CHAPTER I
INTRODUCTION

The use of medicinal plants for treatment of ailments can be traced back over five millennia to written documents of the early civilization in China and India, but it is undoubtedly as old as mankind. There has been an increase attention towards the study of medicinal plants for their conventional utilization, especially in the last decade. According to a report of World Health Organization (Anonymous, 1991), natural drugs are the mainstay of about 80% of the world's population for healthcare requirements. This revival of interest in plant derived drugs is mainly due to the current widespread belief that “green medicine” is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects. Pharmacogonostic investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents (Konig, 1992). The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments. Some common examples of usage of medicinal plants include the use of root extracts of Abrus prectorius (Rosary pea) against krait bite, use of leaf parts of Azadirachta indica (Neem) against viper bites. The use of leaves of A. indica to keep mosquitoes away is an old practice. There are also ample evidences of the usage of Aloe barbadensis (Aloe) in treating skin burns and other epidermal infections. Lawsonia inermis (Henna) has been used for its blood purifying and wound healing properties. Due to better cultural acceptability, better compatibility with the human body, fewer communication means, deficiency and unavailability of new health services, rural people are still relying on plants for treatment of their diseases.

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria fungi or protozoans. Antibiotics provide the main basis for the therapy of microbial infections. In developing countries, synthetic antimicrobial drugs are not only expensive and inadequate for the treatment of diseases but also often associated with adulterations and side effects. In addition, the number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics. Therefore, this is prerequisite to search new infection-fighting strategies to control microbial infections (Sieradzki et al., 1999). It is need of hour to discover new
antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas et al., 2003). Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 50% of prescribed medicines in industrialized countries derived directly or indirectly from plants (Newman et al., 2000). Thus it is a logical approach in drug discovery to screen traditional natural products. The use of plant extracts as antimicrobial agents is of great significance in combating with bacterial infections. In the past few years a number of investigations have been conducted worldwide to prove antimicrobial activities of medicinal plants (Nascimento et al., 1990; Alonso-Paz et al., 1995; Sumathi and Parvathi, 2010; Golam et al., 2011). In order to promote the use of medicinal plants as potential sources of antimicrobial compounds, it is pertinent to thoroughly investigate their composition and activity and thus endorse their use (Nair and Chanda, 2006).

Microbial infections and many other diseases lead to oxidative damage in our body. Medicinal plants treatments are multifaceted. One important aspect is that most of them possess antioxidant compounds as their metabolites. In recent years, the studies on “oxidative stress” and its adverse effects on human health have become a subject of considerable interest. Oxidative stress is caused by the imbalance between oxidants and antioxidants, potentially leading to various diseases in our body. Approximately 1-3% of the oxygen consumed by us is converted to superoxide and other reactive oxygen species called free radicals under physiological conditions (Fridovich, 1986; Halliwell, 1996). Free radicals play role in a number of biological processes including intracellular killing of bacteria by phagocytic cells such as granulocytes and macrophages and certain cell signaling processes (Droge, 2002). Their excess amount is associated with cellular and metabolic injury, aging, cancer, cardiovascular diseases, neurodegenerative diseases, inflammation (Ames, 1983), Alzheimer’s, Schizophrenia and Parkinson’s diseases (Floyd, 1998). To counteract the free radical-induced damage, human body has developed an antioxidant defense system (superoxide dismutases, H₂O₂ removing enzymes, metal binding proteins etc.), but it is inadequate to prevent damage completely. Recent research has proved beyond doubt that antioxidants are significant in the prevention of human illness and may function as free radical scavenger, reducing agents and quencher of singlet oxygen formation.

Currently available synthetic antioxidants like butylated hydroxyanisole (BHA), Butylated hydroxyl toluene (BHT), tertiary butylated hydroquinones and gallic acid esters have been suspected to cause negative health effects. Moreover, these synthetic antioxidants
also show low solubility and moderate antioxidant activity (Barlow, 1990; Kumar et al., 2008).

