Chapter 1

General Introduction

Ayurveda is the practice of well-documented indigenous systems of medicine. Ayurveda uses extracts from various plants for the treatment of various diseases. Modern allopathic medicine did not pay much attention towards the potential of medicinal plants. The medical systems of Siddha, Unani and Homeopathy are also largely plant based. The source texts, the Materica Medica and a variety of numerous other publications are based on the Indian indigenous systems of medicine. They reflect the country's great wealth of medicinal plants and announce the medicinal potential of plants.

Most of the allopathic drugs are synthetic compounds and a lot of side effects have been reported. Plant based drugs are having less side effects when compared with synthetic compounds (Gogtay et al., 2002, Teixeira, 2006). Therefore it is the duty of the phyto-chemists to explore the potential of medicinal plants for providing compounds that can be used as better drugs compared to the existing ones. Once plant-based active compounds are identified and characterized they can be used as 'lead' compounds and chemically modified to produce more powerful drugs with fewer side effects.

1.1 Medicinal Plants and their present day significance

A medicinal plant is defined as any plant which contains substances that can be used for therapeutic purposes or which are precursors of chemotherapeutical semisynthesis (WHO, 1979). Medicinal plant provides health-promoting characteristics, temporary relief to symptomatic problems and has curative properties. With the advent of modern scientific methods many of the medicinally important plants came under chemical scrutiny, leading to the isolation of the active principles. Many of the well known medicinal plants were chemically analysed and characterised for their active principles (Dev, 1983). Soon after their isolation and characterisation these compounds either in pure state or in the form of well-characterised extracts became part of pharmacopoeias of several countries. At present the study on medicinal plants is one of the important areas of biomedical research.

Flowering plants provide chemotherapeutants used traditionally throughout the world to treat many ailments such as diarrhoea, fever and cold as well as for the purposes of birth control, dental hygiene and psychic problems (Mitscher et al., 1987, Deans and Svoboda, 1990). Developing countries depend on plants as the source of medicine where traditional medicine plays a major role in health care (Zakaria, 1991). According to Meyer et al., (1996) between 12 and 15 million South Africans still depend on traditional herbal medicine from as many as 700 indigenous plant species. Since there is an increase in the use of synthetic drugs leading to many side effects and undesirable hazards, there is a global trend to go back to natural resources, mainly plants that are both culturally acceptable and economically viable.

The knowledge of the use of plants (Ethnobotany) for medicine as well as the importance of screening plant species for bioactive compounds recently encouraged several investigators to conduct biological evaluations on plants from different regions including India (Abraham et al., 1986, Naqvi et al., 1991), Somalia

(Samuelsson et al., 1992), Pakistan (Rizvi et al., 1987), Saudi Arabia (Khatibi et al., 1989), New Zealand (Bloor, 1995), Kenya (Githinji and Kokwaro, 1993), Rwanda (Vlietinck et al., 1995), Cuba (Martinez et al., 1996), Nepal (Taylor et al., 1995), Siberia (Kokoska, et al., 2002), Scotland (Kumarasamy et al., 2002), Central America (Camporese et al., 2003) etc.

1.2 Antibiotic resistance acquired by pathogenic microbes

Over the last 50 years, human life expectancy and quality of life have increased dramatically due to improvements in nutrition and the use of antibiotics in the fight against infectious diseases. However, indiscriminate antibiotic treatment resulted in the appearance and spread of resistance among harmful microorganisms. At present, there is great concern that common bacterial populations from food and the gastrointestinal tract of humans and animals, such as lactic acid bacteria and bifidobacteria, could act as a reservoir for antibiotic resistance genes. Resistance could ultimately be transferred to human pathogenic and opportunistic bacteria hampering the treatment of infections (Ammor et al., 2007). *Lactobacillus reuteri* and *Lactobacillus fermentum*, which are commonly, used as food processing aids can potentially act as reservoirs of antibiotic resistance genes. Acquired resistance genes may be transferred via the food chain or in the gastrointestinal tract to pathogenic bacteria (Egervarn et al., 2007)

Aeromonas species were described as being involved in outbreaks of acute gastroenteritis of choleric/dysenteric form or chronic diarrhoea, ulcerative colitis, etc. in normal adults or children. These bacteria exhibit constitutive resistance to certain antibiotics (Balotescu et al., 2003). A higher incidence of resistance to amoxicillin, piperacillin, and trimethoprim/sulfamethoxazole was observed among pathogenic bacteria from inpatients than from outpatients in a study at a university teaching hospital in Yaounde, Cameroon (Pieboji et al., 2004).

