CHAPTER 1

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India is one of the 12-mega biodiversity centres having about 10% of the world’s biodiversity wealth, which is distributed across 16 agro-climatic zones. Out of 17,000 species of higher plants reported to occur within India, 7500 are known to have medicinal uses (Shiva, 1996). This proportion of medicinal plants is the highest known in any other country against the existing flora of that country (Shiva, 1996; Kala et al., 2006). Ayurveda, the oldest medical system in the Indian subcontinent, has alone reported approximately 2000 medicinal plant species, followed by the Siddha and Unani medical systems. The Charak Samhita, an age-old written document on herbal therapy, reports on the production of 340 herbal drugs for curing various diseases (Prajapati et al., 2003).

Currently, approximately 25% of drugs are derived from plants, and many others are synthetic analogues built on prototype compounds isolated from plant species in modern pharmacopoeia (Rao et al., 2004).

Phytopharmaceuticals, also some pharmaceuticals, herbal remedies, dietary supplements, homeopathics, medicinal and herbal teas, liquors, spirits, sweets, aromas, essences, perfumes, cosmetics, colouring agents, varnishes, fireworks, and detergents are plant-based products offered in a wide variety on the market. Whereas in some goods the herbal ingredients are evident, e.g. in teas or in herbal remedies where they are declared on the packaging, in other products the botanical source is more secret: The bitter taste of Campari is based on the Common Centaury (Centaurium erythraea), and the fenugreek (Trigonella foenum-graecum) contains steroid-saponins which are extracted for use in oral contraceptives. The use of botanical raw material is in many cases much cheaper than to use chemical alternative substances.

As a consequence, there is an enormous demand in botanicals resulting in a huge trade, on local, regional, national and international level - for domestic use and for commercial trade. Different aspects are associated with the trade in medicinal and aromatic plant material, the trade structure, trade volumes and values, the herbs used and their production, the ecological and socio-economic impacts of the trade, and the management of the botanical resources.

World-wide, it is estimated that up to 70,000 species are used in folk medicine (Farnsworth and Soejarto, 1991). The WHO reports over 21,000 plant taxa used for
medicinal purposes (Groombridge, 1992). Unfortunately, there is no idea how many species are used in the other areas of use, like cosmetics, spirits or aromas which makes determining exactly the number of all medicinal and aromatic plant species used worldwide impossible. However, it can be stated, that at least every fourth plant is in use, a calculation based upon the estimated total number of 300-350,000 flowering plants.

The number of medicinal and aromatic plant species used in some regions is impressive. In India, which is said to have probably the oldest, richest and most diverse cultural traditions in the use of medicinal plants, about 7,500 species are used in ethnomedicines (Shankar and Majumdar, 1997) which is half of the country’s 17,000 Indian native plant species. In China, the total number of medicinal plants used in different parts of the country adds up to some 6,000 species according to Xiao (1991) and to over ten thousand according to He and Sheng (1997). Of these, approximately 1,000 plant species are commonly used in Chinese medicine, and about half of these are considered as the main medicinal plants (He and Sheng, 1997). In Africa, over 5,000 plant species are known to be used for medicinal purposes (Iwu, 1993). In Europe with its long tradition in the use of botanicals, about 2,000 medicinal and aromatic plant species are used on a commercial basis (Lange, 1998). In Germany, Lange (1996) identified not less than 1,500 taxa as sources of medicinal and aromatic plant material. In Spain, it is estimated that 800 medicinal and aromatic plant species are used of which 450 species are associated with commercial use (Blanco and Breaux, 1997; Lange, 1998).

During the last two decades, the pharmaceutical industry has made massive investments on pharmacological, clinical and chemical researches all over the world in an effort to discover and still more potent plant drugs; in fact, a few new drug plants have successfully passed the tests of commercial screening. However, benefits of this labour would reach the masses when the corresponding support for agricultural studies for commercial cultivation is provided. Infact, agricultural studies on medicinal plants, by their very nature, demand an equally large investment and higher priority. India, in particular, has a big scope for the development of the pharmaceutical and phytochemical industry.

Exploitation of medicinal and aromatic plants as pharmaceuticals, herbal remedies, flavourings, perfumes, cosmetics and other natural products has greatly increased in the recent years. Approximately 80% of the people in the developing
countries rely on traditional medicine for their primary health care needs, and about 80% of traditional medicine involves the use of plant extracts. The global market of drugs has registered a steady increase in recent years. The global market for the medicinal plants and herbal medicine is estimated to be worth US$800 billion, a year.

