INTRODUCTION

“JAGAT EVAM ANOUSHDHAM”
There is nothing in the Universe which is non-medicinal

These were the famous and wise words of the 7th century scholar Vagbhata in Astangahridaya, a compendium of the Ayurvedic system (Anna Moreshwar Kunte and Krishna Navre, 1982). Generations of Botanists have perhaps drawn inspiration from his words which paved the way for the scientific study and exploration of plants for their medicinal values.

Plants, one of the important components of nature have extraordinary healing powers and their curative properties have been exploited in the treatment of various human and animal ailments. The medicinal plants play a crucial role in the existence of life on the earth. Out of approximately 250,000 higher plant species on earth, more than 80,000 are medicinal (Joy et al., 2001). This herbal wealth is used by both developing and developed countries for their health care needs. Particularly, the rural population relies on drug resources of plant origin and the locally collected plants are exploited commercially for the preparation of medicines (Supriya Gaur and Purshotam Kaushik, 2011).

Herbal medicine or herbalism is the study of medicinal plants, their properties and their uses in health care. The scope of the study is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts. Pharmacognosy refers to the study of all medicines derived from natural sources.
Plants have the ability to synthesize a wide variety of chemical compounds that perform important biological functions as also defend against attack from predators such as insects, fungi and herbivorous animals. Many of these phytochemicals have long-term health benefits and can be effectively used to treat human diseases. At least 12,000 such compounds have been isolated so far, a number estimated to be less than 10% of the total (Lal and Roy, 2004; Tapsell et al., 2006). The herbal medicines work in a way similar to the conventional drugs and are as effective as the conventional medicines and at the same time may have the same potential to cause harmful side effects.

**HISTORY OF HERBAL MEDICINES**

The use of plants as medicines was known to mankind from ancient times. Desire and quest for eternal health, longevity, relief from pain and discomfort prompted early man to explore his immediate surroundings to develop a variety of therapeutic agents from natural resources. Ethnobotany, the study of plants in traditional medicine has in fact led to the discovery of many new drugs. In 2001 more than 120 compounds derived from ethnomedical plant sources used in modern medicine were identified by researchers. Many of the pharmaceuticals like digitalis, aspirin, quinine, opium etc. currently used by physicians have a long history of use in traditional medicine as herbal remedies for various human ailments. The clinical success of quinine and quinidine isolated from the *Cinchona* tree bark and recently artemisinin from *Artemisia annua* in the treatment of malaria have rekindled interest in medicinal plants as potential sources of novel drugs (Di Flumeri et al., 2000).

The use of herbal medicines in health care is common in developing nations among non-industrialized societies as it is more affordable than the modern expensive pharmaceuticals. Studies in the United States and
Europe have shown that their use is less common in clinical settings but is increasing in recent years as more scientific evidence regarding the efficacy of the herbal medicine is becoming available.

Many of the herbs and spices used by humans to season and flavour food also yield medicinal compounds (Lal and Roy, 2004; Tapsell et al., 2006). These herbs and spices were used in cuisine mostly as preservatives to combat the threat of food-borne pathogens. Studies have shown that in tropical climates where pathogens are more abundant, recipes are highly spiced. Further, the spices with more potent antimicrobial activity tend to be selected (Billing et al., 1998). In all cultures, vegetables are less spiced than meat as they are presumed to be more resistant to spoilage (Sherman and Hash, 2001). Many of the common weeds found in human settlements like nettle, chickweed etc. have medicinal properties (Stepp et al., 2001).

Archaeological evidences from the Palaeolithic age dating back to about 60,000 years ago indicate that medicinal plants were used by humans to treat diseases. Furthermore, other non-human primates are also known to ingest medicinal plants to treat illness (Sumner and Judith, 2000).

In the written record, the study of herbs traces its origin to the times of Sumerians about 5000 years ago, who created clay tablets with lists of hundreds of medicinal plants such as Myrrh and Opium (Sumner and Judith, 2000). In 1500 B.C., the ancient Egyptians wrote Ebers Papyrus, which contains information on over 850 plant medicines including Garlic, Juniper, Cannabis, Castor bean, Aloe and Mandrake (Sumner and Judith, 2000).

In India, use of many herbs like Turmeric in Ayurveda system of medicine was prevalent as early as 1900 B.C. (Aggarwal et al., 2007). Sanskrit writings from around 1500 B.C. such as the Rig-Veda are some of the
earliest written documents detailing the medical knowledge that formed the basis of Ayurveda system of medicine (Sumner and Judith, 2000). Many other herbs and minerals used in Ayurveda were later described by ancient Indian herbalists such as Charaka and Sushruta. The Sushruta Samhita attributed to Sushruta in the 6th century B.C. describes 700 medicinal plants, 64 preparations from mineral sources and 57 preparations based on animal sources (Girish Dwivedi and Shridhar Dwivedi, 2007).

MODERN HERBAL MEDICINE

Medicinal, herbal and aromatic plants constitute a large segment of the world flora which provide raw materials for use by pharmaceutical, cosmetic, fragrance and flavour industries. Medicinal and aromatic plants play an important role in the economic, social, cultural and ecological aspects of the local communities the world over. They form a part of the traditional healing systems found in numerous tribal communities around the world and comprise a wide range of species which have different sources, characteristics and uses. Since time immemorial the plant products have made a significant contribution to human health and wellbeing as well as being a means of income generation through trade among the local communities. Traditional medicine is defined by the World Health Organization as the sum total of knowledge, skills and practices based on theories, beliefs and experiences indigenous to different cultures, used in the maintenance and improvement of physical and mental health as well as diagnosis, prevention of the various ailments that afflict the humans. The use of herbs to maintain good health, prevent and treat diseases is almost universal among the non-industrialized societies. The World Health organization estimates that 80% of the population of some Asian and African countries presently use herbal medicine for some aspect of primary
health care. In comparison to pharmaceuticals which are prohibitively expensive, herbal medicines can be obtained from nature at little cost.

The medicinal and aromatic plants grow in almost all terrestrial and some aquatic ecosystems around the world in varied environmental conditions. They were mostly collected from the wild particularly by the local communities and thus form an integral component of many local supply chains. But an increasing demand for these valuable plants is threatening their populations from their natural habitats.

India is considered a treasure house of valuable medicinal and aromatic plant species. Wide variation in climatic, meteorological and topographical conditions prevailing in India due to its vastness makes it a rich repository of perhaps the most varied and luxuriant flora growing anywhere on the surface of the earth. India has two major hotspots, namely the eastern Himalayas and the Western Ghats (Dharmendra Singh et al., 2012). It has 15 agro-climatic zones, about 47,000 different plant species and 15,000 medicinal plants known for their curative properties, aroma and flavour. Many of these are used as raw materials in the manufacture of drugs and perfumery products. The world is now looking towards India due to its rich biodiversity of medicinal plants and abundance of traditional medicinal systems (Salahuddin et al., 1998). Nearly 75% of the drugs and perfumery products used in the world are available in their natural state in India where they are extensively used in traditional systems of medicine like Ayurveda, Unani and Siddha. The Charaka Samhita (700 BC) has description of about 1100 species, Sushruta Samhita (200 BC) of about 1270 species, Ayurveda system of medicine uses about 700 species, Unani 700, Siddha 600, Amchi 600 and modern medicine uses about 700 species (Joy et al., 1998). The drugs are derived from the whole plant or from different plant organs like root, stem, bark, leaves, flowers, seeds etc. Even Allopathic system has
adopted a number of plant derived drugs which form an important segment of modern pharmacopoeia. In many of the developing countries, the use of plant drugs is increasing because of the prohibitive costs of the modern, life-saving drugs as also the side effects of several allopathic drugs. As a part of the strategy to reduce financial burden on developing countries, use of plant drugs which offer an economical alternative is encouraged. The scientific study of traditional medicine, derivation of drugs through bioprospecting and systematic conservation of the concerned medicinal plants are thus of great importance.

