Chapter 1.

INTRODUCTION
1.1 General introduction

Ayurveda - the Indian traditional system of medicine, is a science developed mainly based on the experience and wisdom of our ancestors. Ayurveda has made significant contributions to medical science by providing many leads to natural product drug discovery (Patwardhan and Vaidya, 2009). Natural products, herbal medicine, tribal and traditional medicines are being increasingly used in recent years for the prevention and cure of human ailments. Plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide mankind with new remedies. Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. Today according to the World Health Organization (WHO), as many as 80% of the world's people depend on traditional medicine for their primary healthcare needs.

Plants are the source of highly potent drugs for treating diseases like malaria (quinine from cinchona); hypertension and lowering of blood pressure (serpentine from *Rauwolfia serpentine*); headaches (codeine and morphin from *Papaver somniferum*); respiratory ailments (ephedrine from *Ephedra sinica*); heart therapy (digoxin from *Digitalis*), etc. The medicinal value of plants lie in chemicals present in them that produce a definite physiological action on the human body. The most important of these constituents are alkaloids, tannins, flavonoids phenolic compounds, glycosides, steroids, saponins, etc (Hill, 1952). Aspirin, atropine, artemisinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine and vinblastine are a few examples of what medicinal plants have given to us in the past (Sekar et al., 2010).

India is a varietal emporium of medicinal plants and is one of the richest source of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties (Martins et al., 2001). In India, the use of medicinal plants is a centuries-old tradition
and approximately two million traditional health practitioners still use medicinal plants for curing various ailments (Venkatesh, 2002). Medicinal plants were first used in their crude forms as herbal teas, syrups, infusions, ointments, liniments and powders. Natural crude drug extracts and biologically active compounds isolated from plant species used in traditional medicine can be prolific resources for new drugs (Kilani-Jaziri et al., 2011). The crude drugs are always available easily in abundance, are comparatively cheaper, with negligible side effects and are therefore prescribed to patients of all age groups. Modern science provides precise techniques for the extraction, isolation, separation and purification of the active phytoconstituents.

1.2 Phytochemical study

Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers all plant parts to be potential sources of medicinal substances. However a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. Therefore there is an urgent need for documentation of research work carried out on traditional medicines (Dahanukar et al., 2000).

Woody plants and herbs synthesize and accumulate in their cells a great variety of phytochemicals including low molecular phenolics (hydroxybenzoic and hydroxycinnamic acids, acetophenone, flavonoids, stilbenes and lignans) as well as oligo- or polymeric forms (hydrolysable and condensed tannins and lignins). Most of these are potent bioactive compounds that can be used for the synthesis of useful drugs (Sofowora, 1993). Phytochemicals regulate, protect and control many of the diseases in human beings, though the active principles differ from plant to plant because of their diverse biochemical nature.

1.2.1 Phenolics

Phenolic compounds are diverse in nature; they include simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives. They have received a considerable attention because of their free radical scavenging nature and antimicrobial activity (Bandoniene and Murkovic, 2002; Nychas et al., 2003).
Flavonoids belong to a group of polyphenolic compounds found in plants. They include monomeric flavonols, flvanones, anthocyanidins, flavones and flavonols (Waladkhani and Clemens, 2001). In addition to their free radical scavenging activity, flavonoids have multiple biological functions: antibacterial, antifungal and antiviral effects as well as being inhibitors of phospholipase A2, cyclooxygenase and lipoxygenase (Middleton and Kandaswami, 1992). Naturally occurring flavonoids are antiallergic, anticarcinogenic, antiviral and antioxidant (Close and McArthur, 2002). They show anti-inflammatory and anticancer activity (Okwu, 2005). Some isoflavons act as allelochemics widely used in insecticides. They also play a role in disease resistance (Salisbury and Ross, 1992). Isoflavones, which are phytoestrogens, effectively and efficiently, modulate estrogen levels in humans. They are of clinical value in low estrogen states like menopause or imbalanced and toxic estrogen sensitive conditions such as breast, uterine and prostrate tumor growth (Okwu, 2005).

