CHAPTER 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

According to the World Health Organization (WHO), about three-quarters of the world population relies upon traditional remedies (mainly herbs) for the health care of its people. In fact, herbs/plants are the oldest friends of mankind. They not only provide food and shelter but also served the humanity to cure different ailments. The herbal medicine also sometime called as traditional or natural medicine existed in one way or another in different cultures/civilizations, such as Egyptians, Western, Chinese, Kampo (Japan) and Greco-Arab or Unani /Tibb (south Asia) [1]. Traditions are dynamic entities of unchanging knowledge. Traditional medicine is in an evolutionary process as communities and individuals continue to discover new techniques that can transform practices. Drug discovery using natural products remain important issues in the current target-rich, lead-poor scenario [2].

Medicinal plants are harmoniously intertwined with the rich history, culture and culinary tradition of India. It has been recently claimed that 4639 ethnic communities living in different regions of India, utilize locally available medicinal plants to treat various ailments based on their rich and diverse folk knowledge [3]. India is very rich in ethno-botanical and medical anthropology. About 80% of the rural populations rely on traditional medicine or indigenous systems of medicine (ISM) for their primary health needs [4]. Traditional Indian system of medicine has a very old history of human use in various disorders and diseases, but lack in efficacy and safety.
1.2 MEDICINAL PLANTS AND HEALTH CARE SYSTEM

Plants have been a source of medicinal agents for thousands of years and continue to be an abundant source of novel therapeutic agents. It was estimated that approximately 5–15% of the total 250,000 species of higher plants have been systematically investigated and yet the potential of many plants that can be a good source of novel bioactive compounds has barely been tapped [5]. Plant products and herbal medicine play a vital role in the healthcare system, mainly in rural areas of developing countries. Medicinal plants have a great history as the source of potential therapeutic agents, for example reserpine, deserpidine, rescinnamine, vinblastine, vincristine, codeine, morphine, etoposide, guggulsterone, teniposide, nabilone, plaunotol, z-guggulsterone, lectinan, artemisinin and ginkgolides, which have been incorporated into modern medicine [6, 7].

1.3 QUALITY OF HERBAL MEDICINE

The World Health Organization (WHO) defined “herbal medicine” as: a plant derived material or preparation with therapeutic or other human health benefits which contains either raw or processed ingredients from one or more plants [8]. Herbal medicines can be classified into three groups: (i) herbal materials (raw or processed herbal materials, e.g. powder, slice), (ii) traditional herbal products (decoctions, tablets, pills or capsules containing crude herbal materials or crude extracts), and (iii) standardized herbal products (formulations containing standardized extracts or purified substances). From the cultivation of medicinal herbs to the final herbal product, there are many factors which influence the quality of herbal medicines. They are contamination, adulteration and misidentification.

Contaminants most likely to be found in herbal materials or herbal products include but are not limited to heavy metals, pesticides, microbes and mycotoxins [9]. Although the problems are universal, they seem to be more prevalent in Asian countries, especially in China and India, which are the largest producers and users of herbal medicines [10]. Heavy metals have been found in herbal medicines with some regularity. The three most commonly detected toxic metals are mercury, arsenic and lead; others include cadmium, copper and thallium [11]. These heavy metals are often contained in Asian herbal medicines, especially in Chinese patent medicines (CPMs) and Indian Ayurvedic medicines (IAMs) [12]. Contamination can occur due to: (a) the accumulation of heavy metals in the environment
(e.g. from contaminated soil or atmosphere); (b) inadvertent pollution of the production process; (c) or deliberate addition. Minerals, including toxic metals, are commonly used in some traditional medicine systems for specific curative purpose (e.g. traditional Chinese medicines and Indian Ayurveda medicines). The notion that heavy metals have positive health effects is based on ancient concepts which are not sustainable in the light of modern science. Therefore heavy metals have no place in herbal medicines.

Pesticides include insecticides, fungicides and herbicides. To some degree, residues of pesticides including their metabolites and/or degradation products will remain in plants, animals or in the soil. Such residues have become a notable source of contamination for herbal medicines. The WHO and other organizations have established requirements to limit pesticide residues in herbal materials [13]. Contamination with microbes is a common problem in herbal medicines. Pathogenic organisms including enterobacter, enterococcus, shigella and streptococcus have been shown to grow on herbal materials. Mycotoxins include fusarial toxin, aflatoxins, ochratoxin, citreoviridin, penicillic acid etc. Aflatoxins are both relatively common and highly poisonous [14].

