INTRODUCTION
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Ayurveda, the Indian system of medicine, dating back to the vedic ages (1500-800 B.C.), has been an integral part of Indian Culture (Weiss, 1987). The term comes from the Sanskrit root, Ayu (= life) and Veda (= Knowledge). As the name implies, it is not only a science of treatment of the ill but covers the whole gamut of happy human life, involving the physical, metaphysical and the spiritual aspects. According to Susruta, the foremost aim of Ayurveda is to help people maintain health and treating the disease is only secondary.

In India, our divine vedas were compiled much before the commencement of the modern era. They embody a vast treasure of knowledge regarding multifarious uses of plants with major emphasis on medicinal, magico-medicinal, magico-religious and mythological aspects. The Rigveda (3,500-1800 B.C.) appears to be oldest record available on the medicinal plants. Similarly the Vrikshayurveda compiled by Saga Parasara consists of excellent informations about plants and their medicinal properties (Sensarma, 1989). This got propagated further through other Vedas, Samhitas, Brahmans, Nighantus and Modern literature. Plants play a vital role for existence of the life on the earth. They not only provide food and shelter to living beings rather provide medicines too. The major source for the drugs used for the treatment of human ailments is the plants and other products.

Medicinal plants have attracted considerable global interest in recent years. The WHO’s forecast is that the global market for herbal products would be of the order of $5 trillion by year 2050 (Planning Commission, 2000). In USA alone traditional drugs and preparations worth several hundred million dollars are imported from other countries especially from India and China. Medicinal and Aromatic plants play an important role in human life. The international market of herbal products is estimated to be US $ 62 billion.
India’s share in the global market of herbal medicinal plants trade is less than 0.5%.

India accounts for nearly 8.0% of the biodiversity of the world despite having only about 2.4% of the total land area. This makes our country as one among 12 mega diverse countries of the world. Presently India’s export from medicinal and herbal plants is Rs. 446 crore (2000) and efforts are made to increase it to Rs. 3000 crore annually by 2005. Out of 17,000 plants, the classical systems of medicines like Ayurveda, Siddha and Unani make use of only about 2,000 plants in various formulations (Anonymous, 2000a). However, India has a rather small turnover of around Rs. 5,000 crores in medicinal and aromatic plants, which is around 1.0% of global market. Climatically, the Indian sub-continent is best suited for cultivation of various important medicinal plants. There are many opportunities and vast prospects to develop the medicinal plant sector in the country.

Our country has a vast repertoire of plants of medicinal value and the people have been using these bioresources for various therapeutic uses from time immemorial. The varied agro-climatic conditions in the country make it suitable for growing a wide range and variety of medicinal and aromatic plants. The importance of these resources has been well recognized by the people of this country as well as in the other parts of the world. Our ancient scripture and classic literature give an exhaustive account of these valuable plants including their formulations and usage for curing diseases.

The rural masses and tribals in India even now depend largely on the surrounding plants/forest for their day to day needs. The wild source is speedily shrinking day by day. Therefore, there is a need for conservation and sustainable use of herbal and aromatic plants. Though the production of plants being labour intensive but generates increased employment opportunities for the farmers, rural masses and enhances their income.
Plant resources are invaluable assets and socio-economic heritage of our country. Peninsular India is particularly rich in these resources, which include thousands of species of various categories viz. trees, shrubs, herbs and grasses. Medicinal plants constitute an important category and form an integral part of the rural and urban lifestyle. For betterment of health, medicines and medicinal plants become irreplaceable. Constant and unsystematic harvest of medicinal plants, which in majority occur as shrubs and herbs in forests in the form of undergrowth are causing their fast depletion from the forests. Many of the species have reached the stage of “endangered and threatened”, while demand of raw medicinal plant parts is increasing steadily with the coming up of more and more pharmaceutical companies in India and abroad. Hazardous effects of grazing and fire, particularly in Central India, together with over exploitation have created havoc.

Forests have been the chief source of raw material for majority of pharmaceutical industries. With the diminution of these in the forests, it has become essential to restore their overall status by introducing them in other available non-agricultural lands. Moreover, their constant supply is also essential for the existence of industries and security of public health. Further, tribals are, if not fully, but in majority, dependent on locally available medicinal plants for curing their ailments. Very few of them go to the health centers. Some endeavor has been made in this direction to document the medicinal uses of herbs, shrubs, grasses etc. by tribals of Central India (Jain, 1963; 1975, 1981; Prasad et al., 1990).

Only a few medicinal plants of high economic value like *Atropa belladona*, *Catharanthus*, *Cinchona*, *Dioscorea* sps., *Papaver somniferum*, *Ruta graveolens* have been cultivated under field conditions. The majority of plants used for medicines are harvested from the wild. This results in several serious problems such as depletion of resources, extinction of rare species, material not being available in large quantities and throughout the year, incorrect identification and adulteration of the plant material.
If efforts are made for systematic cultivation of medicinal plants instead of collecting them from the wild, many of the problems mentioned above will be minimized. Properly identified and certified planting material can be supplied to the growers. Cultivation of the plants can be planned to meet the needs of the industry in required quantities and at the required time. Unintentional adulteration can be avoided and it will be easier to check deliberate adulteration.