1.2.2 Alkaloids

Many alkaloids are pharmacologically active substances, which exhibit various physiological activities in humans and animals. Alkaloids are very important in medicine and are the constituents of most the valuable drugs. The use of alkaloid containing plants as dyes, spices, drugs or poisons can be traced back almost to the beginning of civilization (Roberts and Wink, 1998). Several alkaloids are still in use. Caffeine, a psychostimulant, is largely obtained from the decaffeination of Coffea species. Codeine (Papaver somniferum) is used as an antitussive (agent that suppresses the coughing reflex). Cocaine (Erythroxylum coca Lam.) is used as a local anesthetic (e.g. in eye surgery). Morphine (Papaver somniferum) is an indispensable analgesic (painkiller), used for treatment of severe pain. Quinine is used for antimalarial activity and remains on the market as an antipyretic (fever suppressant). Alkaloids such as solasodine have been indicated as a starting material in the manufacture of steroidal drugs (Maxwell et al., 1995).
1.3 Antimicrobial study

Antimicrobial drug resistance is defined as “Survival of the fittest” microorganisms in the presence of drugs. Infectious diseases caused by bacteria and fungi are the leading causes of death and disability worldwide. Today, infectious diseases account for one-third of all deaths in the world; the World Health Organization (WHO) estimates that nearly 50,000 people die each day throughout the world from infectious diseases. The emergences of multidrug resistant pathogens threaten the clinical efficacy of many existing antibiotics. After penicillin and the use of other antibiotics between 1929 and 1962, it was believed that infectious diseases have been eradicated. Treating of bacterial infections by antibiotics is beneficial but their indiscriminate use has led to an alarming resistance among microorganisms as well as re-emergence of old infectious diseases (Qadrie et al., 2009). Different antibiotics exercise their inhibitory activity on different pathogenic organisms. In the present millennium, the major issue facing WHO is overcoming resistance to antibiotics (Mbosso et al., 2010).

Antibiotics are powerful bacteria-killing drugs that help our bodies regain the upper hand when a bacterial infection develops. Most antibiotics used for the treatment of bacterial infections may be categorized according to their principal mechanism of action. According to Neu (1992) and Tenover (2006), there are five major modes of action viz:

- a. interference with cell wall synthesis
- b. inhibition of protein synthesis
- c. interference with nucleic acid synthesis
- d. inhibition of a metabolic pathway
- e. Disruption of bacterial membrane structure

Antibiotics that work today may not work tomorrow. It is essential to investigate newer drugs to which the microorganisms are lesser resistant (Chanda and Rakholiya, 2011). Although pharmaceutical industries have produced a considerable number of commercial antibiotics from time to time, resistance in pathogens to these drugs has increased at a high rate and multi-drug resistant microorganisms have
exacerbated the situation (Nino et al., 2006; Sharma and Kumar, 2009). Therefore, the pharmaceutical companies and researchers are searching for new antibacterial agents (Rai et al., 2009). The present situation has been recognized globally as a serious concern and justifies further research to discover antimicrobial agents from natural origins including plant extracts (Nguyen and Graber, 2010; Eldeen et al., 2011).

Several mechanisms have evolved in microorganisms which confer them with antimicrobial resistance. There are two aspects for antimicrobial resistance: biochemical and genetic (Dzidic et al., 2008). Biochemical aspect: (a) Antibiotic inactivation (hydrolysis, group transfer, and redox process), (b) Target modification (peptidoglycan structure, alteration, protein structure interference, and DNA synthesis interference), (c) Efflux pumps and outer membrane permeability changes, (d) Target bypass. Genetic aspects: (a) Mutations (spontaneous mutations, hypermutators, and adaptive mutagenesis), (b) Horizontal gene transfer (plasmids, conjugative transposons, integrons).

