Chapter I

INTRODUCTION

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INTRODUCTION

The use of plants as medicines goes back to early man. Certainly the great civilizations of the ancient Chinese, Indians and North Africans provided written evidence of man's ingenuity in utilizing plants for the treatment of a wide variety of diseases.

The earliest mention of the medicinal use of plants has been found in Rig-Veda, which was written between 4000 & 1600 B.C. Atharva Veda describes varied use of drugs while 'Ayurveda' which is considered as an Upaveda includes utilization of medicinal plants for restoring normal physical fitness. Materialization of 'Sushruta Samhita' and 'Charaka Samhita' (1000 B.C.) incorporates compressive chapters on the therapeutic use of various plant species. Ayurvedic drugs are used in crude forms like expressed juice, powder, decoction or infusion. Ancient healers, developed formulations based on medicinal herbs, were probably not aware about the chemical composition of the herbs. But the advancement they made despite non-availability of scientific procedures is astonishing. The key advantage of natural products over synthetic compound is their greater chemical diversity and intense biological activity as a result of natural selection. Almost one half of the chemical scaffolds from natural products cannot be reproduced by synthetic chemicals.

The association of plants and human beings is an age-old process. Also the relationship between man and environment in general has never been static and it is always under a flux of change. But this is not the case with tribal /aboriginal communities the world over. The life, culture and traditions of these communities have remained almost undisturbed and static for hundred of years and often it is said that they are the living archaeological museums of ancient traditions and cultural heritage of a nation.

The Indian subcontinent is inhabited by over 53 million tribal belonging to over 550 tribal communities that come under 227 linguistic groups (Rao, 1996). They utilize the resources without disturbing the delicate balance of the ecosystem.

Herbal remedies have attained much more popularity in the treatment of minor ailments, due to increasing awareness of personal health maintenance through natural products. Indeed, the market and public demand has been so great that there is a great extinction risk to many medicinal plants. The loss of genetic diversity demands immediate biotechnological interventions for conservation coupled with proficient genotypes for the production of desired metabolites. The total scenario demands sustainable system approach supported by economic viability. And in this direction innovative ways of value addition, quality control and market support are essential along with proficient agro technologies, superior genetic material and down stream processing.

Drugs are derived from a number of sources in a number of ways. As it is already mentioned that the earliest drugs were plant extracts followed by pure natural compounds of known structures. During the last century came synthetic drugs, which were based on the natural products. Now a days people are becoming conscious of the increased potency and harmful effects of synthetic drugs. Disillusioned with synthetic western medicine, more and more people are now realizing that natural medicine is
better and we are returning to the fold of traditional herbal system. The World Health Organization (WHO) also admitted that it will not be possible or even desirable to replace this herbal medicine with western techniques, which leads to a revival of interest in wild medicinal plants. This ‘green wave’ (Tyler, 1986) is likely to gain momentum in the years to come.

It has been reported that nearly 95% of the plant species used traditionally as ingredients in crude drugs are collected from forest and other natural resources. Besides these plants collected as minor forest product show as wide disparity in their values due to lack of information on their life cycle; maturity and regeneration times; all of which changes the quality and quantity of active chemical ingredients (Balick, 1994) and also there are fluctuation in the concentrations and quantities of secondary metabolites in field grown plants as the biosynthesis of secondary metabolites although controlled genetically, is affected strongly by environmental influence. Moreover bio-transformation also takes place once the crop is harvested.

In recent years, there has been a rapid growth in world demand of plant-based raw materials for the manufacture of drugs, pharmaceuticals, food flavours, fragrances, cosmetics and related products. Some of these are high-value chemicals and their demand individually, may command a market of over a billion US dollars annually. Amongst these the corticosteroids, VLB alkaloids, ginsengosides, taxol, artemisinin, lanatocides and other similar phytopharmaceuticals which find use in treatment of circulatory diseases, anti-infectants, antidepressants, sedatives, anti-ageing and endurance providing ones have generated fast growing global markets.

