1. GENERAL INTRODUCTION

Medicinal Plants

Plants provide a variety of resources that contribute to the fundamental needs of food, clothing and shelter. Among plants of economic importance, Medicinal and Aromatic plants have played a vital role in alleviating human sufferings (Baquar, 2001). Plants are utilized as therapeutic agents since time immemorial in both organized (Ayurveda, Unani) and unorganized (folk, tribal, native) form (Girach et al., 2003). The healing properties of many herbal medicines have been recognized in many ancient cultures. Early herbalists believed that the plant part resembling any part of the human body was considered useful for the ailment of those parts and there is no part of the body without its corresponding herb, a hypothesis known as the “Doctrine of Signature” (Baquar, 2001). Medicinal plants have always had an important place in the therapeutic armoury of mankind. Up to 80% of populations in developing countries are totally dependent on plants for their primary health care (Vines, 2004). Recognition of traditional systems of medicines because of their time tested availability at low costs resulted in a great demand for medicinal plants and their products. According to Atal (1995), theoretically there is a possibility of discovering 328 new modern drugs which are lying hidden in 3, 25, 000 species of the tropical rain forest, besides the 47 major modern pharmaceutical plant based drugs which are already available in the world market. Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries derive directly or indirectly from plants (Newman et al., 2000). Thus there is an ever-increasing interest in herbal medicines all over the world.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, mainly based on their use in traditional medicine (Cragg and Newman, 2001). These plants based traditional medicine systems continue to play an essential
role in health care. The application of plants as medicine dates back to prehistoric period. In India, the Ayurvedic system of medicine has been in use for over three thousand years. Charaka and Susruta, two of the earliest Indian authors had sufficient knowledge of the properties of the Indian medicinal plants. The medicinal works, *Charaka Samhita* (Charaka Samhita, 1949) and *Susruta Samhita* (Susruta Samhita, 1968) are esteemed even to this day as the treasures of literature on indigenous medicines. India has 16 agro-climatic zones, 45,000 different plant species and 15,000 medicinal plants that include 7,000 plants used in Ayurveda, 700 in unani medicine, 600 in siddha, 450 in homeopathy and 30 in modern medicines. India is one of the 12 mega biodiversity centers with 2 hot spots of biodiversity in Western Ghat and North Eastern region (Singh and Chowdhery, 2002). There are about 400 families in the world of flowering plants; at least 315 are represented in India (Sharma, 2004). The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000. India is the largest producer of medicinal herbs and is appropriately called the ‘botanical garden of the world’ (Ahmedullah and Nayar, 1999).

The supply base of 90% herbal raw drugs used in the manufacture of Ayurveda, Siddha, Unani and Homoeopathy systems of medicine is largely from the wild. The natural resources how so ever large are bound to diminish, hence need effective strategy for sustainable utilization. The need is to bring these plants under plough to meet the rising demand of the resultant product (Singh et al., 2003). Cultivation of Medicinal and Aromatic plants is constrained due to lack of suitable technology, which has led to low yield and poor quality. Consequently, medicinal herbs are predominantly harvested in sufficient quantities from the wild in an unregulated manner (Shabbir et al., 2003). This wild resource is speedily shrinking day-by-day, many of them are facing extinction. Therefore, there is a need for conservation and sustainable use of medicinal plants (Houghton, 1997). To cope up with this alarming situation, the recent exciting developments in biotechnology have come as a boon. The
powerful techniques in plant cell and tissue culture, recombinant DNA, bioprocess technologies etc., coupled with most sophisticated analytical tools such as NMR, HPLC, GC-MS, LC-MS etc., have offered mankind the great potency of exploiting the totipotent biosynthetic and biotransformation capabilities of plant cells under \textit{in vitro} conditions (Stockigt et al., 1985).