1.3.1 Antifungal infections

Over the past 30 years, fungi have emerged as significant cause of human diseases, with attendant morbidity and mortality (Enoch et al., 2006). A large proportion of fungal infections occur in immunocompromised patients, as these pathogens are readily able to overwhelm the already weakened host defence mechanisms. Hospitalised patients with serious underlying diseases are increasingly exposed to invasive surgical procedures or chemotherapeutic treatments, such as broad-spectrum antibiotics or immunosuppressants following organ transplantation and cancer chemotherapy that readily promote mycotic disease. The treatment with many antifungal drugs is complicated because of high toxicity and low tolerability, ineffectiveness against new or re-emerging fungi, or the development of drug-resistant strains in patients undergoing treatment (Pfaller and Diekema, 2007).

1.3.2 Antimicrobial agents from plants

Medicinal herbs represent a rich source from which novel antibacterial and antifungal chemotherapeutic agents may be obtained (Rates, 2001; Kilani-Jaziri et al.,
There are many advantages of using antimicrobial compounds from medicinal plants, such as fewer side effects, better patient tolerance, economic, acceptance due to long history of use and being renewable in nature (Gur et al., 2006).

1.4 Antioxidant study

There are numerous types of free radicals formed within the body. Reactive oxygen species (ROS) are molecules with high chemical reactivity. ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules. ROS include diverse reactive entities namely superoxide (O$_2^-$), hydroxyl (OH$^-$), peroxyl (ROO$^-$), hydrogen peroxide (H$_2$O$_2$), singlet oxygen (1O$_2$) and reactive nitrogen species (RNS) include diverse reactive entities namely peroxinitrite (•ONOO$^-$), and nitric oxide (NO$^-$) radicals, as well as non free radical species such as nitrous acid (HNO$_2$) and hypochlorous acid (HOCl) (Mavi et al., 2004; Al-Mamun et al., 2007). These free radicals can be formed in different ways. ROS can be produced by endogenous mechanisms like oxidative phosphorylation, action of peroxisomes, activation of inflammatory cells, etc (Klaunig and Kamendulis, 2004; Waris and Ahsan, 2006). ROS can also be produced exogenously by environmental agents such as genotoxic and non-genotoxic carcinogens. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides (Halliwell and Gutteridge, 1989; Robinson et al., 1997).

Oxidation is essential to all living organisms for the production of energy to fuel biological processes such as aerobic respiration, stimulated polymorphonuclear leucocytes, macrophages and peroxisomes and they are the main endogenous sources of free radicals which are generated as by product. However, excessive amounts of free radicals can be harmful to many bio molecules. In fact, oxidative stress results from an imbalance between the generation of ROS and endogenous antioxidant systems. Antioxidants are able to prevent and reduce these abnormalities effectively (Feillet-Coudray et al., 2009).

The aerobic organisms develop antioxidant defense mechanisms that arrest the damage caused by ROS and RNS entities. The defense mechanisms can be enzymatic and non-enzymatic. The enzymatic mechanisms include antioxidant enzymes like...
superoxide dismutase, catalase, glutathione reductase and peroxidase, and nitric oxide synthase, etc. The non-enzymatic mechanisms are comprised of antioxidants such as ascorbic acid, \( \alpha \)-tocopherol, \( \beta \)-carotene, glutathione, flavonoids, uric acid, cysteine, vitamin K, serum albumin, bilirubin, and trace elements such as zinc and selenium, etc (Yeum et al., 2004). Both processes can contribute to prevent the damage caused by oxidative reactions. Since the natural antioxidant mechanism in mammalians under some circumstances can be inefficient, a dietary intake of antioxidant compounds becomes imperative; it has been suggested that there is an inverse relationship between dietary intake of antioxidants and the incidence of diseases caused by their deficiency (Antolovich et al., 2002).

