CHAPTER-I

INTRODUCTION
All life in Universe comprises of the five basic elements: solid (Prithvi), liquid (Jal), gaseous (Vayu), radiant (Agni) and ethereal (Akash) and interacts between these elements influence the action and properties in the plants.

Nature has bestowed us with a large number of diverse types of plants which are grown in worldwide in different vegetations. The history of medicinal plants dates back to the origin of civilization and use of plant resources for medicinal purposes is one of the numerous practices developed by ancient people. About 67 plants have been reported in Rigveda (500 BC), whereas 81 and 290 plants of medicinal use have been reported in Yadurveda and Atharvaveda (4500-2500BC), respectively. Also Charak Samhita (700 BC) and Susruta Samhita (200 BC) have described properties and uses of some 1100 and 1270 plants (Azhakia Manavalan & Manian, 2001), respectively. The number of plants used in traditional and folk remedies has been estimated at about 50,000 species worldwide and majority of them being collected from the wild. With ongoing surveys, research and documentation efforts, the number of plants used in traditional medicines are considerably increasing day by day.

Medicinal plants have occupied an important position in the socio-cultural, spiritual and medicinal arena of urban, rural and tribal lives. Side effects of allopathic drugs, chemotherapy, and radiotherapy have scared people all over the world and the health care systems are going to become more expensive. These remedies, with considerable extent of effectiveness, are socially accepted, economically viable and are the only available source. The folk medicines are the original source that slowly entered to the organized systems like Ayurveda etc. and finally investigated for recognizing among the modern medicines (Ved Prakash, 1998). Most of the Ayurveda based drugs are important in the manufacture of various immunological vaccines and antibodies (Biswal et al., 2007).
With the emergence of ethno-botanical strategies, chemical and pharmaceutical studies of crude plant drugs are being dramatically modified. The advancement occurring in chemical and biological technologies have clearly contributed to the creation of new knowledge possessing the medicinal use of ‘plant extracts’, ‘decoctions’, ‘infusions’ and ‘soft herbal remedies’ instead of the classic pharmaceutical chemical drugs. Medicinal plants possess unlimited and untapped wealth of chemical compounds with high drug potential, which make these plants useful as sources biomedicines (Annexure- II). Immunomodulation, especially using Rasayana drugs, could provide an alternative to conventional chemotherapy under the conditions of impaired immune responsiveness or following organ transplantation and the concept of using Rasayanas for health also gains a little more creditability as herbal antioxidants concurrently exhibit significant immunomodulatory activities (Thatte & Dahanukar, 1997).

Traditional medicines have continued to play an effective role in modern medicine, particularly in revival of interest in the use of medicinal plants both in developed and developing countries because herbal medicines are reported to be safe and do not produce any side effects which are often noticed with the long administration of synthetic drugs.

According to the estimation of WHO, 80% of the population of developing countries rely on traditional medicines, mostly plant drugs for their primary healthcare system (Biswal et al., 2008). In the current strategy document (2000-05) of WHO, it has included the traditional medicine on a broader category of Complementary and Alternate Medicine (CAM). Considering the coverage and effectiveness of the various systems of traditional medicine throughout the world, the Alma-Ata Declaration of the WHO (1978) proposed “Health for all by the year 2000”, the commitment being reaffirmed by the International consultation on conservation of medicinal plants, organized by WHO /
IUCN / WWF in Chiang Mai, Thailand, popularly known as the Chiang Mai Declaration (1988), emphasizing a primary health care approach and the principles of conservation and sustainable development, outlined in the conservation strategy (Bhattarai, 2006).

The International Community’s approach to medicinal plants has broadened much over time, gradually shifting from emphasis on conservation and wild collection to sustainable management and contemporary cultivation. At the same time, various crucial issues related with medicinal plants and traditional medicine practices (Traditional knowledge systems, Indigenous Knowledge Systems, Intellectual Property Rights (IPRs), Patent Rights (PRs), Benefit sharing, Bio-prospects and Bio-piracy, Trade Related Aspects of Intellectual Property Rights (TRIPs), etc.) have been increasingly associated with medicinal plants and plant-based knowledge and tradition.

Herbal industry driven use of plants and phytomedicine increased dramatically in the last two decades in USA. The major producers of medicinal products in the world are China, Brazil, Bulgaria, Poland and India. China is the largest exporter of medicinal plants earning US $5 billion per year from herbal trade due to large scale cultivation, standardization in quality control, safe and regular supplies to the International market, though India has rich medicinal plant diversity (Kirtikar & Basu, 1935; Chopra et al., 1956a, 1969; Asolkar et al., 1992; Patwari, 1992; Rawat & Choudhuri, 1998; Sharma et al., 2000, 2001, 2002). International export trade in medicinal plants is dominated by China, which exports 1, 21, 900 tones of materials a year and India exports 32,600 tons annually (Rajsekharan & Ganeshan, 2002). The position of India is tenth among plant rich countries of the world and fourth among the Asian countries. Recently the World Health Organization (WHO) has compiled a list of 20,000 medicinal plants used in different parts of the globe. However, only about 10,000 plants are used for phytotherapy
in Indian systems of medicine and it has a larger demand and is utilized in major drug markets in the world.

India is a versatile botanical garden of the world with a large reservoir of medicinal and aromatic plants. In traditional systems of medicine in India, although around 3,000 plant species have been used for nearly the last 5,000 years of which, only 1500 species are in use. Currently, out of these species, only 700 species are used in modern medicine system after being investigated for their pharmaceutical and chemical activity (Das et al., 2003). At present in India, total trade of Ayurvedic and other herbal products is estimated to be about 5000 crores, which indicates that, medicinal plants have good potential. India at present nearly 90% of traded medicinal plants are generally collected from wild in India and rest from cultivation. They are either used fresh or in the form of crude drug, which are dried whole plant or their parts such as roots, stems, wood, bark, leaves, flowers, fruits, seeds and their products. The Eastern Himalayas and the Western Ghats of India exhibit approximately 5332 endemic species of higher plants (Anonymous, 1997) which is being lost due to the over exploitations for industrial purposes.