A large number of medicinal plants used in Indian System of Medicine (ISM) have registered fast growth of raw materials. Most of these have shown consistency in drug value on clinical testing and are increasingly utilized as essential of classical or patented drugs. Some if these formulations are manufactured by allopathic medicinal companies in India, raising the demand of raw materials manifold in recent years.

There is no doubt that plants play a dominant role in the introduction of new therapeutic agents, and also drugs from the higher plants continue to occupy an important position in modern medicine (Dev, 1997). Many compounds used in today’s medicine have a complex structure, and synthesizing these bioactive compounds chemically at a low price is not easy (Shimomura et al. 1997). With deforestation, medicinal wealth is rapidly lost, such that any valuable plants are threatened with extinction. Pharmaceutical companies depend largely upon material procured from naturally occurring stands that are being rapidly depleted. To overcome these limitations novel methods have to be adopted that would help enhance the biosynthesis of secondary products so that further loss of plants can be augmentable with the yield.

Natural products, mainly the plant-derived constituents, have long been sources of drugs; and a great part (30%-40%) of the pharmaceuticals available in modern medicine is directly or indirectly derived from natural sources. Plants have been offering valuable and safe natural sources of medicines and agents of therapeutic, industrial and environmental utilities across the varied cultures and civilizations.
Their large diversity in nature, permit the identification of lead molecules of great interest for the development of new therapeutic agents, as well as biochemical and molecular mechanism of action involved in most physiological and pathological processes. The use of traditional medicine and medicinal plants in most developing countries as a normative basis for the maintenance of good health has been widely accepted.

The subject of Phytochemistry or Plant chemistry is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function. Phytochemistry has an established role in practically all branches of plant sciences. The major contribution of phytochemical studies to plant physiology are undoubtedly in determining the chemical structures, biosynthetic origins and modes of action of natural growth hormones.

The actual credit goes to the plant secondary metabolites, perhaps synthesized and accumulated in various tissues for its own defence, making the plant species much more valuable for the biological activities they possesses with therapeutic and/or industrial applications including the aromas, flavours and fragrances.

With upto 50,000 genes estimated in plants, more than 200,000 phytocompounds have been anticipated (Picher sky and Gang, 2000) with already around 50,000 compounds elucidated in plants (De Luca and St. Pierre, 2000).

Plants as pointed out are known to produce numerous secondary metabolites belonging to various classes like alkaloids, terpenoids, phenylpropanoids and their combinations. These molecules singly or in combination have pharmacy and industrial values including aromas, dyes, gums, resins, pulp, fibre, etc. with high bearing on health and commercial sectors.

Furthermore, a growing world-wide interest in the use of phytopharmaceuticals as complementary or alternative medicine, either to prevent or to ameliorate many diseases, has been noted in recent years. It is believed that above 80% of world’s population use plants as their primary source of medicinal agents (Cordell, 1995; Borris, 1996). An increasing reliance on the use of medicinal plants in the industrialized societies has been related to the development of several drugs and chemotherapeutics from plant species as well as from traditionally used rural herbal preparation.

Chemical reactions taking place in plant cells, notably degradation of food substances which provide the energy required by plant cells and biosynthetic reactions leading to the formation of compounds needed by the plant cells. As a result of these metabolic reactions various products are formed, out of which some products are further needed in growth (e.g. amino acids, proteins, carbohydrates, lipids, vitamins, nucleotides etc.). The products that are synthesized by metabolic reactions and are necessary for the growth and development of cell are called primary metabolites. Besides these compounds, plants produce many other compounds, which are not required for normal growth and development by the metabolic pathways common to all plants are referred to as secondary metabolites, which are medicinally important (e.g. alkaloids,
flavonoids, steroids, tannins, lignin, phytoalexins antibiotics, coumarins, resins, cardiac glycosides etc). These active substances are present in storage organs of plants as roots, leaves, bark, seeds etc. Higher plants produce a great variety of secondary products which play a minor role in the basic life processes of the plant but often have and ecological role, such as attractant of pollinators and chemical defense against microorganisms, insects and higher predators. Some of the plants are rich in secondary metabolites which are potential source of drug and essential oils. Biosynthesis of metabolites although controlled genetically is affected strongly by environmental influence. As a result there are fluctuations in the concentration and quantities of secondary metabolites.