Developing countries can ill afford the products of the western pharmaceutical industry due to their high and rising prices. The import of such medicines or payment of royalties for local manufacture of the medicines causes a heavy financial burden for the developing countries. The WHO, therefore, recommends the inclusion of herbal medicines, which are time tested, safe and available at affordable prices in the national health programs. Many of these countries have the necessary land and varied climatic conditions to grow these plants. This is especially true of India where there are several government and privately funded programs to develop wastelands created by deforestation, erosion or neglect.

Besides catering to the needs of the domestic market, medicinal plants can be used by the third world countries to obtain the much-needed foreign exchange. India has been an important exporter of medicinal plants. Some of the medicinal plants exported are Digitalis, Belladonna, Liquorice, Senna and Psyllium. According to the report of the Eximbank, the annual export of medicinal plants has been on the increase. It was only Rs. 35 crores in 1980-1981 whereas it was Rs. 1238 crores during 1995-1996. There is a vast potential for further increase in exports due to the increase in demand for Ayurvedic and Unani medicines in Western countries.

Many programs have been initiated for in situ propagation of medicinal plants in recent times by NGOs (Non Government Organizations) as well as
government and semi-government agencies. However, these programs, though laudable, are limited by several factors. The potential for regeneration of many plants in their natural habitat is poor. The germination of seeds and establishment of seedlings is also poor. There is very little knowledge of the vegetative propagation of these plants.

Biotechnology, a great technical revolution of the century, offers solution to many problems in medicine and health care. Since plants have emerged as an important source of medicine, the interest in their mass propagation has increased. Plant tissue culture techniques are now being used globally for the multiplication of medicinally important plant species and also monitoring of their secondary metabolites. Plant Tissue Culture refers to a cellular mass that is derived from an explant of plant and grown under aseptic conditions on a nutrient medium. Such a mass can be repeatedly grown on a fresh medium to yield stable cell line. By 1950, a methodology was developed to establish tissue cultures from numerous plant species and by manipulation of the growth medium the tissue mass it was demonstrated that the formation of roots, shoots and regeneration of complete plantlets could be obtained. This phenomenon described as the totipotency of plant cells has led to several interesting avenues in many areas such as clonal multiplication of plant, cell transformation and production of plant metabolites. The application of micropropagation technique for medicinal plants give many benefits to the breeders as it enables to increase the rate of rapid multiplication of plants which in a particular climate donot produce seeds or whose seeds have low germination, the availability of plants throughout the year, uniform plants of selected genotypes and thus production of plants with changed genotypes, conservation of genetic resources of species and threatened plants and plant improvement by regeneration technique.

The role that plant metabolites have played through the centuries as medicinal agents, mainly in the form of crude extracts or herbal cures have
chemicals which are not essential for the survival of the organisms but however, plants used these chemicals known as “Secondary Metabolites” in their defense systems.

Secondary metabolites are economically important besides playing important roles in the ecology and physiology of the plants. Investigations in the area of biochemical ecology indicate that some secondary compounds produced by plants are important either to protect these plants against microorganisms and animals, or to enhance the ability of one plant species to compete with other plants in a particular habitat (Bell, 1980). Despite advances in the field of organic chemistry, plants are still an important commercial source of chemical compounds having many pharmaceutical applications. It has been demonstrated that many of these secondary metabolites produced by intact plants could be synthesized by cell cultures (Klein, 1960). Alkaloids, steroids and flavonoids are widespread group of natural compounds.

The plants known as medicinal are rich in secondary metabolites which includes alkaloids, glycosides, amines, steroids and flavonoids and relative active metabolites are of great medicinal value and have been extensively used in drug and pharmaceutical industry (Atal & Kapur, 1982). Besides, these secondary metabolites are also useful in plant systematics.

Plants represent an unlimited source of phytochemicals such as the metabolites of primary and secondary metabolism. The secondary compounds are of major interest because of their different functions and their biological activities ranging from antimicrobial, antibiotic, insecticidal and hormonal properties to highly important pharmacological and pharmaceutical activities. Secondary metabolites are compounds that are biosynthetically derived from the primary metabolites and their distribution in the plant kingdom is restricted. They occur only in a few species or even within a few varieties within a species. The role of secondary metabolites in the function of plant metabolism is not clear but many of
them have an ecological role. They are pollinator attractants, represent chemical adaptations to environmental stresses or serve as a chemical defense against microorganisms and insects. Secondary metabolites accumulate in the plant in small quantities in specialized cells. Their extraction is often difficult and complicated. Some of the important products are medicinal substance, pharmaceuticals, fragrances, food additives, flavouring and aromatic compounds and pesticides. Their compounds are generally detected in lower volume compared to the primary metabolites and also possess significant biological activities. As a result, the secondary metabolites are also termed as the higher value – lower volume products or specialty chemicals. The secondary metabolites are substances, whose structure and metabolic interaction appears to be functionally distinct from those involved in primary metabolism. Those secondary metabolites are not the products of single gene but rather of multigene sequences coding complicated and highly integrated biochemical pathways. The alkaloids, steroids and terpenes are the major secondary metabolites responsible for the pharmaceutical, cosmetic and food industries (Zenk, 1978; Bohm, 1980). Around 2500 species have been thoroughly screened for pharmacological activity. In a recent survey, it is estimated that 25% of the prescription drugs in USA and Europe contain plant components, which generates more than $10,000 M annually.

