CHAPTER 1

INTRODUCTION

"All that man needs for health and healing has been provided by God in nature, the challenge of science is to find it."

By Philippus Theophrastus

1.1 Medicinal plants: integration of primordial tradition to contemporary medicines

Nature has evolved in a very astonishing way to deliver various healing molecules in the form of natural products. These medicinal agents can be derived from microbes to more complex resources like plants and animals. Human dependency on plants to obtain the therapeutic molecules is very ancient. Use of medicinal plants for treatment of various diseases can be traced back to the origin of humans. As human evolved they gradually learnt to make coordination with the nature and was being exploited it for various purposes, use of medicinal plants was one of them. Plants have become the integrated part of the various communities existed in the prehistoric era and this tradition is also assimilated as the essential daily practice of many cultures in the present time also. Plants were the basis of various established traditional system of medicines in ancient times and encouraged development of various modern medicines also. It is the empirical findings of hundreds and thousands of years which make the plant-based drugs safe and reliable. The antiquity of the plant therapy was evident in the writings on a clay tablet found in the most ancient culture of Mesopotamia dated about 2600 BC. They used the Cedar oils, *Cupressus sempervirens* oil, *Glycyrrhiza glabra*, *Commiphora* species and *Papaver somniferum* (Poppy juice). Bishop's weeds (*Ammi majus*) were reported for treating vitiligo in Egyptian culture (Gurib-Fakim 2006) . Many other culture systems and societies have adopted the phytomedicines as their prime healing method to protect their existence and survival. The traditional system of medicine thrived in various countries as Africa, India, China (TCM), Japan (Kampo), Korea, Arab and many European countries especially in Greece (Gurib-Fakim 2006).

Progression in the chemical biology turns the focus towards synthetic drugs which limited the use of phytopharmaceuticals. Chloral hydrate was the first synthetic drug which was used as the sedative (Jones 2011). Chemical synthesis allowed the production of the structurally predefined drugs in the desired dosage form. It was not possible with the naturally derived drugs due to their high level of diversity and minimal amount of biological supply. In addition, the process of drug discovery from the natural resources required time consuming several steps extraction, isolation and structure elucidation procedures, which was simplify by the chemical synthesis (Lahlou 2013). But, on the other hand, some of the natural substances are structurally very complex and hard to mimic by chemical synthesis, economically. In addition, advances in the scientific methods for isolation and determination of new lead compounds from the natural resources retrieved the attention of pharma industry towards traditional medicines (Lahlou 2013). Meanwhile, non-biological origin of synthetic drugs can trigger the side effects.

Thus, the constraints associated with these chemically originated drugs again encouraged the use of natural compounds as the medicines. Medicinal plants have proved as the great repertoire of the therapeutic molecules. Medicinal plants have several advantages to be used as the biological resources of natural products. They fit into the immediate personal need, are easily accessible, inexpensive and show fewer side effects (Kala et al. 2006). Various bioactive molecules are derived from the medicinal plants. Some of the molecules can be directly used as medicines and some of them provide scaffolds, prototype or precursors to develop drug molecules using combinatorial chemical synthesis. Morphine was the first commercial therapeutic plant product introduced by Merck in 1826 (Veeresham, 2012). Aspirin was the first semi-synthetically developed drug originated from salicin, a natural product isolated from *Salix alba*, introduced by Bayer in 1899 (Veeresham 2012). Success in this direction led to the isolation and commercial production of the plant-derived drugs (Sharma et al. 2014). Examples of such blockbuster drugs include anticancer drug Paclitaxel from *Taxus brevifolia*, antimalarial drugs Quinine from *Cinchona cordifolia* Mutis exHumb and Artemisinin from traditional Chinese plant *Artemisia annua*, Silymarin extracted from the seeds of *Silybum marianum* for the treatment of liver diseases, Atropine from *Atropa belladonna* worked on neuronal diseases, Caffeine *Coffea arabica*, antiasthmatic Ephedrine from *Ephedra* species etc (Salim et al. 2008; Veeresham 2012).

Currently, nearly 80% of African and Asian population depends on traditional medicines for their primary healthcare. In other 20% inhabitants of developed countries also, plant products are the prime source of various drugs, nutraceuticals and dietary supplements (Gurib-Fakim 2006). Herbal preparations in these countries are included in the category of '**Complementary and Alternative Medicine' (CAM).** It is estimated that about 25% of the drugs prescribed worldwide are derived from plants and 121 such active compounds are in use. Of the total 252 drugs in WHO's essential medicine list, 11% is exclusively of plant origin (Sahoo et al. 2010). WHO estimates that the present demand for medicinal plants is ~US $14 billion a year and by the year 2050 it would be ~US $5 trillion.

1.1.1 India's contribution

The Indian Systems of Medicine consist of Ayurveda, Siddha, Unani and Homoeopathy, and therapies such as Yoga and Naturopathy. Some of these systems are developed indigenously and due to the influence of foreign invaders over the years have become the part of Indian tradition. Ayurveda is the most ancient and perhaps, the world first systemized and formulated tradition of medicines based on the plants. Ayurveda means the science of life which is based on the holistic treatment of mind and body at physical, mental, social, moral and spiritual level. Including the *Vedas*, *Charak Samhita a*nd *Sushruta Samhita* are the milestones in the ancient Indian system of medicines. These old scripts evident the schematization and rationalization in healing and treatment. Other classical literatures encompassing the philosophies of Ayurveda science are, **Astanga Samgraha** and **Astanga Hridaya, Bhela Samhita** etc (Narayanaswamy 1981). Siddha is also one of the old medicine systems predominantly practiced in the southern part of India especially the Tamil speaking regions. This system of medicine has adopted some concept from Ayurveda but the main emphasis is on the drugs from metals and minerals. Unani system of medicines was originated in the Greece and introduced in India through Arabians and Persians. In the present era also, it is being practiced in various parts of India. Unani system of medicines encompasses various types of treatments and therapies as regimental therapy which refers to treatment through diaphoresis, diuresis, massage etc, diet therapy based on the treatment through consuming specific diets and food and the last one is pharmacotherapy which stands for the

healing of ailments using herbals (Mukherjee 2002). Homeopathy was also originated at foreign land but gradually become an integral part of the Indian system of medicines. It is scientifically founded by a German physician Samuel Hahnemann in 1790. The term “homeo” stands for similar and “pathy” denotes the disease or sufferings. Homeopathy principally follows the idea of consuming medicines in small quantity to generate symptoms similar to any disease. Most of the homeopathic preparations are based on various plant species. Systematics meta-analysis of various Materiae Medica has revealed about more than 800 plants used in homeopathic medicines (Bharatan 2008).

Thus, India have the legacy of well-established ancient system of medicines which is also the inseparable part of the modern society of India. At present, nearly 960 plant species are used by the Indian herbal industry. The turnover of the industry is more than Rs 80 billion and the herbal exports occupy a share of 3% of total Indian pharmaceutical export. Seventy percent of export from the herbal sector consists largely of raw materials and thirty percent of the export consists of finished products (Sahoo and Manchikanti 2013).

Conclusively, The significance of medicinal plants has already evident in the ancient writings and continues in the modern scenario. Inputs of advanced technologies have revealed the structural diversity of plant-derived therapeutic molecules which has infinite prospects to provide ingredients for novel drugs. Synergism of technological advances with traditional medicinal systems has established medicinal plants as resources of more novel therapeutic agents. This renaissance of medicinal plants have brought more opportunities but also fetched various challenges in standardization and scientific validation of the herbal products. The next section cover the complications raised in the translation of old wisdom into modern benchmarks.

