Chapter I
INTRODUCTION
Introduction

Over the centuries, societies around the world have developed their own traditions to make sense of medicinal plants and their uses. All of them are attempts to overcome illness and suffering with an aim to enhance the quality of life. The history of herbal medicines is as old as human civilization. The documents, many of which are of great antiquity, revealed that plants were used medicinally in China, India, Egypt and Greece long before the beginning of the Christian era. Today, herbal remedies are back into prominence. In Ayurveda (Traditional Indian Medicine) about 2,000 plant species are considered to have medicinal value, while the Chinese Pharmacopoeia lists over 5,700 traditional medicines, most of which are of plant origin.

The oriental system of medicine “Ayurveda” which is as old as 5000 years, had classified selected plants under “Medhya Rasayanas”. In Sanskrit, “Medhya” means intellect / cognition and “Rasayana” means “rejuvenation”. These are used both in herbal and conventional medicine and offer benefits that pharmaceutical drugs lack, helping to combat illness and support the body’s efforts to regain good health and intellect. Many medhya drugs have been claimed to improve mental ability (Sharma, 1992). Some of the drugs, which act on the nervous system, include *Bacopa monnieri*, *Withania somnifera*, *Celastrus panniculatus*, *Convolvulus pluricaulis*, *Nardostachys jatamansi*, *Acorus calamus* and *Centella asiatica* (Dash et al. 1996; Sivarajan et al. 1994; Rai et al. 2001). There still remains an urgent need to develop new clinical drugs and this can be exemplified by the numerous diseases, which result from the malfunction of the central nervous system (CNS), e.g. alzheimer’s and parkinson diseases, epilepsy, migraine, pain, schizophrenia, sleeping disorders. Natural products already have a proven track record for CNS activities, e.g. caffeine, codeine, morphine, nicotine, reserpine and it is possible that there are further such drugs still to be found from nature.

The term “herb” refers to a plant used for medicinal purposes. For many of the people of the world, herbal medicines are the only therapeutic agents available. In 1985, the World Health Organization estimated that about 80% of the world’s population relies on herbs for their primary health care needs. Nature has been a source of medicines for thousand of years and an impressive number of modern drugs have been isolated from natural resources based on their use in traditional medicine (Frombi, 2003).

*Bacopa monnieri* L. Wettst belonging to family Scrophulariaceae is locally known as Brahmi or Jalanimba (Chopra et al. 1956). The name Brahmi is derived from the word “Brahma”, the mythical “Creator” in the Hindu pantheon. Because the brain is the centre for creative activity, any compound that improves the brain health is called Brahmi. ‘Brahmi’ also means ‘bringing knowledge of the supreme reality’. It has long been used medicinally and as an aid to meditation. In India, *B. monnieri* is largely treasured as a revitalizing herb that strengthens nervous function and memory and used by Ayurvedic medical practitioners in India for almost 3000 years. *B. monnieri* has been mentioned in several ancient Ayurvedic treatises including the Charaka Samhita (6th century AD), in which it is recommended in formulations for the management of a range of mental conditions including anxiety, poor cognition and lack of concentration and the Bhavprakash Var-Prakarana (16th century A.D.).
Centella asiatica L. Urban, of the Apiaceae (Umbelliferae) family, is also known as Gotu kola, Indian Pennywort and Mandukaparni. C. asiatica has been used since ancient times as a medicinal herb. It has also been referred into the French pharmacopoeia in 1884, in the ancient traditional Chinese Shennong Herbal some 2,000 years ago and in Indian Ayurvedic medicine some 3,000 years ago. The literature reveal that C. asiatica has been used for wound healing, better circulation, memory enhancement, sedative, anti-stress, antianxiety, an aphrodisiac, adaptogen cancer, immune booster, respiratory ailments, treatment of skin disorders (such as psoriasis and eczema), periodontal disease, burn and scar treatment, revitalizing connective tissue, arthritis, treatment of liver and kidneys, detoxifying the body and high blood pressure etc. However, none of these claims have been evaluated by the FDA, but research has been carried out by various research institutes and universities, which concluded that more research is needed to validate this ancient herb.

Convolvulus pluricaulis Choisy of family Convolvulaceae is also known as Shankhpushpi is used in India for hundreds of years for nervous disorders such as stress, anxiety and insomnia. It produces a feeling of peace and calm, reduces stress, anxiety and mental fatigue. Shankhpushpi is a morning-glory like perennial herb that grows on the plains of India. It has been widely used in Ayurvedic medicine to treat the nervous disorders (Ganju et al. 2003). It is only recently that Shankhpushpi has been brought to American stores for medicinal use. Herbalists believe that Shankhpushpi calms the nerves by regulating the body’s production of the stress hormones, adrenaline and cortisol (Kumar, 2006). In Ayurvedic medicine, it is also believed that Shankhpushpi is an anti-aging remedy. Even though Ayurvedic practitioners have used Shankhpushpi for centuries, there is no hard scientific evidence as to the positive effects of this herb, beside few Indian studies performed in the 1970s and ‘80s and most of them were published locally (Shaughnessy, 2002; Husain et al. 2007). Today this herb is still a preferred method for reducing symptoms associated with anxiety, panic attacks, nervousness and insomnia.