Orissa has the oldest and richest tradition culturally associated with use of medicinal and aromatic plants bestowing with a rich biodiversity of medicinal plants due to its diversified topography and variable climatic conditions. The forest area is extensive, constituting about 42 percent of the total area of the state (Saxena and Dutta, 1975). These are seen mainly in forests and hills, plateaus, plains of most of districts including Koraput, Kalahandi, Mayurbhanj, Phulbani, Dhenkanal, Bolangir, Keonjhar and Baragarh (The Gandhamardan range of forests). A large number of medicinal plants are found in the forests of Orissa of which a wide range of medicinal plants are cultivated
for commercial purposes (Annexure-I), which are being used for the production of Ayurvedic, Homeopathic and Advanced systems of medicines.

1.1 Urgency to conserve medicinal plants

Medicinal plants are playing vital role as a raw material for pharmaceutical industries. Majority of the medicinal plants are collected from the wild. Therefore, measures should be undertaken for their conservation, systematic collection and cultivation to meet the demand of raw drug preparation of life saving drugs. Also, there is an urgent need for commercial cultivation of medicinal plants, especially those that are prohibited for trade from wild as well as having good demand in pharmaceutical industries. This natural resource needs to be utilized as an alternative source of income for people, which may lead to socioeconomic development through various activities like conservation, sustainable collection of raw drugs from wild and commercial cultivation.

Several crude drugs are manufactured from the plants available in the wild growth, which leads to extinction due to over exploitation of these traditional healers. The phytochemicals are extracted from various plant parts particularly destructive parts like roots, rhizome, whole plant, and bark, wood and stem which contribute to the extinction of uncared and unexploited medicinal plants. Over harvesting of resources for commercial purposes, destructive methods of harvesting, deforestation and other forms of degradation of habitats and ecosystems are the major threats to medicinal plants. As a result, developing a sustainable conservation and management system has become a global challenge. It was reported that out of 2,800 plants used in traditional medicines practical in different parts of our country, the dry potential of only 5% of these plants have been studied chemically or pharmaceutically (Sabnis & Daniel, 1990). It is a fact that, many plants may be lost from the local floras. The urgency and seriousness of the problem has rightly deserved and drawn worldwide attention. The Government of India
has banned export of medicinal plant species and giving attention to conservation, popularization and systematic cultivation of this wealth of nature through various schemes. For conservation of diversity, Department of Biotechnology (DBT) has set up three gene banks, viz. a) Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow. b) National Bureau of Plant Genetic Resources (NBPGR), New Delhi and c) Tropical Botanical Garden and Research Institute (TBGRI), Thiruvananthapuram. The Ministry of Environment and Forests, Government of India has also adopted very tough policies to confine exploitation of medicinal and aromatic plants from wild sources. Also, National Medicinal Plant Board (NMPB), Department of AYUSH, Ministry of Health and Family Welfare, New Delhi, is coordinating all matters relating to development and sustainable uses of the medicinal plants resources in India.

Many of the species of medicinal and aromatic plants show low rate of fruiting, seed setting and very poor seed germination thereby resulting in extremely slow rate of natural propagation in the wild. Hence the urgency now is to conserve such wild population for future uses and at the same time, produce enough planting materials of desired species by adopting improved and efficient propagation approaches and to facilitate mass cultivation of these species.

The plants or seeds are being collected, characterized and maintained in fields, nurseries, seed banks, gene banks, germplasm collection centers and in vitro conditions through cryopreservation and micropropagation techniques, a solution to afforestation programme for outgrowing demand and future needs. So, increased demand for medicinal plants and dwindling forest resources forces us to look into biotechnology for alternative methods to derive active principles. Biotechnology showed promising results in the field of propagation, secondary metabolite production, and development of new plant varieties through tissue culture techniques.
1.2 Biotechnology of medicinal plants

The conventional methods of propagation are often difficult, time consuming and sometimes even unsuccessful. Modern biotechnological method like the in vitro micropropagation technique, allows achieving large scale multiplication of disease free plants in very short span of time under highly controlled condition. The in vitro propagation of plants is becoming increasingly popular as it enables potentially all species of plants to be cloned rapidly in a large number and used extensively (Annexure-V). It must be admitted that conservation of plant resources is a recent development (Frankel, 1986) and still more recent is the application of tissue culture biotechnology for the purpose of conservation of medicinal plant resources (Bajaj and Simola, 1991).

In vitro technique through plant tissue culture of medicinal plants has distinctly increased for the various reasons like: a) Increase in the propagation rate; b) Rapid multiplication of plants, which do not set seeds in a particular climate or where seeds have low germination capacity; c) Availability of plants throughout the year in all seasons; d) Resistance of plants to pests, diseases and herbicides; e) Production of uniform clones from highly heterozygous plants; f) Production of plants with changed genotypes to avoid danger of monoculture i.e. genetic erosion; g) Conservation of genetic resources of species and threatened plants which are facing extinction; h) Plant improvement by regeneration techniques in conjunction with in vitro cellular manipulation; and i) Identification of fast growing medicinal trees for production of medicines.

Isolated tissues cultured in vitro and subjected to the interacting influences of an array of phytohormones could lead to totipotency. The persistence of the potentiality at the cellular level makes plant tissue culture technology an elegant system in morphogenetic studies where an effective control of exogenous factors could be
advantageously applied. *De novo* organ initiation from a tissue or disorganized mass callus in an aseptic culture medium form a novel approach to understand the factors controlling basic or applied aspects of many plants. They have been relatively less extensively applied to a large number of plants of economic potential, particularly wild or semi wild indigenous medicinal plants, parallel to the plant tissue culture technology (Narayanaswamy, 1994; Gamborg and Nabors, 1987), hence there is another emerging area of ever increasing importance and interest which concerns the elaborate studies in conservation of medicinal plants (Annexure-V). So, the medicinal plants should receive all possible attention and efforts that contribute for conservation, including the use of plant tissue culture technique through *in vitro* micropropagation, which includes shoot multiplication, organogenesis and somatic embryogenesis.

1.3 Objectives and rationale of present study for research

Despite being home for several traditional systems of medicine, the total export of herbal based products from India is below US $100 million. The cause of poor market share may be attributed to lack of standardization, coherent effort, and unplanned collection of medicinal plants from the wild, improper documentation of traditional Ayurved, Unani, Sidha, Tribal, and Folk medicines, etc.

