Chapter 1

INTRODUCTION

The World Health Organization (WHO) has estimated that more than 80% of the world’s population in developing countries depends primarily on herbal medicine for basic healthcare needs. Of the 2,50,000 higher plant species on earth about 40,000-50,000 plant species are known to be used in traditional and modern medicinal system on the global scale (Farnsworth and Soejarto, 1991). India is one of the world’s 12 biodiversity centres with the presence of over 45,000 different plant species. India’s diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes. Of these, about 15,000-20,000 plants have good medicinal values. However, only 7,000-7,500 species are used for their medicinal values by traditional communities (Dhar et al., 2002). In India, drugs of herbal origin have been used in traditional systems of medicines such as Unani and Ayurveda since ancient times. The Ayurveda system of medicine uses about 700 species, Unani 700, Siddha 600, Amchi 600 and modern medicine around 30 species. The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from excretory plant products such as gum, resins and latex. Even the Allopathic system of medicine has adopted a number of plant-derived drugs which form an important segment of the modern pharmacopoeia. Some important chemical intermediates needed for manufacturing the modern drugs are also obtained from plants. Not only, the plant-derived drug offers a stable market world wide, but also plants continue to be an important source for new drugs.
North East India, which occupies only 8% of the total geographical area of the subcontinent, contains about 50% (±8,500 spp.) of the floristic wealth and the richest phytodiversity of the country (Mao, 2006). North East India has been prospering for many centuries due to its own tradition of healthcare system. The Himalayan region including North East India is a rich repository of medicinal plants with a total of 1,748 species. However, this region is still unexplored and informations available about these plants are rudimentary and scanty due to lack of proper biotechnological implementation (Das, 2008).

Medicinal and aromatic plants (MAP) are of global significance, both within the context of the community and the realm of international trade. Plant derived natural products hold great promise for discovery and development of new pharmaceuticals. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments.

Herbal drugs have been extracted from plants cultivated in fields or growing in wild strands. It has been reported that nearly 95% of the plants used traditionally are collected from its natural habitat (Kamboj, 2000). In view of the growing world population, increasing anthropogenic activities, rapidly eroding natural ecosystems etc., the natural habitat for a great number of herbs and trees are dwindling. Endemicity, restricted distribution, small populations, inaccessible areas and anthropogenic pressures have caused a decline in wild populations of many species making their status as rare plants (Kokate et al., 2005). Habitat loss and unchecked commercialization of wild medicinal plants is threatening the future of vital resources, as well as the beauty, diversity and natural heritage of our planet. Unchecked commercialization may render
important traditionally used medicinal plant resources inaccessible and unaffordable to populations that have relied on them for centuries. In addition to the loss of access to traditional remedies by indigenous communities, over collection of species poses a significant threat to some commercially valuable wild species and to their habitats as well. This threat has been known for decades. According to the International Union for Conservation of Nature (IUCN), about 15,000 medicinal plant species may be threatened with extinction worldwide from over harvesting. There is a direct need to standardize herbal drugs to include them in the official pharmacopoeia and to provide genuine raw material for manufacturing standard medicines. The adulteration of material collected from the wild, either deliberate or unintentional, also plays a large role in the efficacy of the final product. There are fluctuations in the concentrations and quantities of secondary metabolites, although controlled genetically; it is affected strongly by the environment. Keeping in pace with modern demand, it has become highly imperative to regenerate, multiply and conserve plants with desirable traits. Although most plants have natural means of doing this, but they have not been able to meet the market demand globally. It is therefore imperative to conserve our medicinal plants wealth on a scientific basis.

