Chapter – I

GENERAL INTRODUCTION

Plants have been major source of medicine in all cultures from ancient times. In the traditional system, various indigenous plants are being used in the diagnosis, prevention and elimination of physical, mental or social imbalance (Manjunath 1990). In India, drugs of herbal origin have been used in traditional system of medicines such as Unani and Ayurveda since ancient times. The drugs are derived from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc., Some drugs are prepared from excretory plant product such as gum, resins and latex. Medicinal plants are a source of important therapeutic aid for alleviating human ailments. With increasing realization of the health hazards and toxicity associated with the indiscriminate use synthetic drugs and antibiotics, interest, in the use of plants and plant-based drugs revived throughout the world. However, a large number of medicinal plants remain to be investigated, for their possible pharmacological value. Most of the pharmaceutical industry is highly dependent on wild population for the supply of raw materials for extraction of medicinally important compounds.

Medicinal plants have been a part of modern life style of a man and these plants are a source of important therapeutic aid for alienating human ailments. Most of medicinal plants, even today, are collected from wild. The continued commercial exploitation of these plants has resulted in receding the population of many species in their natural habitat. Consequently, cultivation of these plants is urgently needed to ensure their availability to the industry as well as to people associated with
traditional system of medicine. In situ conservation of these sources alone cannot meet the ever increasing demand of pharmaceutical industry. It is, therefore, inevitable to develop cultural practices and propagate these plants in suitable agro climatic regions. Commercial cultivation will put a check on the continued exploitation from wild sources and serve as an effective means to conserve the rare floristic wealth and genetic biodiversity. It is necessary to initiate systematic cultivation of medicinal plants in order to conserve biodiversity and protect endangered species. The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health.

Ethno botanical, phytochemical, biochemical in vivo-physiological and clinical studies of the traditional medicinal plants are the areas of research that are essential to promote our efforts in search of new medicines. At the end of any ethno pharmacological study, we aim at either an authentication of a traditional drug or the discovery of a new drug. The traditional aspect of this research provides us with a reference and starting point of our studies without which we could be lost among thousands of plant species. A large number of traditional plants are subjected to tissue culture and pharmacological studies for their plant based drugs and conservation. Baphicacanthus cassia, Aucklandia lappa, Paevaria labata, Agrimonia pilasa, Scopoloa tangentica, Curcuma arometica, Mylabens phalarata, Hes pubescens, Unicaria rhynchophylla, Andrographis paniculata are among the Chinese species in traditional medicines subjected to pharmacological confirmation (Xiao, 1981).

India has one of the richest plant medical traditions in the world (Wakdikar, 2004). It is a tradition that is of remarkable contemporary relevance for ensuring health security to the teeming millions. There are
estimated to be around 25,000 effective plant-based formulations, used in folk medicine and known to rural communities in India. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications. It is estimated that there are over 7800 medicinal drug-manufacturing units in India, which consume about 2000 tones of herbs annually (Ramakrishnappa, 2002).

The health assembly of the WHO passed number of resolutions in response to a resurgence of interest in study and use of traditional medicine in the health care and in recognition of the importance of medicinal plants to the health systems of many developing countries. As a follow-up action, the health authorities and administrators in developing countries have decided to take traditional forms of medicine more seriously and to explore the possibility in primary health care. In this context the conservation and management of medicinal plant resource is very important.

The renewed interest in herbal medicine is likely to continue because of increasing population, better affordability and a paradigm shift in attitude towards alternative medicines. Fresh market demands have not only brought in new opportunities for the herbal industry, but are also posing threats to the phyto-resources, especially in the developing economics. As per estimates released by the International Union for Conservation of nature and Natural Resources (IUCN) 34,000 plant species are facing varying degrees of threat world over (Walter and Gillet, 1998 : Verma et al., 2002) one of the most important tasks for botanist is the conservation of genetic resources of medicinal plants, both in situ and ex situ. Conservationists have so far paid attention mainly to
species that provide food, forages, timber and industrial crops. Attention paid to medicinal plants is still minimum (Natesh, 2001). Therefore, an urgent need to conserve the genetic diversity of medicinal plant resources. Parallel with recent increasing interest in alternative medicine for the prevention and treatment of various illnesses, there is increasing concern about the safety of medicinal plants. The alarming situation has resulted in short supply, high prices and forced substitution and adulteration of crude drugs entering into formulations of many classical, patent or proprietary medicines (Singh et al., 2003). To develop a successful drug to the WHO standard, need for screening of its biological activities to test safety.