In traditional societies, nutrition and healthcare are strongly interconnected and many plants have been consumed both as food and for medicinal purposes (Justin Packia Jacob et al., 2011). The use of and search for drugs and dietary supplements from plants have increased in recent times. The effectiveness of these drugs mainly depends upon the proper use and sustained availability of genuine raw materials. Efforts are on by pharmacologists, microbiologists and botanists to find the phytochemicals that can be used in the treatment of various ailments. There is a growing demand for natural products including items of medicinal value, food supplements and cosmetics in both domestic and international markets. According to World Health Organization, approximately 25% of the modern drugs used in the USA have been derived from plants. Herbal remedies are common in Europe. Prescription drugs are sold alongside essential oils and herbal extracts or Tisanes (herbal teas). Some prefer herbal remedies to pure medical compounds which have been industrially produced. (James Duke, 2000).

Among the 120 active compounds currently isolated from higher plants and widely used in modern medicine today, 80% show positive correlation between their modern therapeutic use and the traditional use of plants from
which they are derived (Fabricant and Farnsworth, 2001). At least 7000 medical compounds in the modern pharmacopoeia are derived from plants (Traditional medicine, Wikipedia). In many medicinal and aromatic plants, significant variations in plant characteristics have been noticed with varying conditions of the soil. Therefore, attention should be given to choose soil and cropping strategies, to obtain satisfactory yields of high quality and best priced products, respecting their safety and nutritional value (Carrubba and Scalenghe, 2012).

BIOLOGICAL BACKGROUND

All plants produce certain chemical compounds as part of their metabolic activities. These phytochemicals are categorized as: (1) Primary metabolites like carbohydrates, proteins, fats etc. found in all plants and (2) secondary metabolites like alkaloids, flavonoids, phenolics, saponins, tannins and essential oils which are found in a smaller range of plants, serving a more specific function (Meskin and Mark, 2002). Some secondary metabolites are toxins, used to deter predators and others are pheromones used to attract insects for pollination. For example, Ipsdienol, a major constituent of the floral fragrance of several orchid species and Azadiractin, present in *Azadiracta indica*, have roles as attractant to bees and defence mechanism against insects respectively (Hill, 1985; Swaminathan and Kochhar, 1989). Many a time, the phytochemicals appear to be non-essential to the plant producing them. For example, Penicillin is of great value to mankind as an antibiotic but, appears to serve no useful purpose in the microorganisms producing it (Mann, 1978; Sofowora, 1984). Many of these natural products play vital roles as mediators of ecological interactions ensuring a continued survival of particular organisms in often hostile environments where there is competition with other organisms (Mann, 1978). These secondary
metabolites could have therapeutic effects in humans and can be refined to produce drugs. For example, Insulin from the roots of Dahlias, Quinine from Cinchona, Morphine and Codeine from Poppy, Digoxin from Foxglove etc. are all plant products extensively used in human health care (Meskin and Mark, 2002).

Medicinal plants are generally used in traditional medicine for the treatment of many ailments (Njoku and Ezeibe, 2007; Ogukwe et al., 2004). They are natural sources of compounds that can be used against many diseases today (Deshpande and Bhalsingh, 2011). The discovery of medicinal plants has usually depended on the experience of the populace based on long and dangerous self experimentation (Chhetri et al., 2008). Many of the indigenous medicinal plants are used as spices and food plants. They are sometimes added to food meant for pregnant and nursing mothers for medicinal purposes (Okwu, 2001).

Plant products have been a part of Phytomedicines since time immemorial. These can be derived from any part of the plant like roots, bark, leaves, flowers, fruits, seeds etc. (Cragg and David, 2001). Some drugs are prepared from excretory products of plants like gums, resins and latex. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers (Mojab et al., 2003; Parekh and Chanda, 2007 and 2008). In fact, the number of chemical substances produced by plants is so enormous and varied that Botanist Walter Lewis and Microbiologist Memory Elvin Lewis (1982) stated in their book Medical Botany, “Nature is still mankind’s greatest chemist and many compounds that remain undiscovered in plants are beyond the imagination of even our best scientists”.
Medicinal plants constitute the main source of new pharmaceuticals and health care products (Ivanova et al., 2005). Due to their inherent chemical and biological properties the plants play a significant role in modern medicine and serve as models in drug development. The use of traditional medicine is wide spread in India (Jeyachandran and Mahesh, 2007) but a scientific approach to their study and use still needs to be developed. The use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicine (Shrikumar and Ravi, 2007).

The phytochemicals are the natural bioactive compounds found in plants which work with nutrients and fibres to form an integrated part of defence system against various diseases and stress conditions (Koche et al., 2010). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, steroids, terpenoids, carbohydrates and phenolic compounds (Pascaline et al., 2011). Extraction and characterization of several active phytocompounds from these green factories have given birth to some high activity profile drugs (Mandal et al., 2007). Antioxidant and antimicrobial properties of various extracts from many plants have recently been of great interest in both research and in the food industry, because their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants and antimicrobials with natural ones (Deba et al., 2008).

Hertog et al. (1993) opined that secondary plant metabolites play critical roles in human health and may be nutritionally important. Those phytochemicals with antibacterial potency have been actively investigated as alternatives to and/or in combination with antibiotics in the therapy of bacterial infections (Sato et al., 1995; Liu et al., 2001). Secondary plant
metabolites also serve as defence mechanisms against predation by many microorganisms, insects and herbivores (Cowan, 1999).

CLINICAL TESTS

Many herbs have shown positive results in in-vitro, animal model or small-scale clinical tests (Srinivasan, 2005) while studies on some herbal treatments have found negative results (Pittler et al., 2000). Herbalists criticize the manner in which many scientific studies make insufficient use of historical knowledge, which has been shown to be useful in drug discovery and development in the past and present (Fabricant and Farnsworth, 2001). They maintain that the traditional knowledge can guide the selection factors such as optimal dose, species, time of harvesting and target population (Eric Yarnell et al., 2002). It, therefore, becomes important that a more scientific approach and in depth study into the various aspects of medicinal uses of plants is carried out along with traditional knowledge for the production of efficacious drugs.

THE ANTI-MICROBIAL EFFECTS OF MEDICINAL PLANTS

Many of the plants used today were known to the people of ancient cultures throughout the world for their preservative and medicinal powers. Scientific experiments on the antimicrobial properties of plants and their components have been documented in the late 19th century (Zaika, 1975). Several plants are used in India in the form of their crude extracts, infusions or plaster to treat common infections without scientific evidence of efficacy (Ahmed et al., 1998). Therefore, it is important to determine the scientific basis for the traditional use of these medicinal plants.