Both wild and cultivated plants are used for drug formulation. For success in cultivation it is necessary to study the conditions under which the plants flourish in the wild state and reproduce these conditions or improve on them. The efficacy of medicinal herbs is affected by different environmental factors like temperature, rainfall and day length and soil characteristics. A plant may grow well in different situations, but fail to produce the same constituents. India is satisfactorily cultivating some wild medicinal herbs. Several research institutes have undertaken studies on the cultivation practices of medicinal plants, which were found suitable and remunerative for commercial cultivation.

The unscrupulous collection of medicinal plants from wild habitats by traders has threatened the very existence of valuable medicinal plant resources. Due to biopiracy and over exploitation, some of the medicinal plants are becoming rare. Reserves of medicinal plants are diminishing and are in danger of extinction due to unfriendly harvesting techniques, loss of growth habitat and their unmonitored trades. Field grown plant material has generally been used but the quality of these products may be affected by different environmental conditions that can alter the medicinal value of plants (Murch et al. 2002). Biotechnologists hope for a bypass to overcome this difficulty, by introducing plant tissue culture technique and further multiplication of important plants by micropropagation technique. The production of useful metabolites
from plant tissue culture has created a new methodology for their commercialization. The culture tissues of plant material can be successfully used to select elite germplasm and to produce an efficient secondary metabolite.

The term plant tissue culture broadly refers to the in vitro cultivation of all plant parts, whether a single cell, a tissue or an organ under aseptic conditions and nutritional components. Any part of a plant, (ex-plant) is used to initiate the culture and the mature cells of the explant revert to their meristematic state and forms undifferentiated callus tissue. Since the multicellular explant comprises cells of diverse type, the callus derived is heterogeneous. These cells are able to differentiate into a whole plant or a plant organ by redifferentiation. These two phenomena are inherent by the plant cell and giving rise to a whole plant is described as cellular totipotency. Since the plants cells are totipotent, all the necessary genetic and physiological potential for natural product formation should be present in an isolated cell (Zenk, 1978). According to this theory cultured cells obtained from any part of a plant might be expected to yield secondary metabolites similar to those of the plant grown in vivo.

For the regeneration of a whole plant from a cell or callus tissue, cytodifferentiation is not enough and there should be a differentiation leading to shoot bud or embryo formation. This may occur either through organogenesis or through somatic embryogenesis. In organogenesis a monopolar structure that has a connection with the preexisting vascular tissue within the callus, while in embryogenesis a bipolar structure with no vascular connection with the maternal callus tissue or an explant is formed.

In somatic embryogenesis the somatic embryos are regenerated from somatic cells/tissue in culture. Induction of somatic embryogenesis requires a single hormonal signal to induce a bipolar structure capable of forming a complete plant, while in organogenesis it requires two different hormonal signals to induce first a shoot organ then a root organ.

Micropropagation represents the optimum efficiency in terms of vegetative plant propagation and allows large scale production in a relatively shorter period of time under controlled conditions throughout the year in a relatively small space. The rate of micropropagation varies greatly from species to species, but it is often possible to produce several million plants in the period of a year starting with any explants.

In recent years, phytotherapy is rapidly evolving throughout the world. Phytochemicals are the naturally occurring biochemicals in the plant that gives plant their color, flavour, smell and texture. They may help to prevent diseases like cancer and heart diseases besides their role to inhibit the microorganisms causing many diseases in human beings.

The naturally occurring plant metabolites have been divided into two groups. Primary metabolites involved directly in growth and metabolism and secondary metabolites considered as end product/byproduct of primary metabolism and in general not involved in metabolic activity. Secondary metabolites are compounds biosynthetically derived from primary metabolites but more limited in occurrence in the plant kingdom and may be restricted to a particular taxonomic group. Secondary metabolites are mostly accumulated by plant cell in smaller quantities than primary metabolites.
These secondary metabolites are synthesized in specialized cells at particular developmental stages, making their extraction and purification difficult as compared to the primary products produced by the whole plant or organ. Secondary metabolites exert in general a profound physiological effect on the mammalian system and thus are known as the active principle of plant. The physiological effects of these active principles are used for curing ailments and therefore these are drug of plant origin or natural drugs.