In modern medicine very small fraction of the potentialities of plants has been utilized. Only 119 pure chemicals extracted from 90 higher plant species are currently used in modern medicine including 90 chemicals isolated from higher plants having ethnic uses (Rawat, 2005). Herbal medicines are popular as remedies for diseases by the majority of world's population. Polyherbal preparations are products from medicinal plants. These are considered as safe since they are natural products. Medicinal plants as therapeutic agents include antihypertensive, anti-cold and cough agents, hepatoprotective agents, anti-inflammatory agents, anti-diabetic, lipid lowering and anticancer...
agents (Kuruvilla, 2002). Prevention is essential and critical in a strategy against Cancer. Allopathic drugs, chemotherapy, radiotherapy, immunotherapy and gene therapy are administered to the patient basing on the type and stage to control the disease. In this critical situation there must be taken a consorted effort to find an alternative therapeutic effort. This concept of using rasayanas for health, also gain a little more creditability when the antioxidants concurrently exhibit significant immunomodulatory activities. There has been about 204 species reported in worldwide (Annexure-III), in Orissa about 160 species are reported (Annexure-IV) for possessing photochemical used in different types and forms of Cancer.

1.4 Review of selected plants

The present study was focused mainly on medicinal plants viz. *Aegle marmelos* (L.) Corr., *Catharanthus roseus* (L.) G. Don., *Citrus reticulata* Blanco., *Citrus sinensis* (L.) Osbeck., *Heliotropium indicum* L., *Syzygium cumini* (L.) Skeels., *Vitis vinifera* L. and *Withania somnifera* (L.) Dunal., for their anticancer activity from the literature survey. Different aspects of these medicinal plants (Plate-1, Fig a-o) were reviewed and mentioned below.

1.4.1 Importance of *Aegle marmelos* (L.) Correa

*Aegle marmelos* (L.) Correa, originated in India, commonly known as Bael, is a member of family Rutaceae, and is the only species in the genus *Aegle*. It grows up to 18 meters tall and a fruit bearing tree bears thorns and fragrant flowers (Plate-1: Fig. a & b). It is also considered as a sacred tree by the Hindus. They offer its leaves to Lord Shiva during worship.

1.4.1.1 Morphology & Distribution

A small to medium-sized aromatic tree, deciduous stem and branches, light brown to green, strong axillary spines present on the branches, and the average height of tree is
10.12 meters. This tree is indigenous to dry forests on hills and plains of Central and Southern India and cultivated throughout India.

1.4.1.2 Taxonomy

Leaves, alternate, pale green, trifoliate, palmate, terminal leaflet, having a long petiole; the two lateral leaflets, almost sessile, ovate to lanceolate having reticulate venation; petiole, 3.2 cm long. Flowers, greenish white, sweet scented, bisexual, actinomorphic, ebracteate, hypogynous, stalked; stalk, 8 mm long; diameter of a fully open flower; flowers, borne in lateral panicles of about 10 flowers, arising from leaf axil; calyx gamosepalous, five-lobed, pubescent, light green, very small in comparison with petals; corolla polypetalous, with 5 petals, imbricate leathery, pale yellow from above and green from beneath, length 4mm; stamens polyandrous, anther numerous, basifixed, dehiscing longitudinally; gynoecium, light green, having capitate stigma and terminal style. Fruits are yellowish green, with small dots on the outer surface, oblong to globose, pulp yellow and mucilaginous, the pulp of dried fruits retains its yellow and also remains intact; rind woody. Seeds, numerous, embedded in the pulp, oblong, compressed, white, having cotton-like hairs on their outer surface.

The flowering is seen from May to July. The fruits take almost one year to mature. The peak fruiting period is April to June.

1.4.1.3 Chemical Constituents

The bark contains tannin and the aegelinol, furocoumarin, marmesin, umbelliferone, alkaloid skimmianine and fagarine, marmin, lupeol and β-sitosterol. Fruit contains psoralein and tannic acid, aegelinol, furocoumarins, furanocoumarin, marmelosin and marmelide. Ripe fruit contains xanthotoxol, marmesin etc. The pulp contains mucilage, pectin, reducing sugars, tannin, a volatile oil and a bitter principle. The leaves contain 0.6% essential oil, mostly composed of d-limolene.
1.4.1.4 Therapeutics

*Aegle marmelos* is more prized for its medicinal virtues than its edible quality. Various parts of the Bael plant are being used in many Ayurveda and Unani patented drugs in India for treatment of various diseases. This plant is also well known for its medicinal properties such as astringent, digestive, stomachic and diarrhea. The pulp of the fruit is used as sweet drink considered being an aromatic summer drink produce soothing and cooling effect. The unripe and half ripe fruits improve appetite and digestion and are valuable in habitual constipation, chronic dysentery and dyspepsia. Ripe fruits are astringent, sweet, aromatic, cooling, febrifuge, laxative and tonic and are good for heart and brain (Sivarajan and Balachandran, 1994; Warrier *et al.*, 1994). The fruits and roots possess antiamoebic and hypoglycemic activity (Chopra *et al.*, 1956b; Ponnachan *et al.*, 1993). The alkaloid ‘aegeline’ present in the leaf is effective antiasthmatic agent (Harvey, 1968). The active principle in this plant is ‘marmelosin’ which acts as a laxative and diuretic (Nadkarni, 1954 and Ambasta, 1986). Parichha (2004), reported its medicinal uses as this plant is very much useful for curing diabetes, treatment of asthma, healing wound, applied to relief swollen joint pain, lower blood pressure, cure jaundice, diarrhea, lowers frequent vomiting nausea during pregnancy, useful in typhoid and sharpens concentration and intelligence. Kamalakkanna and Prince (2003), Kar *et al.* (2003) and Kesari *et al.* (2006) reported the extract of *A. marmelos* fruits shows hypoglycemic activities. It significantly reduces the blood glucose, plasma thiobarbituric acid reactive substances, hydroperoxides, ceruloplasmonic and alphatocopherol. It also shows significant elevation in glutathione and vitamin C. Arui *et al.* (2005) reported the anti-inflammatory, antipyretic and analgesic properties of the leaves of *Aegle marmelos*. The roots and bark of the tree are used in the treatment of fever and malaria. The roots are used to cure pain, fevers and palpitation of the heart. The leaves
are useful as laxative, febrifuge and expectorant, also used as the treatment of ophthalmia, deafness and inflammation, diabetes mellitus and asthmatic complaints.

