CHAPTER -1

Plants are fundamental to almost all life on earth providing protection and sustenance to other organisms. They form the basis for the biological food-web and perform a number of environmental services. Plants have played an integral part in the evolution of human cultures, their physical and chemical properties providing not only an invaluable source of food, but also a whole range of material benefits in the form of shelter, clothing and medicine, thus remaining fundamental to their physical, spiritual and social well-being.

Plants have fed and caused the world since life began. Medicinal plants play an important role in the lives of rural people, particularly in remote parts of developing countries with a few health facilities. Tropical forests are the source of a large proportion of the world’s recognized medicinal plants.

Medicinal plants are defined as those used for human and veterinary application in traditional medicines, phyto-pharmaceuticals, new drugs, intermediates for drug manufacture, industrial and pharmaceutical auxiliary products and for health foods. The efficacy of many plants currently used in traditional herbal medicine are often lacking in reliable clinical evidence. Other plants formerly regarded as official i.e. recognized and listed in national pharmacopoeia, have either been superceded by other products or in the light of research, found wanting and discarded.

The possibilities and potentialities of medicinal plants and vegetable drugs have not been properly and fully satisfied since the chemistry and pharmacology of most of these plants have not been properly investigated.

The importance of studying the subject of Indian medicinal plants has been repeatedly emphasized by several writers (Kirtikar and Basu, 1935) but
for the proper study of the subject, a work exclusively devoted to Indian medicinal plants has been a great lacuna in medicinal literature of India.

Plants exhibit a wide biosynthetic repertoire, indeed many of materials used in food, flavouring, fragrance and pharmaceutical industries are extracted from plants (Collin, 1987). Diseases are as old as mankind and it is not surprising that man, the rational animal he is, would first turn to plants in search of remedies. Nature with the accumulated wisdom of three billion years of evolution, did not disappoint him. Virtually all cultures, throughout history, used a variety of medicines derived from plants for the prevention and treatment of diseases. Evidence of the beneficial therapeutic effects of these medicinal herbs is seen in their continuous use.

Knowledge of the therapeutic properties of plants predates recorded history. Knowledge gained from the use of medicinal herbs and their active ingredients serves as the foundation for the much of modern pharmacology. Drugs such as aspirin, digitoxin, morphine and quinine were all originally isolated or synthesized from materials derived from plants.

Herbal medicine is still the mainstay of about 75-80% of the world population (Naranjo, 1995). It is mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with human body and lesser side effects. Medicines derived from plants, therefore, offer a great potential, as resources for developing total industry that can substitute for costly pharmaceutical imports (Monitor, 1991).

According to the World Health Organization as much as 80 % of world’s population depends on plants for primary health care (Akerele et al., 1992). As many as 3,226 out of 4,752 communities in India, representing 70% of the population depend on traditional plant medicines (Gadgil & Rao, 1998).
Traditional medicine constitutes a rather vague term, used to distinguish any ancient, or culturally based health care practice from orthodox scientific medicine or allopathy. This include those systems which are currently regarded as indigenous, unorthodox, alternative, folk, fringe unofficial and includes both the major Asian systems such as Chinese, Ayurvedic and Unani. Tibb medicinal systems all of which have been well documented since ancient times and less wide spread, largely orally transmitted practices used by traditional communities, elsewhere today.

In India more than 2000 medicinal and essential oil bearing herbs are already recorded in Indian medicinal literature (Chopra et al., 1956). Unfortunately, we know very little or are still unaware of a number of herbs whose potential is yet to be fully exploited. Many pharmas are involved in the manufacture of traditional medicines with or without standardization. However, none of the pharma has standardized herbal medicines using active compounds as marker linked with confirmation of bioactivity of herbal drugs in experimental animal models (Kamboj, 2000).

India has pluralistic, natural systems of medicines and different therapeutic strategies. It is known for its plant resources from time immemorial. Infact it is one of the 12 identified biodiversity centres of the world, having two hot spots, viz., North eastern India and the western ghats.

Diversity of India is unmatched due to the presence of 16 different agroclimatic zones, 10 vegetative zones and 15 biotic provinces. The country has 15000-18000 flowering plants. As per available records, the present global market for herbal medicine has been estimated to be approximately $20 billion (U.S) and is growing at the rate of 15-20% annually (Dev, 1997). The turnover of herbal medicines in India as over the counter products, ethical and classical formulations and home remedies of Ayurveda, Unani and Sidha systems of
medicine is about $1 billion with a meagre export of $80 million considering the huge herbal medicine and nutraceutical market in developed countries, India should reconsider exporting crude herbal drugs.

Plants are important renewable resource commodities. Transgenic plants expressing foreign proteins of industrial or pharmaceutical use represent an economical alternative to fermentation based production systems. A promising concept is the production of specific vaccines in plants as a result of stable or transient expressions of foreign genes. Hence, biotechnological application of medicinal plants has an indispensable role to play in curing the disease.

