General Introduction & Objectives
CHAPTER 1

GENERAL INTRODUCTION AND OBJECTIVES

After a gap of several years, when man depended more on synthetic drugs for curing diseases and health care, great awareness has been brought and now plant based pharmaceuticals are making a significant impact on health care all over the world with Europe leading in the list. Growing interest in scientifically validated alternative and complementary therapies has led the scientific and medical community to full circle back to herbal medicine. Six of the top twenty prescriptions dispensed in 1996 were natural products and the clinical use of drugs such as artemisinin, etoposide, taxol, mevastatin (Keller-Justin, 1971), camphothecin (Wall, 1966) and topotecan (Endo, 1976) has once more focused attention on plants as sources of novel drug entities.

Plants represent a tremendous source of new lead compounds with potentially useful properties. Among the approximately 5,00,000 plant species around the globe, only a small percentage has been systematically investigated for the presence of bioactive compounds (Hostettmann et al, 1997). It has been estimated that more than two third of the world’s population relies on plant derived drugs. Now, in spite of the recent advances in chemotherapy, plant products still remain an integral part of modern medicine and they represent 50% of all drugs in clinical use (Balandrin, 1993). Substances derived from higher plants comprise 25% of the prescribed medicines in USA (Akerale, 1992). Some 7000 medicinal compounds used in the Western Pharmacopoeia are derived from plants (Coe and Anderson, 1996). In fact 77% of the 119 biologically active plant-derived pharmaceuticals used world-wide were discovered as a result of researches on plants used in traditional medicine (Arvigo, 1993; Farnsworth, 1995 and Farnsworth, 1988).

There are several records in Chinese, Sanskrit, Greek and Urdu on natural therapeutics which clearly illustrate their prehistoric origin. As science developed man’s
quest for knowledge urged him to discover the facts behind the utility of particular plants for curing a particular disease (Atta-ur-Rahman et al, 2001). The useful medicinal effects of plant materials result from the combination of secondary metabolites in them.

The first pure chemical substance to be isolated from a plant was benzoic acid in 1560. The German chemist, Karl W. Scheel (1742-86) extracted some simple compounds like glycerol, oxalic acid, lactic acid, tartaric acid and citric acid from plant and animal sources. However, the search for useful drugs of known structures from plant kingdom did not really begin until 1806, when F.W. Serturner (1783-1841) separated morphine (Serturner, 1805 and Goodman, 1958) from the dried leaves of Papaver somniferum. This opened a new door for search of useful drugs from plant kingdom. The isolation, physiological and pharmacological role of strychnine, papaverine, quinine (Jaramillo-Arango, 1949), nicotine and cocaine isolated by Pelletier and Caventor led to more concentrated attention towards the chemical investigation of medicinal plants. Cocaine was the first alkaloid to have its structure established (Giesecce, 1827) and later to be synthesised (Laderburg, 1886).

Ethnopharmacological screening of plants is one of the best ways to select a plant for pharmacological and phytochemical study (Cox and Balick, 1994). For instance, before the discovery of cardiac glycosides from Digitalis purpurea, their leaves were known to be used for the treatment of diopsy by folk healers. Based on this indigenous knowledge, William Withering in 1775 discovered the effectiveness of Digitalis leaves in treating cardiac disorders (Withering, 1775). Later in the twentieth century, the isolation and structural elucidation of chemical constituents from Digitalis purpurea resulted in the discovery of more than 30 cardiac glycosides including digoxin and digitoxin. Every year over 1500 Kg of pure digoxin and 200 Kg of digitoxin are prescribed to thousands of patients with cardiac problem (Cox, 1995).

Another significant discovery was that of Vinca alkaloids, vincristine and vinblastine from Catharanthus roseus (Apocynaceae). This plant was used by physicians for the treatment of hemorrhage, scurvy, toothache, chronic wounds and diabetic ulcers,
because of its hypoglycemic activity but, further studies on the plant showed that (Gorman et al., 1971) the leaves contain components with high proportion of activity against mouse leukemia. The active components vincristine and vinblastine are now used the world over for the treatment of pediatric leukemia and Hodgkin’s disease (Balick and Cox, 1996).

The discovery of taxol (Wani, 1971), a diterpenoid by National Cancer Institute from the leaves of *Taxus baccatta* (Asima Chatterjee, 2003) has become important for cancer therapy in recent years due to its high therapeutic profile. Modern bioassay-guided fractionation of *Homolanthus rutans* (*Euphorbiaceae*), a tree used by Samoan healers to treat yellow fever (a viral disease) resulted in the isolation of prostatin (12-deoxyphorbol-13-acetate), an active compound which stopped cells from becoming infected with HIV-virus. Currently National Cancer Institute (NCI) is considering prostatin as a potential candidate for drug development.

The antimalarial drug artemisinin (Klayman, 1985) obtained from the Chinese herb, *Artemisia annua* is another example (Zhang-Xing, W., et al., 1992). Natural penicillin discovered by Sir Alexander Fleming in 1929 and its chemical modifications such as ampicillin and oxacillin have been a great power for medicine for the last 60 years in fighting against bacterial disease.