Research investigations by Lampronti et al. (2003) observed that extracts from Aegle marmelos are able to inhibit the \textit{in vitro} proliferation of human tumor cell lines, including the leukemic K562, T-lymphoid Jurkat, B-lymphoid Raji, erythroleukemic HEL, melanoma Colo38 and breast cancer MCF7 and MDA-MB-231 cell lines. Aegle marmelos could be considered as potential sources of anticancer compounds, as tested for cytotoxicity by Costa-Lotufo et al. (2005). Clinical evaluation of antimitagenic activity of A. marmelos reported by Annapurani and Priya (1999), antifungal activity of leaves by Rana et al. (1997), antiulcer activity of seeds by Goel et al. (1997) and also anti-diarrheal activity by Shoba and Thomas (2001). The protection against doxorubicin (DOX) induced genotoxicity by Aegle marmelos may be due to inhibition of free radicals and increased antioxidant status reported by Venkatesh et al. (2007). Gangadevi and Muthumary (2008) reported the compound Taxol, an anticancer drug produced by an endophytic fungus Bartalinia robillardoides Tassi, isolated from Aegle marmelos.

1.4.2 Importance of \textit{Catharanthus roseus} (L.) G. Don

\textit{Catharanthus roseus} (L.) G. Don commonly known as Indian periwinkle, belongs to the family Apocynaceae (Plate-I, Fig c & d). It is a wild member of Madagascar that has been distributed throughout tropical and subtropical regions.

1.4.2.1 Morphology and Distribution

It is a perennial herb and about 1.0-1.5 meters in height, found in sandy coastal areas and also available in gardens due to its medicinal values. It is also a commercially cultivated medicinal plant.
1.4.2.2 Taxonomy

Perennial herbs, mesophyte. Tap root system, branched. Stem is cylindrical, solid. Leaves obovate, oblong or oval, rounded at apex, glabrous, polished, base acute, opposite, entire. Inflorescence is axillary, solitary or in paired. Flowers bracteate, white, rose, purple, regular, bisexual, hypogynous, shortly pedicelate or sessile. Calyx, sepals-5, imbricate aestivation, lobes sub-equal, setaceous. Corolla, petals-5, obovate-triangular, apex rounded or obtusely apiculate, imbricate aestivation. Androecium, stamens on the corolla tube, anthers lanceolate, acute. Gynoecium, carpels-2, with two oblong glands altering with the carpels, ovules numerous, 2-seriate, style filiform, distented, many ovuled, stigma with a basal reflected lobed membrane surrounded by 5 tubercles, tip subglobose. Fruit is slender, cylindrical follicles. Seeds are numerous and small. Flowering and fruiting are seen throughout the year.

1.4.2.3 Chemical constituents

Whole plant contains various important alkaloids; including vincristine, vinblastine, pseudoindoxyl alkaloid-rosamine, β-carboline, bannucine and leurosinone which are used as anticancer (vinblastine and vincristine) and hypotensive (serpentine and ajmalicine) agents. Leaves contain alkaloids like serpentine, ajmaline, ajmaline, catharanthine, catharanthineole, vindoline, vindolinine, vincaleucoblastine, leurosidine and vincristine.

1.4.2.4 Therapeutics

*Catharanthus roseus* possesses the largest number of alkaloids in the plant kingdom (Hum-Lin and William, 1972). In *Catharanthus roseus*, more than 100 alkaloids have been isolated from different parts of the plant and many of them possess remarkable pharmacological activities (Svoboda & Blake, 1975; Cordell, 1980). The most important of these are the antileukemic alkaloids vinblastine and vincristine, the antihypertensive
alkaloid ajmalicine and serpentine which have a sedative effect. Satpathy et al. (2003) reported that the leaf buds of this plant are used for curing diabetes. Kurian (1995) also reported that the extract of fresh leaf is said to be anticarcinogenic and also used for diabetes and diarrhea. Narayana et al. (1977) reported that the plant plays as an abortive, hypoglycemic, antidiabetic, antidysenteric, purgative, haemostatic and wound healer. Noble et al. (1958) discovered antineoplastic activities of leaf alkaloids. The alkaloid vincristine sulfate is employed mainly in childhood leukemia and in blood cancer (Conti and Creasy, 1975). Vinblastine is useful in the treatment of Hodgkin’s disease, choriocarcinoma, non-Hodgkin’s lymphomas, renal, testicular, head and neck cancer and similarly Vincristine -2 is used against lung, cervical and breast cancer (Ghosh, 2003).

1.4.3 Importance of *Citrus reticulata* Blanco.

*Citrus reticulata* is commonly known as santra or loose skinned orange belonging to the family Rutaceae (Plate-1, Fig e & f).

1.4.3.1 Morphology & Distribution

An evergreen bushy moderate sized tree with greenish white glabrous branches, widely found in India and other subtropical countries and probably a native of southern-east Asia. It is also cultivated in the hilly areas in Orissa.

1.4.3.2 Taxonomy

*Citrus reticulata* is a perennial, mesophytic, bushy moderate sized tree. Tap-root system. Stem is erect, woody, branched, spiny (1-2 axillary spines), cylindrical, solid. Leaf alternate, exstipulate, petiolate, almost wing less, palmately compound, unifoliate, leaf-let large, ovate-oblong, or elliptic, 5-15x2.5-7cm, crenulate, obtuse, glabrous, base cuneate or rounded, unicostate- reticulate venation. Inflorescence, axillary panicles, solitary, fascicled or in small cymes. Flowers, ebracteate, pedicelate, complete, actinomorphic, bisexual (hermaphrodite), hypogynous. Calyx, sepals-5. small,
polysepalous, valvate aestivation, deciduous. Corolla, petals-5, polypetalous, oblong, white, imbricate aestivation. Androecium, numerous stamens, inserted round the disc. polyadelphous, filaments short, subulate, anthers 2-celled, long, introse. Gynoecium, carpels 4-5, syncarpous, ovary superior, multilocular, 4-many ovules in each locule, axile placentation, style short, stigma small deciduous. Fruit is berry, globose, flattened at the top, pericarp thin, loose, easily separating from the segments, bright orange when ripe, pulp juicy, sweet to mildly acidic, septa membranous and seed numerous. Flowering has been seen from January to June and fruiting is from November to December.

