Chapter 1

Introduction

1.1 Medicinal Plants

Higher Plants are one of the most important sources of phyto drugs. The application of plants as medicines date back to prehistoric period. Now-a-days the medicinal plants are extensively utilized throughout the world in two forms of medicine namely traditional and modern system. The traditional system of medicine drawn through local, folk tribal stream that paves the way to codified and organized medicine systems. The earliest known medical document is a 4000-year-old Sumerian clay tablet that recorded plant remedies for various illnesses. The ancient Egyptian Ebers papyrus from 3500 years ago lists hundreds of remedies. The Pun-tsao contains thousands of herbal cures attributed to Shennung, China’s legendary emperor who lived 4500 years ago. In India, the herbal medicine dates back several thousand years to the Rig-Veda, the collection of Hindu sacred verses. The Badianus manuscript is an illustrated document that reports the traditional medical knowledge of early Greeks and Romans. Western medicine can be traced back to the Greek physician Hippocrates, who believed that the disease had natural causes and used various herbal remedies in his treatments. Early Roman writings also influenced the development of western medicine, especially the works of Dioscorides, who compiled information on more than 600 species of plants with medicinal value in De Materia Medica. Many of the herbal remedies used by the Greeks and Romans were effective treatments that have been incorporated into modern medicine.

Ayurveda, Siddha, Tibetan, Chinese and Unani systems of medicine serve as an important source of health and livelihood for millions of Asian people. Ayurvedic medicine is widely practiced, especially in Bangladesh, India, Nepal, Pakistan and Sri Lanka. Unani medicine draws from the traditional systems of medicine of China, Egypt, India, Iraq, Persia and the Syrian Arab Republic and is also known as Arabic medicine. Asia has abundant medicinal and aromatic plant species due to influence of
tropical and sub-tropical climate conditions. The continent has well-documented traditional knowledge, the longstanding practice of traditional medicine and the potential for social and economic development of medicinal and aromatic plants in primary health care and industrial scale production. Over the centuries, the use of medicinal herbs has become an important part of daily life despite the progress in modern medical and pharmaceutical research. Approximately 3000 plant species are known to have medicinal properties in India (Prakash et al., 2010). It is estimated that 40% of the world populations depending directly on plant based medicine for their health care (WHO, 2003). In India, medicinal plants offer low price and dependable health care results. There are several attempts were made to explore indigenous knowledge on the use of common medicinal plants for the treatment of diseases related to various systems of human beings.

A significant percentage of medicinal plant material is used to make plant extracts. This is carried out either by the end product manufacturers or by extract companies. In addition to the market for medicinal plants, there is an expanding market in developed countries for botanical based products, such as health foods and supplements, herbal drinks and various health and personal care products. The market for herbal products throughout the world is currently worth around an estimated US$60 billion per year with a growth rate of 7 percent (Workshop, 2004). Rising incomes in Asia are likely to raise the standard of living of residents, increasing demand for additional medicinal and aromatic plants as the population suffers from the detrimental effects of ageing, weight gain, and other medical problems that frequently occur in relatively prosperous societies (Gross, 2001). The increase in demand for medicinal and aromatic plants will likely continue to threaten native species in some localities.

Interest in medicinal plants has been revived in recent times and various national and international organizations, including the Food and Agriculture Organization (FAO), the International Development Research Centre (IDRC), the United Nations Industrial Development Organization (UNIDO), the World Health Organization (WHO) and others have been addressing issues concerning medicinal and aromatic plants through support for research, networking and coordination. In almost every Asian country, there is a vast indigenous knowledge of the use of medicinal
plants. Nevertheless, the biodiversity of medicinal and aromatic plants is yet to be studied thoroughly in many countries. The commercial exploitation, unsustainable use, cultural changes and lack of institutional support threaten resources and local knowledge. A growing number of countries are developing national policies on traditional medicine that cover quality and safety. A few countries, including China, India and Sri Lanka have formulated legislation to conserve their natural resources of medicinal and aromatic plants. Many other countries of the region have ceased the practice of wild collection (Chapman and Chomchalow, 2005). Detailed information on the status of medicinal and aromatic plants is important for policy makers, the scientific community and user groups to be able to frame effective policies, formulate projects to advance research and development activities and establish environmentally sustainable and economically viable enterprises.

India is one among the 12 mega biodiversity countries of the globe. It is estimated that of 17,500 species of flowering plants, 140 genera and 5285 species are native to this country. The indigenous flora are concentrated mainly in three major hot-spot centers of flora; the Eastern Himalayas (3,500 species), the Western Ghats (1,600 species), and Andaman and Nicobar Islands (185 species). The Eastern Himalayas and the Western Ghats are identified as a global hot spot of biodiversity. In India species richness is complemented by an enormous genetic diversity found within individual species. The occurrence of several ecotypes, chemotypes and cytotypes even within a single plant species offers ample opportunities to systematically survey, study, characterize and evaluate the economic uses and potentials of the plant resources of India, particularly the medicinal plants. A number of organizations in the country are engaged in the survey, inventory and documentation of medicinal plants of India (Dahanukar, 2000).