\textbf{Essential Oils}

Medicinal plants have their values in the substance or substances present in various tissues which produce specific physiological action in human body. These include alkaloids, glucosides, essential and fatty oils, resins, gums, mucilage, tannins etc. From ancient times plant volatiles have been of considerable significance in relation to flavor and perfumery (Kubeczka, 1982). Essential oils are volatile liquids extracted from aromatic herbs, seeds, leaves, stems, flowers, barks, rhizomes, tubers, roots or other parts of the plant and are known as the ‘life force’ or ‘living essence’ of aromatic plants. The volatile nature and strong aroma distinguishes essential oils from fatty oils. A biologically oriented definition for essential oils is as follows;


\textit{Essential oils are complex mixtures of odorous and steam-volatile compounds which are deposited by plants in the subcuticular space of glandular hairs, in cell organelles (oil bodies), in idioblasts, in excretory cavities or exceptionally in heartwoods.}

Chemically and biogenetically the natural product class “Essential Oils” is heterogeneous. Mono-, sesqui- and di-terpenoids (mevalonic acid-derived constituents); phenylpropanoids (cinnamic acid-derived compounds); and alkane derivatives (alkanes, alkenes, alkynes, alkanols, alkanals, alkanoic acids) are by far the most ubiquitous essential oil components. Usually the bulk of an essential oil is formed by members of one, two or all three of these biogenetic families of compounds. Essential oils are widely distributed throughout the plant kingdom and found in several plant families such as Lamiaceae, Apiaceae, Rutaceae,
Myrtaceae, Geraniaceae, Asteraceae, Lauraceae, Poaceae, Zingiberaceae, Cyperaceae, Cupressaceae, Piperaceae and Leguminosae.

Essential oils are mainly composed of terpenoid compounds and play an important role in the interactions between plants and insects (Whittaker, 1970). Terpenoids share a common characteristic; they all derive from the same building block, the isoprene unit (C5), also called isoprenoids. Some terpenoids are acyclic, but most are cyclic, with a wide variety of carbon skeletons (Kleinig, 1989). For sesquiterpenoids (C15), already more than 300 sesquiterpene carbon skeletons have been identified and thousands of naturally occurring oxidized or otherwise modified derivatives have been isolated from marine, terrestrial plant and microbial sources (Cane, 1999). Two biosynthetic pathways have been described for the production of the terpenoid precursor, isopentenyl diphosphate (IPP): 1) The mevalonate pathway starting from acetyl-CoA via mevalonate (MVA) and 2) The non-mevalonic pathway or DOXP pathway from pyruvate and glyceraldehyde-3-phosphate via 1-deoxy-D-xylulose-5-phosphate (DOXP) (Boucher and Doolittle, 2000). Within plant cells, the terpenoid biosynthesis was observed in the cytoplasm, the mitoplasm (the mitochondrial matrix), and the plastoplasm (the plastid stroma) (Kleinig, 1989). In the cytoplasm, IPP is produced via the MVA pathway, whereas the DOXP pathway is located in the plastids.

The uses for essential oils and their individual components are vast. They include whole industries (paint, petroleum, mining and manufacturing), food (processing and flavoring), drink (alcoholic and non-alcoholic flavorings), pharmaceutical products, perfumes and toiletries, hygiene products and pesticides. The end uses of essential oils are determined by their chemical, physical and sensory properties, which differ greatly from oil to oil. Each of the individual chemical compounds that can be found in an oil contributes to the overall character. In recent years, essential oils have received much attention as resources of potentially useful bioactive compounds. They have been used to
protect crops from pest invasions and are well known to have a range of useful biological properties against pests, fungal, bacterial and viral diseases, and weeds (Rosenthal, 1986). They may be more readily degraded in the environment than synthetic compounds (Plimmer, 1993) and are non-mutagenic and non-toxic to homeotherms (Kulkarni, 1986).

*Artemisia vulgaris* L. (Mugwort)