Biotechnology has opened up new vistas for mass multiplication of the medicinal plants. Development of tissue culture technology offers a great potential for rapid multiplication, large-scale propagation and the reintroduction of the plants in its natural habitats (Bhojwani et al., 1989; Lal et al., 1988). It can also be harnessed for production of plant secondary metabolites. As plant cells are totipotent, cultured cells obtained from any part of the plant might be expected to yield secondary compounds similar to those of the plant grown in vivo. Therefore the exploration of tissue culture technique in medicinal plants for the extraction of important chemical compounds is indeed desirable (Tabata, 1977).
Essential oils are highly concentrated, volatile, hydrophobic mixtures of chemicals extracted from plants. The oils usually consist of a complex mixture of tens to hundreds of low molecular weight terpenoids. Essential oils are composed of more than 70% of terpenes, monoterpenes and sesquiterpenes, (Kulisic et al., 2004) some of which may represent more than 85% of the total content. Essential oils are most commonly extracted by steam distillation, while organic solvent extraction is also sometimes used. Essential oils have characteristic flavor and fragrance properties, and many also possess other biological activities. For these reasons essential oils are used in many industries. The food industry uses them as flavorings (e.g. soft drinks, food additives, confectionary), the cosmetics industry uses them for their fragrance (e.g. perfumes, skin and hair care products), and the pharmaceutical industry uses them for functional properties (e.g. antimalaria, antimicrobial activity). Essential oils are widely used for aromatherapy and in other alternative healthcare products. Some essential oils are also used as insect repellents, and as detergents. About 90% of global essential oil production is consumed by the flavor and fragrance industries. This is mostly in the form of cosmetics, perfumes, soft drinks and food. The largest consumer of essential oils is the USA, followed by western European countries like France, Germany and the UK, and Japan (Holmes, 2005). Approximately 3000 plants are used for their essential oils, with 300 of these being commonly traded on the global market (CBI, 2009a). It is difficult to obtain exact data on the global production of essential oils, but an estimate of 45 000 tonnes was reported in 2004. A recent estimate of the leading 20 essential oils is much higher at 104 000 tonnes (CBI, 2009b). Essential oils can be classified into three groups based on the volumes produced globally. Production of the first group exceeds 100 tonnes per year, the second group is between 50 and 100 tonnes, and the third group is between 1 and 50 tonnes (Shrinivas and Kudli, 2008).
In their natural environment, plants are colonized both by external and internal microorganisms. Some microorganisms, particularly beneficial bacteria and fungi, can improve plant performance under stress environments, and consequently enhance yield (Lazarovits and Nowak, 1997). Mycorrhiza is a mutualistic association between fungi and higher plants (Menge, 1983). Arbuscular mycorrhizal fungi (AMF) belong to a monophyletic phylum, the Glomeromycota (Zhu et al., 2010) and are ubiquitous soil microbes constituting an integral component of terrestrial ecosystems forming symbiotic associations with plant root systems of over 80% of all terrestrial plant species, including many horticulturally as well as medicinally important plants. They are obligate symbionts and acquire carbon from their host plants to complete their life cycle. In return, the fungus provides multiple benefits for the plant, including enhanced mineral nutrition and tolerance to abiotic and biotic stresses (Smith and Read 2008). In general, the symbionts trade nutrients, and the AMF obtains carbon from the plant while providing the plant with an additional supply of phosphorus. As a consequence, the AM symbiosis is of tremendous significance to life on this planet, in both natural and agricultural ecosystems. Hence, the use of AMF can provide an alternative and effective approach for ensuring the proper establishment of in vitro propagated plantlets under field conditions. Excessive production of secondary metabolites has been attributed to establishment of AMF symbiosis in in vitro raised plantlets. Very limited attempts were made towards the improvement of quality and production of secondary metabolites using VAM e.g. enhanced production of Artemisinin by Glomus species (Kapoor et al., 2007). A similar technology can be applied in several important medicinal and essential oil yielding plants for improved secondary metabolite production.

Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from the metabolism of oxygen. ROS, including superoxide radicals, hydroxyl
radicals, hydrogen peroxide etc. are often generated as byproducts of biological reactions or from exogenous factors. Considerable evidence have accumulated to implicate cellular damage arising from ROS, in the etiology and pathophysiology of human diseases such as neurodegenerative disorders (e.g. Alzheimer disease, Parkinson disease, multiple sclerosis, Down’s syndrome), inflammation, viral infections, autoimmune pathologies, and digestive system disorders such as gastrointestinal inflammation and ulcer (Aruoma, 2003). There is a growing demand for natural products in the human diet, both due to the possible negative effects of synthetic food additives on human health and to the increased consumer perception of this problem in recent years. Numerous studies demonstrate that a great number of medicinal and aromatic herbs, as well as fruits and leaves of some berry plants biosynthesize phytochemicals possessing antioxidant activity and may be used as a natural source of free radical scavenging compounds (Blasa et al., 2007). Broadly defined, an antioxidant is a compound that inhibits or delays the oxidation of substrates even if the compound is present in a significantly lower concentration than the oxidized substrate (Halliwell and Gutteridge, 2007).

The natural resources of both potential and established antioxidants are vast. Some antioxidant compounds are extracted from easily available sources, such as agricultural and horticultural crops (maize, buckwheat, grapevine, carrots, beetroot, citrus hesperidia, hops, apples, berries, tea leaves etc.), or medicinal plants such as pine, skullcap, tormentil, and many others. Spices and herbs like rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger, and several Chinese medicinal plant extracts (Lee et al., 2003) are recognized as sources of natural antioxidants.

Nowadays, essential oils and their components are gaining attention because of their relatively safe status, wide acceptance by consumers, and the possibility of their exploitation for potential multi-purpose functional uses (Ormancey et al., 2001). The
antioxidant activities of spice essential oils have been widely demonstrated (Sebranek et al., 2005) although the mechanism of such activity is not fully understood. The bioactive compounds present with antioxidant properties also interfere with propagation reactions (Russo et al., 2000) and inhibit the enzymatic systems involved in initiation reactions (You et al., 1999). The activities of essential oils such as antioxidants depend not only on their structural features but also on many other factors such as concentration, temperature, light, type of substrate and physical state of the system, as well as on microcomponents acting as pro-oxidants or synergists. Aqil et al. (2006) reported about the antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. The overall antioxidant activity of \textit{Lawsonia inermis} was the strongest, followed in descending order by \textit{Ocimum sanctum}, \textit{Cichorium intybus}, \textit{Piper cubeba}, \textit{Punica granatum}, \textit{Allium sativum}, \textit{Delonix regia}, \textit{Terminalia chebula}, \textit{Terminalia bellerica}, \textit{Mangifera indica}, \textit{Camellia sinensis}, and \textit{Trigonella foenum-graecum}. Seven plants, namely \textit{Terminalia chebula}, \textit{Mangifera indica}, \textit{Terminalia bellerica}, \textit{Punica granatum}, \textit{Ocimum sanctum}, \textit{Cichorium intybus}, and \textit{Camellia sinensis} essential oil showed strong free radical scavenging activity with the DPPH method. The tested plant extracts showed promising antioxidant and free radical scavenging activity, thus justifying their traditional use.

The Valerian (Fam: Valerianaceae) originated from Latin word 'Valere' meaning 'to be in good health' is a well-known medicinal family in India and contain only 10 genera and about 370 species worldwide, mostly distributed in north temperate region. Some species are reported from high altitude region of tropical zone only. Valerianaceae occurs naturally throughout the world except Australia and New Zealand. In India about 16 species and two subspecies are reported (Girgune et al., 1980). \textit{Valeriana jatamansi} Jones syn. \textit{V. wallichii}, popularly known as Indian valerian (English), Mushkbala or
Sugandhbala (Hindi) and Tagar (Sanskrit) is distributed in Himalayas from Kashmir to Bhutan at an altitude 1800-3500m, North Western Himalaya 1300-3300, Khasi hills of North East India between 1200-1800m and also found in Afghanistan and Pakistan (Bos et al., 1997).