Other foreign matter, including ash, adjuvant or organic solvents can also lead to external contamination in herbal medicines. To ensure good quality of the end product, it is necessary to minimize this problem. Adulteration is always fraudulent and means ‘‘to make impure by adding extraneous, improper or inferior ingredients’’. Instances of herbal medicines adulterated with orthodox drugs and plant materials have repeatedly been documented [15]. Adulterations can be classified into three categories: addition of orthodox drugs to herbal medicines, substitution (use of fake or inferior plant materials), and addition of foreign materials (non-officinal herb parts, sands, metals).

Contrary to adulteration/substitutions (deliberate behavior), misidentification happens inadvertently. False authentication can occur, when an importer or retailer mistakes one herb for another, due to incorrect labeling and similar appearance of the herbal materials [16]. Confusing nomenclature can be one of the reasons. One herb may be known by many names: one or more common names, a Latin name, Local names, and the brand name. Some different medicinal herbs of different plant species with different constituents may have the similar names.
1.4 STANDARDIZATION OF HERBAL DRUGS

Herbal medicines have distinctive characteristics that make them different from synthetic drugs. They contain more than one active compound and the active principle is frequently unknown. The chemical profiles of medicinal plants are affected by conditions of cultivation, manufacturing, marketing and distribution. Physiological, genetic and environmental variables (photoperiod, climate, soil conditions, nutrient availability and moisture) affect the biochemical profiles and secondary metabolite production in plants. Secondary metabolite content is also dependent on time of harvesting, storage, drying, extraction and processing for final packaging [17].

The development of plant based medicines requires comprehensive understanding of plant systems including biological, chemical, genetic and agronomic aspects. Chemical consistency at all stages of manufacturing processes such as extraction, stability, shelf-life and purity is utmost importance to ensure medicinal efficacy and consumer safety. Substantive evidence is lacking with respect to unique physiology of medicinal plants and their bioactive constituents. Several markers such as taxonomic, chemical, genomic, proteomic markers aid in identification of herbal drug components. Such methods encompass morphological identification (macroscopic), anatomical identification (microscopic), chemical analysis such as IR, HPTLC, HPLC, LC/MS, GC-MS, NMR, protein analysis and the use of molecular markers. Chemical markers are categorized into analytical markers and active markers. Analytical markers are the constituents or groups of constituents that serve solely for analytical purposes, whereas active markers are the constituents or groups of constituents that contribute to therapeutic activities. In cases, where no active constituent or marker can be defined for herbal drug, the percentage extractable matter with a solvent may be used. A total of 282 chemical markers are listed in the secondary metabolites, as markers have been extensively used in quality control and standardization of botanical drugs.

DNA-based techniques have been widely used for authentication of plant species of medicinal importance. This is especially useful in case of those that are frequently substituted or adulterated with other species or varieties that are morphologically and/or phytochemically indistinguishable.
DNA markers as new pharmacognostic tool traditionally, pharmacognosy mainly address quality related issues using routine botanical and organoleptic parameters of crude drugs. Pharmacognosy became more interdisciplinary because of subsequent advances in analytical chemistry. These developments added emphasis on chemo profiling-assisted characterization with chromatographic and spectroscopic techniques. The new pharmacognosy includes all aspects of drug development and discovery, where biotechnology-driven applications will play an important role. Extensive research on DNA-based molecular markers is in progress in many research institutes all over the world. This technique remains important in plant genome research with its applications in pharmacognostic identification and analysis. Chinese researchers have applied DNA markers extensively for characterization of botanicals from the Chinese materia medica. These markers have shown remarkable utility in quality control of commercially important botanicals like Ginseng, Echinacea and Atractylodes.

In India several agricultural universities and research institutes are actively involved in exploring DNA-based techniques in genotyping of medicinal plants. Although considerable progress has been made in DNA marker technology, applications of these techniques for characterizing semi-processed and processed botanical formulations to ensure the desirable quality remain underutilized. Although DNA analysis is currently considered to be cutting-edge technology, it has certain limitations due to which its use has been limited to academia. In order to establish a marker for identification of a particular species, DNA analysis of closely related species and/or varieties and common botanical contaminants and adulterants is necessary, which is a costly and time-consuming process. Isolation of good-quality DNA suitable for analysis from semi-processed or processed botanicals is also a challenge. Another important issue is that, DNA fingerprint will remain the same irrespective of the plant part used, while the phytochemical content will vary with the plant part used, physiology and environment.