The genus *Artemisia* is one of the largest in the Asteraceae family, consisting of more than 800 species which are widespread over the world. Many of *Artemisia* species grow in Europe, temperate Asia, North and Central America and Northern Africa (Judzentiene and Buzelyte, 2006). It was indicated that the plant within the genus *Artemisia* were named after Queen Artemisia of Caria, Asia Minor, and the epithet *vulgaris* meaning usual, common or vulgar (Gledhill, 1990). Mugwort, the common name for *A. vulgaris* is thought to have several derivations including from the Greek Physician Dioscorides, who believed that the plant had the ability to ward off insects, *moughte* (for moth or maggot) with ‘wort’ being an archaic term for an herbaceous plant (Miller, 2000). Mugwort is said to have derived its name from having been used to flavor drinks. It was in common with other herbs such as Ground Ivy, used to a great extent for flavoring beer before the introduction of hops (Grieve, 1972). Andres de Laguna, a famous Spanish Physician of the 16th century, who worked in Netherlands, Rome and Venice said of this plant that, “it is called *Artemisia* from the name of the Greek goddess Artemis (1st century AD), also called Diana since like the goddess the plant also helps women in labor, without ever failing”. Mugwort has always been a plant used because of its effect on the female genitals (George and Roger, 2000). During the European Middle Ages, Mugwort was also known as Cingulum Sancti Johannis. It was believed that John, the Baptist wore a girdle made of Mugwort in the wilderness. Belief grew that Mugwort could prevent misfortune from befalling travelers. Mugwort is sometimes called St. John's Plant because of the tradition of gathering Mugwort
on St. John's Eve to protect against disease and other tragedies. Mugwort was also believed to enhance dreams if placed under one's pillow.

**Classification of Mugwort**

- **Kingdom**: Plantae
- **Subkingdom**: Tracheobionta
- **Superdivision**: Spermatophyta
- **Division**: Magnoliophyta
- **Class**: Magnoliopsida
- **Subclass**: Asteridae
- **Tribe**: Anthemideae
- **Order**: Asterales
- **Family**: Asteraceae/Compositae
- **Genus**: Artemisia
- **Species**: Artemisia vulgaris

**Vernacular Names**

- Sanskrit - Nagadamani; Hindi - Nagadouna; English - Mugwort, Indian wormwood, Felon herb, Fleabane, Armoise, Sailer’s Tobacco, Moxa; Bengali - Nagadonna; Nepalese - Titepatti; Japanese - Yomogi; German – Beiful; Unani – Nagdaun; Arabian – Halyoon; Persian – Marchobah; Tamil – Masipathri

**Habitat**

Western Himalayas, Assam, Western and Southern India

**Phytography**

Mugwort is a tall aromatic perennial herb, pubescent or tomentose. Stems branched, red ribbed, erect, rough. Upper leaves smaller than lower, 3-lobed or entire; lanceolate; lower leaves 2-4 inch long, ovate, pinnately lobed and hairy on both surfaces. Flower heads are small, reddish, yellow or whitish drooping, in branched, wooly spikes, heterogamous, minute. Fruits-achenes, minute,
ellipsoid, oblong, pappus absent (Chopra et al., 1965). A very variable plant regarding the leaves and flower heads.

Chemical Constituents

A volatile oil, inulin, sesquiterpene lactones, flavonoids, coumarins, an acrid resin and tannin are the major constituents. Mugwort is also a good natural source of vitamin C, beta-Carotene, fiber, calcium, zinc, and quercetin. Essential oils make a major contribution to the plant’s biological activity.

Medicinal Properties and Uses

Mugwort has been known not only as an edible plant (mostly as a spice) but also as a folk medicine resource. The investigations of mugwort extracts indicated a hepatoprotective activity and validated the traditional use of this plant for various liver disorders (Gilani et al., 2005). In Oriental medicine, A. vulgaris has been used as an analgesic agent and in acupuncture therapy (Judzentiene and Buzelyte, 2006). The emmenagogic properties of this plant are related to estrogenic flavonoids. Mugwort is commonly used in traditional European medicine as a choleretic and for amenorrhoea and dysmenorrhoea (Teixiera da Silva, 2004). The plant is also useful in the treatment of uterine cancer (Shaik and Hussain, 2004). Infusion of the leaves is given as a vermifuge against intestinal parasites (George and Roger, 2000). In traditional medicine, this plant is being widely used for the treatment of diabetes and extracts of the whole plant is used for epilepsy and in combination for psychoneurosis, depression, irritability, insomnia and anxiety stress (Walter et al., 2003). The crude extract has been used as an antimalarial agent for thousands of years. Sun et al. (1992) found that artemisinin extracted from A. vulgaris had antitumor activity. Leaves are used as inferior substitutes for cinchona in fevers. The plant is used to treat leucorrhoea, threatened abortion, haemoptysis, vomiting, colic, rheumatism and impetigo (Narayan Das et al., 2003). Leaves are cicatrizant for cuts and wounds (Singh et al., 2002). A paste or powder of the leaves is applied
over skin diseases (Kapoor, 2000). In the Asian tradition, Mugwort was used in concoctions to treat rheumatism. The plant is also a highly effective antidote to insect poison (Asima and Satyesh, 1997).