Rapid industrialization has led to an uncontrolled increase in human interference to play a spoilssport, which has resulted in a considerable decline of the habitat. Also, several endemic plant species in peninsular India, highly appreciated for its medicinal value, is at threat in its own land. Delineated as one of the critically endangered species in the red list of the IUCN (2010) needs an urgent attention for conservation.
In terms of the number of the species individually targeted, the use of the plants as medicines represents by far the biggest human use of the natural world. Plants provide the predominant ingredients of medicines in most medical traditions. There is no reliable figure for the total number of medicinal plants on earth, and the number and percentages of countries and regions vary greatly (Schippmann et al., 2002). Estimates for the number of the species used medicinally include: 53,000 worldwide (Schippmann et al., 2002); 11250 in China (He and Gu, 1997). In India actually 4,60,000 medicinal plants were reported in various medicinal systems, among that 30,458 plants have been Institutionally registered and reported as medicinally useful plant that exploration to be continued by various research institutions, in order to obtain the more bioactive compound. In response to the overwhelming interest in alternative therapies, many of the prestigious allopathic medical institutions have also recognized their importance: an example is the National Institute of Health which created the Office of Alternative medicine in 1991 to provide the public with information on alternative treatments and to assess those therapies which have been proven successful (Kolata, 1996).

Metabolomics is the analysis of all metabolites in an organism, and such metabolic profiling aims at measuring a selected group of metabolites in an organism, whereas metabolic fingerprinting aims at measuring a fingerprint of metabolite(s) in an organism but without identifying all of the compounds present. In addition to this basic scientific approach, metabolomics have been applied to a wide array of other fields. For example, it is an important tool for the chemotaxonomy of botanicals or for studying the equivalence of genetically modified and wild-type organisms, especially in the case of plants. Metabolomics also represents a major diagnostic tool for example, in the analysis of urinary metabolites using nuclear magnetic resonance (NMR) spectrometry to diagnose disease. As bioactive phytochemical compounds are used directly as therapeutic agents and also used as starting material for the synthesis of drugs or as models for pharmacologically active compounds, they are very useful for drug development.

There are two main approaches to developing successful drugs from medicinal plants; the phytochemical approach and the phytotherapeutic approach. In the former
approach, the plant materials are subjected to chemical analysis and individual chemical compounds are isolated and characterized. Then the defined chemical entities are tested for their pharmacological activity and therapeutic value. The pharmacological properties are very often predicted based on their chemical structure. Then structure and activity relationships are studied. If it can control, prevent, and/or cure a disease detailed studies were carried out in experimental animals. If it successfully passes animal experiments, detailed clinical trials of chemical entity may be approved as a medicine for human use. In the phytotherapeutic approach, either uses the crude plant preparations (extracts, active fractions, or mixtures of them) or formulations used as drugs with modern standards of safety and efficacy. It is cost-effective and more relevant to the Indian conditions. Such standardized phytomedicines are increasingly accepted in Europe (Ramawat and Goyal, 2008).

1.2 Phytochemical Studies

Phytochemicals are the chemicals extracted from plants, classified as primary and secondary constituents, which depend on their role in plant metabolism. Primary constituents include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlorophylls that are essential for growth of metabolites of plants. Secondary constituents are the plant chemicals such as alkaloids (derived from amino acids), terpenes (a group of lipids) and phenolics (derived from aromatic aminoacids). Unlike primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism’s survivability or perhaps in no significant change at all. Secondary metabolites are often restricted to a narrow set of species within a phylogenetic group. The most common secondary metabolites are saponins, tannins, flavonoids, alkaloids and anthraquinones. Also few primary metabolites conjugates often used for curing various diseases that are cardiac glycosides and cyanogenic glycosides (Ramawat and Merillon, 2008).

Secondary metabolites occur in plants in a high structural diversity. A typical feature of secondary metabolites is their storage as complex in relatively high concentrations, sometimes in organs which do not produce them. Some secondary metabolites are stored as inactive “prodrugs” that are enzymatically activated in case of danger (wounding, infection). Biochemical and physiological features of secondary
metabolism are strongly correlated with its function. Secondary metabolites are not useless waste products, but an important means of plants for defense against herbivores, microbes (bacteria, fungi) and viruses. Some secondary metabolites also function as signal molecules to attract pollinating arthropods or seed dispersing animals. Land plants have evolved secondary metabolites with a wide repertoire of biochemical and pharmacological properties. More than 10,000 secondary metabolites have been identified by phytochemists, including many nitrogen-free (such as terpenes, saponins, polyketides, phenolics and polyacetylenes) and nitrogen containing compounds (such as alkaloids, amines, cyanogenic glycosides, non-protein aminoacids, glucosinolates, alkamides, peptides and lectins). All plants produce secondary metabolites and usually store several major compounds, usually from different structural classes and biochemical pathways, which are commonly accompanied by dozens of minor components. It is typical to find complex mixtures, which differ from organ to organ, sometimes between individual plants and regularly between species. Within a single plant species, 5000 to 20000 individual primary and secondary compounds may be produced, although most of them as tract amounts which usually are overlooked in a Phytochemical analysis.