There are many synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), propyl gallate (PG) and tertbutyl hydroquinone (TBHQ) but their use is restricted by legislation because of their low solubility, moderate antioxidant activity, toxic, carcinogenic effects, etc (Soubra et al., 2007; Ghafar et al., 2010) and also have been suspected to cause or prompt negative health effects. Therefore, there is an increasing interest in searching for antioxidants from natural origin; the need exits for safe, economic, powerful and natural antioxidants.

1.5 Antiulcer study

Gastric hyperacidity and gastroduodenal ulcers are very common ailments today (Chanda et al., 2011a). Gastric and duodenal ulcers affect a considerable number of people in the world and it is considered as the new “plague” of the 21st century (O’Malley, 2003). Gastric ulcers, one of the most widespread diseases, are believed to be due to an imbalance between aggressive and protective factors in the stomach, such as acid-pepsin secretion, mucosal barrier, mucus secretion, blood flow, cellular regeneration, prostaglandins and epidermal growth factors (Lima et al., 2006; Ishikawa et al., 2008). Hyperacidity (hyperchlorhydria) is a pathological condition due to hypersecretion of hydrochloric acid from the parietal cell of the gastric mucosa through the proton pumping \( \text{H}^+\text{K}^+\text{ATPase} \) (Hoogerwerf and Pasricha, 2001).
The major causes of ulcer are infection, certain type of medication and disorders that cause over secretion of stomach juices and balance between some gastroprotective and aggressive factor is lost. Major aggressive factors are acid, pepsin, *Helicobacter pylori* and bile salts. Defensive factors mainly involved are mucus-bicarbonate secretion and prostaglandins (Jainu and Devi, 2006).

The rise in gastric acidity and peptic activity are usually a manifestation of a physiological disturbance affecting one or more mechanisms which normally regulate gastric secretion. Activity of the gastric secretary cells has been found to be stimulated by caffeine, alcohol, hydrochloric acid and sodium chloride. Even the normal rate of acid secretion may cause ulceration in the breached mucosa when some gastroprotective factors are lost (Sannomiya et al., 2005). Infection with *Helicobacter pylori* also plays an important role in the development of gastric ulcers (Correa and Houghton, 2007) and its association with other factors such as ethanol, stress, smoking, nutritional deficiencies and frequent ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs) have contributed to the increase in the gastric ulcer incidence (Barros et al., 2008).

NSAIDs are used worldwide for the treatment of pain, rheumatic arthritis and cardiovascular diseases, and more recently for the prevention of colon cancer and Alzheimer’s disease (Berenguer et al., 2006). Gastrointestinal (GI) symptoms are the most common adverse effects associated with NSAID therapy (Ehsanullah et al., 1988; Singh, 1998). Gastric ulcers, bleeding and perforation are serious side effects which are observed in long term NSAID therapy. Animal models consisting of repeated administration of Indomethacin are found, both macroscopically and microscopically, similar to NSAID-induced ulcers in man (Wallace and McKnight, 1993).

The mechanism by which NSAIDs cause injury to the gastric mucosa is mainly due to the inhibition of cyclooxygenase enzyme (COX) and suppression of prostaglandin (PG)-mediated effects on mucosal protection (Rang et al., 2003). It is known that the inhibition potencies of NSAIDs on COX-1 and COX-2 enzymes are different (Simon, 1999). It is believed that while inhibition of COX-1 by NSAIDs cause side effects as a result of reduced PG synthesis, inhibition of COX-2 is related
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to their anti-inflammatory effect (Wright, 2002; Suleyman et al., 2008). Indomethacin potently damages PG synthesis by inhibiting both COX-1 and COX-2 enzymes (Patrignani, 2000; Botting, 2006). Inhibition of the COX-1 and COX-2 enzymes is responsible for gastric damage (Peskar et al., 2001). Indomethacin and similar NSAIDs, which inhibit both isoforms of the COX enzyme, produce more severe damage in gastric tissue, even gastrointestinal bleeding when combined with antithrombotic agents (Delaney et al., 2007). Inhibition of COX-2 enzyme is thought to be responsible for indomethacin’s anti-inflammatory effect, while inhibition of COX-1 is responsible for its gastrointestinal system (GIS) side effects (Rainsford, 2007). Chronically administrated NSAIDs induce clinically significant gastric mucosal damage by two mechanisms: direct mucosal irritation and prostaglandin inhibition. Four different drugs are currently used for the prevention of GI side effects of NSAIDs: histamine H2 receptor blockers, proton pump inhibitors (PPI), sucralfate and misoprostol (Agrawal, 1995).