Primary products are of prime importance and essentially required for growth of plants e.g. amino acids, ascorbic acid, carbohydrates, enzymes, lipids, nucleic acids and proteins etc. They are of universal occurrence in plants. Amino acids are the building blocks of proteins and many other secondary products. Chemically ascorbic acid is more related to the monosaccharides as it is a hexose derivative. It controls the cholesterol metabolism and helps in the absorption and utilization of iron.

Phytochemical screening of bioactive plants extracts has revealed the presence of alkaloids, tannins, flavonoids, sterols, terpenes, carbohydrates, lactones, proteins, amino acids, glycosides and saponins. These molecules have pharma and industrial values including aromas, dyes, gums, resins, pulp, fiber etc. with high bearing on health and commercial sectors. Most of the high value secondary metabolites are produced in scarce amounts by plant biosynthetic pathways, which are poorly understood in totality. Information is pouring inform the vast research efforts in laboratories across the globe, which is enriching our knowledge of plant secondary metabolites biosynthesis. All the secondary metabolite pathways, producing most of the natural products of use, originate as branch points from primary metabolism with the origin at different places of primary metabolic cycle.

One of the largest groups of chemical compounds produced by plants is alkaloid. Alkaloids are more or less toxic substances. They act primarily on the central nervous system (CNS). They have a basic character, containing heterocyclic nitrogen, and are synthesized in plants from amino acids or their immediate derivatives. In most cases they are of limited distribution in the plant kingdom. Alkaloids are very important in the medicinal world and are used as powerful drugs mainly due to their sedative properties and powerful effect on the nervous system. Alkaloids are produce in actively growing tissue and rarely occur in dead tissue. Researches on production of useful alkaloids by plant tissue culture have also been carried out for more than 25 years. However, industrial production has not yet succeeded because of low producing ability of cultured cells.

Flavonoids are another group of plant secondary metabolites which are present almost universally in higher plants and contribute to the flower and fruit colour. They impart mostly red, yellow, blue and violet colour to plant organs. Chemically they are phenolic compounds and most of them have flavone nucleus with two side aromatic rings. Flavones occur as glycosides in plants. These compounds appear to play vital role in defense against pathogens and predators and contribute to physiological functions (Brenda, 1998). The distribution of flavonoids in plant kingdom is more or less of taxonomic significance. Algae, fungi and bacteria lack any kind of flavonoids, whereas mosses have a few types of them, ferns and gymnosperms have many types of simple flavonoids whereas angiosperms have a whole range of flavonoids. Arun kumar et al. (1987) have screened 24 taxa of Cyperaceae for their flavonoid
composition in assessing the systematic position. Some (1964) have pointed out the biological activities of flavonoids like anticoagulant, estrogenic, antibacterial, molluscsidal, anthelmintic, sedative, analgesic and hypothermal effects.

Triterpenoids can be divided into at least four groups of compounds: True triterpenes, Steroids, Saponins and Cardiac glycosides (Harborne 1973). Many triterpenes are known in plants and new ones are regularly being discovered and characterized (Kulshreshtha et al. 1972). At one time sterols were mainly considered to be animal substances but such compounds have been detected in plant tissues, occurrence of animal estrogen, estrone, in date palm seed and pollen (Bennett and Heftmann, 1966), cholesterol occurs in some plants of Malvaceae (Chauhan, 1984). Steroids are the compounds known as terpenoids or isoprenoids. Terpenes are formed by the polymerization of isoprene units and steroids are triterpenes or triterpenoids. The term triterpenes refers to a group of natural products containing 30 carbon atoms which rederived from six isoprene (5 C) units. Most terpenes possess carbon content in multiples of 5 C. Steroidal sapogenins are of economic importance as main precursors of many medicinally useful steroidal hormones. The sterols are most often discussed steroids in the plant literature. They are crystalline steroids which contain an alcoholic group and may be either saturated or unsaturated. Steroids have at least two functions. As precursor in the formation of other steroids e.g. cholesterol and sitosterol are precursors in the formation of saponins. Depending on their origin, they are call zoosterol (from animals), phytosterols (from plants), mycosterols (from fungi) and marine sterols (from marine organisms e.g. sponges). Phytosterols have been isolated from large number of plant species. Saponins are glycosides of both triterpenes and sterols have been detected in over 70 families of plants (Basu and Rastogi, 1967).