1.2 Quality is the major concern in translation of old wisdom into current prospects

As stated earlier, interrelation of humans and phytopharmaceuticals was established since time immemorial and it is growing continuously across the world in the modern era. In spite of the rapid growth of consumption of herbal remedies, the herbal sector is not devoid of challenges. In

context of India, we shares less than 1% in the global herbal export market. Inspite of high biodiversity as well as an established ancient medicinal system, we are unable to avail the profits (Sahoo and Manchikanti 2013). The issues related to plant-based drugs are dishonoring their reputation and affecting the consumer's belief very negatively. Consistent quality and efficacy is the biggest challenge for the global acceptance of herbal drugs. Quality checking at each level from plant cultivation, collection to processing and marketing is required. Moreover, the challenges related to failure to convince differing regulatory, registration and specific GMP standards, lack of herbal practitioners in overseas, lack of quality in terms of adulteration, substitution and contamination and insufficiency of regulatory guidelines for different aspects of the complete supply chain are pushing back the India's input (Sahoo and Manchikanti 2013). The major issues responsible for the low quality of the herbal drugs are described below **(Figure 1.1)**.

Figure 1.1 Major concern affecting the quality of herbal preparations.

Lack of universality is one of the problems which hinder the global acceptance of herbal drugs. Traditional medicines are the results of therapeutic experiences governed by the indigenous people and the knowledge passes from generation to generation (Sissi & Benzie 2011). These customs and rituals differ in various civilizations corresponding to their local requirements and availability of the resources. Additionally, the descriptions in the local ancient literatures are very difficult to translate and understand in modern scientific language. Differences in the languages can lead to the misapprehension. It is very difficult to reconstruct all the knowledge entrenched in the rituals of indigenous people as well as in the old documents and apply directly for proper laboratory investigations.

Lack of proper toxicity analysis is another major concern related to the quality of herbal drugs. Many reports are there which exhibited that abusing of herbal remedies have acute or chronic adverse effects. Most of the marketed products are launched without any efficacy and toxicological evaluation. Lack of good collection as well as lack of controlled and regulatory manufacturing procedures, an uncontrolled chain of supply, and misidentification of the species may lead to the external mixing of any toxic chemical or plant species, heavy metals, and microbes which would be the reasons for toxic side effects of herbal drugs (Sahoo et al. 2010). Most of the drugs are promoted as food supplements and self-medication without proper prescription without recognizing their hazardous effects (Ekor 2014). Worldwide surveys revealed the cases of organ failures, paralysis, allergic reactions, nausea, vomiting, bleeding as the consequences of herbal drugs toxicity (Aronson 2009). Some of the toxic herbs like *Ephedra*, *Aristolochia,* and *Aconitum* have intrinsic toxic constituents, when taken more than recommended doses can create severe reactions (Panda and Debnath 2015). Some herbal drugs required preprocessing (as in Ayurveda known as ‘Shodhana’) steps to remove the noxious bioactive from the plants. Improper preprocessing due to inadequate knowledge of manufacturers and practitioners causes harmful effects. Interactions of herbal drugs with concomitantly administrated chemical based drugs as well as the active constituents of the other herbs in the polyherbal formulations also potentiate the problem of toxicity (Ekor 2014). These interactions work synergistically or antagonistically which can generate adverse reactions.

Batch to batch variations in the herbal products constrains the high quality of herbal end

products. The integrity of herbal extracts or solid formulations is essential to maintain the reproducibility of pharmaceutical effects. Lack of uniformity in each step from cultivation or wild plant collection to preparation of drugs may affect the homogeneity in the marketed products. The concentration of bioactive constituents in the medicinal plants can vary with the physical conditions of the plants, harvesting or collection time, varied geographical and climatic conditions as temperature, and exposure to light as well as can change with the genetic diversity of the plant species. Physical and chemical stress to the plants as interaction with any microbial organism, pathogens, use of fertilizers and pesticides are also the governing factors. Irregularity in several steps in the processing of raw material as the method of drying, grinding, storage, transportation and extracting also generate the variations in pharmacological activities of the herbal drugs. Irreproducibility in the therapeutic effects and actions of herbal drugs lead to the decline in consumer's belief (Mukherjee et al. 2015).

Adulteration and substitution of genuine raw material with the other plant-derived or synthetic substances is also hampering the production of high quality herbal preparations. Adulteration or debasement is common term include different conditions as deterioration, sophistication, spoilage, admixture, substitution, inferiority. Adulteration may be both unintentional and intentional. Unintentional or accidental adulteration is due to the mixing and substitution with nonprofit motive. It occurs mainly due to the careless collection by unskilled hands having less knowledge about the authentic sources, confusion in the species identity due to same morphology or ambiguous vernacular names or lack of taxonomic key characters during the time of collection. Intentional adulteration is the condition in which mixing and substitution are motivated by some economic benefits. This can be done with the substance having inferior value, with exhausted drugs, toxic materials, synthetic products and other parts of the same species etc. Deliberate adulteration is done with the aim of increasing weight of the material or to gain more profits in lesser investments (Bandaranayake 2006; Blumenthal 2011).

Contamination of herbal preparations with microbes and various chemical agents as heavy metals, organic pollutants, mycotoxins, endotoxins, pesticides etc also downgrade the purity and quality of herbal drugs. Several reports are there exhibited a higher percentage of Asian, Chinese and African herbal (Abba et al. 2009; Alwakeel 2008; Justin-Temu et al. 2009) remedies carrying

the microbial load including bacteria, viruses and fungi in the herbal products. The bacterial species which are predominantly carried by herbal drugs include *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* which are pathogenic in nature. Toxins secreted by these bacteria are also the bottleneck for the higher quality of herbal remedies. The presence of fungus and their resistant spores as well as mycotoxins should also be checked. Evidences are there which reported the presence of toxigenic fungus species as *Aspergillus* and *Fusarium* etc. Besides microbes some parasites as protozoans nematodes etc are also carried by herbal drugs. Withering and presence of moisture content due to improper storage leads to the increase in microbial load and simultaneously convert some metabolites into another which can further react with the endotoxins and mycotoxins and produce adverse reactions (Agarwal et al. 2014).

The physicochemical contaminants include heavy metals, organic pollutants, residual solvents agrochemicals, pesticides and radioactive material. The presence of various elements such as zinc, manganese, chromium, copper, iron, lead, nickel, vanadium, cadmium, mercury, lead and arsenic are reported. In a report by Saper et al. (2009) high prevalence of lead and mercury was found in the US and Indian-Manufactured Ayurvedic medicines. Heavy metal contamination was also found in traditional medicines originated from UAE (Dghaim et al. 2015), Malaysia (Uddin et al. 2013), Brazil (Caldas and Machado 2004), Australia (Denholm 2010) etc. Organic pollutants which are non-biodegradable and persist in soil and environment as DDT, BHC also affect the quality of herbal drugs. Residual agrochemicals and pesticides used during the cultivation can be mixed with the herbal drugs. Detection of these contaminates is mandatory step to provide assured quality and purity of herbal drugs. Various analytical detection techniques as TLC, HPLC, GC combined with MS are recommended for determination of prevalent toxic chemical compounds in herbal products.

1.3 Quality parameters of herbal medicines

The need of regulation of herbal medicines and their roles in health management are recognized by World Health Organization (WHO) which is directing the other countries to join reforming efforts and also helping in establishing their national regulatory policies. WHO provide

guidelines to establish basic criteria to evaluate quality, safety and efficacy of herbal medicines (http://apps.who.int/medicinedocs/pdf/whozip57e/whozip57e.pdf). In compliance with these guidelines WHO also published guidelines related to conservation of medicinal plants, good agriculture and collection practices, good manufacturing practices, quality control methods for evaluation of such medicines, research methodologies using herbal medicine and information on standardization of phytopharmaceuticals.