A good number of our population particularly those living in villages depend largely on herbal remedies. Most of these herbal remedies have stood the test of time, especially for the treatment of allergic, metabolic
and cardiovascular diseases (Igoli et al., 2005). The idea that certain plants had healing potential was well accepted (Rios and Recio, 2005). The medicinal value of the drug plant is due to the presence of some chemical substances (Bhakuni, 1984). These substances can be used for therapeutic purpose or they are the precursors for the synthesis of new drugs (Doherty et al., 2010). Herbal medicine has been practiced worldwide and is now recognised by world Health Organization as an essential building block for primary health care (Wallis, 1985). A number of plants have been used in traditional medicine for many years due to their antimicrobial properties (Sofowora, 1993). Specifically, the medicinal value of these plants lies in some chemical substances that produce definite physiological action on the human or animal body (Edeoga et al., 2005). The active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive (Jigna Parekh and Sumitra Chanda, 2007). However these complementary components give the plant as a whole, a safety and efficiency much superior to that of its isolated and pure active components (Shariff, 2001). The most important of these bioactive constituents which are mainly secondary metabolites are alkaloids, flavonoids, tannins, terpenoids, phenolic compounds and essential oils which have antimicrobial properties (Evans et al., 1986; Cowan et al., 1999). These phytochemicals are toxic to microbial cells (Doherty et al., 2010). As such, plants containing high levels of poly phenols have greater importance as natural antimicrobics (Baravalia et al., 2009). While the medicinal properties of herbs have been recognised since ancient times, there has been a resurgence of interest in the antimicrobial properties of botanical extracts (Anjana Rao et al., 2010) in recent times. It has been well documented that essential oils kill a wide range of pathogenic fungi and bacteria, such as Candida albicans, Staphylococcus aureus and Pseudomonas aeruginosa including their drug resistant variants (Ben Arfa
et al., 2006; Pozzati et al., 2008; De Martino et al., 2009; Pinto et al., 2009). Microorganisms have developed resistance to many antibiotics and this has created immense clinical problems in the treatment of infectious diseases (Davis, 1994). The incidence of resistance in human pathogenic microorganisms in recent years perhaps is due to indiscriminate use of commercial antimicrobial drugs. Gram negative bacteria possess increasing resistance to antibiotics (Alonso et al., 2000; Sader et al., 2002). Further, antibiotics are sometimes associated with side effects and toxicity (Idose et al., 1968; Ahmed et al., 1998). This has forced the scientists to look for new antimicrobial substances from various sources including the medicinal plants (Karaman et al., 2003). Recent works have revealed the potential of several herbs as sources of drugs (Iwu, 2002). Several workers (Mutyala Naidu and Kishor Babu, 2009; Senthil Kumar et al., 2009; Wath et al., 2009; Inampudi et al., 2010; Sathiyantarayan et al., 2010) have screened many plants for their antibacterial properties. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). Many natural antimicrobial compounds can be derived from plants (Gordon and David, 2001). Because of the side effects, drug toxicity and the resistance that the pathogenic bacteria have built against the antibiotics, a lot of attention has been paid to the natural extracts and biologically active compounds from medicinal plants. Ahmed et al., (1998) opined that medicinal plants represent a rich source from which new antibacterial and antifungal chemotherapeutic agents may be obtained. Numerous studies have identified compounds in herbal plants that are effective antibiotics (Basile et al., 2000). They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999; Kokoska et al., 2002). Traditional healing systems around the
world that utilize herbal remedies are an important source for the discovery of new antibiotics (Okpekon et al., 2004). Some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Kone et al., 2004). The results indicate the need for further research into traditional health systems (Romero et al., 2005). It also facilitates pharmacological studies leading to the synthesis of more potent drugs with reduced toxicity (Ebena et al., 1991; Manna and Abalaka, 2000).

The interest in microbicidal plants is ever increasing today since herbal pesticides and fungicides are harmless (Fawett and Spencer, 1970) and easily biodegradable (Mahadevan, 1982). The presence of antibacterial substances in higher plants is now well established (Srinivasan et al., 2001) and they may prove to be more effective herbal protectants than the synthetic and commercial microbicides against a wide spectrum of pathogenic bacteria and fungi as these are eco-friendly and non-toxic. Due to their antimicrobial activities, the Phytomedicines can thus be safely used for the treatment of diseases in the indigenous systems of medicine or it can be the base for the development of a medicine, a natural blue print for the development of a drug (Didry et al., 1998).

THE ANTI-OXIDANT ACTIVITIES OF MEDICINAL PLANTS

One of the most important biological activities of medicinal plants is their ability to produce chemicals which have anti-oxidant activity. Many naturally occurring products are reported to contain large amounts of antioxidants other than vitamin C, E and Carotenoid (Javanmardi et al., 2003). These antioxidants play a vital role in delaying, intercepting or preventing oxidative reactions catalysed by free radicals (Vilioglu et al., 1998). Normally free radicals are generated in the cells at low levels to help in the modulation of several physiological functions but their
destructive effects are negated by the integrated antioxidant system in the body. But if they are produced in excess amount, they prove to be dangerous leading to inflammation, ischemia, lung damage and other degenerative diseases (Halliwell et al., 1992 and Cavalcanti et al., 2006). The free radicals have been reported to be responsible for cataract formation, neurodegenerative diseases and AIDS (Pietta et al., 1998; Lee et al., 2000; Middleton et al., 2000) and development of cardiovascular diseases (Langsethm, 1995; Alho and Leinonin, 1999). Along with the oxidative radicals, they are shown to be involved in many biological processes that cause damage to lipids, proteins, membranes and nucleic acids thus leading to a variety of diseases (Lee et al., 2005; Campos et al., 2006). The role of antioxidants lies in scavenging these free radicals out of the system and protect the body against their harmful effects.

It has been shown that several plants are natural sources of antioxidants. This antioxidant activity of plants could be due to the presence of phenolic compounds such as flavonoids (Pietta et al., 1998), Phenolic acids and Phenolic diterpene (Shahidi and Wanasundara, 1992). It has been observed that plants having polyphenolic compounds like flavonoids possess antioxidant activity (Cook and Samman, 1996; Lu and Foo, 2001; Murthy et al., 2002) which helps to reduce the risk of chronic diseases like cardiovascular disease, cancer and age related neuronal degeneration (Ames et al., 1993; Renaud et al., 1998; Temple, 2000). The radical scavenging property may be due to the presence of hydroxyl groups in the chemical configuration of phenolic and flavonoid compounds.

While discussing about the anti-oxidant properties of plants special mention has to be made about the presence of phenolic compounds in plants. The phenolic compounds which are mainly responsible for the antioxidant properties of plants are one of the largest and most ubiquitous
groups of plant metabolites that possess an aromatic ring bearing one or more hydroxyl constituents (Singh et al., 2007). They possess biological properties like anti-apoptosis, anti-ageing, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of the endothelial function as well as inhibition of angiogenesis and cell proliferation activity (Han et al., 2007). A number of studies have focussed on the biological activities of phenolic compounds, which are potential anti-oxidants and free-radical scavengers (Rice-Evans et al., 1995; Cespedes et al., 2008; Reddy et al., 2008; Chanda and Dave, 2009). Several studies have described the antioxidant properties of medicinal plants, foods and beverages which are rich in phenolic compounds (Brown and Rice-Evans, 1998; Krings and Berger, 2001). Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols etc. (Ali et al., 2008). Several reports suggest that there is a direct correlation between total phenol content and antioxidant activity and that plants having more phenol content show good antioxidant activity (Brighente et al., 2007; Salazar et al., 2008).

Many of the plants with antioxidant properties may therefore be consumed as functional foods as well as utilized in the manufacture of plant based pharmaceutical products.