Continuous exploitation of several medicinal plant species from the wild and substantial loss of their habitats during the past 15 years have resulted in the population decline of many high value medicinal plant species over the years (Kala, 2003; Planning Commission Report 2000). The primary threats to medicinal plants are those that affect any kind of biodiversity used by humans (Rao et al., 2004; Sundriyal and Sharma, 1995). The weakening of customary laws, which have regulated the use of natural resources, is among the causes threatening medicinal plant species (Kala, 2005). These customary laws have often proved to be easily diluted by modern socio-economic forces (KIT, 2003). There are many other potential causes of rarity in medicinal plant species, such as habitat specificity, narrow range of distribution, land-use disturbances, introduction of non-natives, habitat alteration, climatic changes, heavy livestock grazing, explosion of human population, fragmentation and degradation of population, population bottleneck and genetic drift. (Kala, 2005; Kala, 2000; Weekley et al., 2001; Oostermeijer et al., 2003)

There are several stakeholders in the medicinal plants sector, right from herb collectors and growers to manufacturers and consumers. More than 700,000 practitioners of Ayurveda, Siddha, Unani, Yoga, Naturopathy and Homeopathy are registered in the Indian Systems of Medicine and also a sizeable number of practitioners are not registered. Approximately 800 species of medicinal plants are in active trade and still there is a gap of 40,000 metric tonnes in the demand and supply of medicinal plants. The major source of medicinal plants is the forested area and about 90% medicinal plants is collected from the wild, which generates about 40 million man-days 5-14 (Kala, 2003; Sajwan, 2006). In spite of such a huge resource, involvement of manpower and livelihood option, the medicinal plants sector is largely unregulated and not studied properly even at the national level.

The number of organizations, conducting researches and other activities related to the use of medicinal and aromatic plants is large and increasing (Sharma et al., 2006). Plants not only provide access and affordable medicine to poor people but also foreign exchange for developing countries. Thus, the medicinal plants constitute a precious natural wealth of country.
India ranks next to China in export, exporting 32,600 tonnes of medicinal raw materials to the pharmaceutical companies in the developed countries. The quantity of materials annually exported is enormous e.g. 400 tonnes of dried roots of *Madagascar periwinkle*, 500 tonnes of dried root bark of *Rauvolfia vomitoria*, 300 tonnes of dried stem bark of *Pygeum africanum*, 900 tonnes of *Vocanga African* seeds, 300 tonnes of latex (produced by 600 million unripe fruits) of *Carica papaya* and seeds or dried plant materials of various species.

Population explosion has resulted in the increased need for land – for agriculture, urbanization and the resurgence of public interest in plant based medicine and rapid expansion of pharmaceutical industries have necessitated an increased demand for medicinal plants, leading to over-exploitation that threatens the survival of many rare species. Further, the degree of the threat to natural populations of medicinal plants has increased because more than 90% of the plant raw material for the herbal industries in India is drawn from natural habitats which are fast vanishing.

The actual credit goes to the plant secondary metabolites, perhaps synthesized and accumulated in various tissues for its own defense, making the plant species much more valuable for the biological activities they possesses with therapeutic and/or industrial applications including the aromas, flavours and fragrances. Most of the drugs from plant sources are secondary metabolites, like alkaloids, terpenoids, phenylpropanoids and their combinations, which have no role in plant metabolism; but are postulated to play a significant role in the plant defense mechanism. These molecules singly or in combination have pharmacy and industrial values including aromas, dyes, gums, resins, pulp, fibre, etc. with high bearing on health and commercial sectors. However, not much difference is seen in the basic metabolic processes in plants as well as in animals.

With upto 50,000 genes estimated in plants, more than 200,000 phytocompounds have been anticipated (Picher sky and Gang, 2000) with already around 50,000 compounds elucidated in plants (De Luca and St. Pierre, 2000).

In 1985, the World Health organization estimated that about 80% of the world’s population relies on herbs for their primary health care needs and also admitted that it will not be possible or even desirable to replace this herbal medicine with western techniques, which leads to a revival of interest in wild medicinal plants. This ‘green wave’ (Tyler, 1986) is likely to gain momentum in the years to come. Indeed, the market and public demand has been so great that there is a great extinction
risk to many medicinal plants. With increasing pollution and shrinking cultivable land, the potential medicinal plants are becoming threatened. Besides, herbal drugs provided by a practitioner or purchased from a store, do not guarantee for the quality and quantity of the medicinal principle in them.

