CHAPTER I

INTRODUCTION

1.1. Plant as a source of medicine

The traditional medicine system that involves use of plants for curing various human and animal diseases is as old as human society. Most of the people living in rural areas depend on herbal medicines for treatment of some common diseases such as cold, cough, fever, bronchitis, asthma, stomach ache and many more. This is because people in rural and semi urban areas are devoid of proper medical facilities and since plants are easily available for them, they use plant based formulations for curing these common diseases. People accept plant based medicines because of their long history of use and less side effects.

The medicine system that we use in our day to day life falls under two broad categories. One is the modern medicine system i.e allop athy that mostly uses synthetic drugs. Synthetic drugs appeared to be toxic and have side effects for human health. The other medicine system is the traditional medicine system, the best example of which is Ayurveda, Unani and Siddha which includes codified information. There is another medicine system which includes folklore culture and traditional belief where the medicinal information is not well documented and not even tapped. This type of traditional knowledge is confined to some societies and the information is handed down from one generation to the next from many years. This is known as ethnomedicine
which is gaining much interest from second half of the twentieth century. Ethnomedicine basically involves plant based drugs.

In modern times, plant and plant constituents are gaining worldwide interest as a source of drugs and pharmaceuticals and therefore, integrating folklore medicine with modern healthcare system is a current trend with a view to develop novel drugs that could provide effective alternative remedies for different infectious and non-infectious diseases. Plants possess various secondary metabolites that are not required for plant growth and development but to fight against biotic and abiotic factors. For example, artemisinin, an antimalarial drug, derived from *Artemisia annua* L. is a potent antimalarial drug (Tu 2011) which is known to protect the plant from insect and herbivore attack. Youyou Tu, along with various other young researchers started investigating antimalarial activity of various Chinese herbal medicines under a national project from 1967. After screening about more than 250 herbs, finally in 1972, they found an active compound, artemisin from the leaves of the plant *Artemisia annua*. They found that *A. annua* fresh leaves contain abundant artemisinin, the molecule responsible for antimalarial activity. They did various clinical trials, though initial results were not satisfactory, Youyou and her colleague ultimately succeeded to create new antimalarial drug from *A. annua*. A modified form of artemisinin called dehydroartemisinin was found to be more effective antimalarial drug than artemisinin. There are reports on other medicinal uses of artemisinin and dihydroartemisinin (Sun *et al.* 1993, Yang *et al.* 1992).
North East India, known as ‘Mega Biodiversity Hotspot’ because of its rich biodiversity holds approximately 50% of the total Indian flora (Chakraborty et al. 2012). This region of India harbours many ethnic groups with double the number of subgroups who still live in remote and isolated areas maintaining their own custom and rituals. They follow folklore medicinal practices that have been passed generation after generation without any documentation. Therefore, North East India is also rich in traditional knowledge apart from species diversity. Mishimee people of Arunachal Pradesh use Mishimee teeta (*Coptis teeta*) as a folk remedy for jaundice, diarrhea, malaria and fever and malaria cases appeared to be of less occurrence among people using Mishimee teeta. Later scientific investigation revealed that the rhizome of this plant yield berberine, an active ingredient of modern antimalarial drug (Nath 2011). Roots of *Rauvolfia serpentine* popularly known as Sarpagandha in North East India is used traditionally for treatment of mental disorders and insomnia. Resperine, an antihypertensive drug used in modern medicine is prepared from this plant (Nath 2011).

There are many other such examples of folk medicines of North East India that serve as a source of modern drugs.

There are several reports on antibacterial activity of plant derived compounds such as salicylic acid isolated from *Anacardium pulsatilla*, capsaicin from *Capsicum annuum*, nordihydroguaiaretic acid from *Larrea tridentate*, cocaine from *Erythroxylum coca*, fabatin from *Vicia faba*, berberine from *Hydrastis Canadensis*, gallic acid isolated from *Lawsonia inermis* (Cowan 1999). The main classes of antimicrobial compounds include phenolics, terpenoids, alkaloids, lectine and polyetides.
1.2. Antibiotics against microbial diseases