DNA fingerprinting ensures presence of the correct genotype but does not reveal the contents of the active principle or chemical constituents. Hence DNA analysis and pharmacognostic techniques for chemo-profiling such as TLC, HPTLC, etc., will have to be used hand in hand rather than in isolation. Identification of quantitative-trait loci\(^34\), that are closely linked to a biologically active phytochemical will prove to be useful. Several
attempts have been made in recent years, to correlate DNA markers with qualitative and quantitative variations in phytochemical composition among closely related species. Proper integration of molecular techniques and analytical tools will lead to the development of a comprehensive system of botanical characterization that can be conveniently applied at the industry level for quality control of botanicals. The genetic composition is unique for each species and is not affected by age, physiological conditions and environmental factors. DNA based markers are also used in identification of inter- and intra-species variation. Marker based analysis has limitations as the markers are not single compounds and often a combination of methods become necessary for herbal component detection [18].

1.5 GLOBAL SCENARIO AND SAFETY REGULATIONS

Herbal products are not completely free from side effects. Well-controlled randomized clinical trials have revealed that undesirable side effects are possible in the use of herbal drugs. Due to increased reports on adverse effects regulatory/ monitoring agencies in many countries have brought out alert on herbal drugs. The WHO database has over sixteen thousand suspected herbal case reports. The most commonly reported adverse reactions are hypertension, hepatitis, face oedema, angioedema, convulsions, thrombocytopenia, dermatitis and death [19].

Undeclared chemical or synthetic substances or other active ingredients are the adulterants which are common in raw material tradeoff medicinal plants. Adverse event reports are often due to the presence of unintended herbs and this has affected the promotion of herbal products. Adulteration of herbal drugs with one or more synthetic drug is reported from different parts of the world. Fourty-one products out of 3320 Chinese Proprietary Medicines (CPM) screened by Health Science Authority (HSA) Singapore, between 2001 and 2013 were found to contain nineteen synthetic drugs [20].

Substitution involves intentional replacement with another plant species or intentional addition of a foreign substance to increase the weight or potency of the product or to decrease its cost. The use of fake or wrong herbs has generated serious questions about the safety and efficacy of herbal products. Another common problem with use of herbal medicines is the intentional or accidental presence of toxic heavy metals in more than the permissible limit set by national regulatory authorities [21].
Toxic contaminants are reported at all steps beginning from collection of raw materials to manufacturing [22]. Lead, mercury, copper and arsenic are the predominant contaminants. Thirty-one ayurvedic formulations were analyzed for their mercury content. It was found that, with the exception of one remedy, all exceeded the legal limits by more than two magnitudes. Huge variability of mercury content was also observed within one identical remedy manufactured by different companies indicating to the lack of product uniformity and the associated risks [23].

The presence of pesticide residues in herbal materials has seriously affected the development and process of internationalization of traditional herbal medicine. Contamination of crude medicinal plants as well as their products/preparation (infusion, decoctions, tinctures and essential oils) has increasingly been reported. A study with 280 samples of 30 different TCMs for pesticides residues showed that 75.8% of samples contained at least one organochlorine pesticides such as PCNB, aldrin, BHC or DDT [24].

The WHO has established maximum residue limit for pesticides in cultivated or wild medicinal plants as well as appropriate methodologies for their detection [25]. Practices used in harvesting, handling, storage, production and distribution can result in contamination by various fungi. Evaluation of ninety-one medicated herbal samples for the presence of predominant mycoflora and the extent of fungal contamination showed that 54.9% of the samples exceeded the limit determined by the US Pharmacopoeia (2×10² CFU/g of the product is the maximal fungal contamination limit) [26].

These reports on adulteration, contamination with heavy metals, pesticides and microbes in herbal drugs and their effects on health have necessitated the development of effective identification systems for herbal drugs and their components. Methods that ensure the quality and safety of these products have to be developed in order to ensure the quality and purity of herbal drugs.

1.6 TRADITIONAL KNOWLEDGE ON HERBAL DRUGS

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Although some of the therapeutic properties attributed to plants have proven to be
erroneous, medicinal plant therapy is based on the empirical findings of hundreds and thousands of years. The vast majority of people on this planet still rely on their traditional material medica (medicinal plants and other materials) for their everyday health care needs. It is also a fact that one quarter of all medical prescriptions are formulations based on substances derived from plants or plant-derived synthetic analogs, and according to the WHO, 80% of the world’s population primarily those of developing countries rely on plant-derived medicines for their healthcare.