Alkaloids are widely distributed in the plant kingdom (especially angiosperms) and represent the largest group of secondary metabolites that contain one or several nitrogen atoms either in a ring structure (true alkaloids) or in a side chain (pseudo alkaloids). Alkaloids may also be formed from acetate derived polyketides, where the amino nitrogen is introduced as in the hemlock alkaloids, coine. Depending on the ring structures, alkaloids are subdivided into pyrrolidine, piperidine, pyrrolizidine, quinolizidine, isoquinoline, protoberberine, aporphine, morphine, quinoline, acridine, indole monoterpen indole, diterpene or steroid alkaloids. Alkaloids are famous animal toxins and certainly serve mainly as defense chemicals against predators (herbivores, carnivores) and to a lesser degree against bacteria, fungi and viruses.

Non protein amino acids (NPAAs) are abundant in seeds, leaves and roots of legumes (Fabaceae) and in some monocots (Alliaceae, Iridaceae, Hyacinthaceae), but also occur in Cucurbitaceae, Euphorbiaceae, Resedaceae, Sapindaceae and Cycadaceae. They can be considered as structural analogues to one of the 20 protein amino acids.
NPAAs frequently block the uptake and transport of amino acids or disturb their biosynthetic feedback regulations.

Cyanogenic glycosides have been recorded from more than 2000 plant species; they are especially abundant in the Rosaceae, Fabaceae, Euphorbiaceae, Caprifoliaceae, Poaceae, Linaceae, Lamiaceae, Passifloraceae, Sapindaceae, Juncaginaceae and Ranunculaceae. Cyanogens are stored in the vacuole of seeds, leaves and roots as prefabricated allelochemicals ("prodrug" principle). Glucosinolates also function as prefabricated vacuolar defense compounds. They occur in seeds, leaves and roots in a phylogenetically related complex, the Brassicales, which comprises the Brassicaceae, Capparaceae, Tropaeolaceae, Resedaceae, Moringaceae and others. Terpenes have a basic C unit (isopentenyl pyrophosphate or dimethylallyl pyrophosphate) as a building block and can be subdivided into monoterpenes (C10) sesquieterpenes (C1), diterpenes (C20), triterpenes (C30), tetraterpenes (C40) and polyterpenes. Steroids are derived from triterpenes. Mono, sesqui, di and triterpenes occur in most plant families; they are usually highly hydrophobic substances and are stored in resin ducts, oil cells or glandular trichomes. Most of them rapidly interact with biomembranes and membrane proteins.

Saponins are the glycosides of triterpenes or steroids and include the group of cardiac glycosides and steroidal alkaloids. Steroid saponins are typical for monocots, especially for Dioscoreaceae, Melanthiaceae/Trilliaceae, Liliaceae, Agavaceae, Asparagaceae, Ruscaceae, Zingiberaceae, Alliaceae, Poaceae and Smilacaceae; they are less frequent in dicots (Fabaceae, Scrophulariaceae, Plantaginaceae, Solanaceae, Araliaceae). Some saponins have additional functional groups, such as cardiac glycosides (carrying 5 or 6 membered cardenolide or bufadienolide ring), which enable them to inhibit one of the most important molecular targets of animal cells, the Na\(^+\)-, K\(^+\)- ATPase. Na\(^+\)-, K\(^+\)- ATPase builds up Na\(^+\) and K\(^+\) gradients which are essential for transport activities of cells and neuronal signaling.

Flavonoids and phenylpropanoids (including coumarins, furanocoumarins, catechins and tannins are widespread in plants. They exhibit a wide range of biological activities. In several instances, they act as analogues of cellular signal compounds or
substrates. Afflicted mechanisms range from prostaglandin and leukotriene formation, enzyme inhibition estrogenic properties (courmains, isoflavones, stilbenes) to DNA alkylation (e.g. furanocoumarins). These molecules, usually have several phenolic hydroxyl groups in common, which can dissociate in negatively charged phenolate ions under physiological conditions. Phenolic hydroxyls groups form hydrogen and ionic bonds with proteins and peptides. The higher the number of hydroxyl groups, the stronger the astringent and denaturing effect. Tannins inhibit enzymatic activities very effectively; however, most digestive enzymes of herbivores have apparently adapted to tannins during evolution and are less sensitive than other enzymes. Polyphenols are present in most drugs used in phytotherapy and apparently are responsible for a wide array of pharmacological properties, including antioxidant, anti-inflammatory, sedating, wound-healing, antimicrobial and antiviral activities.