1.4.3.3 Chemical Constituents

The raw and ripe orange consists of 14 monoterpenes, 15 sequiterpenes, gibberillic acid, phytol, amyrin, limonin and its glucosides, nomilin derivatives, seventeen flavonoids, carotenoids, thirteen alkaloids, brassinolide, castesterone, sitoste-ol, hydroquinone, sinapic acid, anethole, frurlic acid, etrogol, coumaric acid, citrus ns, caffeic acid, eleven coumarins, pectin and stigmasterol, Proteins like Thiamine (vitamin B₁), Riboflavin (vitamin B₂), Niacin (vitamin B₃), Pantothenic acid (vitamin B₅), Vitamin B₆, Folate (vitamin B₉) and vitamin C. Some common macroelements are calcium, ion, magnesium, phosphorus, potassium and zinc.

1.4.3.4 Therapeutics

Leaves are useful in remedies for internal ailments and fractures and other sicknesses and Crushed leaves of the plant are useful to treat abdominal pains. Leaf and Orange oil consists of 90% d-limoline of orange peel oil, is a significant chemopreventive agent (Crowell, 1999; Patnaik et al., 2004) with potential value as a dietary anticancer tool in humans (Tsuda et al., 2004). Infusion of the bark is used to treat an illness similar to relapse sickness and to treat postpartum sickness. Fruit juice is purgative.
1.4.4 Importance of *Citrus sinensis* (L.) Osbeck

*Citrus sinensis* is commonly known as sweet orange (Kamala or Santra) belongs to the family Rutaceae (Plate-1, Fig g & h). Santra is used as flavouring of food and drink industry as well as in perfume and aromatherapy. Fruit juice is commercially available and contains a rich source of Vitamin C. Oil is a by-product of the juice industry produced by pressing the peel.

1.4.4.1 Morphology and distribution

*C. sinensis*, a small flowering tree are widely grown in warm climates worldwide. It has originated in Southeast Asia (India and China) and cultivated widely in subtropical regions, cultivated in the hilly areas.

1.4.4.2 Taxonomy

Small tree with large spines, branchlets angular when young, armed with straight axillary spines. Petioles narrowly winged, leaflets entire or faintly crenate, acute, obtusely acute, narrowed at base. Flowers white. Fruit globose, greenish yellow to orange when ripe, pericarp thick or moderately thin, tightly adherent, pulp yellowish, sweet or acid with numerous seeds. The flowering is seen from January to June. The fruits are generally seen in November to December.

1.4.4.3 Chemical composition

Chemical constituents are similar to the Chapter I (1.4.3.3).

1.4.4.4 Therapeutics

Therapeutic uses are similar to the Chapter I (1.4.3.4).
1.4.5 Importance of *Heliotropium indicum* L.

*Heliotropium indicum* is commonly known as Hatisur belongs to the family Boraginaceae (Plate-1, Fig i), found everywhere previously but now a days the number is decreased. Pyrrolizidine alkaloids of *H. indicum* are considered for chemotaxonomic and also for pharmaceutical interest.

1.4.5.1 Morphology and Distribution

It is an erect or spreading type, coarse annual herb of 30-60 cm. found throughout the hotter parts of India. It is distributed in the tropics from Africa to Australia under moist conditions.

1.4.5.2 Taxonomy

*Heliotropium indicum* is an annual herb, mesophytic. Tap root system. Stem are aerial, erect, branched, herbaceous, solid, quadrangular, glabrous, hairy, green. Leaves are simple, opposite, sub- sessile, serrate, exstipulate, ovate, acute apex, glabrous, very few and small hairs present, dorsiventral, unicostate, reticulate venation, green. Inflorescence is scorpoid cyme. Flowers bracteate, ebracteolate, sessile, complete, zygomorphic, hermaphrodite, regular, imbricate aestivation, hypogynous. Calyx, sepals-4, gamosepalous, lanceolate, 4 sepals united by thin membrane, 4-sepals are on anterior end and posterior side fully covered by thin membrane, green. Corolla, petals-5, gamopetalous, tubular portion whitish in colour up to the throat and pubescent, exposed portion violet, rotate. Androecium, stamens-2, attached to throat, diyhrceous anther, filament short, epipetalous, dorsifixed, introse, white, polyantrous. Gynoecium, carpel-2, bicarpellary, superior ovary, bilocular, one ovule in each locule, style short, stigma capitate, syncarpous, axile placentation. Flowering and fruiting is seen in most part of the year.
1.4.5.3 Chemical Constituents

Pyrrolizidine alkaloids like indicine, indicinine, acetylindicine, indicine-N-oxide, heliotrine, lasiocarpine, echinatine, supinine and heleurine have been reported from aerial parts of the full-grown plants (Mattocks, 1967; Haque et al., 1976). The proportion of these alkaloids, however, varies in different organs and is dependent upon physiological conditions. Indicine is the major alkaloid in aerial parts concentrated mainly in the inflorescence. Acetylindicine is dominant in the roots (Wirz et al., 1993), whereas heliotrine is the major alkaloid in the seeds (Pandey et al., 1982).

1.4.5.4 Therapeutics

Pyrrolizidine alkaloids of *Heliotropium indicum* are of great pharmacological, biological and chemotaxonomic interest (Reddy et al., 2002; Reina et al., 1998; Catalfamo et al., 1982). These metabolites have been isolated from a wide variety of plants, especially from genera belonging to the *Boraginaceae* family (Birecka et al., 1980; Stemitz et al., 1993; Roeder and Bourauel, 1992). The genus *Heliotropium*, a well-known source of such alkaloids (Reina et al., 1998; Lakshmanan and Shanmugasundaram, 1995; Ravi et al., 1990) and other minor compounds, such as flavonoids and geranyl aromatic derivatives, is constituted of about 250 species represented by herbs and shrubs, distributed throughout the terrestrial globe (Villarreal et al., 2001).