Along with WHO many countries have brought out alerts and established their own national regulations to sustain the quality of plant-derived drugs. Their efforts manifest in the form of their general policy structure, drug registration system, development of pharmacopeia and monographs, the inclusion of traditional medicines into essential medicine list and mode of availability (Over the counter drugs or prescription based) in the markets (Ajazuddin and Saraf 2012; Chaudhary and Singh 2011; Sahoo et al. 2010). India is also obstinate for the protection, promotion, regulation of medicinal plants as well as for auditing quality of herbal drugs. In 1940 and 1945, Drug and Cosmetic act and rules respectively were framed for governing production and marketing of Ayurveda, Siddha and Unani drugs (Sahoo et al. 2010). Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) which was established in November 2003, (formally Department of Indian Medicine and Homeopathy and now ministry of Ayush) is the authorized governmental body for monitoring the system and also to promote education as well as research related to alternative therapies (http://indianmedicine.nic.in/index.asp?lang=1). Establishment of Traditional Knowledge Digital Library (TKDL) was the another combined efforts of Indian government and research institutes (Council of Scientific and Industrial Research) to protect Indian traditional knowledge from illegal patenting by the other countries. It is the digitalized database related to the collection of traditional knowledge from the literature of Ayurveda, Unani and Siddha exist in the public domain. In addition, National medicinal plant board which was established in 2000 is also working for harmonizing all matters related to medicinal plants. This also supports policies and programs for the growth of trade, export, conservation and cultivation of medicinal plants (http://www.nmpb.nic.in/).

The efforts stated above, are taken in order to provide quality and standardized herbal drugs. In

production of herbal drugs (plant or plant parts that have been converted into phytopharmaceuticals), herbs have to pass from various stages as collection, storage, processing to be converted into finished products. Quality assessment of each level is required in order to produce consistent efficacy. Quality of an herbal drug refers to its grade, which is further confined to and govern by the identity, purity, chemical content, and many other chemical, physical, or biological properties, or by the manufacturing processes. The process which involved the maintenance of the quality and validity of a material and the final product is known as quality control (Bandaranayake 2006). The quality control parameters acclaimed for the evaluation and standardization for producing herbal drugs are involved botanical identification, physical assessment, chemical characterization, biological and pharmacological evaluation, and determination of biological and chemical contaminants which are summarized in following sections **(Figure 1.2).** Each evaluation process needs the aid of scientific tools and methods (Annonymus 1998; Kunle et al. 2012; Mangathayaru 2013)

1.3.1 Botanical Identification

Botanical identification is the prime step for quality control of herbal medicines. It is the process of ensuring the authenticity of the plant or the plant parts going to be used as herbal medicines. This is done by evaluating sensory, macroscopic and microscopic characteristics of the plant material. High resolution microscopic techniques as electron microscopes are also useful to assist botanical identification. Additionally, application of various analytical techniques and DNA-based identification are also strongly recommended for the purpose of herb traceability. Voucher specimens of the plants or plant parts should be stored for the future reference.

1.3.2 Physical Assessment

Physical assessment of herbal medicine includes determination of foreign substance, moisture content, ash value. The plant material should devoid of any foreign material as soil, glass, metal, plastics, insect parts or animal excreta. Microscopical techniques can be used to check the presence of any unwanted solid material. Chemical residuals can be checked with chromatographic techniques as TLC/HPTLC. Ash values are calculated by weighing the total

ash, acid-insoluble ash and water-soluble ash after burning the plant material to check the impurity. Extractive value refers to the determination of the amount of active constituents extractable from the given amount of the plant material (Annonymus 1998). Extraction procedures can have a greater impact on the extractive value. The field of extraction methods is also progressing and some advanced scientific approaches have facilitated the extraction of highly potent cost effective active ingredients (Banerjee and Mitra 2012; Choudhary and Sekhon 2011).

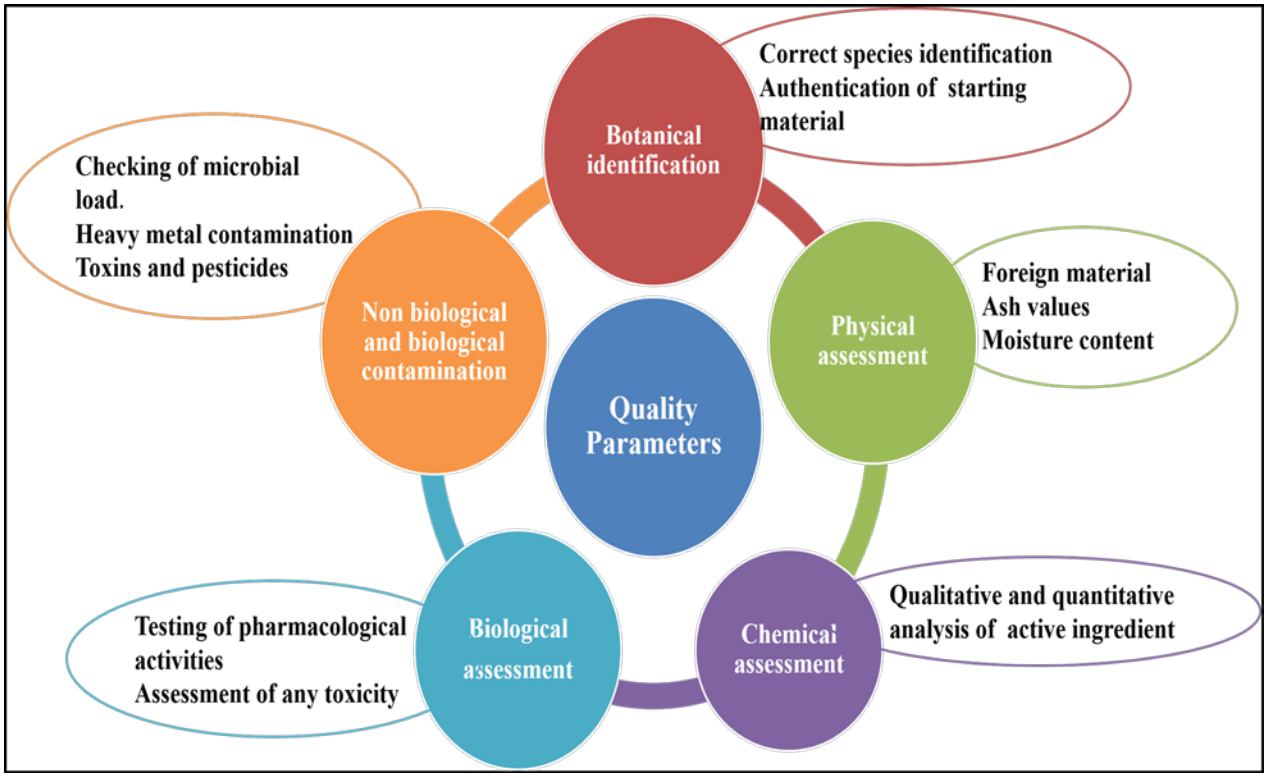

Figure 1.2 Parameters for quality assessment of herbal preparations.

1.3.3 Chemical Evaluation

Chemical evaluation of herbal drugs consists of a qualitative and quantitative analysis of active ingredients for herbal drug standardization and identification of adulteration. Chromatographic techniques independently or along with other advanced chemical analytical techniques as MS,

NMR have contributed significantly to perform the quality analysis of active principles.