Alkaloids form a very heterogeneous group, the heterogeneity exhibited in all aspects at their structure and behaviour. In plants alkaloids may be systemic or restricted to specific organ like roots or rhizomes (*Aconite pecacuanha*) stem barks (Cinchona, Holorrhena), leaves (Tylophora, Adhatoda) fruits (pepper, cumin) or seeds (Areca, Strychnos). The role of alkaloids in plant is not yet understood. They are considered as non toxic excretory material, nitrogen reserves, natural defences or as growth regulators. The important use of alkaloids are as analgesics (codeine), central stimulants (Strychnine), local anaesthetics (cocaine), myotics (physostigmine), antispasmodics (atropine), vermifunges (pelletierine), aphradisias (yohimoinel), antihypertensives (reserpine), muscle paralysers (tubocurarine), cardi represents (quinine) or antileukemic (vincristine).

Flavonoids are members of a class of natural compounds with widespread occurrence in the plant kingdom. Flavonoids have different activities like anti-flammatory, anti-hepatotoxic, anti-tumoral, anti-microbial, anti-viral enzyme inhibiting, anti-oxidant. Bioflavonoids (flavonoids occurring naturally in plants) support microvascular abnormalities of *Diabetes mellitus* by improving the integrity of the blood vessels, decreasing leakiness and breakage of capillaries and improving circulation to the retina. Flavonoids inhibit aldose reductase, the enzyme involved in glycosylation, preventing sorbitol from entering the cell which causes complications in diabetes (Chaudhry, 1983).

In plants, steroidal content is divided into steroid saponins, which are very similar to triterpenoid saponins in the terpenoid group; or, they may be compounds which render them steroid alkaloids, from the alkaloid group (Harborne, 1980).

In our laboratory we have examined a number of medicinal plants in general and have looked into specific groups known to have properties to cure a particular disease. For example we have screened plants for anti-cancer activities and memory plus activities.

*Diabetes mellitus* is one of the oldest diseases known to mankind and yet with the tremendous scientific advances witnessed in this century, medical science cannot claim that it knows all that needs to be known about this disease. This is the main reason for the persistent interest all over the world to explore alternative remedies from the so called alternative system of medicine. The disease was well known to the ancient Indian medical experts. All the renewed classic texts of Ayurveda like Charaka Samhita (1000 B.C.), Sushruta Samhita (600 B.C.) and subsequent works refers to this disease under the term Madhumeha (literally meaning sugar in the urine). Clinically it is a condition characterized by increased blood glucose level.
Hyperglycaemia) due to insufficient or inefficient insulin. As a consequence, the blood glucose level is elevated which spills over into urine.

Scientific interests in alternative traditional system have mainly concentrated on the screening of plants for their blood sugar lowering effects. Plants have always been an exemplary source of diabetes drugs with about 800 plants possessing anti-diabetic potential. In the indigenous Indian system of medicine, good numbers of plants were mentioned for the cure of diabetes and some of them have been experimentally evaluated and their active principles were isolated (Grover et al. 2002). Thus, plant based system plays an essential role in health care.

The plant synthesizes a multitude of phytopharmaceuticals in varying quantities, of which a few, produced in extremely low quantities have industrial value. The use of biotechnology as a tool has shown great utility in producing these compounds in cell lines which these plants in fields take a long growing period (four to six years) to produce these chemicals and growing in field also needs fastidious demands on climatic conditions of growing locality.