During last two decades, it has made massive investments on pharmacological, clinical and chemical researches all over the world in an effort to discover still more potent plant drugs. About 250,000 living plant species contain a much greater diversity of bioactive compounds than any chemical library made by humans but only few plant species have been systematically investigated for the presence of bioactive compounds (Wakdikar, 2004). However, a large number of medicinal plants still remain to be investigated for their possible pharmacological values (Tray and Agrawal, 2005). But systematic efforts were not made to test the efficacy and toxicity of folklore herbs on scientific basis or to standardize their evaluation procedure.

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Plant tissue culture is relatively recent specialization in the field of biotechnology and is being used globally for the ex-situ conservation of plants. The endeavor is to adopt the method to multiply the medicinal herbs and monitor their secondary metabolites. The application of plant cell, tissue and organ culture has proved its potential for the practical application in the improvement of important and threatened medicinal plants (Tiwari et al., 1998; Remashree et al., 1997). For the first time, Dawson (1942) showed the biosynthetic potentialities of plant cell cultures. Knowledge occurring from classical researches on these areas by Tulecks and Mickel (1960), Klien (1960) Staba (1963), Butcher (1977) and others has mostly provided the necessary background information in establishing tissue cultures of number of plants and their utilization for the product of biosynthesis subsequently. Recent year’s research on micro propagation of medicinal plants were studied by in vitro in Adathoda vasica (Gauri Abhyankar et al., 2007); Arundinaria callosa ( Sayanika Devi et al., 2009); Ocimum kilimandscharicum (Soumen Saha et al., 2010); Melissa officinalis L. ( Meftahizade et al., 2010); Bacopa monnieri (L.) Wettst. (Sudhir Sharma et al., 2010); Acacia auriculiformis (Girishankar 2011).

Free radicals contribute to more than one hundred disorders in humans including altherosclerosis, arthritis, ischemia, central nervous system injury, gastritis, cancer and AIDS(Kumpulainen and Salonen, 1999; Cook, 1996). Free radicals due to environmental pollutants,
radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induced abnormal proteins. Oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydroperoxides to non-radical forms and function as natural antioxidants in human body. Due to depletion of immune system, natural antioxidants as free radical scavengers may be necessary (Halliwell, 1994; Kumpulainen and Saloren, 1999; Younes, 1981). Currently available synthetic antioxidants like butylated hydroxyanisole (BHA), tertiary butylated hydroquinone and gallic acid ester, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, those synthetic antioxidants also show low solubility and moderate antioxidant activity (Barlow, 1990).

Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radicals, induced injury (Pourmorad et al., 2006) besides well known and traditionally used natural antioxidants from teas, wines, fruits, vegetables and spices. Some natural antioxidants (e.g., Rosemary and Sage) are already exploited commercially either as antioxidant additives or as biological members are lipids and proteins. Reactive oxygen species can easily initiate the lipids causing damage of the cell membrane constituent i.e., phospholipids and lipoproteins by propagating a reaction cycle (Raja Sudarajan et al., 2006). It has been mention that antioxidant activity of plants might be due to their phenolic compounds (Duh et al., 1999). Antioxidant activities were studied in *Enicostemma L. axillare* (Lam.)
Rayal. (Sharada L Deore et al., 2008); Tridax procumbens Linn. (Habila et al., 2010); Alae vera (Saritha et al., 2010); Selaginella willdenowii (Tsun-Thai Chai et al., 2012); Punica granatum (Ahmed E. Abdel Moneim 2012); Jatropha tanjorensis (Atansuyi et al., 2012).

Many medicinal plants are considered to be potential antimicrobial crude drug as well as a source for novel compounds with antibacterial activity. Microorganisms and insect pests cause massive damage to the crops, horticultural plants, animals as well as humans. In tropical countries like India the damage potential of microorganisms and insects is immense due to congenital atmospheric factors such as temperature and humidity contributing to the growth of microorganisms and insect pests. Although chemical measures are highly effective and have been in fact employed with tremendous success, there has been a renewed interest for botanicals possessing antimicrobial and antibiotic properties (Mankinde et al., 2007; Parekh and Chanda, 2008). In the past few decades, the search for new anti infection agents has occupied many research groups in the field of ethanopharmacology. The antibacterial activity of medicinal plants produced 115 articles from the period between 1966 and 1994. However, in the following decade between 1995 and 2004, this number more than doubled, to 307. In these studies one finds a wide range of criteria related to the discovery of antimicrobial compounds in plants.

Even though pharmacological industries have produced a number of new antibiotics in the last three decades resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Antibiotic resistance has become a global concern in
recent years. This problem is of great significance especially in developing countries. The screening of natural products has been the source for new potential drugs is still largely unexplored and only a small percentage of them has been subjected to phytochemical investigation and the fractions submitted to pharmacological screening. Such screening of various natural organic compounds and identifying active agents is the need of the hour as due to successful prediction of lead molecule and discovery will pay of later in drug development.

Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases. The plant extracts have been developed and proposed for use as antimicrobial substances. Many of the plant materials used in the traditional medicine are readily available in rural areas at relatively cheaper than modern medicine. Thus it is important to characterized different types of medicinal plants for their antioxidant and antimicrobial potential. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs.

Antimicrobial activities of many plants have been reported by the researchers and antimicrobial activities of medicinal plants can be attributed to be the secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids that are present in these plants. Medicinal plants represents a rich source of antimicrobial agents and plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plants parts is used for extracts as raw drugs and they possesses varied medicinal
properties. Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases (Stary and Hans, 1998). The plant extract have been developed and proposed for use as antimicrobial substances (Del Campo et al., 2000). Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases. Medicinal herbs practiced in traditional folk medicine in India were screened for the presence of antibacterial activity. Ghosh et al., (2007) studied antibacterial activity of *Terminalia bellerica, Terminalia chebula, Emblica officinalis, Punica granatum* and *Lawsonia inermis*.

Antimicrobial activities were studied in *Annona muricata* (Pathak et al., 2003); *Nigella sativa* Linn. (Salman et al., 2008); *Mangifera indica* Linn. (Doughri et al., 2008); *Spirulina platensis* (Vinay Kumar et al., 2011); *Adathoda vasica* (Josephin Sheeba et al., 2012).

The liver is the most important organ in the body. It plays a pivotal role in regulating various physiological processes. It is also involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles (Shanani, 1999; Subramonjam and Pushpangadan, 1999). It helps in the maintenance, performance and regulating homeostasis of the body. It involves almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. In addition it aids metabolism of carbohydrate, protein and fat, detoxification, secretion of bile and storage of vitamins (Ahsan et al., 2009). The role played by this organ is the removal of substances from the portal circulation makes it susceptible to first and persistent attack by liver dysfunction (Bodakh 2007). Liver diseases remain one of the major
threats to public. In spite of the tremendous advances in modern medicine, there is no effective drugs available that stimulates liver function, offer protective to the liver from damage or help to regenerate hepatic cells (Chattopadhyay, 2003). It is therefore necessary to search for alternative drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety.

Scientific studies available on medicinal plants indicate that promising phytochemicals can be developed for many health problems (Gupta. 1994). For example Vinca alkaloids (Vincristine and Vinblastin) derived from Catharanthus roseus, Vinca rosea, Lochnera rosea and Ammocallis rosea have been employed for their anti cancer properties. Modern pharmaceuticals still contain at least 25% of drugs derived from plants. Medicinal plants have various effects on living systems. Some are sedatives, analgesics, antipyretics, cardioprotective’s, antibacterial, antiviral and antiprotozoal (Olaleye etal., 2006). Liver protective plants contain a variety of chemical constituents like phenols, coumarins, monoterpenes, glycosides, alkaloids and xanthenes, glycosides, alkaloids and xanthenes (Bhawna and Kumar, 2009).

Liver diseases are the most serious aliments and are mainly caused by toxic chemicals (Excess consumption of alcohol, high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents, peroxidised oil, etc). Inspite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. In India, more than 87 medicinal plants are
used in different combinations in the preparation of 33 patented herbal formulations (Handa et al., 1988; Evans, 1996; Sharma et al., 1991).

Numerous medicinal plants and their formulations are used for liver disorders in ethno medical practices as well as in traditional systems of medicines in India (Subramonium et al., 1998). Hepatic fibrosis is a common condition in which major amounts of liver parenchyma cells are replaced by fibrous connective tissue. Liver diseases remain one of the serious health problems and it is well known that free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury (Babu et al., 2001).

In the absence of reliable liver protective drugs in allopathic medical practices, herbs play an important role in disorders. A number of plants have shown hepatoprotective activity (Scott Luper, 1998) Crassocephalum crepidoioides (Aniya et al., 2004), Epaltes divaricata (Hewawasm et al., 2004), and Aspalathus linearis (Ulican et al., 2003), Ipomoea obscura (L)) Arvind J. Mungole et al., 2010; Morus alba (Linn.) (Hogade et al., 2010) has been well established.

Inflammation is a local response of living mammalian tissue to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation (Mitchell and Cotran, 2000). Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and or the mediators that increase blood flow (Latenti et al., 1995). Several experimental models of paw edema
have been described. Carrageenan induced paw edema is widely used for determining the acute phase of inflammation. Histamine, hydroxytryptamine and bradkinin are the first detectable mediators in the early phase of carrageenan induced inflammation (Di and Willoughby, 1971). Whereas prostaglandern are detectable in the late phase of inflammation (Salvemini et al., 1996).

Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics eg. Opioids or non-narcotics eg. Salicylate and corticosteroids eg. Hydrocortisone. All these drugs possess well known side and toxic effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. Exploring the healing power of plants is an ancient concept. For centuries people have been trying to alleviate and treat disease with different plant extracts and formulations (Cowan, 1999). It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants represent still a large untapped source of structurally novel compounds that serve as lead for the development of novel drugs (Ahmed et al., 1992). Screening of the plants for their biological activity is done on the basis of either their chemotaxonomic investigation or ethno botanical knowledge for a particular disease. Identification of particular compound against a specific disease is a challenging long process. Importance of the plant lies in their biologically active principles. These chemicals exert a significant physiological effects on the mammalian system. Bagul et al., (2005) have reported the anti-inflammatory activity of two ayurvedic formulations containing ‘guggul’. Bhattacharjee et al., (2005) have reported anti-inflammatory potential of methanol extract of Stepenia
glabra of Menispermaceae family. The extract depicted anti-inflammatory activity at the dose of 150mg/kg body weight. Ammar et al., (1997) have revealed the anti-inflammatory activity of bioactive fractions isolated from seeds of Trigonella foenum gracium L., roots of Glycyrrhiza glabra L and fruits of Coriandrum sativum L. Anti-inflammatory activities were studied in Celosia argentea Linn. (Santosh et al., 2008); Crossopteryx febrifuga (Salawu et al., 2008). Eclipta prostrata L. (Arunachalam et al., 2009); Aconitum heterophyllum (Santhosh Verma et al., 2010); Wedelia trilobata (L.) Hitchc. (Govindappa et al., 2011).

Natural products are chemical compounds obtained from plants, animals and insects as well as a plethora of other living organisms. The study on natural products encompasses the investigation into their molecular structure, biogenesis and biological functions in the organism, therapeutic applications and other uses. Studies on natural products have become more and more important with the realization that plants provide a source of useful chemicals that may be used directly or as templates for the development of drugs useful for defense or protection against various diseases. Various workers have made investigations in many medicinal plants of the Asteraceae family with reference to the chemical constituents in the leaves, roots, flowers, seeds etc (Chopra et al., 1956) reported that trace elements, triterpenes, saponins, amino acids, citric acid and helianthic acid in Helianthus annuus (Dev et al., 1982). Emilia coccinea and Tridax procumbens are studied for its phytochemical constituents (Edeoga et al., 2005). Biswas et al., (2005) isolated purified and characterized four pure compounds from the root extract of Pulchea indica.
Phytochemicals are chemical compounds during the plant normal metabolic process. There chemicals are often referred to as “secondary metabolites” of which there are several classes including alkaloids, flavonoids, glycosides, gums, polysaccharides, phenols, tannins, tenpins and terpinoids (Harborne, 1977 ; Okwu, 2004). Phytochemicals are present in a variety of both human and animal diets. These include fruits, seeds, herbs and vegetables (Okwu, 2005). Phytochemical analysis were studied in *Secamone afzelii* (Herve Zabri et al., 2008); *Cassia alata Linn* (El- Mahamood et al.,2008); *Lawsonia inermis* ( Kawo et al., 2011); *Pharthenium hysterophosis* ( Bakhtiar Muhammad et al., 2012); *Sida acuta* and *Euphorbia hirta* (Ibrahim et al., 2012).

The present investigation was envisaged by taking into consideration the present void that exist in the type of research and also the results obtained could be beneficial to a large section of the ailing people.

*Flaveria trinervia* (*Synonym F. australasiaca*)

Plant belongs to the family Asteraceae commonly called yellowtops. It grows easily in many types of wet habitat, including saline and alkaline soil. It is native to parts of the American, including the southeastern and south western United states, most of Mexico, and parts of the Caribbean, but it is known in many other places as an introduced species and often a noxious weed, such as in Hawaii. In India plant is widely distributed in Chengalpattu, Coimbatore, Dharmapuri, Salem, Tiruchirapalli and Tirunelveli (T.N) plant is branched, errect, annual herb usually upto 75 cm tall, leaves opposite, yellowish - green, 1 - 7.5 cm long, lanceolate, elliptic or ovate, petiolaled or upper sessile 3- veined from the base margin more or less finally toothed capitula inflorescence
numerous in congested axillary or terminal heads, yellow with very short ray florets.

Tissue culture protocols have been developed for several plants but there are many other species, which are over exploited in pharmaceutical industries and need conservation. In the present work following objectives have been considered.

1) To establish a protocol on *in vitro* callogenesis on hormone free medium to carry out antioxidant and antimicrobial activities.

2) To carry out hepatoprotective and anti-inflammatory activities *in vivo* and *in vitro* extracts.

3) Phytochemical investigation has to be carried out to identify and characterize secondary metabolites both *in vivo* and *in vitro* extracts.