MEDICINAL AND AROMATIC PLANTS AS SOURCES OF ESSENTIAL OILS

The aromatic plants considered as Chemical gold mines (Skaria, 2007) produce natural essential oils which find extensive use in pharmaceutical, flavour and fragrance industry. Considered as Jewels, the essential oils are secondary metabolites produced by plants to defend them from abnormal/severe changes in the climate and also invasion of insects and animals (Varshney, 2012). They defend the plants and can be considered as
plant immune system boosters. They are called volatile oils or ethereal oils as they evaporate on exposure to air at room temperature (Skaria, 2007). The characteristic odour and flavour of the essential oil is mainly due to the presence of oxygenated organic compounds. They contain a complex mixture of mono and sesquiterpenes, alcohols, esters, aldehydes, ketones etc. (Dorman and Deans, 2000).

The essential oils are used as natural flavour additives to food, as fragrances in perfumery and in traditional and alternative medicine. They are used as medicines to cure organ dysfunction or systemic disorder (Perry et al., 2003). Recent studies have shown that essential oils alone or in combination have therapeutic uses such as prevention of cancer (Pryor et al., 1988), cardiovascular diseases including atherosclerosis, thrombosis, gastritis, peptic ulcer etc. They are known for their bioactivity such as antibacterial, antiviral and antioxidant properties (Alma et al., 2003). In recent years essential oils are used in massages and aromatherapy (Gupta et al., 2009). Various plants are being used in complementary and alternative medicines for management of anxiety. *Origanum* species have been particularly attributed with mood enhancing properties by aroma therapists (Rupesh Kumar et al., 2011). The essential oil obtained from the leaves of Marjoram has antimicrobial properties against several pathogenic bacteria (Yadava and Saini, 1991). The antibacterial activity of the essential oil of Marjoram is due to the presence of biologically active compounds like terpinen-4-ol, α-terpineol and linalool (Olfa Baatour et al., 2012).

Historically, the essential oils from aromatic plants were used by ancient Egyptians as Incense offering to God in sacred rituals. The aroma chemicals were used on auspicious occasions and gifted to the royals. Advent of Christianity and fall of the Roman Empire saw a decline in the
use of perfumes and aromatics (Leelavathi D, 2009). Later Arabs began to use essence and they perfected the art of distillation (Farooqi and Sree Ramu, 2004).

Some of the major essential oil producing countries are India, China, USA, Egypt, Indonesia, Brazil, France, Morocco, Tunisia, Spain, Belgium, Italy, Australia, Turkey, Israel, Russia, Sri Lanka, Mexico etc.

THE DEMAND AND SUPPLY OF ESSENTIAL OILS

The production and trade of essential oils is decided by the demand and supply, fluctuation in price and competitiveness. Major competition for Indian aromatic oils is from China, Brazil and Indonesia. Even in the rest of the world there is a significant increase in the usage and demand for essential oils. The most traded essential oils are mints, citronella, basil, clove, sandalwood, geranium, lavender, tuberose, jasmine, rosemary, eucalyptus, patchouli, cedar wood etc. Many of these are exported from India to the western markets though in recent years there is a slight dip in the export of traditional oils like sandalwood, lemongrass etc. Some of the essential oils for domestic industry are indigenously produced, while some like lavender, geranium, patchouli, rose oil etc. are imported from China, Brazil, Turkey, Australia, Sri Lanka, Indonesia etc. to meet the domestic industrial requirement. The demand for essential oil is increasing on account of their use in pharmaceuticals and aromatherapy. Because of their wide spectrum of activity and safety they are preferred over the modern and synthetic medicines in the treatment of various ailments. The application of essential oils in agriculture as natural herbicides, pesticides, anti-feedants, botanical repellents, growth boosters etc. is attracting the attention of the scientists towards greater research in the field. But, in the cosmetic industry, the synthetic perfumes have outnumbered the natural products perhaps because of the non-availability of the latter in sufficient
quantities as also the fluctuation in their prices. However, natural products are considered safer for human health than their synthetic counterparts and hence the demand for the former is slowly showing an upward trend.

Because of the wide range of usage of the essential oils in the daily life of man, the future of the essential oil industry appears very bright. This increase in demand can be met by a systematic and scientific approach in the production and supply of the raw materials. One method that could be adopted for meeting this need for increased production and supply is through in vitro multiplication and crop improvement.

**EMPLOYMENT OPPORTUNITIES**

The medicinal and aromatic plant collection and marketing have an important role in providing economically disadvantaged groups such as landless labour, marginal farmers and women in rural areas with some income. Further, farmers with small land holding can practice mixed farming by integrating medicinal plants with traditional crops like maize, lentils and vegetables so that there is income throughout the year. Small traders and industries can also benefit by trading dried plant material and processing them into teas, ointments and tinctures for the local markets.

**MARKET FOR MEDICINAL AND AROMATIC PLANTS**

Over 80% of the raw materials used in the preparation of drugs are contributed by medicinal plants. The efficacy of the drugs depends on the genuineness of the raw material and the methods adopted for the preparation. The domestic market of Indian systems of Medicine and Homeopathy is around Rs. 4000 crores (as per year 2000 report in BUSINESS.GOV.IN). Further, there is a growing demand for natural products including pharmaceuticals, neutraceuticals and cosmetics both in the domestic and international markets. The rich biodiversity, the scientific
manpower, a well-established processing industry and huge investments in trade have made India a world leader as far as the production and export of essential oils and their value added products are concerned (Varshney, 2012).

The Ministry of Environment and Forests has identified and documented more than 9500 species of medicinal plants considering their importance in the pharmaceutical industry. Of these, about 65 plants have great demand in world market. India being a rich repository of medicinal plants has a great potential and advantage in the export market. The value of export of medicinal plants and their products from India was Rs. 31,645.13 lakhs and value of import was 3,857.84 lakhs (Ramesh kumar and Janagam, 2011). The EXIM Bank of India, in its report (1997), has stated that the value of medicinal plants related trade in India is around 5.5 billion US dollars and is growing further. Thus, India is emerging as a global leader in the field of herbal medicine, offering great business and investment opportunities. Efforts are on to promote entrepreneurial activities in the medicinal and aromatic plant sector.

The international market of herbal products is estimated to be US $ 62 billion and is poised to grow to US $ 5 trillion by the year 2050. The essential oil and many aroma chemicals constitute a major group of industrial products. The value of world trade of essential oils, perfume and flavour materials (SITC group 551) was 36.7 billion US $ in the year 2009 and in exports India stood at the 9th place with USD 385.1 million (UN Comtrade). The world production of natural essential oils is estimated at 130,000 M. tonnes which includes oil for processing, for the manufacture of fragrances, flavours, natural cosmetics, medicines and aromatherapy (Varshney, 2012). The demand and price of essential oil is increasing
continually in the national and international markets due to their heavy consumption.

GOVERNMENTAL SUPPORT AND REGULATION

In view of the trade and export potential that the medicinal and aromatic plants offer, the Government of India has taken steps to augment and channelize the traditional methods of collection, cultivation, processing and marketing.

In India herbal remedy is so popular that the Government of India has created a separate department- AYUSH- under the Ministry of Health and Family Welfare. The National Medicinal plants Board (NMPB) was also established in 2000 by Govt. of India to deal with the herbal medicinal system (Kala et al., 2007). The NMPB takes care of the overall development of the medicinal plants sector in the country. It is mainly responsible for coordination of all matters relating to medicinal plants, including drawing up policies and strategies for conservation, proper harvesting, cost-effective cultivation, research and development, processing, marketing of raw material with a view to protect, sustain and develop this sector. To take care of the issues related to the development and growth of the medicinal plant sector at the regional level, State Medicinal Plants Boards have also been set up.