Polyketides include anthraquinones, hydro and naphthoquinones. Hydroquinones are typical for Ericaceae, naphthaquinones for Droseraceae, Iridaceae, Bignoniceae, Juglandaceae and Balsaminaceae. Anthraquinones are characteristic for Polygonaceae, Rhamnaceae, Fabaceae, Rubiaceae, Hypericaceae, Scrophulariaceae, Asphodelaceae and Liliaceae. Anthraquinones produce severe diarrhoea in vertebrates by interfering with intestinal Na$^+$, K$^+$-ATPase and adenylyl cyclase. Polyacetylenes are characteristic of Apiaceae, Araliaceae and Asteraceae. Thiophenes, which are sulphur-containing polyacetylenes occur in the Asteraceae genera *Dahlia*, *Eclipta*, *Flaveria*, *Porophyllum*, *Rudbeckia*, *Tagetes* and *Tessaria*. Because of the triple bonds these secondary metabolites are highly reactive (often activated by light) and can interact with biomembranes and proteins.

Many higher plants are major sources of useful secondary metabolites which are used in pharmaceutical, agrochemical, flavor and aroma industries. Plant cell and tissue cultures can be established routinely under sterile conditions from explants, such as plant leaves, stems, roots, meristems etc. for both the ways for multiplication and extraction of secondary metabolites.

In the phytotherapeutic approach, the extraction, fraction, isolation and separation of chemical entities from plant materials are guided by pharmacological
activity (bioactivity). In the activity guided stepwise isolation procedure, if activity is not detected in one step, the next step will not be carried out. This phytotherapeutic approach is employed to find novel compounds as medicines as it enables the development of crude plant materials as standardized medicines. Development of standardized phytomedicines following traditional leads is relatively inexpensive and less time consuming. Pharmacological evaluation is an essential component in the development of herbal drugs (phytomedicine). In traditional medicine, generally entire plants or plant parts including leaves, roots, flowers, seeds, bark and stem, fresh or dried, are used as crude homogenates, extracts, decoctions and tinctures. These herbal drugs are mixtures of numerous phytochemicals of which only a few may be involved in exhibiting the pharmacological property in a given herbal medicine and some of the phytochemical compound exhibit toxicity that needs to be eliminated from the phytodrug source (Mendonça-Filho, 2006).

Many pharmacological methods are available to evaluate most of the pharmacological properties in phytodrugs that demonstrating activity in an in-vivo bioassay is the first step in the verification (validation) of herbal drugs. Animal experimental models simulating human diseases are used, to a large extent, to determine efficacy. Efficacy and toxicity determinations of many potential herbal drugs consist of orally administering the crude drugs into experimental animals and watching changes in key physiological, biochemical and behavioral parameters (Salim et al., 2008). After establishing in-vitro effects, attempts are made to set up simple in-vitro assays to reflect in-vitro effects. These in-vitro assays can be used for setting up pharmacological standards for efficacy. Isolated organs such as smooth muscle, pieces of intestine, heart and lung may be used to test plant extracts for muscle contraction, heartbeat, secretion, and other functions. Unraveling the mechanism of action of herbal drugs could help to devise a simple test system to assess the efficacy (Mendonça-Filho, 2006).

Current research in drug discovery from medicinal plants involves a multifaceted approach combing botanical, phytochemical, biological and molecular techniques. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets, including cancer, HIV/AIDS, Alzheimer,
malaria and pain. Although drug discovery from medicinal plants continues to provide an important source of new drug leads, numerous challenges are encountered, including the procurement of plant materials, the selection and implementation of appropriate high-throughput screening bioassays and the scale up of active compounds. The first major step in the development of a phytomedicine is to prepare a therapeutically valuable extract or active fraction (Mendonça-Filho, 2006; Salim et al., 2008).

The process of medicine development has to begin with identification of promising plants for combating a particular disease. The plants may be selected based on ethnomedical leads. The identified plant has to be evaluated for its likely efficacy in suitable experimental animal models. To start with, the part of the plant used in ethnomedical practices need to be tested. The crude homogenate preparation used in ethnomedical practice may be evaluated in two or three doses, including one reasonably high dose. If there is any sign of bioactivity (therapeutic action) three different extracts may be tried [eg. N-hexane (non-polar), alcohol and water (polar)]. It is preferred to do each of these extractions separately from the original plant material (dry powder). Some investigators prefer a sequential extraction. These extractions may be done at room temperature because biological activity of some of the compounds could be destroyed by heat. The yield of the extract has to be determined in each case. One or two doses of each of these extracts may be tested for pharmacological activity. Dose selection may be guided by the activity in the original homogenate and the yield of each extract. Based on the bioactivity one or more of the three extracts may be selected for further studies. The appropriate extract (active extract) prepared from different parts of the plant has to be tested to determine the most suitable plant part (Mendonça-Filho, 2006; Salim et al., 2008).

