CHAPTER 1

INTRODUCTION
Medicinal plants are the touching Stone of Modern Science and India is a store house of it. During last few decades there has been an increase in the study of medicinal plants and their traditional use in different parts of the world (Lev, 2006). Prior to the development of modern medicine, over the centuries within various communities, the traditional systems of medicine that evolved are still maintained as a great traditional knowledge base in herbal medicines (Mukherjee et al, 2006).

Even today plants are almost exclusive source of drugs for the majority of world population. People in developing countries still rely on traditional medicine for their primary health care need. This is also true in India that only a small percentage of plants have been evaluated for antibacterial activity against human pathogen (Patwardhan et al., 2004). The general population is increasingly using herbal medicines as dietary supplements to relieve and treat many different human disorders. Herbs and spices are an important part of the human diet. They have been using from thousands of years to enhance the colour, flavour and aroma of food.

The use of medicinal plants has low cost and has been used for several generations for scientific studies on the chemical and Pharmacological properties of medicinal plants allowing scientists to indicate their proper use, that is how toxicity when correctly employed is considered as one of the main advantages (Ccabuana vasquez et al, 2007). Diseases that have been managed traditionally using medicinal plant include malaria, epilepsy, infantile convulsion, diarrhoea, dysentery, fungal and bacterial infections (Sofowora, 1996).

According to World Health Organization (WHO) (Santos et al., 1995) medicinal plants are the best source to obtain a variety of drugs. Therefore, medicinal plants should be investigated to better understand their properties, safety and efficiency (Ellof et al., 1998). Thus considering the vast potentiality of plant as a source of new therapeutic agents hence detail investigations were conducted to test the efficacy of the plant extracts against human pathogenic bacteria.
Characterization of Respiratory Tract Infecting Bacteria

Respiratory tract infection refers to any number of infectious diseases involving the respiratory tract. Acute respiratory infections are the single most common infective cause of death worldwide. Nasopharynx is not only the primary settlement of opportunistic pathogen but also the chief carrier of common respiratory pathogens like *Staphylococcus aureus, Streptococcus pneumoniae, Neisseria meningitides, Klebsiella pneumoniae, Pseudomonas aeruginosa* etc.

Urbanization, climate change, tobacco consumption and specific diseases such as HIV/AIDS have contributed to a rising prevalence of the respiratory infections.

Respiratory tract infection is normally further classified as an upper respiratory tract infection (URI or URTI) or a lower respiratory tract infection (LRI or LRTI). Lower respiratory infections, such as pneumonia, tend to be more serious conditions than upper respiratory infections, such as the common cold.

Although some disagreement exists on the exact boundary between the upper and lower respiratory tracts, the upper respiratory tract is considered to be the airway above the glottis or vocal cords. This includes the nose, sinuses, pharynx, and larynx.

Typical infections of the upper respiratory tract include tonsillitis, pharyngitis (*Streptococcus pyogenes*), laryngitis (*Haemophilus influenza, Streptococcus pneumoniae*), sinusitis (*Moraxella catarrhalis, Haemophilus influenza, Streptococcus pneumoniae*), certain types of influenza, and the common cold (Eccles et al., 2007). Symptoms of URIs can include cough, sore throat, runny nose, nasal congestion, headache, low grade fever, facial pressure and sneezing.

The lower respiratory tract consists of the trachea (wind pipe), bronchial tubes, the bronchioles, and the lungs. Lower respiratory tract infections are generally more
serious than upper respiratory infections. LRI are the leading cause for death among all infectious diseases (Robert Beaglehole, 2004), caused by *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Chlamydia spp*, *Legionella*, *Coxiella burnetti* and viruses. The two most common LRIs are bronchitis and pneumonia. (Antibiotic Expert Group, 2006).