1.3.4 Biological Evaluation

Biological parameters related to standardization of herbal medicines which include testing of pharmacological activities, assessment of toxicity and effectiveness. Biological assays are performed to test the activity or toxic behavior of the herbal drugs. These assays are done using specific cell lines models (*in vitro*) or the animal models (*in vivo*) (Kunle et al. 2012). Scientific evaluation of drug metabolism, drug retention and pharmacokinetic behavior of the drug has flourished the production of standardized herbal medicines.

1.3.5 Determination of biological and non-biological contaminants

Determination of various contaminants such as heavy metal, pesticides residue and microbial toxins is also the part of quality control of herbal drugs. Presence of toxic heavy metals like arsenic, lead, copper, cadmium and mercury can cause clinical ailments. Various methods have been mentioned in the pharmacopeias to determine level of toxic heavy metals. Simple color reactions or advanced chromatographic and spectroscopic techniques as atomic absorption spectroscopy, optical emission spectrometry or mass spectrometry, X-ray fluorescence spectrometry etc have contributed to check the presence of any toxic metal in the herbal drugs (Bandaranayake 2006; Yuan et al. 2011). Meanwhile, herbal drugs can also carry various soil born microbes inherently and in addition, poor handling can increase the microbial load. The presence of any microbe and their toxins are determined by simple laboratory practices as total aerobic count, total fungal count and total enetrobacteriaceae count. Specific tests are recommended to check the contamination of *Salmonella, E. coli, Staphylococcus aureus, Shigella* and *Pseudomonas* (Bandaranayake 2006). Moreover, the herbal drugs should be evaluated for the presence of any pesticide and radioactive contaminants which can be done using various analytical techniques. Pesticide residues can also be traced principally with column/ gas chromatographic techniques (Annonymus 1998).

1.4 Authentication/identification of botanicals is the first step in quality management

In general, quality parameters can be divided into three essential components 1) Botanical identity of raw material, 2) Purity of herbal preparations 3) Content of active compounds in certain limits (Bandaranayake 2006). Correct identification of the starting material is the very first step among the various quality parameters. The original plant species should be reliably distinguished from their close relatives, inferior substitutes, adulterants, filler materials and counterfeits (Chen et al. 2014). Case reports are there evidencing the serious ill effects, due to misidentification of the medicinal plant species. As, in 2004, aristolochic acid nephropathy was diagnosed in three patients. It was reported that they ingested Aristolochiae Mollissimae Herba (Xungufeng) which was derived from the *Aristolochia mollissima* Thunb, in place of non-harmful genuine anti-cancer herb Solani Lyrati Herba (Baiying), which was *Solanum lyratum* Hance. Safety issues regarding the use of Periplocae Cortex (Xiangjiapi) derived from *Periploca sepium* Bge. have also been reported. It was due to the replacement of original *Periplocae Cortex* with *Acanthopanacis Cortex* (Wujiapi) which was derived from *Acanthopanax gracilistylus* W.W. Smith (Chen et al. 2014). In addition, mixing of any inferior or irrelevant filler materials reduces the effective dosage of original drugs. Properly authenticated and scientifically validated plant species are the prime requirement for the preparation of evidenced based drugs. The authenticity of the material should be well established, whether it is required for the research or the clinical evaluation or for the consumption in the market (Smillie and Khan 2010). In the Indian prospect, as mentioned in the **section 1.1.1** most of the part export by Indian herbal sectors is in the form of the raw material and there are the reports that most of the adulteration and contamination has been testified in the herbal drugs from India along with China (Posadzki et al. 2013). A survey of 150 herbal drug companies revealed that more than 50% of companies are facing problems in collecting and authenticating raw material and 54 companies (36%) admitted that adulteration of raw materials is very common (Sahoo and Manchikanti 2013). These facts have raised the alarm to all the personnel's associated with the herbal drug sector to find the scientific solutions.

1.4.1 Reasons for misidentification and plant derived adulteration

Many factors as exemplified below are responsible for the misidentification of the herbs as well as plant-derived adulteration.

1.4.1.1 Careless Collection by unskilled collectors

Most of the times, plant collection rely upon the persons who are not expert in the taxonomic skills. Collections from the wild resources enhance the problem intensity. The persons who are employed as collectors, lack proper training for species identification in a scientific way. Mostly the persons are picked from the local communities who do not have appropriate knowledge of botanical names of the species. They know the plants from their indigenous names. This increases the chances of wrong identification and collection of the species. Cultivation of medicinal plants also depends the farmers who are not literate to the extent that they can grow right species. WHO recommended some GAP for medicinal plant collection from wild as well as from the fields. Each and every person involved in the plant collection should be trained and skilled for the procedure of collection in a scientific way. All the personnel as growers, collectors, producers should receive adequate knowledge of agriculture, plant science and their conservation.

1.4.1.2 Less knowledge about the authentic source

Sometimes the collection place or the sources are hard to explore or inaccessible for searching the authentic species. Easy accessibility to the other areas for collection of the any other similar species can lead to the procurement for analogous species from these areas. As for example authentic source of Nagakesar is *Messua ferrea* which is found abundantly in the Western Ghats of the Himalayan region but the unawareness of the collectors or suppliers and the restriction in collection in the forest areas, the drug is adulterated with the *Callophylum inophyllm* (Shah and Seth 2010).

1.4.1.3 Confusion in species identity

The method of description of medicinal plants is different in the modern era and in the ancient medical literature. In ayurvedic literature, plants were classified on the basis of their therapeutic

properties. Lack of apposite synchronizations between Sanskrit and botanical names, lead to the incorrect identification of plant species. Wrong interpretation of the plant description may cause the confusion which is sometimes influenced by the local languages and indigenous culture. The similarity in vernacular names based on the belief of indigenous people also one of the reasons of erroneous identity of the plants. These drugs were considered as 'Sandigdha dravyas' or 'Controversial drugs' (Dixit 2011). Jivanti is one of such example. Three different species *Lepatdenia reticulata, Desmotrichm fabricatum, Cimifuga foetida* all are known as Jivanti (Dixit 2011). Some other example include Ashoka and Brahmi. The three species *Shorea robusta, Polyalthia longifolia* and *Saraca asoca* all are confused as 'Ashoka drug' (Khatoon and Mehrotra 2009), while *Bacopa monnieri* and *Centella asiatica* both are traded under the same vernacular name Brahmi (Kumar 2007).

Sometimes the herbs which are available abundantly in different regions can be used as adulterant or substituent. As for example the two species, one is *Fumeria parviflora* which is known as Parapatta and the other is *Mollugo pentaphylla* which is used in Siddha medicines as Parpadagam. In the north Indian region the former plant species is traded both as Parapatta and Parapadgam and in the south India the later species is sold (Shah and Seth 2010). Similarly, Ginseng a very important drug used in traditional system of medicine. Different species are traded in different countries. *Panax ginseng* known as Chinese/Asian ginseng, *Panax quinquefolius* is American ginseng, Siberian ginseng is *Eleutherococcus senticosus*, *Withania somnifera* and *Acanthopanax senticoccus* known as Indian and Russian ginseng respectively (Kumar 2007).