Acinetobacter infections have increased and gained attention because of the organism's prolonged environmental survival and propensity to develop antimicrobial drug resistance, the multidrug-resistant *Acinetobacter* infection in inpatients caused increased length of stay in hospital and intensive care unit (Sunenshine et al., 2007). In the United States alone the cost of treating hospital acquired drug resistant infection is estimated to be US $ 4.5 billion annually (McGowan, 1991). It is commonly assumed that infections caused by multiple resistant strains occur throughout the developing world (Gibbons, 1992). The problem of multidrug resistant microorganism is arising simultaneously in a number of places in the world (Cohen, 1992, Neu, 1992, Murray, 1994, Gould, 1994, Hryniewicz, 1994, Casellas et al., 1994). Multidrug resistant *Acinetobacter baumannii*, methicillin resistant *Staphylococcus aureus*, vancomycin resistant beta- lactamase producing *Enterococcus* species etc. have been observed in hospital acquired pathogens (Kaatz et al., 1990). There are reports of antibiotic resistant bacteria emerging in animal populations and these bacteria are appearing with increased frequency (Nijsten et al., 1993, Bates et al., 1993). The causes of antimicrobial resistance may be due to the excessive consumption of antibiotics, the use of broad-spectrum antimicrobial agents instead of agents with a narrower spectrum, procuring antibiotics without prescription etc.

According to Idose et al., (1968) antibiotics are sometimes associated with adverse effects on hosts, which include hypersensitivity, depletion of beneficial gut, and mucosal microorganisms, immuno-suppression and allergic reactions. Similarly several antibiotics were found to effectively prevent dental caries, but many resulted in derangement of oral and intestinal bacterial flora (Fitzgerald, 1972). The search for new antibacterial substances exhibiting minimal side effects are required since many of the drugs currently in use result in adverse side effects and some are harmful.

1.3 Secondary metabolites of plants and their medicinal values

In addition to primary metabolites, plants also contain a large variety of substances, called secondary metabolites, with no apparent metabolic function. Certain secondary metabolites are restricted in a few plant species, where they fulfil specific ecological functions, such as attracting insects to transfer pollen grains or attracting animals to consume fruits and in this way to distribute seed. Some secondary metabolites are also acting as natural pesticides. By and large the pharmacological activity of a medicinal plant resides in the so-called secondary metabolites which are comparatively smaller molecules in contrast to the primary metabolites such as the peptides, proteins and carbohydrates.

Secondary metabolites accumulate in the plant in small quantities in specialised cells. These compounds are generally detected in lower concentration compared to the primary metabolites and so these are termed as the higher value-lower volume products or speciality chemicals. Secondary metabolites are bio-synthetically derived from the primary metabolites and their distribution in plant kingdom is restricted (Buchanan et al., 2000).

General pathways by which secondary metabolites formed are (1) The shikimic acid pathway that leads to the formation of flavonoids, alkaloids, lignins, coumarins, tannins, phenols and various aromatics, (2) The acetate malonate pathway that forms the precursors to fatty acids, phospholipids, glycerides, waxes, glycolipids etc. and (3) the acetate mevalonate pathway – that results in the synthesis of sterols and certain sesquiterpenes. The secondary metabolites possess various biological activities, ranging from antimicrobial, antibiotic, insecticidal and hormonal properties, to highly important pharmacological and pharmaceutical activities. These are major sources of food additives, carotenoids, anthocyanins, vanillins and perfumes. Also these compounds help plants to survive in their environment by acting as attractants of pollinators and chemical defences against microorganisms, insects and predators (Heldt, 2005).

Alkaloids comprise a variety of heterocyclic secondary metabolites. Phenylpropanoids include coumarins, lignin, suberin, cutin flavonoids tanins etc. Terpenoids and glycosides represent other groups of secondary metabolites (Heldt, 2005).