So there is currently immense interest in natural antioxidants and their role in human health and nutrition (Aruoma, 1994). Plants are most susceptible to damages caused by active oxygen species. Resultantly, they develop several antioxidant defense systems forming numerous potent antioxidant compounds. Thus, there has recently been a resurgence of awareness in antioxidants from plants, especially from those of the developing countries that have a rich heritage of botanical ethno-pharmacopoeia. Scientific information on antioxidant properties of various plants, particularly those that are less widely used in culinary and medicine is still scarce. Therefore, the assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and nutraceuticals.

Zhou et al., 1991 mentioned that plants having polyphenolic compounds such as flavonoids possess antioxidant activity. Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it is reasonable to determine their total amount in the selected plant extracts. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Polyphenols are also well documented to have microbicide activities against a large number of pathogenic bacteria and fungal species (Scalbert, 1991; Field and Lettinga, 1992; Cowan, 1999). Antioxidant activities of polyphenols have been suggested to exert beneficial pharmacological effects on neurological disorders on the basis of in vitro observations (Moosmann and Behl, 1999; Parr and Bolwell, 2000). So, a systematic search for antioxidants from medicinal plants is now considered to be a rational approach in pharmaceutical and drug research.

Pathogenic bacteria present an astonishing stash of virulence factors that enable them to conquer many different niches during the course of infection and enhance their potential to cause disease. Four general classes of virulence factors include bacterial toxins, adhesins, extracellular enzymes and antiphagocytic factors. Particularly fascinating virulence factor are microbial enzymes which play a major role at various stages of infection. Several enzymes like leukocidins, hemolysins, lecithinase, proteases, phospholipases, neuraminidases, collagenases and hyaluronidase have been implicated in microbial virulence by destroying white blood cells, red blood cells, plasma membrane of cells and damaging tissues, making the host permissive for microbial infection (Wilson et al., 2002; Konopka et al., 2003). Other enzymes, such as urease, contribute to virulence by facilitating survival inside phagocytic
cells (Cox et al., 2000). Innovative pharmacological methodologies to control the expression of these bacterial enzymes are thought to be beneficial to combat the bacterial infections. Targeting the virulence factors is one of the alternative approaches to find new molecules to treat infections due to resistant bacteria (Rasko and Sperandio, 2010). Because of their great pathogenic significance and virulence nature, the present investigation deals with two bacterial enzyme-urease and collagenase.

Urease (EC 3.5.1.5), a large nickel containing enzyme from the super family of amidohydrolases and phosphotriestrases, is the most prominent protein component of some bacteria (Dunn et al., 1997). It is produced by bacteria, fungi, yeast, and plants where it catalyzes the urea degradation to supply these organisms with a source of nitrogen for growth. More than 200 bacterial species such as Helicobacter pylori, Clostridium perfringens, Klebsiella pneumoniae, Proteus sp., Salmonella sp., Staphylococcus aureus, Staphylococcus saprophyticus, Pseudomonas aeruginosa, Ureaplasma urealyticum, and Yersinia enterocolitica are responsible for various diseases like gastritis, urolithiasis, pyelonephritides, ammonia and hepatic encephalopathy, hepatic coma, urinary catheter encrustation (Andrews et al., 1984; Mobley et al., 1995; Burne and Chen, 2000), and Parkinson’s disease (Amtul et al., 2002). Chronic bacterial kidney infections can directly cause kidney stones (Griffith et al., 1976; Thomas et al., 2008) due to hydrolysis of urea, producing ammonium and hydroxyl ions. The resulting alkaline urine, in combination with ammonium and phosphate ions, leads to the development of magnesium ammonium phosphate stones, also known as struvite stones (Rahman et al., 2003). These stones are always associated with urinary tract infections with urea splitting bacteria namely Proteus sp., K. pneumoniae, P. aeruginosa, and S. aureus (Griffith et al., 1976; Rahman et al., 2003; Belhadji et al., 2004; Juszkiewicz et al., 2004). Many urease inhibitors have been described in the past decades, such as phosphorodiamidates (Pedrazzini et al., 1987), polyhalogenated benzo- and naphthoquinones (Ashiralieva and Kleiner, 2003), imidazoles (Tanaka et al., 2003) and α-hydroxyketones (Tanaka et al., 2004). But until now, only acetohydroxamic acid has been clinically used for the treatment of infections caused by bacterial urease (Juszkiewicz et al., 2004). Acetohydroxamic acid (AHA) is a synthetic drug derived from hydroxylamine and ethyl acetate, is similar in structure to urea. In the urine, it acts as an antagonist of the bacterial enzyme urease. AHA has no direct antimicrobial action and does not acidify urine directly. It is used, in addition to antibiotics or medical procedures, to treat chronic urea-splitting urinary infections. AHA reversibly inhibits the bacterial enzyme
urease. This inhibits the hydrolysis of urea and production of ammonia in urine infected with urea-splitting organisms, leading to a decrease in pH and ammonia levels.