This system provides the advantage of allowing biotransformation and bioconversion in cell cultures at normal temperatures which chemical produces seldom destroy in laboratories. Several useful techniques are invented to improve their synthesis, like modification in cultures media, growing conditions, illumination hours, stress and use of growth hormones.

Given the demands of the market for a continuous and uniform supply of raw materials and the increasing depletion of the forest resource base, expanding the number of medicinal plants in cultivation appears to be an important strategy for research and development.

Plant tissue culture is an alternative method of propagation (George and Sherrington, 1984) and is being used widely for the commercial propagation of a large number of plant species, including many medicinal plants (Rout et al. 2000). Tissue culture technique could play an important role in the production of active phytochemical substances i.e. secondary metabolites and can reduce the pressure on natural resources. The production of useful compounds by plant cell cultures has become increasingly significant in the field of biotechnology. There are two important problems that have to be overcome for in vitro production of useful compounds. These are the selection of specific cells that produce high amounts of the desired compounds and the development of an adequate culture medium for the production of such useful compounds. The successful selection of cells producing high amounts of secondary metabolites has been made possible because of the heterogeneity associated with cultured plant cells. It has therefore been possible to select cells and develop cell line with desirable characteristics. For example high vitamin producing cells (Matsumoto et al.1980; Yamada and Watanabe, 1980; Watanabe and Yamada, 1982) high alkaloid producing cells (Zenk et al. 1977; Ogino et al. 1978; Yamada and Hashimoto, 1982) have each been obtained in various plant species.

Callus cultures are large aggregates of undifferentiated plant cells usually grown on solidified nutrient media. The state of undifferentiated growth is maintained by the precarious phytohormone balance, mainly the auxin and cytokinins added to the
Callus consists of an amorphous mass of loosely arranged parenchymatous cells arising from the proliferating cells of the parent tissue from functional viewpoint. Callus tissue from different plant species may be different in structure and growth habit viz. white or coloured, soft or hard, friable or compact. The callus growth within a plant species may also depend on factors such as the original position of the explant within the plant and the growth conditions.

Callus culture can be extremely impotent in plant biotechnology. Callus tissue also has proved suitable material for secondary metabolise extraction. It has been found to be advantageous as it provides as continuous and reliable source of natural product year around without the destruction of the entire plant. High quality and desired compounds can be obtained through cell line selection and or addition of the precursor into the production medium. Callus culture can also be used to initiate cell suspension which is used in variety of ways in plant regeneration studies. Manipulation of the auxin to cytokinin ratio leads to the development of shoots, roots or somatic embryos from which whole plant can be produced.

Micropropagation allows the production of large numbers of plants from small pieces of the stock plant in relatively short periods of time. Depending on the species in question, the original tissue piece may be taken from shoot tip, leaf, and lateral bud, stem or root tissue. In most cases, the original plant is not destroyed in the process. Once the plant is placed in tissue culture, proliferation of lateral buds and adventitious shoots or the differentiation of shoots directly from callus, results in tremendous increases in the number of shoots available for rooting. Rooted "micro cuttings" or "plantlets" of many species have been established in production situations and have been successfully grown on either in containers or in field plantings.

In the present studies we have concentrated on plants known in the literature as well as herbals used by various Ayurveda practioners in the treatment of diabetes. We wanted to also include studies on understanding the mechanism of blood sugar regulation but in absence of required facilities on a rat model we concentrated our efforts on tissue culture studies in terms of physiological studies and phytochemical screening for metabolites with the following objectives of the following plants known for the treatment of diabetes.

**OBJECTIVES:**

1. To develop protocol for the establishment of explant for production of callus and plantlets of *Clerodendron phlomidis* L., *Bougainvillea spectabilis* rosea L., *Tabernaemontana coronaria* L. and *Enicostemma littorale* Blume

2. To optimize callus growth for the production of primary metabolites

3. To optimize callus growth for the production of secondary metabolites

4. To compare *in vivo* and *in vitro* produced phytoactive substances

and the results are presented in this thesis.