Antibiotic medications are prescribed to patients suffering from infectious diseases. Antibiotics help to fight bacterial infections either by killing the pathogen or by preventing their reproduction. The contribution of antibiotics to human health is remarkable. Many bacterial diseases that once fatal to human can be now cured effectively by the use of antibiotics. Penicillin was the first discovered antibiotics in 1929. Since then different antibiotics have been developed for curing bacterial infections. In present time different classes of antibiotics are commercially available for treatment of infectious diseases such as fluroquinolones, rifamycines, β-lactams, tetracylines, macrolides etc. Quinolones were discovered in 1960s as a chloroquine byproduct for the urinary tract infection treatment. Rifamycins used against mycobacterial infections were first introduced in 1950s. Rifamycin was isolated from the bacterium Amycolatopsis mediterranei. Teteracyclines derived from Streptomyces aureofaciens and S. rimosus are used against aerobic Gram positive and Gram negative species. Macrolide class of antibiotics derived from Saccharopolyspora erythraea and Streptomyces ambofaciens are used against both aerobic and anaerobic Gram positive and Gram negative species.

1.3. Mechanism of action of antibiotics

Antibiotics can be mainly classified into two categories (a) bactericidal antibiotics (directly cause cell death) and (b) bacteriostatic antibiotics (inhibit cell growth). Antibiotics like macrolides, tetracylines, chloramphenicol and spectinomycins are
bacteristatic in nature, however they may become bactericidal in certain species or under specific conditions (Kohanski et al. 2010). The mechanism of action of most of the bactericidal antimicrobials involves inhibition of DNA synthesis, RNA synthesis, protein synthesis or cell wall synthesis (Walsh 2003). Quinolones are a class of antibiotics that interfere with DNA synthesis. Quinolone drugs are known to trap topoisomerase enzymes, required for strand breakage and rejoining in DNA synthesis. This results in formation of quinolone-topoisomeras-DNA complex eventually leading to cell death (Drlica et al. 2008, Sugino et al. 1977). Rifamycins inhibit RNA synthesis by binding to RNA polymerase enzyme (Hartmann et al. 1967, Campbell et al. 2001, Naryshkina et al. 2001) and antibiotic like vanomycins are known to inhibit bacterial cell wall synthesis (Kahne et al. 2005). Another class of antibiotics like erythromycin, clindamycin, tetracyclines inhibit bacterial protein synthesis by blocking either 50S or 30S ribosome (Katz and Ashley 2005, Chopra and Roberts 2001). Reactive oxygen species generation is a common mechanism of antibiotic action leading to cell death (Kohanski et al. 2007).

The mechanism of action of plant antimicrobial agents is poorly understood. There are some reports on mode of antimicrobial action of some plant derived compounds. Oxidized phenolic compounds induce microbial toxicity by inhibiting enzyme activity probably through binding to sulphydryl groups or some nonspecific groups on the enzyme molecules (Mason et al. 1987). The highly reactive quinone molecules are found to induce production of free radicals and also quinones have tendency towards forming covalent interaction with nucleophilic amino acids leading to protein
inactivation (Stern et al. 1996). Flavonoids are plant secondary metabolites synthesized in response to microbial attack. Their mode of action involves disruption of bacterial cell wall or membrane or formation of complex with extracellular and soluble proteins leading to loss of function (Cowan 1999). The proposed mechanism of action of alkaloids like berberine and harmane involves blocking of DNA synthesis by intercalating with DNA. Peptides isolated from plants mostly contain disulphide bonds (Zhang and Lewis 1997) and their proposed mechanism of antimicrobial action involves ion channel formation in the microbial membrane. Most of the terpenoids isolated from plants have activity against bacteria but their mechanism of action is not yet fully understood.
Figure 1.1. A common mechanism of action of bactericidal antibiotics involving ROS production (Kohanski et al. 2007)