Biotechnological applications of medicinal plants is particularly indispensable to cure the disease, for example jaundice (Hepatitis) where there is no effective synthetic drug available. It is being a fatal disease which claims about a million lives every year. Jaundice or icterus is commonly mistaken to be a disease. In reality it is only the external manifestation of symptoms that is common to a large number of diseases. It refers to yellow appearance of skin and mucous membrane. Jaundice is most often seen in association with infectious hepatitis caused by viruses, bacteria and protozoa. In addition, the other causes of jaundice are industrial toxins, drugs, alcohol, etc. It has been estimated that about 90 percent of the acute hepatitis is due to infectious viruses (Thyagarajan and Subramanian, 1987).

Different plants require different climatic conditions. Natural factors like optimum rainfall, humidity and temperature poses a major problem. Apart from these, natural calamities like flood, drought and wind affect the growth and availability of these plants. In natural occurrence the plants are found scattered and it is difficult as well as uneconomical to collect and process these plants for supply from natural sources. Since curing effect to these plants are quite high and cost effective than the allopathic systems of medicines, various
pharmaceuticals, including medical and research institutions are showing interest on these indigenous plants. The increasing demand and inadequate supply of these plants have resulted in the increase of prices. For example ‘Livomyn’ a premium hepatoprotective stimulant and liver regenerative drug formulation (Charak pharmaceuticals (India) Ltd. Mumbai) containing the extracts of *Solanum nigrum, Adathoda vasica, Eclipta alba, Phyllanthus amarus, Swertia chirta, Plumbago zeylancia, Tinospora cordifolia, Rosa damascene, Andrographis paniculata, Picrorhiza kurroa, Lawsonia alba, Boerhaavia diffusa, Amoora rohituka* and many other plants costs Rs. 80 for 200ml. This necessitates the mass propagation of these plants on a large scale in economically valuable terms. Therefore, it is very essential to establish and support a broad based medicinal industry having rapid micro-propagation methods.

Ethnobotanical, phytochemical, biochemical, in vivo-physiological and clinical studies of the traditional medicinal plants are the areas of research that are essential to promote our efforts in search of new medicines. As the end result of any ethno-pharmacological study, we aim at either an authentication of a traditional drug or the discovery of a new drug. The traditional aspect of this research provides us with a reference and starting point for our studies, without which we would be lost among the hundreds of thousands of plant species.

A large number of traditional plant based drugs from China, Africa, South America and India have been subjected to pharmacological studies.

*Baphicacanthus cassia, Aucklandia lappa, Puevaria labata, Agrimonia pilosa, Scopoloa tanguitica, Curcuma aromatic, Mylabens phalarata, Hex pubescens, Unicaria rhynchophylla, Andrographis paniculata* are among the Chinese species in traditional medicine subjected to pharmacological confirmation (Xiao, 1981).
Tubocurarine, the muscle relaxant compound in the traditional arrow poison from *Chondrodendron tomenosum* from South America, is an indispensable aid in modern surgery. Studies of these species are hailed as an example of success of ethnopharmacology A number of arrow poisons that were investigated were discussed by Bisset (1995).

Oliver (1986) has cited several examples of West African species of plants, including *Erythroxylum coca*, whose traditional therapeutic properties were evaluated pharmacologically.

Currently, the surge in demand for these natural products is beyond the production capacity of the extraction laboratories, which has resulted in increase of prices and ruthless collection of these plants from natural sources have resulted in their rapid depletion (Fowler, 1986). There is particular strong demand for secondary plant products that have medicinal applications (galenicals).

Plant Tissue Culture is relatively recent specialization in the field of biotechnology and is being used globally for the *ex situ* conservation of plants. The endeavour is to adopt the method to multiply the medicinal herbs and monitor their secondary metabolites. The application of plant cell, tissue and organ culture has proved its potential for the practical application in the improvement of important and threatened medicinal plants (Tiwari *et al.*, 1998; Remashree *et al.*, 1997; Satish *et al.*, 2003 and Rajashekar *et al.*, 2006). The regeneration of whole plants through tissue culture is popularly called “micropropagation”, by which a large number of plant species can be propagated all round the year and the plant breeder is no longer restricted by season in the production of large number of plants. The plants synthesize various medicinally important compounds such as alkaloids, glycosides, steroids, flavonoids etc. Similarly, *in vitro* derived calli can also synthesize
these compounds. Hence, *in vitro* culture is used as an alternative to whole plants for the production of useful secondary metabolites (Ammirato, 1987).

Many rare and endangered species can now be quickly propagated from a small plant material and with low impact on wild population using *in vitro* propagation technology (Cuenca *et al.*, 1999).