At the international level, the WHO, the specialized agency of the United Nations that is concerned with International Public Health, published Quality Control Methods for Medicinal plant materials in 1998 in order to support WHO member states in establishing quality standards and specifications for herbal materials, within the overall context of quality assurance and control of herbal medicines (Breakspear, 2006).
EXTINCTION OF MEDICINAL PLANT SPECIES AND THE NEED FOR THEIR CONSERVATION

The medicinal and aromatic plants used in health care were originally collected from the wild. Due to over exploitation, disappearance of natural habitats, on account of human interference and urbanization as also unscientific and unsustainable methods of collection of plant material there is gradual depletion in the population of medicinal herbs and the threat of extinction looms large. In fact, the forests which are a treasure house of plant and animal diversity are fast disappearing due to urbanization and many of the rare medicinal plants are on the verge of extinction. About 50% of the tropical forests have already been destroyed and in India, the forest cover is disappearing at an annual rate of 1.5 mha/yr. What is left at present is only 8% as against a mandatory 33% of the geographical area (Joy et al., 1998).

Belinda Hawkins quoting experts reported in 2008 on BBC News that hundreds of medicinal plants are at risk of extinction, threatening the discovery of future cures for diseases. As over 50% of prescription drugs are derived from chemicals first identified in plants, researchers warned that “cures for things such as cancer and HIV may become ‘extinct before they are ever found’.” The expert group from Botanic Gardens Conservation International representing Botanic Gardens across 120 countries identified 400 medicinal plants at risk of extinction from over collection and deforestation, threatening the discovery of future cure for diseases. The report said that five billion people still rely on traditional plant-based medicine as their primary form of health care.

Further, the demand for the herbal medicines, health products, pharmaceuticals, food supplements and cosmetics from plant sources is increasing steadily in both developed and developing countries due to the
growing recognition that the natural products are non-toxic, have lesser side effects and are easily available at affordable prices (Ripa et al., 2010). It has been estimated that in developed countries, plant drugs constitute as much as 25% of the total drugs, while in developing nations, the contribution is as much as 80% (Joy et al., 1998). Indeed, the market and public demand has been so great that the medicinal plants today are facing the risk of either extinction or loss of genetic diversity (Misra, 2009). Hence there is a need to conserve the source material, cultivate and propagate it to meet the increasing demand.

In recent years several programmes have been initiated by Government, Semi-Government and Non-Governmental agencies to promote in-situ propagation of medicinal plants. Though the efforts are laudable there are certain limitations. The potential for regeneration in their natural habitat is poor in many plants. The germination of seeds and subsequent establishment of the seedlings is also difficult. Further, there is little knowledge about vegetative propagation in many of these plants. Under such circumstances, tissue culture techniques are employed for the mass propagation and conservation of selected, economically useful native plants thereby promoting ex-situ conservation. In vitro regeneration is recognised as an efficient means of ex-situ conservation of medicinal plants (Fay, 1994). During the past few decades, there has been a great interest and progress in in vitro propagation of medicinal plants using the technique of organ, tissue, cell and protoplast culture. The primary objective of tissue culture of medicinal plants has always been mass clonal propagation of the most desirable genotypes.

**NEED FOR IN VITRO MULTIPLICATION**

*In vitro* multiplication or Micropropagation is the most significant method widely used in commercial production of plants (Morel, 1960). It gives
many benefits to the breeders such as, increase in the propagation rate of plants, availability of plants throughout the year and conservation of genetic resources (Bajaj et al., 1988). Most of the dicotyledonous and Monocotyledonous plants have been cultured or micropropagated by in vitro technique and over 1000 species have been conserved using this technique (Brown and Thorpe, 1986; George and Debergh, 2008 and Kane et al., 2008)

In vitro multiplication is generally practiced wherever conventional methods of propagation are not able to meet the large scale requirement of genetically uniform planting material. Tissue culture techniques are able to very effectively exploit the regenerative potential of the plants because of which they are employed in the multiplication of plants particularly those that are economically important and in great demand. As in vitro techniques are practiced under aseptic conditions, there is an added advantage of the plantlets being free from pests and diseases that are commonly encountered in the normal methods of propagation through seeds and vegetative propagules. Further, with the demand for herbal medicines increasing steadily both in developing and developed countries, there is a need for large scale production of the useful herbs. The pharmaceutical and cosmetic industries also require large quantities of the source material for the preparation, processing and testing of the products. This demand could be met through tissue culture which also serves as an efficient biotechnological approach towards genetic improvement of valuable plants. In vitro Micropropagation is now established as an alternate strategy for the mass multiplication of economically important plants (Murashige, 1974). It helps in not only large scale production of plants but also in the conservation of over exploited and endangered species of plants. The technique has been accepted as a useful method of
plant propagation, which could be harnessed for qualitative and quantitative improvement of morphological and phytochemical characters. It could also be used to induce beneficial variations resulting in better clones.

Multiplication under *in vitro* conditions commonly occurs through Organogenesis. It is a process of differentiation by which plant organs like shoots, roots, leaves, flower buds etc. are formed. The process of Organogenesis occurs either directly from the explants (Direct organogenesis) or from the callus developed from the explant (Indirect organogenesis). Differentiation of plants from callus cultures has been suggested as a potential method for rapid propagation and for induction of variations (Martin, 2002).

The plants are also sources of a large spectrum of natural products, the secondary metabolites. Though these compounds are not important for the primary metabolism of plants, they are of great help for the survival of the plant in their natural environment as most of them act as chemicals of offence and defence protecting the plants from the attack by the predators. Many of these compounds are used by man as spices, flavouring agents, food additives, dyes, cosmetics, medicines etc. But in recent years, obtaining sufficient quantity of plant material for the isolation of the appropriate secondary metabolite has become quite expensive and difficult due to their non-availability and gradual disappearance on account of human interference. Thus there is an urgent need for developing and applying alternative methods for a continuous and sustainable supply of these useful compounds and plant tissue and organ cultures have proved to provide such alternatives. *In vitro* culture techniques have gained importance as methods of enhancing the production of secondary metabolites (Vanishree et al., 2004) and there are several reports on the
enhancement of secondary metabolism in micro-propagated plants than the source of the explants (Giulietti and Ertola, 1999; Leal et al., 2009).

**APPLICATION OF AM FUNGI FOR CROP IMPROVEMENT**

Vast majority of plants take up nutrients through interactions with root symbionts. Of these root symbionts, the most common are the Arbuscular Mycorrhizal Fungi (AMF) which form associations with many plant species. Since the association is mutualistic, both organisms derive benefit from the association (Frank, 1985). The fungus receives carbohydrates from the plant, which in turn receives many benefits, including increased nutrient absorption (Kaushik, 1988). In this association, the fungus takes over the role of plant’s root hairs and acts as an extension of the root system (Turk et al., 2006). The AMF are considered important because they facilitate plants’ uptake of Phosphorus, a limiting nutrient in many soils (James et al., 2001). Associations with Arbuscular Mycorrhizal Fungi are said to increase plant access to scarce or immobile soil minerals, especially Phosphorus particularly in infertile soils and under dry conditions thereby increasing plant growth rate (Quilambo et al., 2010). Several works have shown that AMF increase plant uptake of Phosphate (Bolan, 1991), micronutrients (Burkert and Robson, 1994), nitrogen (Barea et al., 1992) and act as antagonists against some plant pathogens (Duponnois et al., 2005). It has been demonstrated that the plants inoculated with AMF utilize more soluble phosphate from rock phosphate than non-inoculated plants (Antunes and Cardoso, 1991) which is due to the development of extrametrical mycelium increasing the root phosphate absorbing sites (Bolan, 1991).