1.4. Development of antibiotic resistance

Since the discovery of antibiotics (1928), there is considerable development in the treatment of infectious diseases. Although antibiotics served as an effective measure for treatment of many contagious diseases, microbial resistance to antibiotics became a
major threat by the late 1940’s (Martin 1998). Researchers are trying to find out
different alternative sources of medicine and also to change the structure of traditionally
used antimicrobial agents to combat microbial drug resistance. There may be various
ways by which resistant bacteria may arise in the environment. For example,
antimicrobials present in manure and biosolids pollute the aquatic sources used for
drinking water through diffusion which may increase the selection of antibiotic resistant
bacteria (Rooklidge 2004). Antibiotics are now integral part of modern health care
system and various antimicrobial agents are also used in food items, household products
as well as in agriculture. Most of the time people use antibiotics without medication.
Overconsumption of antibiotics is one of the main causes of development of resistant
bacteria although some bacteria can acquire resistance naturally. When antibiotics are
consumed, only those cells which are sensitive to the particular antibiotic die and those
showing insensitivity remain alive. With the continuation of treatment the cells which
remained survived in the last dose used will attain stronger resistance against the
antibiotic either through gene mutation or through gene exchange (Walsh 2000).

Use of plant for curing infectious diseases is an ancient practice. Although plant
medicines cure diseases most often, rate of poisonings is also very high. Many
medicinal plants have been proved to be potential source of antimicrobial compounds
(Friedman et al. 2006). There are many reports on antimicrobial activity of medicinal
plants (1966 to 1994) and the number increase three folds from the period between
1995- 2004 (Sumthong 2007). Researchers usually focus on isolating the compounds
present in plant extract responsible for antimicrobial activity. From the crude extract,
active compounds are usually obtained by bioassay guided fractionation. Plant derived bioactive molecules are either directly used as drug e.g. taxol, resperine, digoxin, digitoxin, atropine or used as lead compounds to produce more active and less toxic (semi) synthetic novel molecules e.g. cocaine isolated from morphine, taxotene from taxol, verapamil from khellin, metformin from galegine, nabilone from Δ–tetrahydrocannabinol, oxycodon from morphine and teneposide from podophyllotoxin (Sumthong 2007).

Figure 1.2. Process of development of antibiotic resistance (Walsh 2000)
1.5. **Infectious diseases**

Infectious disease caused by microbes is one of the major causes of death worldwide (Mahady 2005, Cos *et al.* 2006). Infectious disease could be caused by either primary pathogens or by opportunistic pathogens. Rapid emergence of various infectious diseases has threatened the near future. Emerging infections not only affect health but also challenges the global economic growth and development. For example, severe acute respiratory syndrome (SARS), an infectious disease first emerged in China in 2003 and then spread to different countries leading to hundreds of deaths within a couple of months (Dikid *et al.* 2013). Emergence of SARS risked human lives as well as affected the economy with approximately USD 10-30 billion global economic losses. Another case of infectious disease is H1N1 influenza, caused by H1N1 virus. It was first reported in Mexico in 2009, which was later spread all over the world including India, taking more than 17000 lives globally (Dikid *et al.* 2013).

People with weak immune system might develop most severe form of disease caused by primary pathogen. Opportunistic pathogens mainly infect persons with weekend immune system. If not treated and recognized promptly, infectious disease could be fatal. Various infectious diseases caused by bacteria include tuberculosis, respiratory tract infections, bacterial meningitis, helicobacter pylori infection, urinary tract infections and various others. In recent times, fighting drug resistant microbes is the major concern of the scientists all over the world as resistant microbes have complicated the treatment of infectious diseases. There are various approach to combat bacterial infection which include development of new class of antibiotics either
synthetic or natural origin. An alternative therapy is the development of antimicrobial agents from plant sources. Most plant antimicrobials with broad spectrum activity are known to contain “phytoalexins”. Another strategy is the use of combination therapy which involves combining of different plant extracts together or combining antibiotics with plant extracts or antibiotics with different chemicals to enhance antibacterial effects. Extracts of *Mangifera indica* L. mixed with antibiotics erythromycin, norfloxacin and tetracycline showed four-fold increase in antibacterial activity than the plant extracts when used alone (Souto de Oliveira *et al.* 2011).

1.5.1. Respiratory tract infections

The respiratory tract is involved in gaseous exchange and thus it is exposed to the gaseous environment constantly, which contains different foreign material like bacteria, viruses and various other pathogens. Lungs of healthy individuals filter out most of the foreign particles and therefore rarely have any infections. But those with weak immune system may encounter microbial infection in the respiratory tract because of the ability of respiratory pathogens to overcome host weak defense mechanism. Some bacterial species like *Streptococcus pneumoniae* and *Haemophilus influenza* can resist phagocytosis by producing an enzyme (Ig A protease) that can disintegrate mucosal Ig A. Respiratory tract infection is divided into upper respiratory tract infection and lower respiratory tract infection.