Secondary metabolites often protect plants from pathogenic microorganisms and herbivores. The major groups of secondary metabolites are alkaloids, isoprenoids and phenylpropanoids, all of which include natural pesticides that protect plants against herbivores and pathogenic microorganisms. In some plants natural pesticide amount is 10% of the dry matter. Plants form phytoalexins which are defence substances against microorganisms, especially fungi. Phytoalexins are synthesised in response to infections. Phytoalexins include isoprenoids (terpenoids), flavonoids, and stilbenes, many of which act against a broad spectrum of pathogenic fungi and bacteria. Alkaloids belong to a group of secondary metabolites that are synthesised from amino acids and contain one or several 'N' atoms as constituents of heterocycles. Many of these alkaloids act as a defence substances against animals and microorganisms (Heldt, 2005).

Plant isoprenoids act as antibiotics to protect the plant from pathogenic microorganisms. Plant isoprenoids are utilised as pharmaceuticals. Higher plants have two different pathways for isoprenoid synthesis. In the first pathway isopentenyl pyrophosphate is synthesised by acetate-mevalonate and in the second pathway the pyruvate and D-glyceraldehyde –3 phosphate are precursors for the synthesis of isopentyl pyrophosphate (Heldt, 2005).

Acetyl-CoA is the precursor for the synthesis of steroid (isoprenoids) in the cytosol. Two molecules of acetyl CoA are converted into melvolonate and finally converted into isopentenyl pyrophosphate, which is responsible for the synthesis of sterols (Heldt, 2005). Plants contain a large variety of phenolic derivatives. As well as simple phenols, these comprise flavonoids, stilbenes, tannin, lignans, and lignin. All these substances are derived from phenyl alanine and tyrosine by Shikimate pathway. Since the phenolic compounds are formed from phenyl ring with a C_3 side chain, they are collectively termed as phenylpropanoids (Heldt, 2005).

Flavonoids have multiple functions in plants. A precursor for the synthesis of flavonoids is chalcone, synthesised by chalcone synthase. From p-coumaryl CoA and three molecules of malonyl CoA, this reaction is also called malonate pathway. Some plants including pine, grape wine and peanuts possess stilbense synthase. Stilbenes are very potentent natural fungicides (Heldt, 2005).

Chalcone is converted into flavanone by chalcone isomerase. Flavanone is the precursor for a variety of flavonoids. Flavones and flavonols have absorption maximum in UV Region (Heldt, 2005). Flavonoids possess free radical scavenging activity and hence act as antioxidants (Ami et al., 2003).

Flavonoids are becoming very popular because they have much health promoting effects. Some of the activities attributed to flavonoids include anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral. The flavonoids quercetin is known for its ability to relieve hay fever, eczema, sinusitis and asthma. Flavonoids have been shown to have antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic and vasodilatory activity. The potent antioxidant activity of flavonoids—their ability to scavenge hydroxyl radicals, superoxide anions, and lipid peroxy radicals may be the most important function of flavonoids, and underlies many of the above actions in the body (Miller, 1996). For this reason nutrients containing flavonoids in green tea, soy sauce and red wine have been regarded as beneficial for health (Heldt, 2005).

1.4 Antibacterial properties of plant products

According to Eisa et al., (2000) crude extracts of *Dichrostachys cinerea* fruits and leaves exhibited antibacterial activity justifying its traditional use. The results of preliminary screening of *Indigofera dendroides* leaves for antimicrobial activity showed a wide spectrum of activity against tested bacteria and fungi (Esimone et al*.,* 1999). Studies on antimicrobial activity of *Ficus racemosa* Linn. leaf extract (Mandal et al., 2000) and essential oils of five *Eucalyptus* species (Oyedeji et al., 1999) exhibited considerable activity against tested microbes. Adamu et al., (2000) screened four selected medicinal plants in Nigeria and proved their efficacy against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Promising results were obtained when extracts of fifty-six widely used dried Chinese medicinal plants were screened for their antimycotic activities (Blaszezyk et al., 2000).

Since plants are storehouses of chemotherapeutants and provide an inexhaustible source of several bio-chemicals, these plant materials can be used to develop natural antibiotics. Hence, in the present study, the effect of crude plant extracts as well as purified plant product on multidrug resistant microorganisms was investigated to detect antibacterial properties.