The world is now witnessing scientific and commercial interests in medicinal plants mainly due to their immense economic potential and due to the widespread cultural acceptability of plant based products because of the fewer side effects and increased efficiency due to synergistic action.

Some of the drugs which are progressing to be commercialized include bilobalide (terpene lactone) isolated from *Gingko biloka* extract, for its neuroprotective property. Others include podophyllotoxin, a lead compound from *Podophyllum peltatum* and its derivatives etoposide and teniposide used as antineoplastic agents, the antiviral agent,
castanospermine, a tetrahydroxy indolizidine from the Australian tree *Castanospermine australe* and its O-acetyl derivatives as inhibitors for replication of HIV.

Several studies have pointed to allicin as the primary constituent responsible for antimicrobial, hypolipidemic, antithrombotic, antioxidant and hypoglycemic effects of garlic (*Allium sativum*). It also contributes partly to garlic’s anticancer action. Bergenin and neo bergenin (isocoumarin group) which were isolated from leaves and roots of *Fluggeo micararpa* (*Euphorbiaceae*) demonstrated significant protection against aspirin induced peptic ulcer and pylorus-ligation in chemical models.


*Eichinacea purpurea* is one of the top selling herbs in US used for a variety of purposes like treating immunostimulating action and for treating acute bronchitis in children who recovered faster than those treated with antibiotics (Baetgen, D., 1988). Three major categories of chemical compounds appear to contribute to the immunostatic action, namely, arabinogalactan (Dorsch, W., *et al*, 1994), cichoric acid and alkamides.

These are just a few examples from the vast treasures of herbal drugs (Standin, O.D., 1963; Ladmier, D., 1984).
1.2 Role of flavonoids

Science has shown that humans can live at least 150 years. But why are many dying at the age of 60, 70 or 80 when they can live twice as long? Though it sounds impossible, the science of nutrition and immunology can make it possible.

The high intake of red wine by French might explain why they suffer less from coronary heart disease, though they take cholesterol rich food. This might be due to the presence of a high content of flavonoids in red wine. Epidemiological studies have illustrated that heart diseases are inversely related to flavonoid intake. Flavonoids prevent the oxidation of low density lipoprotein thereby reducing the risk for the development of atherosclerosis.

Flavonoids whose presence accounts for the wide range of colours and shades in flowers have antioxidant activity and they are becoming very popular these days because they have many health promoting effects. Besides their antioxidant activity, some of the other activities attributed to flavonoids include antiallergic, anticancer, anti-inflammatory and antiviral. The flavonoid quercetin is known for its ability to relieve hay fever, eczema, sinusitis and asthma. Though there are many herbal anti-inflammatory drugs, the most potent one in chamomile \textit{(Matricaria recutita)} is the flavonoid apigenin which is more active than indomethacin. Gossypin, a bioflavonoid obtained from yellow petals of \textit{Hibiscus vitifolius} reduces rat paw edema and chronic arthritis in rats (Parnar, N.S., 1978).

1.3 Flavonoids as antioxidants

Antioxidants are vital in combating the free radicals that are constantly forming in our bodies due to oxidation. Free radicals damage the cells in our bodies and an imbalance of free radicals causes oxidative stress which can cause grave disturbances in cell metabolism. By curbing the activity of free radicals in our body, we can slow down the processes that cause disease and ageing and live a longer, healthier life. Many
conditions that have been linked to oxidative stress include alzheimers, cancer, cardiovascular disease, cataract, Parkinson's disease etc.

Antioxidants play an important role to avoid oxidative stress which results in a number of reactive oxygen species (Vaya and Aviram, 2001). Diets rich in fruits, vegetables, legumes and grains reduce risks of cancer, heart diseases and other illness. Vitamin E (phenolic benzochroman derivatives) (Packer and Cadenas, 2002), ascorbic acid (Feri et al, 1989; Shahidi, 1997), carotenoids (polyisoprenoid C₄₀ carbon chain), β-carotene (Polyakov et al, 2001) are all effective oxygen quenchers (Percival, 1998). Phenolic phytochemicals with one or more hydroxyl group such as caffeic acid, catechin, ellagic acid have important antioxidant property (Wiseman et al, 1997).

Flavonoids are some of the most powerful and effective antioxidant compounds available. Many biological effects countering inflammatory, bacterial, viral, microbial, hormonal, carcinogenic, neoplastic and allergic disorders (Middleton, 1996) have been reported for flavonoids in both the in vivo and in vitro systems. Flavonoids exert their antioxidant effects by neutralizing all types of oxidizing radicals (Bors et al, 1998) including the superoxide (Robak et al, 1998) peroxyl and hydroxyl radicals (Hussain et al, 1987) and by chelation. A chelator binds to metal ions in our bodies to prevent them from being available for oxidation. Flavonoids can also act as powerful chain breaking antioxidants due to the electron-donating capacity of their phenolic groups. Recent works have highlighted the role of polyphenolic compounds such as flavanols (Hertog et al, 1993), anthraquinones (Salah et al, 1995), xanthones (Yen et al, 2000) and proanthocyanidines (Minomi et al, 1994) in quenching these free radicals.