In young children, most of LRTIs occur during the seasonal respiratory viral epidemics, which are generally caused by parainfluenza virus, influenza virus, adenovirus, metapneumovirus or respiratory syncytial virus. Viral pneumonia caused by cytomegalovirus and herpes simplex virus should be considered even without a suspicion of maternal history. Pneumocystis pneumonia is generally limited to immunocompromised infants while Cryptococcus neoformans may be found in patients with HIV infections. In infants, LRTIs can be even caused by milk aspiration or by a foreign body.

Regional variations in respiratory disease prevalence have important implications for diagnosis, treatment and prevention particularly where medical facilities are limited. Despite advances in the development of strategies to prevent respiratory tract infections, the availability of newer, safer and more potent antimicrobials and effective vaccines, it continues to be a leading cause of morbidity and mortality for children of all age groups. (Baqui *et al* 2007).

**Plant Material**

The use of plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. Nowadays, medicinal plants receive attention to research centers because of their special importance in safety of communities

Medicinal plants are resources of new drugs. It is estimated that there are more than 2, 50,000 flower plant species. Studying medicinal plants helps to understand plant
toxicity and protect human and animals from such natural toxins. Cultivation and preservation of medicinal plants protect biological diversity. Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin. Plants are directly used as medicines by a majority of cultures around the world, for example Chinese and Indian medicine. Many food crops have medicinal effects, for example ginger, garlic.

_Gymnema sylvestre_ is one of the most important medicinal plants of the Asclepiadaceae family. It is native to central and western India, tropical Africa and Australia. It is very often called “gurmar” (destroyer of sugar), as chewing the leaves causes a loss of sweet taste (Gloria _et al._, 2003). It is one of the local herbs that is claimed to possess various physiological effects and it occupies an important place in the indigenous system of medicine.

It is used in folkloric medicine since time immemorial. The leaves of _Gymnema sylvestre_ have been used for centuries in the traditional Indian system of Ayurvedic medicine for various ailments like the way the leaf powder is used which stimulates the heart and the circulatory system, increases the secretion of urine, and activates the uterus. In the Ayurvedic system of medicine, _Gymnema sylvestre_ R.Br. is referred to as “mesasrngi,” and both the dried leaf (mesasrngi leaf) and dried root (mesasrngi root) are used therapeutically.

Recent studies on _Gymnema sylvestre_ (especially leaves) shows a number of compounds isolated, like Gymnemic acid A, B, C, D, gymnemagenol, deacyl gymnemic acid, quercitol, lupeol, β-amyrin and stigmasterol etc. that tend to possess potential medicinal properties against diabetics, obesity, cancer, inflammation, snake venom etc. Recent studies has shown that Gymnemic acid molecules fill the receptor location in the absorptive external layers of the intestine thereby preventing the sugar molecules absorption by the intestine, which results in low blood sugar level (Sahu _et al._, 1996). In addition, it possesses antimicrobial, anti hyper cholesterolemic, sweet suppressing and
hepatoprotective activities. (Rana et al., 1992). It also acts as feeding deterrents to caterpillar, Prodenia eridania (Granich Mark, 1974), prevent dental caries caused by Streptococcus mutans (Hiji Yasutake, 1990) and in skin cosmetics.

**Antibacterial Activity**

Various medical plants have been used for years in daily life to treat diseases all over the world. Medicinal plants are gifts of nature to cure limitless number of diseases among human beings (Bushra Beegum et al., 2003). The abundance of plants on the earth’s surfaces has led to an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents (Bonjar et al., 2004). A large number of plants in different locations around the world have been extracted and semi-purified to investigate individually their antimicrobial activity. Among the different plant derivatives, secondary metabolites were proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity (Ahmed et al., 2002).

Researchers have shown that all different parts of the plants which include; stem, root, flower, bark, leaves possess antimicrobial property. Microorganisms have potential to cause human diseases.

The discovery of antibiotics in the early twentieth century provided an increasingly important tool to combat bacterial infections. As increasingly, antibiotics are used and misused, the bacterial strains become resistant to antibiotics rapidly. Keeping in view the importance of diverse medicinal flora, many research works have been conducted to investigate ethno botanical uses and create awareness about the plant species for the welfare of human communities throughout the world.