*H. indicum* is traditionally used as diuretic, in ulcers, boils, wounds, insect bites and eye infections (Singh et al. (1983). Wound healing effects was reported by Reddy et al. (2002), family control and birth control (Tiwari et al., 1982), used in patients with advanced cancer (Ohnuma et al., 1982), antitumor activity of other species of *Heliotropium* have also been reported (Jain and Purohit, 1986), antimicrobial activity of
Heliotropium bursiferum (Marquina et al., 1989) and antituberculosis activity against Mycobacterium tuberculosis (Machan et al., 2006). It most frequently used against vomiting and also used against amenorrhoea, baby thinness, ocular infections and high blood pressure (Togola et al., 2005). Leaf powder is applied to dermatitis and especially to suppurating eczema and impetigo in children (Kerharo and Adams, 1974), also the decoction of leaf is used in poultices for herpes and rheumatism. The dried leaf powder is taken up by the nose as decongestant in colds and sinusitis (Burkill, 1985). Indicine-N-Oxide, Indicine oxide, Lasiocarpine have been shown to possess significant against cancerous tumours (Kugelman et al., 1976; Ghosh, 2003).

1.4.6 Importance of Syzygium cumini (L.) Skeels

Syzygium cumini, popularly known as Jammun tree is belongs to the family Myrtaceae (Plate-1, Fig j & k). It has great importance in the food as well as wood industry and is useful in social forestry programme (Anonymous, 1992).

1.4.6.1 Morphology & Distribution

S. cumini, a medium sized tree, usually forks into multiple trunks a short distance from the ground. The bark on the lower part of the tree is rough, cracked, flaking, and discolored and further up it is smooth and light-grey. It ranges up to 100 ft. (30 meter) in height in India.

It is native in India, Burma, Ceylon and the Andaman Islands. These trees grow well up to 6,000 ft above the sea level, develop most luxuriantly in regions of heavy rainfall, prosper on riverbanks and have been known to withstand prolonged flooding and are cultivated as shade trees along roadsides. Dry weather is desirable during flowering and fruiting periods. It is sensitive to frost.

In Southern Asia, the tree is venerated by Buddhists and commonly planted near Hindu temples because it is sacred to Lord Krishna. The leaves and fruits are employed in
worshipping the God Ganesha on the personification of “Parvana”, the apex of Hindu religion and philosophy.

1.4.6.2 Taxonomy

A medium sized to large tree, 15-30 m in height, bark rough, twigs 4-angular. Leaves broadly elliptic obovate, acuminate, secondary nerves irregular. Flowers white, sessile, in ternate trichotomous lateral panicles mostly from old leaf scars. Calyx tube long and broad, lobes 4, subacute to obtuse, deciduous. Petals suborbicular, pseudo-calyprate. Connective of anthers with a large gland. Berries globose or ovoid, glabrous, dark purple with pinkish juicy pulp, one-seeded. The flowering is seen from April to May. The fruits generally set in July to August.

1.4.6.3 Chemical constituents

Seeds contain glycoside jambolin, tannin, gallic acid, chlorophyll, fatty oil, starch, resin, sugar, and traces of oil. Flowers give acetyl oleanolic acid, triterpenoids, ellagic acid, isoquercitrin, quercetin, kaempferol and myricetin, chlorine, vitamin A, thiamine, riboflavin, niacin, ascorbic acid, choline and folic acid. The plant possesses acetyl oleanolic acid, triterpenoids, ellagic acid, isoquercitin, quercetin, kaempferol and myricetin in different concentrations (Rastogi and Mehrotra, 1990).

1.4.6.4 Therapeutics

The bark is astringent, sweet, sour, acrid, refrigerant, carminative, diuretic, digestive, anthelmintic, febrifuge, constipating, stomachic and antibacterial. It is useful in diabetes, leucorrhoea, stomachalgia, fever, gastropathy, strangury and dermatopathy. Decoction of bark is taken in cases of asthma and bronchitis and is gargled or used as mouth wash for the astringent effect on mouth ulcerations and also for stomatitis.

The leaves are antibacterial, used for strengthening the teeth and gums. The tender leaves are used for vomiting. Leaf juice is effective in the treatment of dysentery.
The fruits and seeds are sweet, acrid, sour, tonic and cooling, astringent, stomachic, carminative, antiscorbutic and diuretic. It is also used in diabetes, diarrhoea, pharyngitis, splenopathy, urethrorrhea and ringworm. The juice of the ripe fruit or decoction of the fruit is used in enlargement of the spleen, chronic diarrhea and promotes urine retention capacity. Alcoholic extract of seeds reduce blood sugar level and glycosuria in patients. It is popularly used in the treatment of insulin dependent diabetes mellitus (Schossler et al., 2004). Medicinally, the bark is stated to be astringent, digestive, anthelmintic, constipating, stomachic and antibacterial. It is useful in diabetes, leucorrhoea, fever, gastropathy, stomachalgia and dermatopathy. The fruits and seeds are sweet, acrid, sour, tonic and cooling and are used in diabetes, diarrhea, pharyngitis, splenopathy, urethrorrhea and ringworm (Warrier et al., 1994). The antidiabetic properties of the seeds have been clinically checked (Purohit and Daradka, 2000). Antioxidant activity was also reported by several authors (Ruan et al., 2008; Rastogi and Mehrotra, 1990; Takana et al., 1998; Banerjee et al., 2005; Benherlal et al., 2007).

1.4.7 Importance of \textit{Vitis vinifera} L.

\textit{V. vinifera} is a large woody climber commonly known as grape belongs to the family Vitaceae (Plate-1, Fig 1 & m), found in cold regions throughout globe except for Antarctica. It has many varieties i.e. seedless and with seeds, whitish green and also violet in colour.