After identification of the promising extract, it is subjected to detailed studies and may be fractionated by sequential solvent extraction, column chromatography, or other appropriate techniques to obtain an active fraction. In rare cases, the fraction or even the extract can develop toxic side effects. Therefore, a therapeutic dose of crude homogenate extract and active fraction may be compared for their possible toxicity in general toxicity evaluation in experimental animals. Development of toxicity indicates that the extract contains one or more compounds, which attenuates or abrogates the
toxicity of therapeutically valuable compounds present in the extract. The bioactivity screening of the plant part is done to evaluate the pharmaceutical importance of the medicinal plants. The presence of bioactive compounds in the various organic solvent extracts is determined by using different separation techniques, such as extraction of active principles isolation of bioactive compounds using techniques such as thin layer chromatography, column chromatography, HPLC, HPTLC, gas chromatography and mass spectroscopy. Prediction of the structure and chemical nature of the isolated compound is done using NMR techniques (Ganesan, 2002; Ju and Howard, 2003).

The elucidation of the chemical structure is critical to avoid the rediscovery of a chemical agent that is already known for its structure and chemical activity. Mass spectrometry is often used to determine structure of the individual compound based on their mass/charge ratio after ionization. Chemical compounds exist in nature as mixtures, so the combination of liquid chromatography and mass spectrometry (LC-MS) is often used to separate the individual chemicals. Databases of mass spectra of known compounds are available. NMR spectroscopy is used for determining chemical structures of natural products. NMR yields information about individual hydrogen and carbon atoms in the structure allowing detailed reconstruction of the molecules architecture (Awad et al., 2003).

1.3 Secondary Metabolites Production from Plants

The search for new plant-derived chemicals should thus be a priority in current and future efforts towards sustainable conservation and rational utilization of biodiversity (Philipson, 1990). Biotechnological approaches, specifically plant tissue culture play a vital role in the search for alternatives to produce the desirable medicinal compounds from plants (Ramachandra Rao and Ravishankar, 2002). Discoveries of cell cultures capable of producing specific medicinal compounds at a rate similar or superior to that of intact plants have accelerated in the last few years. New physiologically active substances of medicinal interest have been found by bioassay. It has been demonstrated that the biosynthetic activity of cultured cells can be enhanced by regulating environmental factors, as well as by artificial selection or the induction of variant clones. Some of the medicinal compounds localized in morphologically specialized tissues or organs of native plants have been produced in culture systems not
only by inducing specific organized cultures, but also by undifferentiated cell cultures. The possible use of plant cell cultures for the specific biotransformations of natural compounds by using callus, suspension culture and hairy root culture methods (Ramachandra Rao and Ravishankar, 2000). Due to these advances, research in the area of tissue culture technology for production of plant chemicals has bloomed beyond expectations.

The major advantages of a cell culture system over the conventional cultivation of whole plants are:

1. Useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions.
2. Cultured cells would be free of microbes and insects.
3. The cells of any plants, tropical or alpine, could easily be multiplied to yield their specific metabolites.
4. Automated control of cell growth and rational regulation of metabolite processes would reduce labor costs and improve productivity.
5. Organic substances are extractable from callus cultures.
6. Organogenesis can be obtained from desired explants for the identified compounds.

Pharmaceutical companies depend largely upon materials procured from naturally occurring stands that are being rapidly depleted. Plant tissue culture is an alternative method of commercial propagation as well as production of phytochemical compounds through various forms like callus, cell suspension culture and genetic manipulation. Experimental approaches used for propagation of medicinal plants through tissue culture can be divided into three based categories. The most common approach is to isolate organized meristems like shoot tips or axillary buds and induce them to grow into complete plants. This system of propagation is commonly referred to as micropropagation. In the second approach, adventitious shoots are initiated root, leaf and stem segments or on callus derived from those organs. The third system of propagation involves induction of somatic embryogenesis in cell and callus cultures.
This system is theoretically more efficient as large numbers of somatic embryos can be obtained once the whole process is standardized.

The induction of callus growth and subsequent differentiation and organogenesis is accomplished by the differential application of growth regulators and the control of conditions in the culture medium. With the stimulus of endogenous growth substances or by addition of exogenous growth regulators to the nutrient medium, cell division, cell growth and tissue differentiation are induced. Plant tissue culture is now a well-proven technology. Like many other technologies, it has gone through different stages of evolution; scientific curiosity, research tool, novel applications and mass exploitation. Initially, plant tissue culture was exploited as a research tool and focused on attempts to culture and study the development of small, isolated segments of plant tissues or isolated cells. Around the mid twentieth century, the notion that plants could be regenerated or multiplied from either callus or organ culture was widely accepted and practical application in the plant propagation industry ensued. Tissue culture and plant regeneration are an integral part of most plant transformation strategies, and can often prove to be the most challenging aspect of a plant transformation protocol. The key to success in integrating the plant tissue culture into plant transformation strategy is the realization that a quick and efficient regeneration system must be developed. However, this system must also allow high transformation efficiencies from whichever transformation technique is adopted. Not all regeneration protocols are compatible with all transformation techniques. Some crops may be amenable to a variety of regeneration and transformation strategies, others may currently only be amenable to one particular protocol. Advances are being made all the time, so it is impossible to say that a particular crop will never be regenerated by a particular protocol. However, some protocols, at least at the moment, are clearly more efficient than others. Regeneration from immature embryo-derived somatic embryos is, for example, the favoured method for regenerating monocot species.