There has been an explosion of scientific information concerning plants, crude plant extracts, and various substances from plants as medical agents during last 20-30 years. Although herbal medicine has existed since the dawn of time, our knowledge of how plants actually affect human physiology remains largely unexplored. (Sathiynarayanan and Arulmozhi, 2007)

The loss of genetic diversity demands immediate biotechnological interventions for conservation coupled with proficient genotypes for the production of desired metabolites. The total scenario demands sustainable system approach supported by economic viability. In this direction innovative ways of value addition, quality control and market support are essential along with proficient agro technologies, superior genetic material and downstream processing. Many compounds used in today’s medicine have a complex structure, and synthesizing these bioactive compounds chemically at a low price is not easy (Shimomura et al., 1997). Pharmaceutical companies depend largely upon material procured from naturally occurring stands that are being rapidly depleted. To overcome these limitations novel methods have to be adopted that would help enhance the biosynthesis of secondary products so that further loss of plants can be augmentable with the yield.

Given the demands of the market for a continuous and uniform supply of raw materials and the increasing depletion of the forest resource base, expanding the number of medicinal plants in cultivation appears to be an important strategy for research and development.

Tissue culture technique could play an important role in the production of active phytochemical substances i.e. secondary metabolites and can reduce the pressure on natural resources. Since plant cells are totipotent all the necessary genetic and physiological potential for natural product formation should be present in isolated cell (Zenk, 1978) that means cultured cell obtained from any part of a plant is capable to yield secondary compounds similar to that of the plant in vivo and also capable to regenerate the whole plantlet. The production of useful compounds by plant cell cultures has become increasingly significant in the field of biotechnology.

There are two important problems that have to be overcome for in vitro
production of useful compounds. These are the selection of specific cells that produce high amounts of the desired compounds and the development of an adequate culture medium for the production of such useful compounds. The successful selection of cells producing high amounts of secondary metabolites has been made possible because of the heterogeneity associated with cultured plant cells. Callus provides a continuous and reliable source of natural product (both secondary and primary metabolites) year round without the destruction of the entire plant. High quality and desired compounds can be obtained through cell line selection and/or addition of the precursor into the production medium. Regenerated plants from selected callus (or somatic embryo) line can act as source material for high quality product. It has therefore been possible to select cells and develop cell line with desirable characteristics. For example high vitamin producing cells high alkaloid producing cells (Zenk et al. 1977; Yamada and Hashimoto, 1982) have each been obtained in various plant species.

The production of secondary metabolites in-vitro can be possible through plant cell culture (Barz and Ellis, 1981; Deus and Zenk, 1982). Successful establishment of cell lines capable of producing high yields of secondary compounds in cell suspension cultures has been reported by Zenk (1978). The accumulation of secondary products in plant cell cultures depends on the composition of the culture medium, and on environmental conditions (Stafford, 1986). Strategies for improving secondary products in suspension cultures, using different media for different species, have been reported by Robins (1994).

The production of secondary metabolites in plant cell suspension cultures has been reported from various medicinal plants. The production of solasodine from calli of Solanum eleagnifolium, and pyrrolizidine alkaloids from root cultures of Senecio sp. are examples (Nigra et al., 1987; Toppel et al., 1987). Cephaelin and emetine were isolated from callus cultures of Cephaelis ipecacuanha (Jha et al., 1988). Scragg (1992) isolated quinoline alkaloids in significant quantities from globular cell suspension cultures of Cinchona ledgeriana. Enhanced indole alkaloid biosynthesis in the suspension culture of Catharanthus roseus has also been reported (Zhao et al., 2001; Kumar et al., 2011).

Emblica officinalis family- Euphorbiaceae has been selected for the present study, as it has immense medicinal value. In vitro studies on Emblica officinalis are
several, yet nevertheless insufficient to meet the target. Keeping above mentioned strategic points in view following investigation was planned to be carried out in the present study-

Development of protocols for induction of callus from different explants using a range of hormonal combinations in the nutrient media.

- Comparison of biochemical attributes of explants and calli under various hormonal combinations.
- Identification of explants and hormonal combinations suitable for good yield of ascorbic acid content from calli.