CHAPTER- 1

INTRODUCTION

1.1 DRUG RESISTANCE

Infectious diseases have remained a major cause of death in the history of mankind. Present time is also affected by infectious diseases like cholera, influenza, cryptosporidiosis, hepatitis, HIV/AIDS, meningitis etc. and account for almost 1/3 of all the deaths in the world. The causal microorganisms are bacteria, fungi and viruses (Frean and Blumberg, 2008). As we take a look on the history of antibiotics, the preparations derived from various resources were used to heal the wounds till 19th century. Then the time came when Pasteur noted antagonistic action of some bacteria on the Bacillus anthracis, this was the first step towards the discovery of antibiotics. In the early 1920s, the British scientist Alexander Fleming reported that a product in human tears could lyse bacterial cells and same time German chemist Paul Ehrlich developed the concept of selective toxicity, which states that there are specific chemicals secreted by the host to effectively destroy the disease causing organisms without harming the host. Alexander Fleming in 1928 had analyzed that a common mold, Penicillium notatum had destroyed Staphylococcus bacteria which was the major turning point in the field of antibiotics and first antibiotic penicillin was isolated. After that there were various antibiotics discovered throughout the world. Among them Howard Florey has developed the manufacturing process for Penicillin G in 1942. Due to which Fleming, Florey, and Chain shared Nobel Prize in medicine for their work on penicillin in 1945. In developed nations, such as USA, the graph of deaths caused by infectious diseases has been declined since the ‘antibiotic era’ has begun in early 1950s. In 1955 tetracycline, in 1957 nystatin and in 1981 amoxicillin or amoxicillin/clavulanate potassium tablets were patented (Kokate et al., 2012) and different generations of antibiotics were developed, to fight against different pathogenic infections; 1) Penicillin: It has a narrow spectrum of clinical use; 2) Ampicillin and amoxicillin: they have an extended or broad spectrum of clinical use; 3) Carbenicillin and ticarcillin: they have broader spectrum of use than the second-generation penicillins, and is in use for serious infections; 4) Mezlocillin sodium and piperacillin: was even more potent (Eliopoulos and Moellering, 1982; King et al., 2000).
Development of antimicrobial resistance to antibiotics is one of the major challenges to combat infectious diseases caused by bacterial and fungal pathogens. The microbes carry genes for resistance and can be passed on to other microbes by various natural processes (conjugation, transformation etc). Steady increase in antibiotics resistance and decrease in the availability of new antibiotic have led to the difficulties in treatment of infections (Alanis, 2005). In fact, with the continuous use of penicillin for four years in treatment, resistant infections were reported. However, in the 1950s and 1960s, resistant bacterial strains were not very common. This was breakthrough for modifications by altering existing antibiotics to reduce toxicity and to design drugs to which bacteria has developed resistance. The worldwide spread of pneumonia was started in 1970s due to Penicillin resistant strain, *Streptococcus pneumoniae*. A completely new class of antibiotic wasn’t developed for another 30 years. In 1999 the new drug Zyvox was developed, which was a new class of antibiotics called the Oxazolidinones. It was effective against multi-resistant strains of bacteria. From 1970 to late 1990s the antibiotics were derived from existing classes, e.g., on the basis of chemical structure of penicillin, many synthetic variants have been produced which keeps the pharmacologically active part of the chemical and overcome resistance by modifying other part of the structure (Figure 1.1).

Extensive use of antibiotics in healthcare has led to the co-evolutionary emergence of dangerous pathogens that are resistant to traditional antibiotics, as well as the re-emergence of some old infectious diseases. In 1961, methicillin-resistant *Staphylococcus aureus* (MRSA) was discovered. On the other hand, nosocomial infection with multidrug resistant *S. aureus* (MRSA) has become a major problem during 1990 (Rybak and LaPlante, 2005). Vancomycin-resistant *S. aureus* (VRSA) was believed to acquire the resistance genes horizontally from vancomycin-resistant enterococci (VRE) reported in the US (Tenover, 2006). The resistance of *Psuedomonas aeruginosa* to many antimicrobial agents is topic of worry nowadays, because it has developed resistance against all classes of antimicrobials i.e. carbapenems, quinolones and aminoglycosides (Saga and Yamaguchi, 2009). A recent study showed that 5 out of 264 soil isolates obtained from different natural habitats in Hyderabad were found to be resistant for 10 antibiotics (Sengupta and Chattopadhyay, 2012).
Thus, clinical medicine is posed with this additional challenge. Presently, five generations of cephlosporins are present to overcome bacterial resistance along with different classes of aminoglycosides, penicillins, ansamycins, carbacephem, carbapenems, lincosamides, lipopeptide, macrolides, nitrofurans, oxazolidonones, penicillin combinations etc. Due to the development of antimicrobial resistance, higher dosages for longer time (Opatowski et al., 2010) or the combination of different drugs are recommended (Doring et al., 2000; Iseman, 1993), which may result in the emergence of multi-drug resistant pathogens. This therapy may lead to the increase in the side effects and toxicity to the patients like neurotoxic, nephrotoxic, ototoxic or hypertensive causing severe damage to the liver and bone marrow (Chong and Pagano, 1997). As a result, there is a demand to search new drugs which are more potent at low dosage and patient friendly.
1.2 TRADITIONAL MEDICINAL PLANTS IN TREATMENT OF DISEASES