The morphological similarity is also one of the reasons for the use of misidentified herbs. Lots of confusion associated with the genus Phyllanthus. The three morphologically near species *P. fraternus, P. amarus, P. urinaria* are considered as Tamalaki or Bhuiamalaki. Moreover, *P. amarus* and *P. niruri* sometimes considered as synonyms or the previous species is considered as a subspecies of the other. *P. fraternus* also considered as synonyms of *P. niruri* (Khabiya 2012; Theerakulpisut et al. 2008). Similarly, the source of drug Puunernava that is *Boerhavia diffusa* also has conflicts of identification with other *Boerhavia* species (Selvaraj et al. 2012). *Tribulus terrestris* also share similar morphology with *T. lanuginosus* and *T. subramanyamii*. Fruits of all

the three are known as goksura (Balasubramani et al. 2010).

Some of the medicinal plant species have more than one name. The more numbers of synonyms poses difficulty and can create misunderstanding. As for the example *Cassia acutifolia* also vernacularly known as Alexandiran senna and have different synonyms as *Senna Alexandria*, *C. senna*, *C. obtusata*, *C. sophera* (Kumar 2007).

1.4.1.4 Absence of taxonomic key characters during collection

In certain plants identification is possible only at a particular stage of life or may be relied upon particular organs which may or may not be present during the time of plant collection. As for example leaves of *Saraca asoca* and *Polyalthia longifolia* have close resemblance to each other (Begum et al. 2014). It would be difficult to differentiate them on the basis of leaves for untrained collectors. Flowers can help somehow as a character for identification. The problem becomes more prevalent in lower taxa as in algae, fungi, bryophytes, pteridophytes and conifers in which reduce taxonomic characters are present in much reduced form. As for instances, *Equisetum arvense* known as horse tail is the member of pteridophytes and can be admixed with the toxic *E. palustre* (Mills and Bone 2005).

1.4.1.5 Deficiency of the authentic species

Increasing demands of herbal drugs leads to their overexploitation. This condition is responsible for the collection of species other than authentic one. Some authentic species are endemic to a particular area, in such circumstances the species found abundantly in other areas are preferred for gathering. As for example *Hypericum perforatum* is the species of European region but in India *H. patulum* is sold as *H. perforatum*, which can be found copiously in the Indo-Nepal region (Shah and Seth 2010).

1.4.1.6 Intentional mixing of other vegetative parts of the same plant

Many secondary metabolites synthesized in the plants in tissue-specific manner. As in *Withania sommnifera* withaferin A is accumulate in leaves whereas withanolide A is root specific metabolite, which are used as the anticancer compounds and the neuroprotective compounds respectively (Gupta et al. 2013). In some medicinal plant species, specific plant part or organ is

consumed as the drug. As for example bark of *Cinchona, Saraca asoca,* fruits of *Terminalia chebula,* and roots of *Clerodendrum multiflorum* (Burm F.*)* etc. Different plant parts show different pharmacological activities. Mixing or substitution of organs and plant parts with the therapeutically important plant tissue of the same plant can produce negative impact.

1.4.1.7 Lack of working taxonomic knowledge

The other issue which is related to misidentification of the species is the lack of proper working taxonomic knowledge among the scientific community, deals with the plant-based drugs, as ethnopharmacologist, phytochemist, toxicologist, clinicians. A systematic survey of the research papers was done to know the problem and impact of erroneous botanical nomenclature in phytomedicinal research. The factors identified in these investigations may lead to the wrong interpretation of the research, irreproducibility in the results as well as in the application of a particular medicinal plant species for claimed health benefits (Bennett and Balick 2014; Rivera et al. 2014).

1.4.2 Methods for traceability of botanicals

Previous section was related to the factors responsible for the plant derived adulteration. Various methods are available to inhibit the use of incorrect starting material and to ascertain the identity of any herbal material. Conventionally, this is done by examining morphological characters, anatomical characters and chemical analysis using chemical fingerprinting or by evaluating specific markers. These methods have some limitations which can be overwhelmed by the adoption of DNA-based technologies. The subsequent sections encompass the description of scientific methods used for authentication of herbal material **(Figure 1.3).**

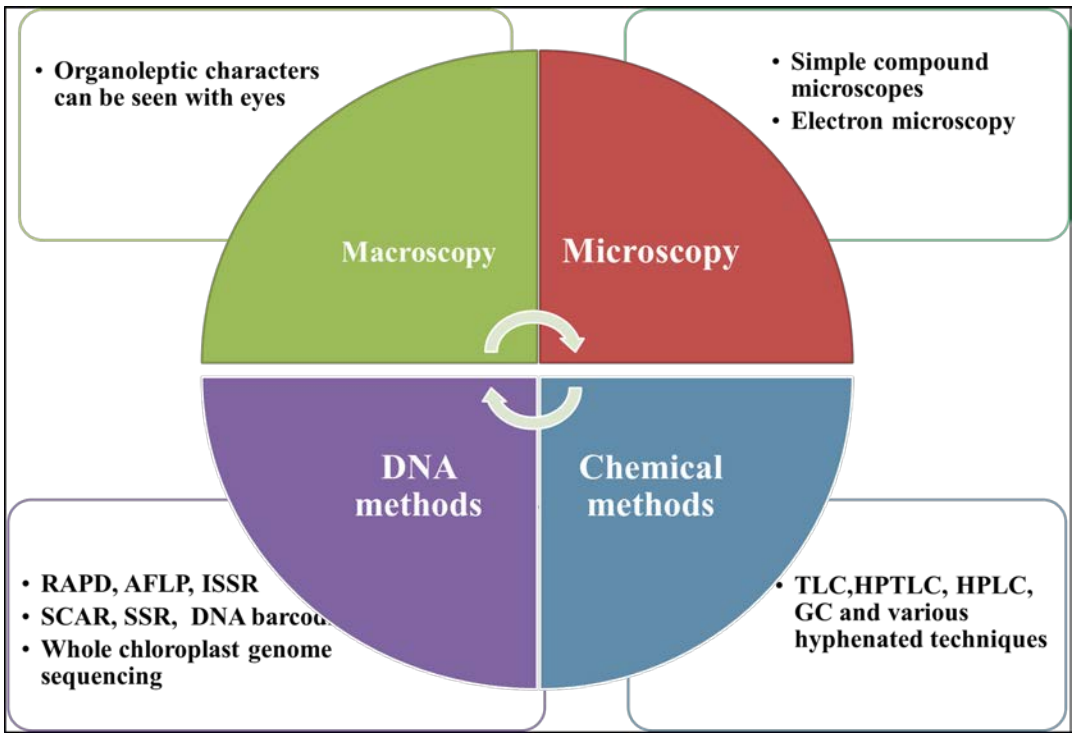

Figure 1.3 Scientific methods for correct identification of the medicinal plant species.

1.4.2.1 Morphological Identification

Morphological identification methods comprise an examination of the taxonomic key characters which can be easily visualized with eyes. Macroscopic characters depend on the analyses of organoleptic characters of whole plants or the plant part. For example shape, size, color, texture and odor of leaves, flowers and fruits, leaf type, leaf margin, leaf tips, flowers and their characteristics, inflorescence etc. Identification of the unknown sample can be possible by comparing it with the plant reference material in the form of herbarium or the voucher specimens. Morphological identification is the easiest way to validate the identity of any plant material in its intact form. But, it becomes impossible when the sample for identification is not complete and when the taxonomic key characters are reduced. The plant samples devoid of key taxonomic characters (as, the absence of flowers) also present challenge in the identification with the classical botany. In addition, impact of the personal expertise cannot be avoided. These methods are not sensitive enough for intraspecific gradations as ecotypes and chemotypes (Techen et al. 2004).