The capacity of a plant cell, tissue, and organ cultures to produce and accumulate many of the same valuable chemical compounds as the parent plant in nature has been recognized almost since the inception of in vitro technology. The strong and growing demand in today’s marketplace for natural, renewable products has
refocused attention on *in vitro* plant materials as potential factories for secondary phytochemical products, and has paved the way for new research exploring secondary metabolites. The deliberate stimulation of defined chemical products within carefully regulated *in vitro* cultures provides an excellent forum for in-depth investigation of biochemical and metabolic pathways, under highly controlled micro environmental regimes. Plant-produced secondary compounds have been incorporated into a wide range of commercial and industrial applications because of much selective advantage order the *in vivo* sources. They are a) Production can be more reliable, simpler, and more predictable, b) Isolation of the phytochemical can be rapid and efficient, as compared to extraction from complex whole plants, c) Compounds produced *in vitro* can directly parallel compounds in the whole plant, d) Interfering compounds that occur in the field-grown plant can be avoided in cell cultures, e) Tissue and cell cultures can yield a source of defined standard phytochemicals in large volumes, f) Tissue and cell cultures are a potential model to test elicitation, g) Cell cultures can be radio labeled, such that the accumulated secondary products, when provided as feed to laboratory animals, can be traced metabolically.

Indeed, in plant cell culture the secondary metabolites can be produced and continuously the year-round, without any seasonal constraints. The production is also reliable and predictable. At least in some cases, the yield per gram fresh weight may exceed that which is found in nature. Furthermore, valuable product is found in a wild or scarce plant species, intensive cell culture is a practical alternative to wild collection of fruits or other plant materials. Extraction from the *in vitro* tissues is much simpler than extraction from organized, complex tissues of a plant. Plant tissue culture techniques offer tailor made chemical profile of a phytochemical product, by manipulation of the chemical or physical microenvironment, to produce a compound of potentially more value for human use.

Plants and/or plant cells *in vitro*, show physiological and morphological responses to microbial, physical or chemical factors which are known as ‘elicitors’. Elicitation is a process of inducing or enhancing the synthesis of secondary metabolites by the plants to ensure their survival, persistence and competitiveness (Namdeo, 2007). The production of secondary metabolites in callus, cell suspension and hairy roots.
Plants generally produce secondary metabolites in nature as a defense mechanism against pathogenic and insect attack. The study was applied in several abiotic elicitors to enhance growth and ginseng saponin biosynthesis in the hairy roots of *Panax ginseng* (Jeong et al., 2007). Generally, elicitor treatments were found to inhibit the growth of the hairy roots, although simultaneously enhancing ginseng saponin biosynthesis.

### 1.4 Silver nanoparticle Synthesis from Plants

Nanotechnology is a way for research and development in the field of herbal and medicinal plant biology. Nanoparticles can be easily synthesized using various methods by various approaches available for the synthesis of silver nanoparticles include chemical, electrochemical, radiation, photochemical methods and Langmuir-Blodgett and biological techniques (Sun et al., 2002). But most of the chemical methods used for the synthesis of nanoparticles involve the use of toxic, hazardous chemicals that create biological risks and sometime these chemical processes are not ecofriendly. This enhances the growing need to develop environmentally friendly processes through green synthesis and other biological approaches. Sometimes the synthesis of nanoparticles using various plant materials and their extracts can be beneficial over other biological synthesis processes which involve the very complex procedures of maintaining microbial cultures (Sastry et al., 2003). A lot of literature has been reported till to date on biological synthesis of silver nanoparticles using microorganisms including bacteria, fungi and plants; because of their antioxidant or reducing properties typically responsible for the reduction of metal compounds in their respective nanoparticles. Although, among the various biological methods of silver nanoparticle synthesis, microbe mediated synthesis is not very suitable for industrial feasibility because of requirements of highly aseptic conditions and their maintenance. So, the use of plant extracts for this purpose is potentially advantageous over microorganisms due to the ease of improvement, the less biohazard and elaborate process of maintaining cell cultures (Kalishwaralal et al., 2010). It is one of the best platforms for synthesis of nanoparticles as it is free from toxic chemicals as well as provides natural capping agents for the stabilization of silver nanoparticles. Now, plant mediated synthesis of metal nanoparticles is receiving lots of attention due to its
simplicity, speedy synthesis of nanoparticles of attractive and diverse morphologies and elimination of detailed maintenance of cell cultures and eco-friendliness.