Medicinal plants have been used in many forms over the years to treat, manage or control infections by folklore (Nadeem et al., 2012; Bano et al., 2014). Therefore any effort to further explore the medicinal or natural products from the botanical flora towards improving health care delivery deserves attention (Aiyegoro and Okoh, 2009). In USA the various plants have been utilized for dietary supplements such as *Echinacea purpurea*, *Panax ginseng*, *Serono repens*, *Ginkgo biloba*, *Hypericum perforatum*, *Valeriana officinalis*, *Allium sativum*, *Hydrastis canadensis*, *Matricaria chamomilla*, *Silybum marianum*, *Trigonella foenum-graecum*, *Tanacetum parthenium*, *Ephedra sinica* and *Cimicifuga racemosa* (Raskin et al., 2002). The medicinal plants produce secondary metabolites such as alkaloids, essential oils, fatty oils, resins, mucilages, tannins, gums etc. having antimicrobial activity (Quiroga, et al., 2001). Medicinal plants have been the subject of man’s curiosity since time immemorial (Constable, 1990). Almost every civilization has a history of medicinal plants usage (Ensminger et al., 1983). Approximately, 80% of the people in the World’s developing countries rely on traditional medicine for their primary health care needs, and about 85% of traditional medicine involves the use of plant extracts (Vieira and Skorupa, 1993). In the early twentieth century, the pharmaceutical industry synthesized wide variety of medicinal drug molecules and allowed the treatment of incurable and life-threatening diseases. Interest in phytomedicine has exploded in the last few years and about 500 different plant species are used as key-ingredients and many are still being collected from the wild (Balick and Mendelsohn, 1992).

Herbal medicines are now globally accepted as a valid alternative system of therapy in the form of pharmaceuticals, functional foods, etc., which has been recognized and advocated by the World Health Organization (WHO). Various studies around the World, especially in Europe have been initiated to develop scientific evidence based rational herbal therapies. Though ancient medical treatises have documented a large number of medicinal plants, most have remained undocumented and uncharacterized, the knowledge of their use being passed down from generation to generation by word of mouth (Ahmad et al., 2006). The ancient scriptures and the mythology are replete with a lot of references about the medicinal plants and their medicinal importance from the Himalayas. The Himalayan region is home to many medicinal plants (Gaur, 1999), with 250-300 km across stretches over 2,500 km from Jammu and Kashmir in the West to Arunachal Pradesh in the East spreading between 21°57´-37°5´ N latitudes and 72°40´-97°25´ E
longitudes. This great chain of mountains in Indian territory extends all along the Northern border of the country from the Eastern border of Pakistan on the west to the frontiers of Myanmar in the East covering partially/fully twelve states of India, i.e., Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Sikkim, Arunachal Pradesh, Nagaland, Manipur, Mijoram, Tripura, Meghalaya and hills of Assam and West Bengal. The medicinal plants of Himalayan region were known for antibacterial and antioxidant activity (Kumar et al., 2010; Kumar and Singh, 2011). Some plants with high medicinal value like Aconitum heterophyllum, Aconitum ferox, Dactylorhiza hatagirea, Picrorhiza kurroa, Nardostachys jatamansi, Arnebia euchroma, Valeriana jatamansi, and Angelica glauca comes under endangered species of this region. Some plants namely Dioscorea deltoidea and Berberis aristata are also under threats (Chauhan, 1999).

Plants like Picrorhiza kurroa, Aconitum heterophyllum and Valeriana jatamansi plays dominant role in the introduction of new therapeutic agents and also a drug from these plants continue to occupy an important niche in modern medicine. These medicinal plants contain phyto-constituents like alkaloids, tannins, saponins, steroid, terpenoid, flavonoids, phlobatannin, antioxidants and glycoside (Edeoga et al., 2005; Ayoola et al., 2008), which are responsible for higher medicinal values of these plants. Living organisms produce thousands of different organic compounds. Many of these have no defined function in the basic processes of growth and development and have been historically referred to as natural products or secondary metabolites.