1.4.7.1 Morphology & Distribution

Large woody climbers with bifid tendril, a native over a broad area extending from Spain in the west to the eastern Mediterranean, Southern and parts of Central Asia. It is a major fruit crop throughout the temperate regions of the world and cultivated in different parts of India specifically in Hyderabad.
1.4.7.2 Taxonomy

*V. vinifera* is a large woody, climber with bifid tendrils, mesophyte. Tap root system, branched. Stem is woody, branched, cylindrical, solid. Leaves are more or less deeply 5-lobed, margins unequally cut into acute teeth, stipules-2, deciduous. Inflorescence arranged in umbel-like cymes which form large pyramidal compound panicles. Flowers, bracts caducous, polygamo-deciduous in leaf-opposed thyrses, regular, bisexual, hypogynous. Calyx, sepals-5, copular, with 5 obscure teeth. Corolla, petals-5, coherent by their tips and deciduous as a calyptra, valvate aestivation. Androecium, stamens-5, filaments slender, long in male, shorter in bisexual flowers, 2-celled, introse. Gynoecium, carpels-2, bicarpellary, ovules 2 per cell, style very short, stigma obtuse. Fruit is berry, globose to oblong, yellow green to dark purple depending on the variety cultivated. Seed, 4 or less. Flowering is seen from December to February and fruiting is seen from February- May.

1.4.7.3 Chemical Constituents

Main constituent is grape-sugar, gum, tannin, tartaric, citric, racemic and malic acids, chlorides of potassium and sodium, sulphate of potash, tartarate of lime, alum, iron and some albumin.

1.4.7.4 Therapeutics

Leaves are being used to stop bleeding, pain and inflammation of hemorrhoids. Unripe grapes are used to treat sore throats and raisins are used for the consumption of tuberculosis, constipation and thirst. It is also used for the treatment of cancer, cholera, smallpox, nausea, skin, eye infections as well as for kidney and liver diseases. It is also used as an important drug in curing bronchitis, heart diseases, jaundice, enlarged spleen and liver (Joshi, 2000).
Modern research on ‘resveratrol’, a chemical found in grape skins, as a tool against cancer (Patnaik et al., 2004), cardiovascular diseases, metabolic disorders and aging has begun to back up some of the assertions of the folk healers. The compound ‘Resveratrol’ has shown to modulate the metabolism of lipids and to inhibit the oxidation of low-density lipoproteins and the aggregation of plantlets (Chan and Delucchi, 2000).

1.4.8 Importance of *Withania somnifera* (L.) Dunal

*Withania somnifera* (L.) Dunal is indigenous to India and commonly known as Indian Ginseng (Aswagandha) belongs to the family Solanaceae (Plate-1, Fig n &o).

1.4.8.1 Morphology and distribution

It is a middle-sized under shrub, branching and perennial and about 30 cm to 150 cm in height. It is found in drier regions of India and other subtropical countries. Due to its heavy pharmaceutical and therapeutic demand, it is grown in the medicinal plant gardens and also cultivated for commercial purposes.

1.4.8.2 Taxonomy

*Withania somnifera* is a much-branched perennial herb, partly shrubby at the base, all the plant parts emit characteristic odour of horse. Tap-root system. Stem erect, herbaceous, semi- woody, branched, cylindrical, glabrous, solid, covered densely with stellate hairs. Leaves alternate below opposite near inflorescence, exstipulate, petiolate, simple, entire, unciostate- reticulate venation, margin entire, apex acute or obtuse, ovate-oblong. Inflorescence, sub-sessile, axillary umbellate cyme (usually each possessing 5 flowers). Flowers, ebracteate, ebracteolate, pedicelate, complete, regular or actinomorphic, bisexual (hermaphrodite), hypogynous, pentamerous, lurid-yellow or greenish. Calyx, sepals-5, small, gamosepalous, five-toothed, campanulate, valvate aestivation persistent (thin pappery calyx encloses the fruit), tomentose, persistent, covering the fruit. Corolla petals -5, gamopetalous, campanulate, five-toothed, valvate
aestivation, lurid-yellow or greenish. Androecium, stamens-5, polyandrous, epipetalous, alternate to petals, filament short, anthers dithecous, introse. Gynoecium, carpels-2, syncarpous, ovary superior, bilocular, many ovules in each locule, axile placentation, style short, stigma bilobed, capitate. Fruit is berry, enclosed in enlarged. Seed, numerous, small, endospermic, smooth, curved embryo small and embedded in endosperm. Flowering & Fruiting is seen during October-May.

1.4.8.3 Chemical constituents

The chemical constituents are the important alkaloids, those include withanone, withaferin A, withanolides I, II, III, A, B, C, D, E, F, G, H, I, K, L, M, WS-I, P and S, withasomidienone, withanolide C and alkaloids viz. cuscohygrine, anahygrine, tropine, psudotropine, anaferine, isopellatierine and 3-tropyltiglate. Roots contain several pyrazole alkaloids viz. withasomnine and steroidal lactones, withaferin A and withanolides. They also contain starch, reducing sugars, hentriacontane, glycosides, dulcitol and withanol.

1.4.8.4 Values

*W. somnifera* is one of the valuable medicinal plants and its root is the source of drug ‘Aswagandha’, which is used in more than 100 herbal formulations under different trade names. Leaves have extensively demand for making herbal tea. The whole plant is used commercially as raw materials for different formulations and exhibits wide range of therapeutic actions.

1.4.8.5 Therapeutics

*W. somnifera* is one of the most extensively used medicinal plants in Ayurvedic and Unani medicines (Roja et al., 1991). In Ayurveda the roots are prescribed for gynaec disorders, bronchitis, arthritis, rheumatism, inflammation, fevers and skin diseases because they contain a number of alkaloids like somniferine, withasomnine, etc. reported