The nanoparticles used so far, the metallic nanoparticles considered as the most promising as they contain remarkable antimicrobial properties due to their large surface area to volume ratio, which is of interest for researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains. In nanotechnology, the silver nanoparticles are the most promising one. It is observed that silver nanoparticles do not affect living cells, so not able to provoke microbial resistance. It is believed that silver nanoparticles can attach to the cell wall and disturb cell-wall permeability and cellular respiration (Singh et al., 2008). Green synthesis of nanoparticles has been an emerging research area now a day. The advancement of green synthesis over chemical and physical methods is: environment friendly, cost effective and easily scaled up for large scale synthesis of nanoparticles, furthermore, there is no need to use high temperature, pressure, energy and toxic chemicals. The use of plants for the production of silver nanoparticles has received lots of attention due to its rapid, eco-friendly, nonpathogenic, economical protocol and providing a single step technique for the green synthesis processes (Huang et al., 2007). The reduction and stabilization of silver ions by a combination of biomolecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpinoids and vitamins, which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures (Kulkarni and Muddapur, 2014). A large number of plants are already reported to facilitate silver nanoparticles synthesis.

Flavonone and terpenoid components of leaf broth are being predicted to stabilize the formation of nanoparticles in comparison to high molecular weight proteins of fungal biomass (Shankar et al., 2004). The polyol components and the water soluble heterocyclic components are mainly responsible for reduction of silver ions ($\text{Ag}^+$) as well as stabilization of nanoparticles. Synthesis of silver nanoparticles using plant extracts is beneficial as it is an economical, energy efficient, low cost and supplemented to that it protects human health and environment leading to lesser waste and safer products. Green synthesized silver nanoparticles have significant aspects of
nanotechnology through unmatched applications and synthesis of nanoparticles using plants can be beneficial over other biological entities which can overcome the time consuming process of employing microbes and maintaining their culture which can lose their potential during biosynthesis of nanoparticles.

1.5 Antibacterial Activity

Nanotechnology is reliable, non-toxic, clean and eco-friendly experimental protocols for the synthesis of metal nanoparticles of controlled size, shape and monodispersity, which is possible through ambient biological resources. The first report of bacteria synthesizing silver nanoparticles was back in 1984 when Haefeli reported Pseudomonas stutzeri AG259, a bacterial strain originally isolated from silver mine capable of synthesizing silver nanoparticles. In addition, the in-vitro production of secondary metabolic compounds the emerging trends in nanotechnology supported the phytodrug investigated research in the recent years. Plant mediated synthesis of metal nanoparticles is gaining importance owing to its simplicity, rapid rate of synthesis of nanoparticles of diverse morphologies and elimination of elaborate maintenance of cell cultures and ecofriendliness (Ghosh et al., 2012). The mechanism for synthesis of nanoparticles in principle remains same for both microorganisms and plants. Metal salts comprising of metal ion is first reduced to atoms by means of a reducing agent. The obtained atoms then nucleate in small clusters that grow into particles. Shanker (2004) has reported the presence of proteins and secondary metabolites in the water-soluble fractions of geranium leaves and postulated that terpenoids contributes to the reduction of silver ions and oxidized to carbonyl groups. Fourier transfer infrared spectroscopy (FTIR) analysis of the study suggested ester C=O group of chlorophyll acting as a reducing agent and a protein involved in the surface capping of gold nanoparticles synthesized using geranium leaf extract. There is growing interest in the synthesis of metal nanoparticles by ‘green’ methods, biomass or extracts of different plants have been tried with success as reducing agents (Singh et al., 2010).

The antibacterial activity exhibited by silver nanoparticles depends on AgNO₃ concentration. It is inversely proportional i.e. less metal concentration more is the activity and vice versa. This is because smaller particles have a larger surface area available for interaction and will give more bactericidal effect than the larger particles.
(Baker et al., 2005). Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. The cell membrane of microorganisms is negatively charged and silver nanoparticles are positively charged and when these positively charged silver nano particles accumulate on the negatively charged cell membrane, it brings about a substantial conformational change in the membrane and it ultimately loses permeability control which leads to cell death (Ramamurthy et al., 2013). Mubarak Ali et al. (2011) stated that, once silver nanoparticles enter the bacterial cell, they would interfere with the bacterial growth signaling pathway by modulating tyrosine phosphorylation of putative peptides substrates critical for cell viability and cell division. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Sondi et al., 2004). Zodrow et al. (2009) stated that silver nanoparticles preferable attack the respiratory chain, cell division finally leading to cell death. According to Amro et al. (2000), metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by progressive release of lipopolysaccharides and membrane proteins or perhaps DNA loses its replication ability and expression of ribosomal subunits proteins as well as some other cellular proteins and enzymes essential to ATP production becomes inactivated. The other mechanism proposed by Kim et al. (2007) is the formation of free radicals which subsequently induces membrane damage leading to efficient antimicrobial property of silver nanoparticles. The other mechanism proposed is involved of interaction of silver nanoparticles with biological macromolecules such as enzymes and DNA through an electro-release mechanism. The nanoparticles get attached to the cell membrane and penetrate inside the bacteria. The bacterial membrane contains sulfur containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. Their interaction may cause damage to DNA and proteins resulting in cell death. Ag+ binds to functional groups of proteins, resulting in protein denaturation. The silver nanoparticles show efficient antimicrobial property due to their extremely large surface area, which provides better contact with microorganisms. It is reasonable to state that the binding of the nanoparticles to the bacteria depends on the interaction of the surface area available. Smaller
particles having a larger surface area available for interaction will have a stronger bactericidal effect than larger particles (Guzman et al., 2012).