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has
been increasing. Therefore, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are used as therapeutic agents (Cohen, 1992).

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is uncertain. Therefore, action needs to be taken to reduce this problem, for example, to control the use of antibiotic, to develop research to better understand the genetic mechanisms of resistance, and to continue the studies to develop new drugs, which can be synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

From a long period of time, plants have been a valuable source of natural products for maintaining a good health. Documenting the indigenous knowledge through ethno botanical studies is important for the conservation and utilization of biological resources. Ethno botanical survey has been found to be one of the reliable approaches to drug discovery. Thus efforts were made to introduce many of these drug plants into Agriculture, and studies on the cultivation practices were undertaken for those plants which were found suitable and remunerative for commercial cultivation.

**Antioxidant Capacity**

There is increasing evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs and medicinal plants (Osawa, 1994; Noda et al., 1997)

Antioxidants are molecules of synthetic or natural origin and have ability to stabilize or deactivate free radicals. Highly reactive free radicals are major causative agents for different cellular injury including liver damage (Halliwell, 1994). Many dietary constituents and phytochemicals of medicinal plants are being investigated as antioxidants for its protective effects (Maria et al., 2010; Nakasa et al., 2011; Gupta et al., 2011; De Oliviera et al., 2011)
Oxygen is essential for the survival of all on earth. During the process of oxygen utilization in a normal physiological and metabolic process, approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All these radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering each cell to face about 10,000 oxidative hits per second (Mondal et al., 2006).

Antioxidants are added as redox systems possessing higher oxidative potential than the drug that they are designed to protect or as chain inhibitors of radical induced decomposition. In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule (Lachman et al., 1986).

It has been suggested that fruits, vegetables, natural plants contain a large variety of substance called phytochemicals which are present in plants and are the main source of antioxidants in the diet, which could reduce the potential stress caused by reactive oxygen species. The natural antioxidants may have reducing agents, free-radical scavengers, potential complexers of prooxidant metals, quenches of singlet oxygen etc (Ebadi, 2002). The antioxidants can interfere with the oxidation process by reacting with free radicals (Gupta et al., 2004).

Recently interest has increased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity (Kumaran et al., 2007). Antioxidants principles from natural resources possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance (Shriwaikar et al., 2006). Food industry uses natural antioxidants as a replacement of conventional synthetic antioxidants (Govindarajan et al., 2003).
Antioxidant constituents of plant materials are important in the maintenance of health and protection from coronary disease because they possess the ability to protect the body from damage caused by toxic free radical induced oxidative stress (Ahmad et al., 2010a, 2010b; Ahmad et al., 2011a, 2011b).

**Phytochemical Activity**

The use of plants as medicine is as old as human civilization. People of different ages in both developing and undeveloped countries use plants in an attempt to cure various diseases and to get relief from physical sufferings.

The average annual foreign trade in crude drugs and their phytochemicals is between 60 and 80 million rupees and these accounts for a little more than 0.5 per cent of the world trade in these commodities.

The use of plant extracts and phytochemicals, both with knowledge of antimicrobial properties, can be of good significance in therapeutic treatments. In the past a few years ago, a number of studies have been conducted in different countries for proving such efficiency. Many plants have been used because of their antimicrobial characters, which are due to compounds synthesized in the secondary metabolism of the plant. These products are recognised by their active substances, for example, the phenolic compounds which are part of the essential oils (Jansen et al., 1987), as well as in tannin (Saxena et al. 1994).

The curative properties of drugs are due to the presence of complex chemical substances of varied composition (present as secondary plant metabolites). Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga et al., 2005; Mann, 1978). These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically diverse compounds
with obscure function. They are widely used in the human therapy, agriculture, veterinary, scientific research and countless other areas (Vasu, 2009).