1.4.2.2 Microscopic Identification

Microscopic identification methods go a step further and can be applied to establish authenticity and identity of original botanicals and adulterants in the broken or grounded state, in which morphological characters are indistinguishable. Microscopy allows examining the sample identity using distinguished characters as histology, cell morphology, cell type and cell contents. Advanced microscopy techniques as the application of phase contrast microscopes, fluorescence and confocal microscopes, scanning electron microscope has increased the accuracy and precision of identification (Smillie and Khan 2010). However, cost factor limits the use of high-end microscopes over light microscopes. Microscopic identification methods for various medicinal plant species were illustrated in various reports (Zhang et al. 2015). However, microscopic analysis for identification also requires specialized trained persons. Moreover, it is difficult to found distinguishable characteristics in two closely related or co-generic species because most of the time they share similar anatomical features (Smillie and Khan 2010).

1.4.2.3 Analytical chemical methods

Analysis of phytochemistry and detection of chemical phytoconstituents of medicinal plants is the other most preferred approach in order to ascertain the identity of particular species. This can be done in two ways by generating chemical fingerprints (pattern oriented) or through identification of specific chemical /biological marker compounds (compound oriented) (Govindaraghavan et al. 2012). Many analytical methodologies as TLC, HPTLC, HPLC and GC along with various hyphenated detection modes can be used for authentication of botanicals using the either approaches (Smillie and Khan 2010; Steinmann and Ganzera 2011).

Chemical fingerprint analysis and more complex complete metabolic profiling have demonstrated their potential in various research (Gao et al. 2012; Li et al. 2011). However, to generate the established and properly validated chemical fingerprints for a particular plant species, a number of authenticated reference plant materials from the multiple populations are required. As exposure to differing environmental conditions and genome structure can bring the spatial and temporal variations in the chemical profile, even in the same species, so statistically significant number of representative samples are must (Dhami and Mishra 2015; Smillie and Khan 2010).

Amalgamation of chemical fingerprinting data with the statistical techniques as hierarchal cluster analysis and principle component analysis to generate chemometrics can provide automation and improve the quality of analysis through chemical profiling (Bansal et al. 2014). This multicomponent approach including chemical fingerprinting followed by chemometric analysis, was illustrated for the authentication purpose of Cassia seeds (Lai et al. 2010), licorice roots (*Glycyrrhiza glabra*, *G. uralensis*, and *G. inflate*) (Simmler et al. 2015), analysis of formulas containing *Taraxacum officinalis*, *Cynara scolimus*, *Silybum marianum*, *Hypericum perforatum*, *Chelidonium majus* and *Lycopodium clavatum* (Pop et al. 2013).

Chemical fingerprinting based on one or more known characteristic chemical or biological markers enhance the specificity over uncharacterized fingerprinting. Chemical markers can be defined as the fully characterized chemical constituent/s of the plants which can be used for qualitative as well as quantitative quality control of herbal drugs including species authentication. The chemical markers having any therapeutic value, are considered as biological markers (Li et al. 2008). Some other categories for chemical markers were proposed by various scientists. They also proposed their selection criteria to be optimally used in the standardization of herbal drugs (Li et al. 2008). The major issues related to widespread adoption of chemical markers for authentication is that, very few markers are well defined for very less number of herbal drugs and sometimes two different plants share the same markers. Commercial availability of the markers in lower costs is also a limiting factor because chemical synthesis or the extraction and isolation of the each identified marker compound is not possible. Environmental variations can cause phytochemical variability even in the same species. Universally applicable optimizations are required for methodological approaches for isolation and preparation of reference compounds and should be regulated properly to minimize the experimental variations (Govindaraghavan et al. 2012; Li et al. 2008; Smillie and Khan 2010).

1.4.2.4 DNA based methods- from genome profiling to DNA barcoding

Traditional methods from morphological assessment to chemical analysis for authentication and identification of medicinal plants have contributed immensely since the advent of pharmacognosy by Austrian physicist Schmidt. These techniques are the primarily mentioned in

the pharmacopoeias for the quality control of the herbal drugs including their authentication (Chen et al. 2014). Limitations of these approaches provoked the need for additional methods. DNA-based methods are prominently drawing the attention of scientific communities due to their various benefits over conventional methods. Genetic composition of a particular species is generally not affected by age, type of tissue, diverse physiological and environment conditions. DNA is very rigid molecule so chances of affecting the detection due to physical state of the samples are less. These approaches are not completely dependent upon the personal expertise as macro and microscopy. Small amount of experimenting material is required for detection. DNA-based methods can be used as stand-alone or as strengthening tool to the conventional methods (Joshi et al. 2004). Patents were granted in this field proves the need and progress of these techniques for authentication (Shaw et al. 2009). In a recent report by Palhares et al. (2015), it was recommended that authenticity of the plant samples must be evaluated with the DNA based methods. They observed that the samples which were identified as the substituents by the DNA methods, were passed in the quality checking by chemical marker analysis. They raised concerns about the deliberate mixing of synthetic chemical markers in the drugs which can enable them to be passed during chemical authentication. Genome-based approaches for herbs traceability and molecular identification of the medicinal material are progressing (Galimberti et al. 2013; Sucher and Carles 2008). Various DNA techniques have been using for medicinal plant species identification which can be broadly classified as 1) DNA fingerprinting and 2) Sequence-based methods

(A) Non-targeted DNA fingerprinting/genome profiling

In general, DNA fingerprinting here stands for the techniques which are arbitrarily/specifically primed to generate DNA banding patterns through *in vitro* amplification/ PCR. These techniques are very helpful when small genetic information of a particular species is available. These are the multilocus practices which cover the variations spreading in the whole genome and not dependent upon the prior information of genome sequences. These methods are relatively cheap and generate multiple markers in one run (Poczai et al. 2013). RAPD (Randomly amplified polymorphic DNA), AFLP (Amplified fragment length polymorphism), ISSR (Inter simple sequence repeats) are some of the examples of such basic techniques applied for molecular

identification of medicinal plant species and their discrimination with the plant-derived adulteration.

RAPD is based on the amplification of genome with arbitrary oligonucleotides of 10-15 bases in length. The amplification is carried out at low annealing temperatures. The primers bind several priming sites on the complementary sequences in the template genomic DNA and produce discrete DNA products if these priming sites are within an amplifiable distance of each other. Banding patterns thus generated are analyzed on agarose gels. RAPD was reported for molecular authentication of various medicinal plants (Guo et al. 2010; Khan et al. 2011; Lam et al. 2015). However, robustness and reproducibility is the major problem in RAPD. The technique is prone to changes in experimental conditions, for instances, poor template quality, presence of too much RNA, inconsistent interpretation of mixed-intensity banding patterns, competition between primer target sites, generation of heteroduplex molecules and generation of primer-derived, nonspecific amplification products, change in the thermocyclers units (Weising et al. 2005).

ISSR or Inter simple sequence repeats is the technique based on the variations detection between the microsatellite regions. Primers for amplification are designed based on the sequence repeats which resulted into the fingerprints, belong to the genomic regions between two repeat sites, oriented in the opposite direction (Godwin et al. 1997). ISSR are considered as more reproducible than RAPD due to longer primer length which allowed amplification on the higher annealing temperatures (Reddy et al. 2002). However, reproducibility may be negatively affected by sensitivity of the detection methods and can lead to the wrong interpretations (Ganie et al. 2015). ISSR markers have been used for genetic diversity measurement and to authenticate plants as *Sida* spp.(Thul et al. 2011), *Tribulus terrestris* (Sarwat et al. 2008) etc.