The success of commercially available drugs in the treatment of diseases caused by bacterial urease is overshadowed by the various side effects associated with these drugs. So there is a need to explore more effective urease inhibitors, possessing enhanced efficacy against infectious bacteria while exhibiting less toxicity to human cells. Natural compounds extracted from plants, particularly higher plants, have been suggested as alternative sources for synthetic anti-urease products. This approach is alluring, in part, because they constitute a potential source of bioactive compounds that have been professed by the general public as comparatively safe and often act at multiple and novel target sites, thereby reducing the potential for resistance (Raskin et al., 2002).

Collagenases (EC 3.4.24...) are endopeptidases, capable of degrading the triple-helical region of native collagen, which is susceptible to attack by other peptidases only after initiation of cleavage by collagenase (Madhan, 2007). Uncontrolled proteolysis by collagenase contributes to abnormal development and to the generation of many pathological conditions including wrinkle formation, skin ulceration, metastasis, arthritis, chronic inflammation, osteoporosis, periodontal disease, tumor invasion, cardiovascular disease, nephritis, neurological disease, breakdown of blood brain barrier, gastric ulcer, corneal ulceration, liver fibrosis, emphysema, fibrotic lung disease, etc. (Nagase, 1999). Bacterial species such as Clostridia produce collagenase (Wilson et al., 2002) that breaks down protein collagen and helps in spreading of bacteria in infected body tissues and organs. It is also a potential virulence factor expressed by Porphyromonas gingivalis associated with periodontal disease (Olsen and Dahlen, 2004). Therefore, collagenase represents an attractive pharmacological drug target and its inhibition has become a promising therapeutic strategy for the treatment of such diseases (Nagase, 1999). Most of Collagenase inhibitors discovered are synthetic peptides, chemically modified tetracyclines, bisphosphonates, hydroxamate, carboxylate, sulfdryl, sulfoximine, sulfodiimine, sulfonamide or thiol (Browner et al., 1995; Sang et al., 2006). However, these drugs are reported to exert side effects such as musculoskeletal pain in tendons and joints (Nelson et al., 2000). So, seeking novel and efficacious collagenase inhibitors with good bioavailability and low toxicity is very substantial. Several hundred genera of plants and relevant prescriptions are used medicinally for treatment and prevention of various disorders in different countries. But the full potential of both urease and collagenase inhibitors from natural sources has not yet been fully explored.
Natural products have been used as sources for the discovery of new drugs on the basis of their widespread application in traditional medicine to cure several human diseases. So, apart from nutritional value, plants also contribute to the protection from oxidative stress by scavenging free radicals, inducing antioxidant enzymes, modulating protein kinase signaling pathway, inhibiting cyclooxygenase-2 (COX 2) and matrix metalloproteinases enzymatic activity (Firdaus et al., 2013).

Though antibiotic strategies are highly effective in the management of bacterial infections, they have been responsible for emergence of drug and multi-drug resistant strains of bacteria. A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in pharmaceutical and drug research.

In effort to discover the new inhibitors of therapeutically important enzymes (urease and collagenase) and to correlate the antibacterial and antioxidant activities of plants, the objectives of this study were taken as following:

1. To evaluate if any correlation exists between antibacterial and antioxidant activity.
2. To evaluate and correlate phenolic concentration and antioxidant activity of various medicinal plants.
3. To evaluate and correlate antibacterial activity with enzyme inhibitory effects of medicinal plants.