It is likely that, the profound knowledge of herbal remedies in traditional cultures developed through trial and error over many centuries, and that the most important cures were carefully passed on verbally from one generation to another. People who use traditional remedies may not understand the scientific rationale behind their medicines, but they know from personal experience that some medicinal plants can be highly effective if used at therapeutic doses. Since we have a better understanding today of how the body functions, we are thus in a better position to understand the healing powers of plants and their potential for their potential as multi-functional chemical entities for treating complicated health conditions.

Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health. A single plant may, for example, contain bitter substances that stimulate digestion, anti-inflammatory compounds that reduce swellings and pain, phenolic compounds that can act as an antioxidant and venotonics, anti-bacterial and anti-fungal tannins that act as natural antibiotics, diuretic substances that enhance the elimination of waste products and toxins and alkaloids that enhance mood and give a sense of well-being. In most societies today, allopathic and traditional systems of medicine occur side by side in a complimentary way. The former treats serious acute conditions while the latter is used for chronic illnesses, to reduce symptoms and improve the quality of life in a cost-effective way [27]. Ayurveda is perhaps, the most ancient of all medicinal traditions is probably older than the traditional Chinese medicine. It is considered to be the origin of systemized medicine. It is actually a practical and holistic set of guidelines to maintain balance and harmony in the system. Dioscorides (who influenced Hippocrates) is thought to have taken many of his ideas from India. Ancient
Hindu writings on medicine contain no references to foreign medicines whereas Greek and Middle Eastern texts refer to ideas and drugs of Indian origin.

Ayurveda is derived from the Indian words Ayar (Life) and veda (Knowledge or Science) and hence means the Science of Life. Following the system would help to ensure a long life, which is considered to be the instrument for achieving righteousness (dharma), wealth (artha) and happiness (sukha). In India, knowledge and wisdom have been passed on from one generation to the next through songs and poems, which scholars and physicians had to learn by heart and recite. The Veda is an ancient text in four parts (Rig Veda, Sama Veda, Yajur Veda and Atharva Veda), the earliest of which date back to 2000 years BC.

The principles of Ayurvedic medicine and the medicinal plants uses of herbs are contained in thousands of poetic hymns in the Rig Veda. The first school to teach Ayurvedic medicine was at the University of Banaras in 500 BC and the great Samhita (or encyclopedia of medicine) was written. 700 Years later, another great encyclopedia was written and these two together form the basis of the Ayurveda. Ayurveda is similar to Galenical Medicine in that, it is based on bodily humours (dosas) and the inner life force (prana) that is believed to maintain digestion and mental activity. The living and the non-living environment, including humans, is composed of the elements earth (prithvi), water (jada), fire (tejac), air (vaju) and space (akasa). For an understanding of these traditions, the concept of impurity and cleansing is also essential. Illness is the consequence of imbalance between the various elements and it is the goal of the treatment to restore its balance [28].

Plants with complex phytochemical mixtures have advantage over single molecules in treating such diseases, with an added advantage of being devoid of toxic side effects. The World Health Organization (WHO) encourages the use of plant-based medicine, especially in developing countries, even if the rationale is to reduce the financial burden on the respective governments. In view of the increasing demand for herbal products in Western countries, well-defined herbal products of proven efficacy and safety have been introduced in the last two decades. In the draft of the National Policy on the Indian Systems of Medicine, priority is being given to research on standardization, pharmacology, toxicology and clinical trials of herbal drugs [29].

Ethnopharmacology and natural product drug discovery remains a significant hope in the current target-rich, lead-poor scenario. Many modern drugs have origin in traditional
medicine and ethnopharmacology. Traditional Indian Medicine – Ayurveda has a long history and is one of the great living traditions. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on Ayurvedic medicinal plants. Several preclinical and clinical studies have examined cytoprotective, immunomodulatory and immunoadjuvant potential of Ayurvedic medicines [30]. The ethnopharmacology knowledge, it’s holistic and systems approach supported by experiential base can serve as an innovative and powerful discovery engine for newer, safer and affordable medicines.