*W. somnifera* has been reported to have a wide range of biological activities. It possesses anti-inflammatory, anti-tumor activity (Chopra et al., 1958; Suffness and Douros, 1982; Al-Hindawi et al., 1992, 1989; Sethi et al., 1970), antimicrobial activity (Sethi et al., 1974; Dhar et al., 1968; Sastry and Singh, 1982), anti-stress, anti-arthritic (Kirtikar and Basu, 1969; Bector et al., 1968), antioxidant (Dhuley, 1998a; Bhattacharyya et al., 1997, 1987, 2001), cardio-protective agent (Dhuley, 2000; Mohanty et al., 2004), central nervous system (Singh et al., 1990; Mehta et al., 1991; Ahumada et al., 1991a, Karnick, 1992; Jayaram et al., 1993; Kulkarni et al., 1993; Kulkarni and Ninan, 1997; Elsakka et al., 1990), aphrodisiac (Nadkarni, 1954; Lohar et al., 1992; Boone, 1998), adaptogen (Malhotra et al., 1960, 1965), growth promoter activity (Budhiraja and Sudhir, 1987; Janaki, 1980; Venkataraghvan et al., 1980), inhibitor of drug-induced urotoxicity, immunomodulatory activity, cytokine production and stem cell proliferation (Davis and Kuttan, 2000, 1999; Dhuley, 1998b; Ziauddin et al., 1996; Agarwal et al., 1999; Diwanay et al., 2004), enhancer of white blood cell and platelet counts (Agarwal et al., 1999), used for cataract blindness (Thiagarajan et al., 2003), neuroprotective effects (Ahmad et al., 2005), diabetes mellitus (Muralik et al., 2005), hemopoetic and its dry root powder is used extensively as rejuvenating as well as tonic for general debility, having little or no associated toxicity. Indian Ginseng is useful as it shows relaxant and antispasmodic effects against several plasmogens on intestinal, uterine, bronchial, tracheal and blood vascular muscles (Bhakuni and Sudha, 1995). Kuboyama et al. (2006) reported the secondary metabolites like withanoside IV and its active metabolites, sominone, at enuate Aβ (25-35)-induced neurodegeneration of this species. Its roots are source of drug.
‘Aswagandha’, which is used in more than 100 herbal formulations under different trade names (Tripathi et al., 1996). Medicinal properties associated with this plant are largely attributed to the presence of withanolides and alkaloids (Power and Salway, 1911; Majumdar, 1955; Dhalla et al., 1961a & 1961b; Khanna, 1963; Sharma and Dardiya, 1992).

The leaves contain withanolides like Withaferin-A that exhibit anti-bacterial and anti-tumor properties (Devi et al., 1993; Kurup, 1956; Kupchan et al., 1965; Uma Devi et al., 1992 and 1993).

It has been used for 4000 years in traditional Indian medicine (Khanna et al., 2006). It is the drug of choice among Ayurvedic physicians in the treatment of rheumatic pains, inflammation of joints and certain paralytic conditions (Dutta, 1977). It is used in several Ayurvedic preparations along with other herbal drugs such as ‘Laksha Guggulu’, ‘Raktawardak’, ‘Abana’, ‘BR-16A’, etc. in the treatment of hypercholesterolaemia, mental disturbances, convulsions, etc (Panda, 1990; Poehlmann, 1993; Shah and Fatkar, 1993; Kulkarni and Verma, 1993). It is prescribed for all kinds of weakness and is supposed to promote strength and vigour (Kumar et al., 1980). There are a number of proprietary preparations of this drug used for all types of nervous disorders and as sedatives in the treatment of insanity and in hypertension (Singh and Kumar, 1988). It is used throughout India as a safe home remedy as a tonic in geriatrics, being efficacious in relieving hand and limb tremors of elderly people (Kuppurajan et al., 1980). It is an appropriate remedy in the treatment of asthma, leucoderma and bronchitis (Dey, 1980; Anabalagan and Sadiques, 1981; Ghosal et al., 1988). A decrease in blood glucose, cholesterol and triglycerides was also reported by Andallu and Radhika (2000).

Anticancer activity of this plant was also reported by several authors (Menon et al., 1997; Devi, 1996; Ahumada et al., 1991b; Devi et al., 1992, 1993; Sharad et al.,
1996; Khanna et al., 2006; Lavie et al., 1965; Shohat et al., 1970; Davis and Kuttan, 1998; Kuttan, 1996). Presence of alkaloids like withanolides (Nittala and Lavie, 1981; Ali et al., 1997) and withaferins (Devi et al., 2000) from Leaves, roots and stems of this plant. Root has chemotherapeutic efficacy against Forestomach and Skin-carcinogenesis (Padmavathi et al. 2005), angiogenesis (Mathur et al., 2006; Mohan et al., 2004), lung cancer (Senthilnathan et al., 2006) and also the plant is useful in Alzheimer’s disease (Bhattacharya et al., 2006).

1.5 Micropropagation studies of these species

Micropropagation of medicinal plants have been developed for shoot multiplication, organogenesis and rooting of individual shoots are continuously increasing. The present study was undertaken to standardize the novel micropropagation technique of eight elite medicinal plants viz. Aegle marmelos (L.) Corr., Catharanthus roseus (L.) G. Don., Citrus reticulata Blanco., Citrus sinensis (L.) Osbeck., Heliotropium indicum L., Syzygium cumini (L.) Skeels., Vitis vinifera L. and Withania somnifera (L.) Dunal. These species were selected basing upon the emerging demand of pharmaceutical industries and requirement for the conservation with their multiplication.

1.6 Biochemical studies of these valuable species

There is an increasing demand for pharmacologically active and novel photochemical from important medicinal plants. In this context alternative propagation methods like cell culture and tissue culture are becoming popular methods to produce secondary bioactive metabolites for new drug formulation and design (Williams and Thankamani, 2007). Micropropagation of elite plants is capable of biosynthesizing primary products like carbohydrates, fats, amino acids, proteins etc. to some extent like intact plants.
Increased demand for medicinal plants and dwindling forest resources forced us to look into biotechnology for alternative methods to derive active principles. Biotechnology comes into rescue in the field of propagation and secondary metabolic production through cell cultures. Application of cell culture methods assures the possibilities of continuous production of biochemical.

*Aegle marmelos, Catharanthus roseus, Citrus reticulata, Citrus sinensis, Heliotropium indicum, Syzygium cumini, Vitis vinifera* and *Withania somnifera* are well known for their multidimensional medicinal properties and specific in anticancer properties.

Therefore, objective of the present study was to develop efficient protocol for callus induction and obtain plants via shoot organogenesis. This callus mediated plant regeneration cannot only ensure availability of a rapid method for plant regeneration but can also provide an effective means for inducing somaclonal variation among regenerated plant populations. These medicinal plants can serve as ideal source of pharmaceutically important secondary metabolites.