The upper respiratory tract infections mainly include cold, sinusitis, otitis, pharyngitis, epiglottitis and laryngotracheitis. Otitis, infection of the ears is common in
young children. Otitis is caused by several bacterial species such as *Staphylococcus aureus*, *S. epidermis*, *P. aeruginosa*, *Streptococcus pneumoniae* and *Haemophilus influenzae*. *P. aeruginosa* is responsible for causing malignant otitis externa which is a severe necrotizing infection (Dasaraju and Liu 1996). Sinusitis is an acute inflammatory infection caused by *S. pneumoniae*, *H. influenzae*, *Moraxella catarrhalis*, *S. aureus*, *S. pyogens*.

Lower respiratory tract infection can cause severe illness leading to death while upper respiratory tract infections are usually not fatal. Lower respiratory tract infections mainly include bronchitis, bronchiolitis and pneumonia. Although viruses, fungi and mycoplasmas can also cause lower respiratory tract infections, bacteria are the dominant causative agents.

Pneumonia is a lung infection that can be diagnosed by X-ray or physical examination. Pneumonia can occur in individuals with weak immune system or those undergoing long term health care facilities as well as individuals with some other infections like cold or flu. The type of pneumonia occurs in individuals outside hospital or healthcare system is called community acquired pneumonia (CAP) which is the most common type and affects people of all ages. CAP is most commonly caused by *S. pneumoniae*, *H. influenza*, *C. pneumoniae* and *M. pneumoniae*. CAP due to *S. aureus* is not very common and occurs in patient suffering from staphylococcal bacteremia or influenza. Although *S. aureus* rarely cause CAP, in older and sicker patients it may be fatal. *S. aureus* is also one of the causative agents of bronchitis. In children with less than 5 years *S. pneumoniae*, *S. aureus* and *S. pyogenes* are most common causes of CAP. *Klebsiella pneumoniae* and *H. influenzae* cause pneumonia in elderly patients.
suffering from lung diseases. Some viruses and few fungi can also cause CAP. Pneumonia that occurs in hospitalized patients is known as nonsocomial or hospital-acquired pneumonia. Nonsocomial pneumonia is most commonly caused by aerobic gram-negative bacteria including \( P. \) aeruginosa, \( E. \) coli, Klebsiella and Enterobacter species (Dasaraju and Liu 1996). \( S. \) aureus is the gram positive bacteria responsible for causing hospital-acquired pneumonia and ventilator associated pneumonia (Rello et al. 1990 and Rubinstein et al. 2008). Nonsocomial pneumonia has more tendency of causing serious illness than other lung diseases because patients are too weak to fight off the pathogens and also the germs in hospitals have greater chance of being antibiotic resistant. There is another type of pneumonia, aspiration pneumonia that occurs due to inhalation of foreign materials such as vomit, food or liquid into the lungs. Aspiration pneumonia is caused by the bacteria that usually inhabit the oral and nasal pharynx. Lungs weak defense mechanism results in pneumonia. Fever, shortness of breath, chest pain, cough, and sputum production are the common symptoms of pneumonia patients. Other symptoms may vary depending on the age of the patient and type of causative agent. Pneumococcal vaccines are used as preventive measures for elderly and immunocompromised patients. Different types of antibiotics like macrolides, tetracyclines, fluoroquinolones, penicillines, vanomycines are mostly used for pneumonia treatment. But bacteria are resistant to most of these drugs in present time, making pneumonia treatment more challenging.

Discovery of new antimicrobial agents from natural sources has shown remarkable progress. Carvacrol and thymol derived from plant sources showed broad spectrum antibacterial activity both in vivo and in vitro (Dorman & Deans 2000, Lambert et al.
2001, Adam et al. 1998; Manohar et al. 2001). Phytoalexins such as scopoletin (derived from tobacco), camalexin isolated from *Arabidopsis thaliana*, nomilactone B isolated from rice, and glucosinolates extracted from *Brassicaceae* are some examples of antimicrobial compounds that have gained much attention recently (Lamothe et al. 2009). Similarly “phytoanticipin” another class of plant derived antimicrobial agents which include avenacin isolated from oat roots and α- tomatine from tomato are reported to show antimicrobial activity (Lamothe et al. 2009).