The expanding universe of the chemistry of natural products is indicative of the organic chemist’s interest in the plant kingdom for finding new phyto-constituents of therapeutic value, precursors for the synthesis of complex chemical substances, or new sources of compounds of economic value. Wide phytochemical surveys of alkaloids, saponins, tannins, cardenolides etc., have been carried out in different countries. In India, Badhwar et al. (1944) surveyed 306 plants for vegetable tannin material, Chakrabarthy et al. (1961) examined 38 species for saponins, Bhatnagar et al. (1961) screened 175 plants belonging to families alleged to posses medicinal properties in the Ayurvedic and Unani systems of medicine. Out of estimated 250,000 different plant species only 15% have been subjected to phytochemical analysis. By the year 2000 about 100,000 different secondary metabolites were isolated from plants. The biosynthesis of secondary metabolites are in lesser amount compared to primary metabolites e.g. 1 g taxol is obtained from 7 kg dried bark of Taxus in the same way many other compounds are extremely valuable e.g. codeine from Papaver somniferum, vincristine from Catharanthus roseus, diosgenin from Discocrea deltoidea, ajmalicin from Catharanthus roseus but their low yields from plant tissues prevent their commercial application. Scientists are looking towards biotechnology to increase the production of require secondary metabolites through tissue culture. Looking to their importance in preparing drugs efforts have been made to increase their content in plant cells (Laxmi and Gupta 1983; Ehmke, 1995; Bose and Tripathi 1996; Alcatara et al. 1996; Hamud, 2004; Jelesko and Halzious 2005)
However, many reports have described that yields of desired products were very low or sometimes not detectable in dedifferentiated cells such as callus tissues or suspension cultured cells. In order to obtain products in concentrations high enough for commercial manufacturing, many efforts have been made to stimulate or restore biosynthetic activities of cultured cells. These factors include media components, phytohormones (growth regulators), pH, temperature, aeration, agitation, light, etc. There are many reports and patents concerning optimization of cultural conditions in order to improve growth rates of cells and/or higher yield of desirable products.

Secondary product involved in plant defense through cytotoxicity towards microbial pathogens could prove useful as antimicrobial medicines in humans, if not too toxic. The wide distribution of antibiotic principles has comprehensively been discussed (Nickell 1959). Important characteristic of chemical antimicrobial substances is their capability of inhibiting bacterial colonization. Plant products are very powerful antimicrobial agents (Cowan, 1999). Antimicrobial activity also had been shown in tissue culture produce metabolites (Nickell, 1958; Veliky, 1974). Many possible sources of antibiotics are being explored these days, yet a scientific study to determine the antimicrobial of broad spectrum is lacking.

Different approaches to drug discovery using higher plants can be distinguished: random selection followed by chemical screening; random selection followed by one or more biological assays; biological activity reports and ethnomedical use of plants (Eloff, 1998). The latter approach includes plants used in traditional medical systems; herbalism, folklore, and shamanism; and the use of databases. The targeted objective is isolation of new bioactive phytocompounds. When an active extract has been identified, the first task to be taken is the identification of the bioactive phytocompounds, and this can mean either a full identification of a bioactive phytocompounds after purification or partial identification to the level of a family of known compounds (Miles et al. 1998). The analysis of crude drugs for their pharmaceutical content is performed for several reasons, namely, for the estimation of total and individual ingredients, the determination of pharmacological potency and conformity to purity, study of contents as a function of plant growth conditions and time for harvesting or studies of the distribution of contents at various portion of plant. The classical chemical methods, besides the special conditions, demand larger amount of starting materials and are also time consuming. The development of modern methods such as chromatography and spectroscopy simplified investigation. The amount of samples for analysis is reduced, time of analysis is shortened, and even more information is obtained. The manipulation of facts is further improved by combining the methods with the computer and the other electronic devices. Of the many methods presently available Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) become widely adopted for the rapid and positive analysis of drugs and drug preparations. In addition to qualitative detection, TLC also provides semi quantitative information on the chief active constituent of a drug or drug preparations thus enabling an assessment for drug quality. HPTLC provides a chromatographic drug fingerprint. It is therefore suitable for monitoring the identity and purity of drug. With the aid of appropriate separation procedure HPTLC can be used to analyze drug combination and phytochemical preparation and also can be documented (Scott, 1984).
In the view of the forgoing it is evident that we need more studies on these plants with the following objectives:

1. To standardize protocols for the optimum production of callus.
2. To develop protocols for large scale regeneration of plantlets (micropropagation) using explants / callus.
3. To assess physiological status of metabolites and enzymes in *in vivo* and *in vitro* produced non-embryonic callus and embryonic callus.
4. To screen identity and quantify of alkaloids, flavonoids, steroids and saponins of *in vivo* and *in vitro* produced materials and their comparisons.
5. To screen the above secondary metabolites for their antimicrobial activity.
6. To enhance biomass of these plants and increase in amount of secondary metabolites under natural conditions.