AFLP or amplified length polymorphism is the more accurate and reproducible fingerprinting technique. AFLP is based on the amplification of specifically restricted genomic DNA. It combines the reliability of the RFLP technique with the flexibility and rapidity of PCR technique. The technique involves three steps: (i) Restriction of the DNA with two enzymes rare and frequent cutter (ii) Ligation of oligonucleotide adapters (iii) selective amplification of restriction fragments having one to three random oligonucleotides at the 3’ends. AFLP have been used to

ascertain the identity of *Panax ginseng* and *Panax quinquefolius* (Ha et al. 2002), *Chlorophytum* and *Asparagus* (Misra et al. 2007), *Embelia ribes* burm.F and *Embelia tsjeriam*- Cottam A. DC.(Gowda et al. 2010), *Swertia* species (Misra et al. 2010), *Crocus sativus* (Busconi et al. 2015) etc.

(B) Sequence-based methods

The above mentioned description are the examples of DNA fingerprinting techniques used for the molecular identification of the medicinal plants. The advantage of these techniques comprises no requirement of prior knowledge of genome. However, their universal application may be hampered because their reproducibility and robustness changes in some extent with reaction conditions, template concentration, ratio of primer and template DNA, methods of DNA isolation, tissue source of DNA and quality as well as integrity of DNA. Results may be varying based upon the local lab expertise and difference in analysis can cause the errors (Boiteux 1999; Crawford et al. 2012; Jones et al. 1997). Genetic diversity of a particular species, hybridization and polyploidization history can also create a negative impact on the analysis especially when the purpose of the study is species authentication (Galimberti et al. 2013). In a report by Peng et al. (2016), ISSR markers were found insufficient to discriminate *Tetrastigma hemsleyanum* from the species of the same genus.

The advancement and cost reduction of sequencing procedures increase their popularity and fondness over gel based DNA fingerprinting, for various genome-based analysis integrating authentication (Mishra et al. 2015). These techniques need access to a sequence of the particular genomic region and can give more precision, robustness and improve cost/effectiveness ratio in comparison to discontinue fingerprinting techniques mentioned above (Galimberti et al. 2013). Sequence-based approaches have added smartness to the studies and can be used to obtain information directly from a defined locus with more transferability among labs than random fingerprinting techniques. Access to the sequence information in various databases eases the identification processes and makes them approachable and fast. Regarding DNA sequence-based approaches different techniques like SSR, SNP and SCAR are used widely for the authentication and several other genomic applications. These approaches are described below.

SCAR or sequentially characterized amplified region is the advance single locus targeted version of random markers. Dependent upon PCR they give rapidity, stability, reliability with specificity. These markers originated from the fingerprints, so give the advantage to be utilized where no any prior knowledge of genome sequence is available. To generate SCAR markers distinct bands were selected, eluted, cloned and then sequenced. The resulted sequence can be utilized to develop primers for the development of both singleplex as well as multiplex species-specific PCR-based identification assays. Most of the SCAR markers were derived from the RAPD due to its easy setup. Derivation of SCAR from the more robust techniques like AFLP can give the benefit of more reproducibility and fineness. SCAR markers were developed to authenticate three species of *Phyllanthus* (Theerakulpisut et al. 2008), *Angelica decursiva* (*Peucedanum decursivum*), *Peucedanum praeruptorum* and *Anthricus sylvestris* (Choo et al. 2009), *Panax notoginseng* (Kwon et al. 2009), *Crocus sativus* (Marieschi et al. 2010), *Ipomoea mauritiana* (Venkatasubramanian et al. 2011), Liriope and Ophiopogon (Li and Park 2012), *Cuscuta reflexa* and its adulterant *Cuscuta chinensis* (Abdin et al. 2012), *Knema andanamica* (Sheeja et al. 2013), *Bulbus fritillariae* (Xin et al. 2014).

Simple sequence repeats (SSR) or microsatellites are the markers having repeated units of nucleotides which are known as motifs. Each repeating unit may contain mono, di to six nucleotides and number of repetition of each unit can be polymorphic, hence it is a type of length polymorphism. These repeating units may be spread both in the coding or noncoding part of the nuclear as well as in the organelles genome (chloroplast and mitochondria). They are advantageous in terms of codominance, high allelic polymorphism and reproducibility. Many microsatellites markers were identified using different methods and for different purposes as genetic characterization, diversity analysis at the genomic level, cultivar identification, marker assisted selection breeding and mapping of medicinally important plants. Microsatellites markers were isolated from medicinally important plant, *Dendrobium officinale* in 2007 by Gu et al. , for authentication purpose along with an analysis of genetic diversity, population structure. SSR markers were also used to authenticate *Bupleurum chinense* (Sui et al. 2009), *Picrorhiza kurrooa* and to detect its adulterant *Lagotis cashmiriana* (Hussain and Bedi 2012).

DNA barcoding is the most advance concept developed for molecular identification of the animal or plant species which have been potentially extended to molecular standardization of herbal material. The above-mentioned techniques (SCAR, SSR) showed great potential to ascertain the identity of botanicals. However, lack of universality and standardized experimental procedures limit their use for authentication and simultaneously, transferability of these procedures is confound to a particular taxon and their close relatives (Galimberti et al. 2013). They are not sufficient for exact taxonomic annotation of a species, therefore, needed a more broadly accepted commercial tool which should be universally applicable to each species on the earth (Mishra et al. 2015). DNA barcoding has potential to define species boundaries very precisely. Cryptic species can also be identified. The concept of DNA barcoding was given by Hebert (Hebert and Gregory 2005) at first in animals which meant for utilization of variations present in the sequence of the single gene or intronic region as a unique identifier of a particular species; such that one sequence represents one single species. The standardized experimental procedure for DNA barcoding includes amplification of barcode region with universal primers, sequencing and then sequence comparison with databases. This is universally applied procedure and not limited to particular species and any particular stage of the life cycle for species identification. The sequence recommended for barcoding should contain some attributes as they
should universally amplifiable with a single set of primers, should not suffer during sequencing and should diverge enough between species than within species. The genes endorsed for barcoding in animals (gene for cytochrome oxidase) cannot be immediately used for plants due to its comparatively slow evolution pace (Kress et al. 2005). The inherent properties of plants make them harder to discriminate and species delimitation is somewhat difficult for this kingdom (Rubinoff et al. 2006). Different scientists recommended different regions for plant DNA barcoding both from chloroplast and nuclear genome **(Table 1.1)**.

Table 1.1 Various DNA barcode regions proposed and their characteristics (Vijayan and Tsou 2010; Hollingsworth et al. 2011)

Barcode	Genome location	Function	Approximate amplicon length in bp using different primer sets
nrITS	Nuclear	Transcribed Spacers between rRNA genes	683-724
nrITS2	Nuclear	Transcribed Spacers between rRNA genes	492-506
rbcL	Plastid	Protein coding, large subunit of Rubisco enzyme	550-734
trnH-psbA	Plastid	Intergenic spacers	296-1120
matK	Plastid	Protein coding for maturase K	734-930
rpoB	Plastid	Protein coding, Chloroplast Ribosomal protein B	298-510
rpoC1	Plastid	Protein coding, Chloroplast Ribosomal protein C1	467-564
trnL-F	Plastid	Intron and intergenic spacer	254-767
trnL (p6 loop)	Plastid	Intron	10-173
atpF-H	Plastid	Intergenic spacer between ATPase synthase genes	196-573
psbK-I	Plastid	Intergenic spacer between genes for low molecular weight proteins for photosystem II	444-492
Ycf5	Plastid	Protein coding	221-382

None of the barcoding candidates given in the **Table 1.1**, ideally cover all standards established for a DNA barcode. The highlighted barcoding regions are rbcL, matK, trnH-psbA from plastome and ITS/ITS2 from nuclear genome. Each candidate has own experimental, structural and functional benefits and limitations as well. RbcL gene is universally amplifiable (except in some algae) and can be sequenced easily but possess fewer variations between the species. Matk does not fulfill the criteria of cosmopolitan PCR amplifiability, in spite of having most variability. Variability between species is also attributable to psbA-trnH but due to the presence of nucleotides repeats sequencing part has the problem. However, chloroplast genome has its own constraints due to single parent inheritance. These regions can't identify the boundaries of species if hybridization, introgression and chloroplast capture was present in the evolutionary history.