### 1.6 Natural sources of antioxidants

The adverse effects of oxidative stress on human health have become a serious issue. The World Health Organization (WHO) has estimated that 80% of the earth’s inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components (Craig, 1999). Medicinal plants have great antioxidant potential which is due to their contents of variable phyto constituents. A large number of experiments have been carried out concerning the antioxidant activity of several plant extracts and powders. The results of these experiments reveal that, the activity is due to several secondary metabolites especially, e.g., phenolic compounds (tannins, flavonoids, anthrocyanins, chalcones, xanthones, liganans, depsides, and depsidones), terpenes (sesquiterpens and diterpines), alkaloids, and organic sulfur compounds (Marzouk et al., 2006; Al-Jaber et al., 2011).

### 1.7 Cancer Studies

Cancer is a major public health burden in both developed and developing countries. It is an abnormal growth of cells in the body that can lead to death. Cancer cells usually invade and destroy normal cells. These cells are born due to imbalance in the body and by correcting this imbalance, the cancer may be treated. Billions of dollars have been spent on cancer research and yet we do not understand exactly what cancer is. Every year, millions of people are diagnosed with cancer, leading to death. According to the American Cancer Society, deaths arising from cancer constitute 2–3% of the annual deaths recorded worldwide. Thus, cancer kills about 3500 million people annually all over the world. Environmental factors which, from a scientist’s standpoint, include smoking, diet, and infectious diseases as well as chemicals and radiation in our homes and workplace along with trace levels of pollutants in food, drinking water and in the air. Other factors which are most likely to affect are tobacco use, unhealthy diet, not enough physical activity, however the degree of risk from pollutants depends on the concentration, intensity and exposure. The cancer risk becomes highly increased where workers are exposed to ionizing radiation, carcinomas chemicals, certain metals and some other specific substances even exposed at low levels.
Natural therapies, such as the use of the plants or plant derived natural products are being beneficial to combat cancer. The search for anti-cancer agents from plant sources started in the 1950s, when discovery and development of the vinca alkaloids (vinblastin and vincristine), and the isolation of the cytotoxic podophyllotoxins was carried out (Cragg and Newman, 2005). It is documented that medicinal herbs have the rich anticancer potential, and on the forefront whenever we talk about anticancer remedies, are a significant source of synthetic and/or herbal origin. Natural products discovered from medicinal plants have played an important role in the treatment of cancer. They have exhibited anticancer activity in animal models of leukemia, skin cancer and sarcomas. Through generating awareness regarding usage of herbs and exploring natural product properties, healthcare professionals, can play significant clinical roles as knowledge resources for the masses. From information of this review, health care professional can initiate discussion with colleagues to determine whether a patient may benefit from taking a specific herb or natural product.

Current developments in tissue culture, technology indicate that transcription factors are efficient new molecular tools for plant metabolic engineering to increase the production of valuable compounds (Gantet and Memelink, 2002). *In vitro* cell culture offers an intrinsic advantage for foreign protein synthesis in certain situations since they can be designed to produce therapeutic proteins, including monoclonal antibodies, antigenic proteins that act as immunogenes. The natural products for medicinal purposes have increased by metabolic engineering can alter the production of pharmaceuticals and help to design new therapies. At present, researchers aim to produce substances with antitumor, antiviral, hypoglycaemic, anti-inflammatory, antiparasite, antimicrobial, tranquilizer and immunomodulating activities through tissue culture technology. Exploration of the biosynthetic capabilities of various cell cultures has been carried out by a group of plant scientists and microbiologists in several countries during the last decade. In the last few years, promising findings have been reported for a variety of medicinally valuable substances, some of which may be produced on an industrial scale in the near future. In the light of the above, the present study focused on the following objectives.
1.8. Objectives

- To standardize the *in vitro* propagation of *Trichosanthes cucumerina* L. using shoot tip and nodal explants.
- To increase the compound cucurbitacin B through callus production.
- To characterize the phytochemical compounds through TLC, HPTLC, HPLC and NMR.
- To synthesize and characterize the silver nanoparticles using leaf and callus extracts of *Trichosanthes cucumerina* L.
- To find out the efficacy of silver nanoparticles against some human pathogenic bacteria, antioxidant activity and anti-cancer activity on ME 180 cervical cancer cell lines.