards the margin. The bisexual flowers are greenish white in colour as terminal and axillary spikes having offensive smell. The bracts are 2-3 mm long with calyx tube which is constricted above the ovary is 1.5-2.5 × 0.8-1 mm, having five lobes which is creamy and triangular. The ten stamens are in two rows, with filaments 4-6 mm and five lobed discs. The inferior ovary is densely villous, one celled with terminal stigma. Fruit is a greenish-yellow drupe 3-4 × 2-2.5 cm, obovoid, woody, obscurely 5 angled, glabrous, having one seed.

2.10.2 Parts used and properties

T. arjuna: Bark has medicinal properties like astringent, antioxidant, aphrodisiac, cooling, tumors, ulcers, diabetes, leucorrhoea, spermatorrhoea, in fractures, cough, asthma, excessive perspiration and skin related problems (Warrier, 1994).

T. bellirica: Fruits, roots and leaves have medicinal properties. It is used in Ayurveda and Siddha for constipation, chronic diarrhoea, ulcer, gastroenteritis, asthma, cough, dyspnea, dyspepsia, hemorrhoids, malabsorption syndrome, parasites, candidiasis, hepatomegaly, renal calculi, urinary discharge, tumours, skin disease, memory loss, epilepsy, diabetes, cardiovascular disease, anorexia and wounds (Nadkarni, 1976).

T. chebula: Unripe fruits are used as purgative, ripe fruits are more astringent, antibilious and stomachic. It is prescribed for treating diarrhea, dysentery, constipation and flatulence, vomiting, digestive disorders, cyst, enlarged liver and spleen, bronchial asthma and cough and for metabolic harmony. Bark is diuretic (Warrier, 2002).

2.10.3 Diversity

Diversity DNA isolation from leaves of *Terminalia* species is cumbersome due to high amount of carbohydrates precipitated with DNA (Sarwat *et al.*, 2006; Deshmukh, 2007; Sairkar *et al.*, 2013; Bharti, 2018). Warude *et al.* (2003) standardized DNA extraction protocol for RAPD based identification of *Terminalia bellerica* and *T. chebula*. A common protocol was developed for the isolation of genomic DNA from different tree species of Combretaceae family (Gupta *et al.*, 2011). Dangi *et al.* (2011) reported variations in 28 accessions of *Terminalia bellerica* based on RAPD markers. Gowda *et al.* (2012) estimated genetic diversity and relatedness among *Terminalia chebula* trees from five provenances of Karnataka using RAPD markers while Sankanur (2013) used twenty five RAPD and twelve ISSR primers to reveal polymorphism in the species from the same region. In *T. arjuna*, low genetic diversity was recorded in the germplasm of Madhya Pradesh (Sairkar *et al.*, 2017) RAPD markers have also been used to diagnose genetic diversity and species relationship among *T. arjuna*, *T. bellirica*, *T. chebula*, *T. tomentosa* and *T. catappa*. RAPD markers clustered *T. arjuna*, *T. bellirica*, *T. chebula* in one group and the remaining two species were clustered together in another group (Deshmukh *et al.*, 2009). Further, genetic diversity and species correlation using AFLP, ISSR and RAPD clustered these three species in three different groups (Sarwat *et al.*, 2011a, b).

To assess genetic diversity and species relationships of five taxonomically critical *Terminalia* species, 31 random primers were used. Among these primers, 26 primers amplified across all species with 90 per cent of the bands polymorphic. Each primer on an averaged scored 12.92 bands. The dendrogram showed that the five *Terminalias* were grouped into two distinct clusters (Deshmukh *et al.*, 2009).

Studies using chloroplast DNA barcodes (rbcL, matK, and trnH-psbA) and a nuclear DNA barcode (ITS2) for species identification by Nithaniyal and Parani (2016) revealed that trnH-psbA was the best marker for species identification in *Terminalias*. With regard to species relationship studies, ITS2 was found to be better. However, Sharma and Shrivastava (2016) used internal transcribed spacer (ITS) regions to ascertain the identity of *T. arjuna* as well as detection of mixing of *T. bellirica* and *T. chebula*. A similar result

was obtained by Mishra *et al.* (2017), who used rbcL, matK, ITS and psbAtrnH. They report that combination of matK+ITS to be an ideal barcode to differentiate *Terminalias*.

3. Conclusion

A global shift from illness care to wellness care is driving growth in foods as medicine. The demand for Ayurvedic products and traditional medicines is also putting pressure on the indigenous Ayurvedic industry which is witnessing higher demand for crude drugs. The total estimated market size of Indian Ayurveda industry products is US \$ 2.27 billion and is envisioned to grow to reach US \$ 9 billion by the year 2022 (CII, 2017; 2018). Given this scenario, there is a need to encourage and support small-scale cultivation of herbs in the rural regions, which would also open employment opportunities for youth. Mass cultivation of medicinal herbs is a potential market in the future. This will also help the industry for a sustainable collection of raw materials.

Medicinal plants can be prioritized as : (i) with high export potential, (ii) required for manufacture of classical formulations, and (iii) required in proprietary medicines. Species mentioned in this article fall into category two, and need to be brought from the wild to cultivated status to prevent depletion of these resources. The demand and supply situation for these bioresources will see drastic changes. It would be wise to prepare for this change, by adopting and evolving a workable formula in consultation with stake holders and regulators which would be beneficial to them and also help promote development of the herbal medicine industry. It would also supplement the cause of sustainable utilization of these bioresources promoting conservation alongside.

Conflict of interest

The authors declare that no conflict of interest exists in the course of conducting this research. All authors had final decision regarding the manuscript and the decision to submit the findings for publication.

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