Being existent in the nuclear genome ITS region can disentangle these problems as it is biparentally inherited, have low functional constraints, flanked by highly conserved genic sequences, high evolutionary rates give more discriminatory power and can be amplified from degraded DNA also (Hollingsworth 2011). Concerted evolution is the reason for the existence of identical copies of this array, but sometimes incomplete concerted evolution leaves the nonhomogenized copies within the genome. The presence of multiple copies can mislead the analysis. Orthology is needed for correct assessment of species resolution and presence of paralogous copies can hinder the investigation (Alverez & Wendel 2003). These factor disgraces the value of ITS. Despite, many scientists proposed ITS as a solid candidate for barcoding in different groups of plants with more emphasis for flowering plants (Chen et al. 2010; Group et al. 2011, Techen et al. 2014; Tripathi et al. 2013; Yao et al. 2010). In the view of the failures of single loci barcoding, many scientists recommended inclusion of two or three barcode candidates (multilocus approach) (Li et al. 2014). The plant working group of the consortium of the barcode of life stamped the two-locus system rbcL + mat K as suitable DNA barcode for plants (Group et al. 2009). Other barcode combinations as rbcL with and/or the internal transcribed spacers and ITS+ trnH– psbA, have also been found equally potential (Kress et al. 2005; Kress and Erickson 2008; Pang et al. 2012; Tripathi et al. 2013). In addition to the multiloci barcoding, two-tiered approach was proposed by Newmaster et al. (2006) in which two barcode loci should be used sequentially. Particularly, for medicinal plants rbcL can be used as first barcoding loci followed by ITS/ITS2 (Mishra et al. 2015).

Various international and national institutes (CBOL, iBoL) are working together to catalog each species on the earth with DNA barcoding and also provide a platform for sequence deposition which is freely accessible. Particularly for medicinal plants, MMDBD or Medicinal Materials DNA Barcode Database **(http://137.189.42.34/mherbsdb/index.php)** is the database provides sequence information related to medicinal plants materials recorded in the Pharmacopoeia of China and USA. This database, updated in May 2014 with 1661 species and 51,375 sequences available related to plants, fungi and animal. Furthermore, an online DNA barcoding database specific to herbal materials has been created **(http://www.tcmbarcode.cn)** based on the ITS2 and psbA–trnH as the core and supplementary DNA barcodes, respectively. This database also contains barcoding data related to adulterants, substitutes and closely related species (Chen et al. 2014).

(C) Future developments towards NGS

The advent of high throughput sequencing technologies termed as next generation sequencing or NGS expand the boundaries of DNA barcoding beyond the single or two locus barcodes. Sequencing of whole chloroplast genome is represented as the potent alternative of single or multilocus barcodes (Li et al. 2014). Consideration of whole chloroplast genome for plant species identification can increase the discrimination power and resolve the closely related species. In comparison to nuclear genome chloroplast genome is smaller in size so easy to be applied. However, unavailability of high-quality DNA, finished assembled sequence, lack of reference sequences or still high cost of sequencing restrict the use of whole chloroplast genome as barcode in routine practice (Li et al. 2014; Ivanova et al. 2016). Another futuristic advancement is the DNA based identification method, is the amalgamation of DNA barcoding with NGS technology which is demonstrated by various researchers and mentioned in the **Chapter 2 in section 2.3.7**.

Medicinal plants have their glorious past and still used as the medicinal resources. But, admiring the magnificent therapeutic potential of these plants is not sufficient to make them a reliable source of medicines. Scientific validation of phytopharmaceuticals is essential to make them consistently safer and efficacious. Quality in the herbal products is the major concern in their global acceptance. This has raised the need for scientific evaluation of herbal drugs from the initial step from plant collection or cultivation to the preparation of formulation. Correct identification of the species or the raw material is the first step for the production of standard herbal drugs. This is possible only through imposing strict legislations assisting with various scientific methods from morphological analysis to DNA-based methods. DNA-based methods are the most advanced approach and can be used stand alone. Furthermore, these can be combined with the other conventional practices to develop comprehensive approaches for authentication of botanicals. The project derives the advantages of DNA-based method for the purpose of correct identification of herbal material and discrimination of originals from the plant-derived adulterants.

1.5 The project

As mentioned in above section biotechnology has provided various sophisticated molecular techniques for authentication of botanical materials at the DNA level. The project is designed to develop DNA marker based on ITS region for the authentication and rapid identification of correct plant material. Two medicinally important plants were selected for the project one is *Terminalia arjuna* which is a cardioprotectent and can be adulterated with other *Terminalia* species. Secondly, *Convolvulus microphyllus* vs *Evolvulus alsinoides* which have controversial identity due to ambiguous vernacular name 'Shankhpushpi'. These plants are included in the highly traded and exported drugs (Ved and Goraya 2007). ITS region was selected due to its several merits biparental inheritance, universality, simplicity, intragenomic uniformity, intergenomic variability, low functional constraint and high copy number (Feng et al. 2010).

1.5.1 Scope of the project

Macroscopic and microscopic evaluation and chemical profiling techniques have their own limitations for authentication of plant species as discussed earlier. DNA-based markers give the new directions to pharmacognosy. It is important to distinguish accurately the authentic plant sources for herbal drugs because adulteration and substitutions diminish their efficacy and reduce the trust of users as well as it is necessary to convert botanical materials into scientifically valid herbal drugs. Proper integration of molecular techniques as analytical tools with conventional knowledge will lead to the development of a comprehensive system of identification of medicinal plants that can be conveniently applied at the research and industry level for quality control of botanicals.

1.5.2 Hypothesis

On the basis of above lines it was hypothesized that the ITS-based markers can be potentially used to develop DNA methods for unequivocal identification of medicinal plants material and can be distinguished from its adulterants.

1.5.3 Objectives

In order to carry the project some objectives were set which are described below:

- Collection of plant material including their adulterant species
- Optimization of technique for high-quality DNA isolation
- Amplification of genomic DNA with different universal primers
- Sequencing and development of barcode
- Primer designing for specific band
- Amplification with species-specific primer
- Data collection, analysis, and interpretation of results
- Report preparation

1.6 Organization of the thesis

The thesis comprises five chapters. The first chapter regarding introduction is followed by the **Chapter 2 Review of the literature**. The second chapter includes the details of Internal transcribed spacer region as DNA barcode as well as various tools and molecular techniques based on the ITS region used for correct identification of medicinal plant material. Description of the plants selected for the project is also given in the second chapter with the details of the research efforts done to authenticate these plants. The **Chapter 3 Materials and methods** include the details of various equipment, instruments, chemicals and reagents used in the study. Various methods and protocols followed for the completion of the project are also described. The **Chapter 4 Results & Discussion** deals with the various outcomes of the experiments in the form of tables and figures done to fulfill the objectives. In **Chapter 5** titled as **Summary and conclusion** major results are mentioned in summarize form and a conclusion is drawn. This is followed by the references cited in the work and appendices.