In the development of sustainable crop production practices, the use of microbial inoculants as a replacement for chemical fertilizers and pesticides is receiving a lot of attention (O’Gara, 1996). One group of
microorganisms important to the development of long term community structure is mycorrhizal association (Barea et al., 1992). Presence of these fungi has been shown to be essential for the sustained growth and competitive ability of plants (Janos, 1980; Allen and Allen 1990; Hartnett et al., 1993; Koide et al., 1994). AM (Arbuscular Mycorrhiza) is a symbiotic or mutualistic association between the roots of about 90% of the species of plants including Angiosperms, Gymnosperms, Pteridophytes and Bryophytes, and a fungus (Mishra et al., 1980; Williams et al., 1994). AM fungi are a major component of rhizospheric micro flora in natural ecosystems and play a significant role in the decomposition of the soil organic matter, mineralization and cycling of plant nutrients (Beare et al., 1997; Bagayoko et al., 2000; Pare et al., 2000). The mycorrhizal mycelia are much smaller in diameter than the smallest of roots and thus can effectively explore a greater volume of the soil, providing a larger surface area for absorption. The fungus enhances the absorption of inorganic nutrients, especially phosphorus which are translocated into the plant in exchange for photosynthetically fixed carbon. In leguminous plants, AM associations improve Phosphorus nutrition resulting in better nodulation, nitrogen fixation and growth. The bidirectional transport leads to enhanced plant growth, improves its physiological status, at the same time helping the fungus complete its life cycle. The mycorrhizal associations improve the uptake and translocation of N, K, S, NH$_3$, Cu, Mg and poorly mobile micronutrients like Zn, Bo, Cl and Mn (Smith and Read, 1997). The fungal colonization not only influences plant growth and reproduction but also helps overcome competition particularly in nutrient limited soils.

In recent years, application of mycorrhizal technology for better growth and establishment of micropropagated plants is being tried with a lot of success. The micropropagated plants go through a period of transition and
hardening before they get acclimatized to the external environment. Several sterile substrates like perlite, vermiculite, soilrite, sand etc. are used for hardening under controlled conditions before they are transferred to the field. The process of transfer is quite difficult because the in vitro plantlets are not well adapted to the in vivo environment (Pierik, 1988). The period of acclimatization of the micropropagated plants can be shortened by the application of the AM fungus (Salamanca et al., 1992). The absence of a compatible mycorrhizal fungus may slow down the subsequent growth of the micropropagated plants. The AMF play a significant role in ensuring the health of the plantlets (Gianinazzi and Gianinazzi-Pearson, 1988). The role of Mycorrhizae in enhancing the growth of the host plant is well established in several taxa. Further, it has also been observed that the AMF associated plants perform better and yield more active principles than the control plants. The effect of Mycorrhizal fungal association in enhancing growth, biomass production, content of nutrients and secondary metabolites has been demonstrated in several medicinal plants (karthikeyan et al., 2009; Hemashenpagam and Selvaraj, 2011; Tejavathi et al., 2011; Aditya kumar, 2012; Tejavathi and Jayashree, 2013). The AM Fungi are also known increase essential oil production in aromatic plants (Khaosaad et al., 2006; Morone-Fortunato and Avato, 2008; Mir Hassan Rasouli-Sadaghiani et al., 2010).

Plant-Mycorrhiza interaction is believed to play an important role in the production of Secondary metabolites and in some plants fungus symbiosis induces changes in the root accumulation of secondary compounds some of them acting as signal molecules (Akiyama and Hayashi, 2002; Peipp et al., 1997; Smith et al., 2006). Though there is enormous data on the yield and growth increase in ornamental, vegetable crops as well as trees, limited research has been conducted to investigate the contribution of such
microorganisms on the quantitative and qualitative profile of secondary metabolites typical of the plants (Strack et al., 2003; Xin et al., 2006). Only a few studies have been carried out to establish the role of Arbuscular Mycorrhizal Fungi in enhancing the productivity of essential oil bearing plants (Copetta et al., 2006; Khaosaad et al., 2006). Study by Khaosaad et al. (2006) on Oregano showed that colonization by AMF increases the amount of shoot biomass and has an effect on oil yield. Morone-Fortunato and Avato (2008) studied the influence of Mycorrhizal inoculation on micropropagated plant growth, development of secretory structures and production of essential oils in *Origanum vulgare* ssp. *hirtum* inoculated with AM fungus, *Glomus viscosum*. The positive effect of AM inoculation was evident on all growth parameters suggesting that the performance of plants was improved by the combination of two technological approaches, Micropropagation and Mycorrhization.

The use of AMF drastically reduces the dependence on fertilizers and promotes sustainable crop production. Thus, the technology can be applied not only for the better establishment of the micropropagated medicinal plants but also for the enhancement of biomass, primary and secondary metabolites which are in great demand in the pharmaceutical, flavour and fragrance industry.

The potential for manipulating mycorrhizal associations to increase productivity in plantation forestry, or plant establishment during ecosystem recovery after severe disturbance are the focus of major research initiatives (Saleh Al-Garni, 2006; Sameera Bafeel, 2008; Khosla et al., 2008). There is also much interest in their potential utilization in medicinal, agricultural and horticultural crops (Kaushik, 1983; Akond et al., 2008).
THE PLANT SELECTED FOR STUDY: MAJORANA HORTENSIS MOENCH

Majorana hortensis Moench is a valuable medicinal and aromatic plant which needs to be conserved and cultivated on a commercial scale because of the demand for its essential oil in the market. The warm and pleasant aroma of the essential oil makes it an ideal component of many perfumes either used singly or in combination with other essential oils. The plant with its exotic aroma is extensively used in flavour, cosmetic and pharmaceutical industry which requires large quantities of the source material for the preparation, processing and testing of the products and hence the need for mass propagation on a commercial scale.

Majorana hortensis Moench, an aromatic perennial herbaceous shrub is an important spice and food additive common in European cuisine. Indigenous to Mediterranean countries, the plant was known to the ancient Egyptians, Greeks and Romans (Tainter and Grennis, 1993). Greeks and Romans dedicated it to Aphrodite, the goddess of love, fertility and beauty. It was gifted to the newlyweds as a token of god fortune and was used in garlands to symbolize love and honour. In death, it was used as a funerary herb to wish restful peace. It was a very popular herb among the Greeks and was widely used in medicine and perfumes. During the 16th century the plant used to be strewn on the floor to mask the unpleasant odour and purify the indoor atmosphere. Fresh sprigs of the plant are used in garlands, bouquets as also for garnishing and flavouring food. The dried flowering tops are used for sachets and potpourri for decoration (Farooqi and Vasundhara, 1997).

EXTRACTION OF THE ESSENTIAL OIL

Steam distillation of leaves and flower heads yields a volatile oil known commercially as the Oil of Sweet Marjoram. The oil may be colourless,
pale yellow or yellow green depending on the material used for distillation. The oil content in fresh leaves ranges from 0.3 to 0.5 per cent (Farooqi and Vasundhara, 1997). The dried plant gives higher yield of essential oil up to 1%, but it is better to distil fresh leaves and flowering heads than dried herbage as there is a possibility of oil with flat note in case of dried leaves (Ramachandraiah et al., 1984). The oil has a warm woody scent with spicy undertones.