1.4 A brief introduction on essential oils

Essential oils, ethereal oils or volatile oils are the pleasant smelling, volatile constituents of a plant which can be obtained by steam distillation. They are the oily material, insoluble in water but soluble in alcohol and ether and can be distinguished
from the fixed oils by their volatility (Guenther, E., 1948 and British Pharmacopoeia 1998).

About 200 essential oils are known commercially among which 25 are produced on a large scale such as citrus, peppermint, spearmint, orange, lemon and some spice oils such as clove and nutmeg. The essential oils are widely used in different industries, for example, as ingredients in many pharmaceutical products, antiseptics and flavouring agents, as analgesics and as antimicrobial components in mouth washes or gargles. They are also important ingredients in fragrances, perfumes and different foodstuffs (Husain, A., et al, 1988 and Pushpangadan, P., 1993).

The recent methods for the extraction of essential oils are steam distillation and hydrodistillation with Clevenger apparatus, extraction with solvents (fewer applications) or extraction using super critical fluid extraction. Essential oils comprise mostly of volatile terpenes and esters with phenols, alkanes, aldehydes etc. The sulphur and nitrogen containing compounds which are steam distilled are also considered as components of essential oils (Jarvis, A.P., et al, 1997).

The method adopted for the identification of components of essential oils is gas chromatography equipped with FID and then its combination with GC/MS in which even a trace amount of a compound can be identified. The GC/MS data are then compared with literature values (Vilegas et al, 1994 and Masada, 1976). The other methods include coupling of GC with IR and coinjection with authentic samples.

Hence, natural product screening and isolation can lead to novel molecular structures not foreseen through other techniques. The unique peroxy-bond in artemisinin, the ring system of taxol and the stereochemistry of erythromycin-A are synthetically challenging and are unlikely to have been discovered through a synthetic approach. Thus, it is clear that natural products continue to represent a rich and largely untapped reservoir (resource) for the discovery of drugs with potential applications for treatment of contemporary diseases. Current research in natural products indicates that the future
holds great promise through the use of recently developed technologies (Griffo, 1997) which includes correlation of structure and activity on selected secondary metabolites. Thus the phytochemical study on medicinal plants can serve the dual purpose of bringing up new therapeutic agents and providing useful leads for chemotherapeutic studies directed towards the synthesis of drugs modeled on the chemical structure and physiological activity through functional variations in active constituents of plant materials.

*Acalypha fruticosa* belongs to the *Euphorbiaceae* family and is of wide therapeutic use. The plant *Aristolochia tagala* belongs to *Aristolochiaceae* and is reputed to possess profound medicinal properties. Taking into account the facts stated above, the work undertaken for the present research entails the isolation and characterization of the chemical constituents of two medicinal plants—*Acalypha fruticosa* and *Aristolochia tagala*.

### 1.5 Objectives

Phytochemicals derived from plants remain the basis for a large proportion of the commercial medications used today for the treatment of a wide variety of diseases, and as Dr. Norman.R. Farnsworth, a leading phytochemist rightly puts it,

‘For every disease that affects mankind, there is a treatment or cure occurring naturally on this earth.’

Despite this, the world of plants is still virtually an untapped reservoir of novel bioactive agents. Considering the extensive bioactive potentiality of the family *Euphorbiaceae* which is a large and diverse family of flowering plants and a good source of rubber (*Hevea*), tung oil (*Aleurites*), castor oil (*Ricinus*), casava (*Manihot*) and also rich in bioactive polycyclic diterpenoids of the tigiliane and ingenane type (present in the milky latex), triterpenoids, polyphenols and anthocyanidins, much work is being carried out in this plant family. Different parts of the plant belonging to this family are used in
folk medicine. These plants are chiefly used to cure skin diseases, gonorrhea, migraine, intestinal disorder, warts etc. Of late, there are reports of its antitumour and anti-HIV activities. So the plant undertaken for the present study is *Acalypha fruticosa*—an unexplored plant from the family *Euphorbiaceae*. Similarly, the second plant chosen was *Aristolochia tagala* which is an endangered species and has good medicinal properties. So the objectives of the present research are

1. Isolation of the secondary metabolites from the two medicinal plants by chromatographic methods.
2. Checking the homogeneity and purity of the compounds.
3. Identification of the compounds by colour reactions and spectral methods (UV, IR, PMR, CMR, 2D NMR-COSY, HETCOR, DEPT and Mass spectrometry).
4. Isolation of essential oil from the leaves of *Acalypha fruticosa* (Forsk) and the aerial parts of *Aristolochia tagala* (Cham).
5. Characterization of the essential oil by GC and GC- MS method and study of its antimicrobial activity.

The manifold uses of the plants chosen have created wide interest in their phytochemistry. So the isolation and structural elucidation of the phytoconstituents will form an integral part of the thesis.