1.6. Ethnomedicinal survey and isolation of active compound

Ethnomedicinal survey one of the oldest strategies of natural product research involves selection of plant material based on information on some specific traditional uses for treatment of certain diseases. It helps to identify plants which are more likely to have bioactive components. Folk knowledge and indigenous cultures of healthcare are the source for many of the drugs that are commercially available in the market (Prance 1994). But most of these drugs are being used in a different way than those used in traditional cultures. Ethnomedicine system has been a useful indicator of many of the pharmaceutically active substances that are used in modern medicine. Utilization of traditional knowledge in research and development could help to improve the health care system. Study of ethnomedicine is gaining much importance in recent time in order to conserve the traditional knowledge of ethnic people. Therefore, the ethnobotanical information from local communities such as medicinal properties of the plants, reaction of the human body to the plant based medicines, distribution of the plants and relation
of the plants with ecosystem must be gathered for conservation of the medicinal plants and the traditional knowledge. Much emphasis should also be given on conservation of biodiversity in order to prevent extinction because industrialization, urbanization, habitat destruction are the major factors that resulted in decreasing diversity of plant species. There are various reports on ethnomedicinal survey of medicinal plants in India (Meenakshi et al. 2016, Jadhav 2015, Raju and Reddy 2005, Yesodharan and Sujana 2007, Chandra et al. 2007, Mondal and Rahaman 2012).

North eastern region of India is well known for its diverse flora and fauna with unique ecosystem. The ethnic groups in North East India follow various traditionally used plant based medicine systems for curing different infectious diseases. In recent years, research on folk medicine system followed by the ethnic groups and local people of villages of North East India showed significant progress. Saikia (2006) reported the ethnomedicinal use of different plants by the local Assamese and various ethnic communities including Bodo, Mishing and Santhal communities of Sonitpur District Assam. Choudhury et al. (2011) reported various medicinal plants with poisonous and medicinal properties used by the local people of Cachar District of Assam. Namsa et al. (2011) reported use of plants with anti-malarial activity by the local people of Sonitpur District, Assam. Tangjang et al. (2011) documented the ethnomedicinal plants used by ethnic tribes namely Nyishi, Nocte and Adi in Arunachal Pradesh. Das (2016) reported the biodiversity of various medicinal plants used tradionally by the ethnic communities of Barpeta district of Assam. Bailung and Puzari (2016) reported the use of various plants by Ahom community in Assam in health care management. Medicinal plants
used in treatment of piles by the local people of Betbari area of Sivasagar District, Assam was reported by Gogoi (2016). The documentation of folk medicinal uses of plants is required for carrying out evidence based research and also to conserve the traditional knowledge.

Isolation and identification of a potent bioactive lead compound from plants have become easy and quick due to improvement of various new highly specific screening techniques through *in vitro* bioassays, development of different chromatographic methods and spectrographic techniques. There are different extraction methods involved in plant active compound isolation depending on the plant material and compounds of interest. Different extraction methods include maceration, boiling, soxhlet, supercritical fluid extraction, sublimation and steam distillation. The crude extract is separated into different fraction using chromatographic techniques like thin layer chromatography (TLC), column chromatography (CC), size exclusion chromatography (SEC) or vacuum liquid chromatography (VLC). For isolation of plant active compound bioactivity guided isolation is used which involve step by step separation of the crude extract to active lead molecule followed by bioassay at each step. Serial dilution method is generally used for quantitative assessment of bioassay.
1. 7. **Research objectives**

Based on the above scenario emphasis has been given to accomplish the following objectives:

- Survey and collection of medicinal plants based on ethnomedicinal knowledge
- Antibacterial assay of the plant extracts against pathogenic bacteria
  - To study the mechanism of action of the potent plant extract on bacterial strains
  - Bioassay guided fractionation of the most potent bioactive extracts and characterization of the active compound.