**Systematic position**

Kingdom : Plantae  
Division : Magnoliophyta  
Class : Magnoliopsida  
Order : Dipsacales  
Family : Valerianaceae  
Genus : Valeriana  
Species : *jatamansi*

*V. jatamansi* is a perennial, erect herb; 60-100 cm high indigenous to temperate climate of India. It has horizontal root stock, thick, tuberculated rhizome, thick descending fibres. The roots are yellowish brown, 1.5-7 cm long and 1-2 mm thick. The rhizome is yellowish to brownish, 4-7 cm long and 1 cm thick, sub-cylindrical. Leaves are radical, persistent, stalked, cordate-ovate, acute, toothed. The flowers are white or tinged with pink in a terminal corymb 2.5-8 cm across, often unisexual, and the male and female on different plants. Fruits are small, smooth, without hairs. The market samples of the rhizomes are unbranched. The odour is valerianous, and the taste bitter and camphoraceous (Kapoor, 1990).

The species has been claimed to possess sedative, neuroprotective, anxiolytic, anticonvulsant and antistress activities (Bhattacharyya et al., 2007; Rehni et al., 2007, Wasowski et al., 2002) for which used in several Ayurvedic preparations (i.e., Sudarshan
Churna, Pipalayasava, Dasan galep, etc.), and known to cure obesity, skin diseases, insanity, epilepsy and snake poisoning (Prakash, 1999). The sedative, antispasmodic and tranquilizing properties of the plant are due to the presence of essential oil and nonglycosidic iridoid esters known as valepotriates (Gupta et al., 1986). Essential oil from rhizomes itself exhibited antifungal and antibacterial activities (Girgune et al., 1980). Few recent studies evaluated the high antioxidant activity (Das et al., 2011) and antidepressant like effect of essential oil of *V. jatamansi* in both acute and chronic treatment (Sah et al., 2011).
Fig. 1 (a) A flowering plant of *V. jatamansi* in wild condition (b) Flower of *V. jatamansi* (c and d) seeds of *V. jatamansi* with dispersal matter
V. jatamansi is one of the most exploited plants by the modern pharma and perfumery industries as substitute of V. officinalis and Nardostachys jatamansi (Kaul and Handa, 2000). In India the industrial demand of this plant is met almost exclusively through collection from its natural habitat. Recently V. jatamansi has been listed as an endangered and threatened species in India (Kaul and Handa, 2000). Over the years its indiscriminate collection has led to its large scale depletion in the wild and has necessitated its replenishment and cultivation. However, Indian valerian is not yet cultivated anywhere in India for large scale production (Kaur et. al. 1999).

Conventionally the herb is propagated through seeds, despite being the common method of its propagation, it is not an attractive practice, since the seeds germinate slowly and remain dormant for a long time (Kaur et. al. 1999). Besides this continuous anthropogenic activity has posed verymuch stress on its multiplication in natural habitat. Therefore an urgent need of mass propagation of this particular plant is needed for its conservation as well as sustainable utilization.

The present research work is broadly based on two fold objectives viz. to develop an efficient, reproducible and rapid in vitro propagation protocol of V. jatamansi for the mass production and bioprospecting the particular species towards the characterization and improvement of essential oil production. This is the first attempt towards the bioprospecting of Indian valerian for the production of valerian oil. Therefore, in order to meet the ever-growing demand of pharmaceutical industries, mass propagation and cultivation of this plant is of commercial significance.

**Objectives of the research**

1. To develop a rapid and efficient in vitro propagation technique for V. jatamansi.

2. To study the chemotypic variation of Indian valerian oil in North East India.
3. To study the seasonal variation on the production and terpenoid compositions of valerian oil.

4. Comparative analysis of chemical profiling from *in vitro* raised plantlets and *in vivo* plantlets of *V. jatamansi*.

5. To improve the essential oil production by mycorrhization

6. To study the antioxidant potential of *V. jatamansi*.