The drugs of plant origin especially of herbaceous nature are used as whole plant and are identified with their origin, common name, scientific nomenclature, family, geographical source, cultivation, collection, preservation, storage, macroscopy, microscopy, chemical composition, identity, purity, strength and assay, substitute and adulterants etc.

Interest in plant secondary metabolites has risen dramatically in recent years among plant molecular biologists and plant breeders because of their diverse effects. Few recent developments include the efficiency of conversion of plant protein into animal protein (increase rumen undegradable protein and thus increase protein availability post ruminally), reduce greenhouse gases, and reduce gastrointestinal parasites.

Phytochemical screening of plants has revealed the presence of numerous chemicals like alkaloids, flavonoids, steroids, tannins, glycosides and saponins etc. Secondary metabolites of plants serve as defence mechanisms against predation by many microorganisms, insects etc.

Due to the ever increasing prices of drugs especially in developing countries, the side effects and development of resistance by a pathogen to any of commonly used antibiotics provides an impetus for further attempts to search for new antimicrobial agents to combat infections and overcome the problems of resistance and side effects of the currently available antimicrobial agents.

**LC-MS Analysis**

Liquid chromatography–mass spectrometry (LC-MS, or alternatively HPLC-MS) is an analytical chemistry technique that combines the physical separation
capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry. LC-MS is a powerful method used for several applications which has very high sensitivity and selectivity. Its application is oriented mainly towards the general detection and potential identification of chemicals in the presence of other chemicals (in a complex mixture).

**Liquid chromatography**

Similar to gas chromatography MS (GC/MS), liquid chromatography mass spectrometry (LC/MS or LC-MS) separates compounds chromatographically. It differs from GC/MS in that the mobile phase is liquid, usually a mixture of organic solvents and water, instead of gas, as a result the ion fragments cannot yield predictable patterns. An electrospray ionization (ESI) source is used most commonly in LC/MS. Electrospray ionization is the ion source of choice to couple liquid chromatography with mass spectrometry. The electrospray voltage has been identified as an important parameter to consider in ESI LC/MS gradient elution (Marginean *et al.*, 2009).

**Mass spectrometry**

Mass spectrometry works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios. In a typical Mass spectrometry procedure a sample, which can be solid, liquid, or gas, is ionized. The ions are separated according to their mass-to-charge ratio. (Sparkman, 2000).

A mass spectrometer consists of three components: ion source, mass analyzer, and detector. (Chhabil, 2007).

- The ionizer converts some portion of the sample into ions. There are various types of ionization techniques, depending on the phase (solid, liquid, gas) of the sample, and the efficiency of various ionization mechanisms for the target species.
Mass analyzers separate the ions according to their mass-to-charge ratio. There are many different mass analyzers that can be used in LC/MS.

The detector measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present.

LC-MS is very commonly used in pharmacokinetic studies of pharmaceuticals and is thus the most frequently used technique in the field of bioanalysis. These studies provide information about how quickly a drug will be cleared from the hepatic blood flow, and organs of the body. Mass spectrometry is used for this due to high sensitivity and exceptional specificity compared to UV (as long as the analyte can be suitably ionised), and short analysis time. (Hsieh, 2006; Covey et al., 1986).

LC-MS is also used in the study of proteomics where again components of a complex mixture must be detected and identified in some manner. Profiling of secondary metabolites in plants or food like phenolics can be achieved with liquid chromatography-mass spectrometry (Stobiecki et al., 2006). LC-MS is frequently used in drug development at many different stages including Glycoprotein Mapping, Peptide Mapping, Natural Products Dereplication, Bioaffinity Screening, quantitative bioanalysis, and quality control. (Lee et al., 1999).

Therefore, Liquid chromatography-mass spectrometry (LC-MS) is an analytical technique that couples high resolution chromatographic separation with sensitive and specific mass spectrometric detection. It is a powerful and widely applied method for the study of biological systems, pharmacological interventions and biomarker discovery.