1.5 Pteridophytes and their importance

The name 'pteridophyta' was derived from the Greek words, 'pteron' denoting feather and 'phyta' plant, due to feather-like leaves. The ferns and fern-allies together form the pteridophytes. The ferns are considered as primitive in the evolutionary point of view. They are characterised by the circinate vernation (young fronds being coiled like a watch spring), the spore bearing structure (sporangia), usually with well developed and copiously veinated leaves. The internal structure of the rhizome, the vascular cylinder (stele), is characterised by the presence of leaf gaps. On the other hand, 'fern-allies' lack the circinate vernation and leaf gaps (Rashid, 1976).

Pteridophytes are primitive vascular plants, which can adapt well in terrestrial habitat. They are found scattered all over the globe but more abundant in hills and mountains at high altitude. This group of non-flowering plants is considered less important. However, with the introduction of ethnobotany by Hershberger (1896) for the study of relationships which exits between peoples of primitive societies and their plant environment, many attempts are made on the study of relationships of pteridophytes with man, and more particularly for their medicinal value.

Pteridophytes constitute an important part of the flora of the world and our country, next to the flowering plants. Pteridophytes have complex internal structural organisation and occupy an intermediate position between bryophytes and higher land plants such as gymnosperms and angiosperms (Rashid, 1976). Not much has been known regarding the economic value of this group of plants. This is not because of the misunderstood fact that they lack any economic utility, but because of the real fact that enough attention has not been paid towards assessing the potentialities of ferns and fern allies towards human welfare (Vasudeva, 1999). Medicinal ferns of India were studied and listed by Nayar (1959). Kaushik and Dhiman (1995) listed some common medicinal pteridophytes. Joshi (1997), Dhiman (1998), Singh et al., (2001) and Nwosu (2002) reported ethnobotanical potential of pteridophytes.

1.6 Biological activity of Ferns

Flavonoid of *Drynaria fortunei,* a fern used against renal failure (Long et al., 2005). There are reports of *Drynaria quercifolia* against *Neisseria gonorrhoeae* (Shokeen et al., 2005). Friedelin, epifriedelirol, beta amyrin, beta sitosterol, 3-beta-D-glucopyraroside, and naringin were isolated from dried rhizome of *Drynaria quercifolia.* The methanol extract showed broad and concentration dependent antimicrobial activity (Ramesh et al., 2001). Cytotoxic & antioxidant effects by *Drynaria fortunei* (Liu et al., 2001). *Gleichenia linearis* show antibacterial properties (Vasudeva, 1999).

Five different approaches of selecting plants for pharmacological screening are recognised, namely (1) random approach, (2) phytochemical approach, (3) ethno-directed sampling approach, (4) chemotaxonomic approach and (5) a method based on specific plant parts such as seeds (Cotton, 1996). Of these, ethno – directed sampling approach is adopted for the present study and for this preliminary ethno-medical information is collected from surveys at clinics, local elderly people, traditional healers and herbalists.

Traditional medicine uses intact plants for treatment of diseases. The crude plant extracts contain several compounds like alkaloids, terpenoids, flavonoids, tannins, saponins etc. Consequently, there is no possibility for attributing the efficacy to any specific compound. Hence, in the present study an attempt was made to isolate, purify and characterise the active principle after identifying the plant showing potential antibacterial activity. The secondary metabolites in different plants differ greatly in their basic chemistry as well as physical and chemical properties. Also they possess a wide range of biological activities. The active principle responsible for antibacterial activity can be isolated using chromatographic methods and characterised using spectroscopic methods. The present study was therefore assigned to:

- Conduct a preliminary *in vitro* screening for verifying the existence of antibacterial property in pteridophyte plants especially in ferns common in Kottayam and nearby areas.

- Select a plant that is not a threatened species and the crude sample of the plant shows prominent antibacterial activity against pathogenic strains.

- Isolate, purify and characterise the antibacterial principle from the selected plant.

- Determine the structure of the compound by using various spectroscopic techniques.

- Study the antibacterial properties against different strains by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

- Study the similarity of the isolated active compound with known antibacterial compounds and antibiotics.