Traditions are dynamic entities of unchanging knowledge. Traditional medicine is in an evolutionary process as communities and individuals continue to discover new techniques that can transform practices. Ethnopharmacology and drug discovery using natural products remain important issues in the current target-rich, lead-poor scenario. Many modern drugs have their origin in ethnopharmacology. Globally, there is a positive trend in favor of traditional and integrative health sciences both in research and practice. There are common approaches to drug discovery including use of chemical biology, serendipity, chemical synthesis, combinatorial chemistry and genomics. However, the innovative approaches involve ethnopharmacology, reverse pharmacology, holistic, systems biology and personalized medicine. There are clear trends to show that the mainstream in pharmaceutical research is moving away from single molecule or single target approach to combinations and multiple target approaches [31].

1.7 MEDICINAL PLANTS OF WETLANDS

Medicinal plants of the wet lands in India are exploited to a very less extent, despite the availability of rich traditional knowledge and also greater possibilities of offering novel bioactive compounds. In recent decades the resurgence of focus is slowly moving towards the wild and aquatic plants of wet land ecosystem to meet the increasing demand for novel herbal drugs [32, 33]. The wild or underutilized plants are experiencing more attention, due to their contribution, in meeting the greater demand for vegetable protein, carbohydrate, antioxidants, polyphenols etc., [33]. The plants of wet land ecosystems, played an important role in the life of human beings in earlier days, as food, fodder, medicine etc., but with the advancement of agriculture and urbanization, the uses of wet land aquatic herbs are neglected and they are treated as noxious weeds and the wet lands as a menace, aquatic
plants face a great threat of extinction, due to the lack of awareness on their nutritional values in favor of the exotic ones.

Wet land plants are rich in dietary nutrients such as proteins, carbohydrates, fats, oils, vitamins, amino-acids and minerals [34]. The ethnic communities of different states of India are well acquainted with wet land plants and are expertise in utilizing them as drugs for various ailments in crude form. Wet land plants are classified as floating, submerged and emergent. Emergent aquatic macrophytes are defined as plants that are rooted in shallow water with vegetative parts emerging above the water surface. It is thought that, emergent macrophytes are the most particularly productive of all aquatic macrophytes, since they make the best use of all three possible states, with their roots in sediments beneath water and their photosynthetic parts in the air [35].

*Monochoria vaginalis* C. Presl and *Monochoria hastata* (L.) Solms, belonging to Pontederiaceae (Water-Hyacinth) family are emergent aquatic macrophytes, commonly found throughout India. *Monochoria vaginalis* is a small annual aquatic plant and is one of the two species within the genus, the other being *Monochoria hastata* [36]. Both the species are gregarious in habit and are similar in morphology, *M. vaginalis* is an emergent aquatic herb with short, sub-erect spongy root-stocks commonly found in rice fields, throughout India and widely distributed in Asian countries like South Korea, Japan, China etc., [35]. This aquatic plant, which is claimed to be a noxious weed, has numerous medicinal properties. The kattunaiykar, Paniya and Chetti tribes of Wayanad district of Kerala, use the entire plant, except the rootstock as vegetable [37] and the juice of the fresh leaves for the treatment of diabetes [38]. Traditional healers of Tamilnadu use the powdered rootstock as dentifrices [39], whereas, the ethnic communities of Assamese in Assam consume the decoction of rootstock for the treatment of toothache and asthma [40].

*M. hastata* is an emergent aquatic herb with elongate, creeping, spongy rootstock found in the margins of tanks and ponds, swamps, ditches and brackish water, almost throughout India, Srilanka and South East Asia [41].Tender stalks and leaves of the plant are eaten as vegetable; ethnic communities of Tinsukia District of Assam consume the juice of the leaf as digestive [42], the plant extract is applied locally on boils for quick healing by Tripuri tribes [32]. Even though, these plants were claimed to be medicinally rich in terms of
ethno-medicinal practices, considerably very less research has been carried on systematics and as well as for its medicinal and nutritional properties [43], due to the difficulty in identification of the aquatic herb from their morphologically similar species.

Hence the present study is an initialization for the first time to explore and compare the detailed Pharmacognostical, Phytochemical and Biological properties of these plants. Identification and standardization of therapeutic elements of these medicinal plants will enhance the health care system and nutritional status of ethnic communities. WHO has strongly highlighted the need for safety evaluation and quality assurance of traditional medicine. Thus it becomes compulsory that all herbal preparations and raw materials acquired from the wild and cultivated source has to be screened for the presence of heavy metals to guarantee quality, efficacy and safety of herbal preparation.