**Antiulcer Activity**

Medicinal plants play a vital role against a number of diseases. Variety of herbal plants and plant extracts has significant antiulcer activity in animal models. It should be noted that substances such as flavonoids and tannins that possess antiulcer
INTRODUCTION

activity are of particular therapeutic importance. Antiulcer activity of the drug can be attributed to free-radical scavenging property, inhibition of acid secretory parameters and strengthening of gastric mucosal barrier.

An ulcer is basically an inflamed break in the skin or the mucus membrane lining the alimentary tract. Ulcer occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance. About 19 out of 20 peptic ulcers are duodenal. Gastric ulcers, occurring in stomach wall are less common. The gastric mucosa is continuously exposed to potentially injurious agents for example, acid, pepsin, bile acids, food ingredients, bacterial products (Helicobacter pylori) and drugs. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced pepsin and gastric acid secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility (Grossman, 2009).

Peptic ulcer disease is a very common global health problem today. Peptic ulcer is a lesion of gastric or duodenal mucosa. Duodenal ulcers are more common in adult males. Gastric ulcers occur commonly at old age and in lower socio-economic class of individuals. Although the exact cause of ulceration is not known, hydrochloric acid and pepsin are responsible for maintaining the lesion once it is produced.

Peptic ulceration occurs only in areas which are bathed by the acidic gastric juice. Therefore, the term peptic ulcer refers to ulceration of the areas which might be acted upon by acid peptic juice namely the stomach and the first portion of duodenum (Dey et al., 2002).

The formation of peptic ulcers depends on the presence of acid and peptic activity in gastric juice plus a breakdown in mucosal defences. The major factors that can disrupt the mucosal resistance to injury: Non-Steroidal Anti Inflammatory Drugs (NSAIDs) example, aspirin and Helicobacter pylori infection. Many natural products have been evaluated as therapeutics for the treatment of a various diseases, including
peptic ulcer. There is considerable pharmacological investigation going on, into the antiulcer activity of some compounds.

Peptic ulcer is also found to occur at the lower end of the esophagus, on the jejunal side of a gastroenterostomy and in Meckel’s diverticulum (Boyd, 1970).

- In peptic ulcers drug treatment is targeted at either counteracting aggressive factors or stimulating the mucosal defences;
- Peptic ulcer disease is treated with the goals to relieve pain, heal the ulcer and prevent ulcer recurrence.

In ancient system of medicines, herbal preparations were used for treating duodenal ulcers (Chatterjee, 2000). In the last few years, efforts have been taken to identify new antiulcer drugs from natural sources like plants (Jana et al., 2005)

A large number of spices and herbs have been evaluated by various researchers for their antiulcer effects to achieve a favourable outcome. Large numbers of medicinal plants and dietary nutrients have been shown to possess gastro-protective activities such as Terminalia Chebula, Aloe, Vetiveria Zizinoides, Capsicum, Ginseng etc.

In recent years, focus on plant research has increased worldwide and several studies had showed immense potential of medicinal plants (Dahanurkar, 2000). The herbal medicines derived from various plant extracts, are being recognized increasingly in treating various clinical diseases, with relatively a little knowledge of their modes of action (Mayuren et al., 2008)

A number of drugs are available for the treatment of peptic ulcer but its clinical evaluation shows the side effects, incidence of relapses, and drug interactions. This has been the rational for the development of new antiulcer drugs and search for novel molecules. Plants have been an invaluable source of therapeutic agents to treat the various disorders including peptic ulcer disease.
OBJECTIVES OF THE STUDY:

- Isolation and characterization of respiratory tract infecting bacteria.
- Collection of *Gymnema sylvestre* plant material and preparation of leaf extract.
- Antibacterial activity of *Gymnema sylvestre* Leaf extracts.
- Determination of Antioxidant capacity in different solvent extracts of *Gymnema sylvestre*.
- Screening the Primary Phytochemical Constituents followed by Secondary Metabolites in leaf extracts of *Gymnema sylvestre* using different solvents.
- LC-MS analysis.
- Antiulcer activity of *Gymnema sylvestre* Leaf extracts.