**THERAPEUTIC PROPERTIES AND USES**

The oil is widely used in Ayurveda and Unani systems of medicine to cure various human ailments. The plant is reported to possess antibacterial properties (Ben et al., 2001 and Farooqi and Sreeramu, 2004). Components from Marjoram oil have shown therapeutic effects like analgesic, aphrodisiac, antioxidant, antiseptic, antispasmodic, antiviral, antibacterial, digestive, carminative, diuretic, expectorant, sedative, hypotensive, vasodilator. An infusion of the plant is used as a stimulant, sudorific, emmenagogue, and galactogogue; It is reported to be useful against asthma, hysteria and paralysis (Anon, 1985). Being anti-spasmodic, it is used against spasms of the respiratory system, intestine, muscle spasms, pulls and cramps. The oil is anti-fungal and cures fungal infections of the skin. It helps hyperactive people calm down while boosting blood circulation. Because of its soothing effect on mind and body it is used in Aromatherapy. Used either alone or blended with other essential oils like Lavender, Eucalyptus, Cedar wood, Chamomile etc., Marjoram oil is used in Aromatherapy to treat anxiety, depression, insomnia and emotional trauma. The effect is relaxing rather than stimulating. It is a muscle relaxant, pain reliever and anti-inflammatory hence used in ointments for pains and swellings. A steamy infusion for a foot bath is very effective in relieving pain.
Marjoram oil is non-toxic, non-irritant, and non-sensitizing but should not be used during pregnancy and nursing. It is also not recommended for children.

**USES IN THE FOOD INDUSTRY**

The sweet scented leaves of the herb or its essential oil are used as spice in sausages but its use in baked goods, processed vegetables, condiments, soups, snack foods and gravies is also reported (Burdocek, 1990). It is used to flavour salads, meat products, cheese, poultry dishes and for dressing and garnishing stews and soups. The dried seeds are used in confectionery.

**USES IN THE COSMETIC INDUSTRY**

It finds use as a fragrance ingredient in soaps, detergents, perfumes, face packs, hair oils, body massage oils, skin creams, shaving lotions, body lotions etc. Studies have shown that Marjoram can prevent premature ageing.

**PLANT PROFILE**

**Systematic position:**

- **Class**: Dicotyledonae
- **Subclass**: Metachlamydeae
- **Order**: Tubiflorae
- **Family**: Lamiaceae
- **Genus**: *Majorana*
- **Species**: *M. hortensis* Moench

**Synonym**: *Origanum majorana* L.

Derived from the Greek word Oregano meaning Joy of the mountains and they are indeed a joy to grow (Harvala and Kaltra, 1986).
Common names:

Marjoram, Sweet marjoram, Knotted marjoram (Because of the small scaly heads which look like knots from which the white flowers arise).

Vernacular names:

Kannada : Maruga
Sanskrit : Marwa
Telagu : Maruvamu
Tamil : Marvu, Marrau
Konkani : Mijrikamvil
Bengali : Marru
Hindi : Marwa
French : la Marjolaine; l’origan
German : Maigram, Majoran
Italian : Maggiorana
Spanish : la mejorana

There are also other Marjorams like

Common marjoram or Oregano - *Origanum vulgare*
Dittany of Crete - *Origanum dictamnus* or *Amaracus dictamus*
Golden marjoram - *Origanum aureum*
Winter marjoram - *Origanum heracleoticum*
Pot marjoram variegated or - *Origanum onites variegatum* or
French marjoram - *Origanum prismaticum*
Botanically, the *Origanums* differ from the other labiates, in that the flowers are collected into condensed little cylindrical heads called spicule. Depending on the shape of the calyx, the species are divided into three sections.

1. Section Origanum has calyx with five even teeth.
   Ex. *O. vulgare* and *O. heracleoticum*

2. Section Marjorana has one lipped deeply slit calyx
   Ex. *O. majorana* and *O. onites*

3. Section Amaracus has a two lipped calyx (Amaracus - the original name of Marjoram)
   Ex. *O. dictamnus*

**Taxonomic description:**

*Majorana hortensis* Moench is a perennial aromatic herbaceous shrub cultivated for its fragrant leaves, flowers and seeds. The plant is an upright bush growing to a height of about 20-40 cms. The main stem is woody but the branches are soft. (Plate 1- Fig.1 and 2)

Leaves are simple, petiolate, opposite-decussate, elliptic, matt green and aromatic.

Inflorescence of axillary and terminal spiklets, globose, giving a knotted appearance and hence the name Knotted Marjoram.

Flowers are small, white, produced in spikelets and protrude out of overlapping bracts, zygomorphic, bisexual, complete and hypogynous.

Calyx of five sepals, gamosepalous, deeply slit, hairy along the margin and abaxial surface, persistent.
Corolla of five petals, gamopetalous, bilobed with a posterior lobe of 3 petals and an anterior lobe of 2 petals.

Androecium of 4 epipetalous, didynamous stamens, anterior pair shorter than the posterior pair, anthers dithecous and introse.

Gynoecium bicarpellary, syncarpous, superior, ovary four-partite by a secondary division with one ovule in each locule on axile placenta. Style gynobasic, stigma bifid. Hypogynous disc fleshy.

Fruit, four one-seeded nutlets enclosed in the persistent calyx. Seeds are oblong, brownish about 3 mm long and 1 mm wide; testa thin, endosperm scanty; embryo with flat cotyledons parallel to the fruit axis and a short inferior radical (Prasanna kumar, 1994).

**DISTRIBUTION**

The plant is native to Southern Europe, Northern Africa and Asia Minor. It is cultivated throughout Europe, Mediterranean region, South and North America. In India, Marjoram is grown in Tamil Nadu, Andhra Pradesh, Karnataka, Kerala and some northern states.

**CLIMATIC AND AGRONOMIC NEEDS OF THE PLANT**

**Climate:**

It is a plant of the warmer regions and is sensitive to cold climate. It is grown as an annual wherever the winters are very cold. It grows luxuriantly in sunny areas during summer when the photoperiod is long and the temperature is modest to high. Heavy and prolonged rainfall is not suitable for the growth of the plant and the quality of the oil.
Soil:

Marjoram grows best in well drained, fertile loam soil rich in nutrients. Germination of seeds is very slow in soils which form crusts and hence not suitable for the growth of Marjoram. The quality of oil is dependent on the type of soil, its nutritional content and aeration. The amount of sunshine and water the plants receive will determine the quality of the oil. Under drought conditions those plants raised on nutrient rich soil possess strong aroma and flavour. It is one of those rare crops which can be grown even on saline or alkaline soil and can withstand high salt content (Pandey et al., 1978). The plants can thrive even under drought conditions but prolonged periods of drought are harmful for the plant.

Planting time:

Raising the crop in the right season is very essential to ensure proper growth and maximum yield per unit area. It has been suggested that under Bangalore conditions November is the best season for planting Marjoram to ensure maximum growth and yield attributes (Anon, 1993).

PROPAGATION

1. Nursery raising:

Marjoram can be propagated through seeds. Sowing is done twice a year during June-July and September-October perhaps corresponding to the monsoons to ensure a higher rate of survival. They are sown in well prepared nursery beds in rows spaced about 15 cms apart. About 50 gms of seeds are enough to raise seedlings to plant in one hectare area. As the seeds are very small they are mixed with dry sand in the ratio of 1:10 and broadcasted in rows in the nursery beds. A thin layer of sand is spread uniformly over the seeds to cover them (Farooqi and Vasundhara, 1997).
The germination takes about 10 days. The seedlings are watered carefully using a sprinkler. The seedlings can be transplanted after about two months.