Mugwort essential oils are used for their insecticidal, antimicrobial and anti-parasitical properties (Judzentiene and Buzelyte, 2006). It can also repel mosquitoes and other insects. *A. vulgaris* essential oils have been reported to have a significant fumigant and repellent effect against *Musca domestica* (Judzentiene and Buzelyte, 2006). The essential oil of *Artemisia vulgaris* was reported to exhibit 90% mosquito repellency against *Aedes aegypti*, a mosquito that transmits yellow fever (Hwang et al., 1985; Ram and Mehrotra, 1995; Nentwig, 2003). Repellant and fumigant activity of *Artemisia vulgaris* against the stored-product insect pest *Tribolium castaneum* (Herbst) was also reported (Wang et al., 2006). The chief compounds of volatile oils include camphor, 1,8-cineole, α-thujone, camphene and β-caryophyllene. They exhibit activities such as analgesic, anesthetic, antiacne, antidiarrheic, antineuralgic, antiseptic, antispasmodic, CNS-stimulant, cancer-preventive, cosmetic, decongestant, expectorant, larvicidal, insect repellent, insectifuge, nematicide, pesticide, antiasthmatic, antibacterial, anti-inflammatory, antispasmodic, sedative and flavor and perfumery (Teixiera da Silva, 2004).

**Plant Status**

*Artemisia vulgaris* is at low risk [LR] of extinction as evidenced from the First Red Data List of South Indian Medicinal Plants (IUCN version) prepared by the Conservative Assessment and Management Plant (CAMP). Source: Foundation for Revitalization of Local Health Traditions (FRLHT) (Mandal and Choudary, 2002).

**Micropropagation of Medicinal and Aromatic Plants**

The most widely used *in vivo* methods of cloning plant species include cuttings of vegetative parts, layering, grafting and budding. However, the *in vivo* clonal propagation of plants is often difficult, expensive and even unsuccessful.
Tissue culture methods offer an alternative means of plant vegetative propagation. Clonal propagation through tissue culture popularly called micropropagation can be achieved in a short period of time and space. Thus it is possible to produce plants in large numbers starting from a single individual. Use of plant tissue culture for micropropagation was initiated by Morel (1960) and he found that the only commercially viable approach for orchid micropropagation. The introduction of cell and tissue culture techniques has allowed studying the problems previously inaccessible and has turned the "dreams" of Haberlandt, White and Gautheret into realities. In the past few decades, cell and tissue culture techniques has emerged as a tool for the study of an increasing number of fundamental problems in Plant Sciences, viz., cell and tissue developmental biology, biochemistry, physiology, pathology, genetics, molecular biology and general applied aspects such as clonal propagation, haploid production and cell transformation with an ultimate objective of improving economically important plants.

The techniques of plant tissue culture has found applications in in vitro rapid clonal multiplication and micropropagation (Murashige, 1977); for raising disease-free and disease-resistant plants (Kartha and Gamborg, 1978); somatic embryogenesis for synthetic seed production (Datta and Potrykus, 1989); secondary metabolites of immense value (Staba, 1977) and genetic engineering for the production of transgenic plants (Uchimiya et al., 1989). In vitro techniques are important tools for modern plant improvement programs used in conjunction with classical breeding methods. In vitro techniques offer powerful tools for germplasm conservation and the mass multiplication of many threatened plant species (Murch et al., 2000). An efficient in vitro regeneration system could accelerate cultivar development programmes. In vitro cell and tissue culture methodology is envisaged as a mean for germplasm conservation to ensure the survival of endangered plant species, rapid mass propagation for large-scale revegetation and for genetic manipulation studies (Nalawade et al., 2003).
The application of micropropagation techniques for medicinal plants gives many benefits to the breeders as it enables to increase the rate of rapid multiplication of plants which in a particular climate do not produce seeds or whose seeds have low germination, the availability of plants throughout the year, uniform plants of selected genotypes and thus production of uniform clones from highly heterozygous plants, production of plants with changed genotypes, conservation of genetic resources of species and threatened plants and plant improvement by regeneration technique. Though there are in vitro research reports on other species of the genus *Artemisia* (Mackay and Kitto, 1988; Benjamin et al., 1990; Mathe and Laszloffy, 1991; Nin et al., 1996; Sharief et al., 1997; Mozetti and De-Donato et al., 1998; Saxena, 2001; Liu et al., 2003, 2004), there is no systematic cultivation of this plant and no published reports on tissue culture of *Artemisia vulgaris* are available. Therefore, there is a need to develop a means for rapid regeneration of *Artemisia vulgaris* for conservation, large-scale propagation and for further research on the biochemical composition and medicinal importance of this valuable plant.