Recent technique of hairy root culture has enabled to increase the production of many biologically active substances. These hairy root cultures have the same metabolic features as normal root cultures, yet are as rapid growing as suspension cultures. High forskolin production in hairy roots of *Coleus forskolkii in vitro* was achieved by Sasaki *et al.* (1998). Large scale production of secondary metabolites such as alkaloids, terpenes, flavconoids,
glycosides, cardenolides etc, from field grown plant as has its own limitations to the dependency of the metabolism to season and environmental constrains during the cultivation. Emergence of secondary metabolite production by cell a culture methods fascinated the production of both the existing as well as novel compounds (Tharasaraswathi et al., 1998) useful as new drugs availed very much to the pharmaceutical industries (Geerlings et al., 1999). Plant tissue culture system offers the best way to exploit our bio richness of medicinal and aromatic plants and thus to strengthen our economy without effecting the national heritage.

Techniques of micropropagation or in vitro cultivation have emerged as alternatives for species that do not have the property of producing viable seeds, that is, species that cannot germinate and develop adequately in their natural environment (Gonzalez et al., 2004). Among the micropropagation techniques somatic embryogenesis or the regeneration of embryos based on the vegetative tissue of N. alpine is an efficient technique that allows for the mass propagation of selected genotypes, for productive and conservation purposes (Castellanos et al., 2004). The success of this technique depends on the development of a series of processes that influence the genotype of the mother or donating explants and the concentration of exogenous growth regulators, which in adequate combinations would allow for obtaining a determinant embryogenic response for the production of somatic embryos (Guerra et al., 2001).

Somatic embryogenesis is the process by which somatic cells, under induction conditions, generate embryogenic cells, which go through a series of morphological and biochemical changes that result in the formation of a somatic embryo. Somatic embryogenesis differs from zygotic embryogenesis in that it is observable, its various culture conditions can be controlled, and a lack of material is not a limiting factor for experimentation. These characteristics have converted somatic embryogenesis into a model system for the study of morphological, physiological, molecular and biochemical events.
occurring during the onset and development of embryogenesis in higher plants: it also has potential biotechnological applications. The focus of this review is on embryo development through somatic embryogenesis and especially the factors affecting cell and embryo differentiation.

In this regard, synthetic seed technology offers an excellent scope for conservation of rare hybrids, elite genotypes and genetically engineered patchouli plants. During the last four decades, synthetic seed technology has gained considerable importance in plant biotechnology as a potential, viable and valuable system for ex situ conservation of commercially important plants (Kavyashree et al., 2004). Encapsulation and storage of the buds at freezing temperatures offers a long-term storage capability, maximum space and maintenance. Low production costs, ease of storage and transport are the additional advantages (Ghosh and Sen, 1994).

Studies on the in vitro germplasm conservation by encapsulation of somatic embryos is reported in many plant species, including cereals, vegetables, fruits, ornamentals and medicinal plants (Onay et al., 1996 and Castillo et al., 1998). In recent years encapsulation of in vitro derived shoot tips or axillary buds has become a suitable alternatives in place of somatic embryos (Bapat et al., 1987; Sharma et al., 1994; Sarkar and Naik, 1997 and Adriani et al., 2000). This technology is quite promising as developing somatic embryos system is difficult for many plant species. Kageyama et al., (1995) has reported encapsulation and regeneration of patchouli protoplasts, however encapsulation of in vitro derived nodal segments is being reported for the first time in the present study, which is a simpler and easier technique.

The present investigation was envisaged by taking into consideration the present void that exist in the type of research and also the results obtained could be beneficial to a large section of the ailing people. One medicinal plant
was selected and was subjected to plant tissue culture technique for their conservation, propagation and identification.

**Artemisia vulgaris** L. (Compositae)

*Artemisia vulgaris* L. has a hot, sharp, pungent taste. It is alexiteric, appetiser, cures “kapha”, asthma and itching. It is useful for stomachic, deobstruent, and antispasmodic.

The plant is prescribed in infusion and electuary in cases of obstructed menses and hysteria. It is used in fomentation given in skin diseases and foul ulcers as an alternative. The specially prepared juice is used in diseases of children.

The leaves and shoot tips are administered in nervous and spasmodic affections connected with debility in asthma and disease of the brain. The plant shows antispasmodic and anthelmintic activity. The plant is prescribed in the treatment of snake-bite and scorpion-stings (Kirtikar and Basu, 1935).

It is perennial, shrubby, aromatic, 0.6-2.4m high, pubescent, stem leafy, paniculately branched. Lower leaves 5-10 by 2.5-5cm., petioled, ovate in outline, with stipule like lobes at the base, deeply pinnatisect, white-tomentose beneath, upper leaves smaller, 3-fid or entire, lanceolate. Head 3-4mm long, ovoid or *Artemisia vulgaris* L. has a hot, sharp, pungent taste. It is alexiteric, appetiser, cures “kapha”, asthma and itching. It is useful for stomachic, deobstruent, and antispasmodic.

Perusal of literature reveals that tissue culture studies on *Artemisia vulgaris* L. have not been studied. Hence the present investigation was undertaken with the following objectives.
Objectives

- To develop protocol for the regeneration *in vitro* from leaf and stem explants and to understand the development of embryoids through histological studies
- Induction of somatic embryos via suspension culture and preparation of synthetic seeds and their germination.
- To study the cytological variations in cultured cells.