This method of raising seedlings through seeds is rather cumbersome and time consuming. Therefore it is not usually followed.

2. **Vegetative propagation:**

The most common method of propagation is through cuttings. The crop grown by this method is not only true to type but also shows faster growth initially. The stem cuttings obtained from the second ratoon are most ideal for planting to raise the crop. Herbaceous cuttings of about 15 cms length are planted in sand in seed pans. They are shifted to shade or mist-propagation chambers and watered regularly twice a day. It takes about a month for the cuttings to develop the roots. Once rooted, they can be transplanted into the field for establishment.

Spacing of plants in the field has a very significant effect on the oil content. Plants spaced at 30x30 cms have recorded maximum oil content while it is minimum when spaced at 15x7.5 cms though the amount of herbage and oil yield are quite high (Farooqi and Vasundhara, 1997). This may be attributed to the reason that the closer spacing accommodates more number of plants per unit area which ultimately results in higher herbage and oil yield per hectare when compared to wider spacing having less number of plants per unit area (Anon, 1993).

Application of NPK fertilizers is said to boost vegetative growth and oil content. It is advisable to apply Nitrogen at 240 Kg/ha, Phosphorus at 40 Kg/ha and Potash at 80 Kg/ha in three equal split doses in order to obtain maximum herbage and oil yield in marjoram (Farooqi et al., 1994).

Marjoram cultivation can be rotated with certain monsoon crops like cumin, methi and coriander.
IRRIGATION AND INTERCULTURE

Once in the field, the plants are watered by a sprinkler for 5-6 days. Then a light surface irrigation is given. Subsequently, watering is done sparingly once in 4-8 days depending on the season and the soil type (Farooqi and Vasundhara, 1997). Regular weeding and hoeing are done to ensure luxuriant growth of the plants.

INSECTS, PESTS AND DISEASES

Marjoram is quite a hardy plant and is not commonly attacked by insects and pests. Rare attacks by termites can be controlled by spraying Termex 20% E.C. During cloudy weather the plants become prone to leaf rusts which can be easily be controlled by spraying 0.2 % Blitox at weekly intervals (Farooqi and Vasundhara, 1997).

HARVESTING

The first harvesting is done after 90 days of planting when the plants start flowering. The plants are cut about 10 cms above the ground to ensure further growth of the plant. Subsequent harvests are carried out after 45 days of the first harvest. About 4-5 harvests are done annually, though under very good management up to 7 cuttings can be taken. After every harvest, nitrogenous fertilizers, preferably urea are applied to the crop to ensure good yield. To obtain oil of export quality, the first harvest should be done only after 90 days of planting (Anon., 1993). On an average, Marjoram may yield about 16 -18 tonnes of fresh herbage per hectare (Farooqi and Vasundhara, 1997).

PRODUCTION OF THE OIL

Steam distillation of fresh herbage yields the aromatic oil of marjoram. One complete distillation requires about 4-5 hours. The oil should be
completely free from moisture, suspended matter and sediment before storage. The oil recovery may be about 0.3 - 0.4 per cent on fresh weight basis.

**COMPOSITION OF THE OIL**

The oil of sweet marjoram contains more than 31 components including terpinen-4-ol, γ-terpenene, α-terpenene, α-terpeneol, linalool, cis-sabinene hydrate, carvacrol, p-cymene, thymol etc. The composition of the oil depends not only on the soil, climate and the variety of the plant but varies with the harvesting stage of the raw material used for distillation. It has been observed that plants harvested at 90 days yield oil that close to the international standard needed for export. Most South Indian varieties show oil composition similar to that of the oils from USA and Europe.

Based on the major constituents of the essential oil, two chemotypes have been identified: Terpinen-4-ol/sabinene hydrate chemotype (Banchio et al., 2008) and thymol (carvacrol) chemotype (Baser et al., 1993). In the first chemotype, the two major constituents, Terpinen-4-ol and sabinene hydrate are responsible for the characteristic flavour and fragrance of marjoram oil (Vagi et al., 2005).

In medicinal and aromatic plants, the biosynthesis of secondary metabolites such as essential oils and their constituents is strongly influenced by environmental factors (Stutte, 2006). One of the most important environmental constraints that affect almost 50% of the irrigated areas is salinity (Flagella et al., 2002). This constraint generally modifies essential oil biosynthesis and its secretion (Heuer et al., 2002).

The essential oil yield of some species can change with age, growth cycle, climatic conditions, soil type and cropping pattern (Hamrouni et al., 2009). Culturing conditions can affect the quality of the essential oil (Viljoen et
al., 2005). The age of the plant also has a significant effect on the essential oil composition (Bayder and Bayder, 2005). Study by Olfa Baatour (2012) showed that in *Origanum majorana* the essential oil yield and composition depend on the culturing and climatic conditions. The quality and yield of essential oil could also improve with the use of AM Fungi, compost and bio-fertilizers.

**MARKET FOR OIL**

France and Egypt are major exporters of Marjoram oil in the world. The USA consumes about 400 tonnes of oil annually while the requirement is more than 500 tonnes in France and Germany. According to a 2011 estimate, under the category of exotic essential oils for fragrances/ natural medicines/ pharma, Morocco and Egypt produce 40 tons of Marjoram oil (Varshney, 2012).

**WHY MICROPROPAGATION IN MAJORANA HORTENSIS**

*Majorana hortensis* Moench is an important medicinal and aromatic plant whose curative properties are yet to be fully exploited though the plant is used extensively in flavour, cosmetic and pharmaceutical industry. Increasing demand for the essential oil by these industries has necessitated large scale production of the plant. The plant is usually propagated through seeds and stem cuttings with poor rooting. These conventional methods are cumbersome and time consuming. Through micro propagation it is possible to obtain large quantities of healthy planting material in a relatively short span of time throughout the year, irrespective of the season. Further variations could be introduced through tissue culture studies and the elite variants can be selected for better yield. There is scope for qualitative and quantitative enhancement of the essential oil of the aromatic plant through tissue culture technique. As the demand for the
essential oil is quite high in the west, there are a lot of opportunities for the export of quality plant material and hence, the cultivation of the plant on a commercial scale would benefit the farming community.

Further, efforts can be made to improve the biomass and essential oil content of micropropagated plants by AM Fungal treatment.
OBJECTIVES OF STUDY

The present study was undertaken in *Majorana hortensis* Moench with the following objectives

1. To develop and standardize repeatable protocols for *in vitro* multiplication. Various explants and different media combinations can be tried for Micropropagation of *Majorana hortensis*
   a. Through direct/adventitious proliferation
   b. Through indirect regeneration
2. To evaluate the morphogenetic response of the regenerates.
3. To establish the regenerates in the field.
4. To study the effect of AMF treatment on Normal and micropropagated plants in pot cultures.
5. To determine the phytochemicals in the Normal and Micropropagated plants with and without AM fungi by preliminary qualitative screening.
6. To quantify the Phytochemicals in Normal and Micropropagated plants with and without AM fungi.
7. To extract and analyse the essential oil - the Oil of Sweet Marjoram from Normal, Micropropagated and Micropropagated plants treated with AMF.
8. To investigate the anti-microbial activity of the Normal and Regenerated plants.
9. To investigate the anti-oxidant activity of the Normal and Regenerated plants.
10. To statistically analyse the results of the experiments.