By 1970, reports of secondary metabolites in plant tissue culture had appeared and the idea of manipulating the plant cells like microorganisms to accumulate the metabolites in yield comparable or superior to those from the field grown plants gathered momentum. The tissue culture technique can help solve some basic hurdles associated with the biosynthetic pathway of certain medicinally important compounds and also how the production of secondary metabolites is linked with organogenesis, as is evident from the celery tissues where flavour compounds were lacking in undifferentiated callus but were produced by embryoids (Collin & Watts, 1983; Nakagawa et al., 1982). In several cases secondary products of mature embryos and plantlets are comparable to those of intact plants.
The developments in plant cell and tissue culture techniques have provided an alternative to whole plant cultivation for obtaining several plant-derived chemicals. Using this method, the compounds can be obtained under precise controlled conditions unaffected by seasonal conditions within a short period of time. In the cultivated plants the secondary metabolites are often accumulated in the roots, stems, bark or to some extent in the leaves of the tree after several years of growth. Extraction of the compounds involves destruction of the entire tree, which may result in their extinction. The need to conserve and cultivate the desired plant species for the production of high value compounds is therefore imperative. Plant tissue and cell cultures are also advantageous for understanding the biosynthesis of several compounds. The production of secondary products in plant tissue culture is controlled by various regulatory factors.

Initial efforts in the area of production of secondary metabolites by in vitro cultures did not meet with success since a majority of the tissue culture failed to synthesize the characteristic constituents of the parent plant. However, subsequent research on growth, differentiation and metabolism of plant cells gave further insight into the function of a plant cell and to date several secondary metabolites belonging to various groups have been isolated from tissue and cell suspension cultures of higher plants (Ellis, 1988). There are also examples of certain natural products formed by plant cell suspension cultures in remarkable yields compared to the differentiated plant.

*Vitex negundo* L. (Verbenaceae) is an important medicinal plant and its roots and leaves are used as a tonic in various ailments (Gupta, 1973). The extract of leaves is used for catarrhal fever and vermifuge (Chadha, 1976; Hussain *et al.*, 1992) and used extensively in Ayurvedic and Unani medicine (Kapur *et al.*, 1994). The plant was found to possess anti-arthritic (Tanhankar & Sarf, 1994), hepatoprotective (Kapur *et al.*, 1994), anti-inflammatory, anti-allergic (Chawla *et al.*, 1991), insecticidal (Aswal *et al.*, 1996), anti-fungal
(Rusia & Shrivastava, 1998) as well as mosquito repellent activities (Hebbalkar et al., 1992).

*Tinospora cordifolia* (Willd.) Miers ex Hook. f. & Thoms is a large, glabrous, deciduous climbing shrub belonging to family Menispermaceae. In Hindi, the plant is commonly known as *Giloya*, which is a Hindu mythological term that refers to the heavenly elixir that have saved celestial beings from aging and kept them eternally young. The plant has been reported to possess anti-inflammatory, anti-allergic, hypotensive, hepatoprotective, immunostimulant and diuretic properties (Nayampalli et al., 1986; Rege et al.; 1984; Pathak et al., 1995). In Ayurvedic system of medicine, it is known to possess tonic and vitalizer properties and as a remedy for diabetes, arthritis and metabolic disorders (Nadkarni, 1954; Chopra et al., 1958).

*V. negundo* and *T. cordifolia* suffer from poor seed set and germination in natural habitat. Stem cuttings, though useful for propagation, are dependent upon weather conditions for proper growth. Also, attempts at improving the quality of plants multiplied through vegetative means suffer because of lack of genetic variability. Tissue culture technology not only reduces the duration for propagation and selection of desirable traits but also facilitates raising plantlets in a limited space (Kumar et al., 2003). Therefore these two plants were selected for present investigations with following objectives:

1. To initiate shoot bud culture from different explants of *Vitex negundo* and *Tinospora cordifolia*.

2. To find suitable medium for explant establishment, shoot proliferation and rooting of microshoots for cultures derived from different explants of *V. negundo* and *T. cordifolia*. 
To study the effects of subculturing on shoot proliferation of microshoots of *V. negundo* and *T. cordifolia*.

To find out suitable medium for callus formation from different explants of *V. negundo* and *T. cordifolia*.

To make attempts for inducing differentiation in callus initiated from different explants of *V. negundo* and *T. cordifolia*.

Estimation of primary metabolite (carbohydrates and protein) from wild and tissue cultured plants of *V. negundo* and *T. cordifolia*.

Estimation of chlorophyll content in wild and tissue cultured plants of *V. negundo* and *T. cordifolia*.

Extraction of some of the important secondary metabolites present in *V. negundo* and *T. cordifolia*.