**Hairy Root Cultures**

A new route for enhancing secondary metabolite production in tissue culture system is by transformation of desirable plant species using the natural vector system *Agrobacterium rhizogenes*. It is the causative agent of hairy root disease in plants (Giri and Narasu, 2000; Bourgaud et al., 2001). In nature, the gram-negative soil bacterium *A. rhizogenes* genetically engineers dicotyledonous plant species into chemical producers of an *Agrobacterium* food source (opines). This transformation process leads to the emergence of “hairy roots” at the site of infection of the plant (Shanks and Morgan, 1999). These genetically transformed (hairy) roots are capable of unlimited growth in culture media free of growth hormones. Hairy root cultures of a number of dicotyledonous/monocotyledonous plants have been established and found to produce the same secondary metabolites as natural roots and hence offer a promising system for secondary
metabolite production (Mukundan et al., 1997; Rudrappa et al., 2005). The greatest advantage of hairy roots is that they often exhibit about the same or greater biosynthetic capacity for secondary metabolite production as compared to their mother plants (Kim et al., 2002). Hairy root cultures have turned out to be a valuable tool to study the biochemical properties and the gene expression profile of metabolic pathways. Moreover, hairy root culture can be used to elucidate the intermediates and key enzymes involved in the biosynthesis of secondary metabolites (Hu and Du, 2006).

Transformed roots arise as a result of the integration of TL-DNA and TR-DNA into the plant genome by the bacterial strains and its expression induces root differentiation and subsequent growth (Christey, 2001). *Rol A, B, C* and *D* genes have been identified as the main determinants of hairy root disease caused on dicotyledonous plants by the soil bacterium *Agrobacterium rhizogenes*. Individual *rol* genes have different phenotypic effects that can be ascribed to modifications in the endogenous hormone equilibrium in plants (Bettini et al., 2003). Transformation with *rol A* gene results in plants with a highly aberrant phenotype, characterized by wrinkled, intensely green leaves, long internodes, dwarfism or semidwarfism and retarded senescence (Schmülling et al., 1993). The *rol B* protein on the other hand, has been shown to have a tyrosine phosphatase activity and therefore a possible role in signal transduction pathway (Filippini et al., 1996). Estruch et al. (1991) have demonstrated that *rol C* can be involved in the release of active cytokinins from their inactive glucosides due to its cytokinin-β-glucosidase activity. *Rol D* gene functions as an ornithine cyclodeaminase enzyme, catalyzing the conversion of ornithine to proline (Trovato et al., 2001). Though a number of research reports are available for hairy root production in the two closely related species, *A. annua* (Qin et al., 1994; Jaziri et al., 1995; Chen et al., 1999; Giri and Narasu, 2000; Kim et al., 2003) and *A. absinthium* (Nin et al., 1997), there is no study being carried out on *A. rhizogenes* mediated hairy root culture and secondary metabolite production in *A. vulgaris*. 
Objectives

In view of the afore mentioned data, the pioneering research on mugwort was proposed with the following objectives.

- Standardization of procedure for *in vitro* seed germination
- Standardization of reproducible protocols for micropropagation through solid and liquid culture system.
- Standardization of protocols for indirect organogenesis from various explants
- Standardization of protocols for the production of root cultures
- Standardization of reproducible protocols for the establishment of *Agrobacterium rhizogenes*-mediated hairy root cultures
- Analysis of essential (volatile) oils harvested from leaves, non-transformed and transformed roots using GC-MS.
- Preliminary screening for larvicidal effect of the transformed root volatile oil against the dengue vector, *Aedes aegypti*