The mechanisms which trigger gastric lesions have been studied in various experimental models (Matsuda et al., 1998; Rodriguez et al., 2006; Sanchez et al., 2006). Ethanol has been shown to produce gastric damage by impairing gastric defensive factors, such as mucus and mucosa circulation (Szabo et al., 1992). Moreover, oxygen radicals and lipid peroxidation are thought to be involved in the ethanol-induced gastric damage. As a consequence of their extreme chemical reactivity, ROS can cause severe changes at the cellular level which can culminate in cell death. At the molecular level, they attack essential cell constituents, such as proteins, lipids and nucleic acids, which can cause the loss of their biological function and formation of toxic compounds (Kaharaman et al., 2003). ROS especially hydroxyl radicals plays a major role in causing oxidative damage of mucosa in all types of ulcers (Das et al., 1997).

The maintenance and repair of gastric mucosa is a dynamic processes associated with proliferation and migration of epithelial cells and connective tissue to maintain/regain mucosal architecture (Johnson, 1994). It involves a complex host of mechanisms which work in tandem (1) to protect the gastric mucosa from damage and (2) to trigger repair mechanisms of the underlying mucosal defects by proliferating and migrating epithelial cells and connective tissue, resulting in reconstruction of the
mucosal architecture (Holzer, 2001). Plants contain bioactive chemical constituents, which are capable of scavenging free radicals, reducing oxidative stress and offer gastroprotection (Thompson et al., 2006); by scavenging the free radicals, the metabolites might protect the gastric mucosa from oxidative damage or accelerate healing of gastric ulcers (Hahm et al., 1997).

In traditional systems of medicine, several plants and herbs have been used to treat gastrointestinal disorders, including gastric ulcers (Gracioso et al., 2000; Hiruma-Lima et al., 2001). A large number of medicinal plants and dietary nutrients have been shown to possess gastro-protective property (Dharmani and Palit, 2006; Kath and Gupta, 2006; Malairajan et al., 2007a; Zahra et al., 2009). Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers (Logan and Walker, 2001; Schmeda-Hirschmann and Yesilada, 2005). Herbal drugs obtained from plant sources are relatively less expensive, safe and well tolerated even in higher doses (Goel and Sairam, 2002).

1.6 Selection of the plants for present study

When selecting the plants for pharmacological activities, four basics methods are usually followed (Suffness and Douros, 1979):

a) Random choice of plant species
b) Choice based on ethnomedical use
c) Follow up of existing literature on the use of the species
d) Chemotaxonomic approaches

Comparison of the four methods showed that the choice based on folklore has given about 25% more positive leads than other methods. Based on the second and third approach, sixteen plants were selected in this study.
Considering the above, the objectives of the present study are as mentioned below:

- Review of literature:
  - Reported antimicrobial activity of some medicinal plants
  - Reported antioxidant activity of some medicinal plants
  - Reported antiulcer activity of some medicinal plants
- Plant description of selected sixteen plants with their reported activities
- Screening of sixteen plants for their antimicrobial activity
- Screening of sixteen plants for antioxidant activities
- Phytochemical analysis of screened plants
- Selection of the plant showing best antimicrobial and antioxidant activity
- Pharmacognostic study of the selected plant
- Phytochemical and physicochemical analysis of the selected plant
- Fractionation of the best solvent extract of the selected plant
- Antimicrobial activity of isolated fractions
- Antioxidant activity of isolated fractions
- Acute toxicological study of the selected plant
- Antiulcer activity of the selected plant