The importance of natural products in medicine, agriculture and industry has led to numerous studies on the synthesis, biosynthesis and biological activities of these substances. Yet we still know comparatively little about their actual roles in nature. Such knowledge is especially lacking for terpenoids or isoprenoids, the largest group of natural products (Gershenson and Dudareva, 2007).

Taxol, derived from the bark of Taxus brevifolia, has been used to cure cancer of various types (Gibson et al., 1993). Picroliv, isolated from rhizome of Picrorhiza kurroa, showed hepatoprotective activity (Puri et al., 1992) and phenol glycoside androsin isolated from this plant was observed to be antiasthmatic (Dorsch et al., 1991). Many compounds used in today’s medicine have a complex structure and synthesizing these bioactive compounds chemically at a low price is not easy (Shimomura et al., 1997). To overcome this problem the herbal medicine is the best alternative to fight against the resistant pathogenic infections and there is a large demand for medicinal herbs all over the world for herbal formulations.
As common belief, all the bioactive phytocompounds are not safe because they have hidden risks just like all active chemical compounds. Research is still in progress to elucidate the side-effects, calculate appropriate dosages, identify the bioactive components and define the best methods of extraction. Besides these, legal aspects regarding the prescription and trade in medicinal plants are a matter of debate all around the world (Ahmad et al., 2006). The synergistic effect from the association of antibiotic and plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. Plant antimicrobials observed to be synergistic enhancers or bioenhancers in that though they may not have any antimicrobial properties alone, but when they are taken in combination with standard antibiotics they enhanced the effect of that drug (Kamatou et al., 2006). The phytochemicals in very low concentration in the complex mixture (oil and extract) can act as synergist (Hummelbrunner and Isman, 2001). Drug synergism between known antimicrobial agents and bioactive plant extracts is a novel and growing concept and has been reported by different research groups (Nascimento et al., 2000; Aburjal et al., 2001; Shimizu et al., 2001; Aqil et al., 2005; Junior et al., 2005). This process exists either by inhibiting the modified targets or exhibit a synergy by blocking one or more of the other targets in the metabolic pathway (Ge et al., 1999; Lambert, 2005; Zimmermann and Keith, 2007). Cyclosporine a powerful immunosuppressant (Laupacis, 1982), was observed to be synergistic in combination with fluconazole against Candida albicans, as cyclosporine alone was not antifungal (Marchetti et al., 2000). The compound (piperine) isolated from P. nigrum, in combination with rifampicin and isoniazid showed synergistic effect against M. tuberculosis, the new drug formulation against tuberculosis named as ‘resorine’ which contains reduced dose (200mg) of rifampicin + isoniazid (300mg) + piperine (10mg) (Chawla, 2010). However, the mechanism of plant extracts to potentiate antibiotics is not understood yet. In addition, the chemical diversity in plants with potential in improving the clinical efficacy of antibiotics still remains largely un-investigated.

1.3 ESSENTIAL OILS AND THEIR USES

Essential oils isolated from plants Ageratum conyzoides, Zingiber officinale and Cymbopogon citratus contain diverse and fragrant odors (Okigbo et al., 2009). The isolation of oils was done by gentle heating and later by steam distillation. The oil thus isolated was named as “essential oil”. The importance of these steam volatile oils gained significance in the sixteenth
century when the oils of anise, spike, cinnamon, clove etc., were used for medicinal purposes in various pharmaceuticals. The knowledge of using flowers, fruits, leaves, barks and roots of many plants containing essential oil is in practice since ancient times. Essential oils, which were once considered inactive waste products of plants metabolism and have no significant biological function, are now being realized for their importance as a means of chemical communication through which the plants protect themselves against competitors, predator and pathogens (Mahindru, 1992).

As many traditional medicinal plants are still to be explored, there are various opportunities for the discovery of novel modifying compounds for antibiotics. Our aim was to identify and characterize the plant derived compounds that exhibit synergism with commercial antibiotics against clinical pathogenic bacteria. The Himalayan biosphere is very rich in medicinal plants containing such compounds, source of potent bioactive enhancers for traditional antibiotics to fight against various pathogenic resistant microorganisms.

The Colebrookea oppositifolia (extracts and volatile oil) of Lamiaceae family was selected to pursue the study, as it was the only plant showing best synergistic effect among all the listed plants screened for synergism with erythromycin (Table 4.4) against E. coli and S. aureus. In traditional medicine, the leaves are used for the treatment of wounds and fracture and cataract by folklore (Pande et al., 2007). The leaves used to treat infertility and oil to treat fungal infections. The various phytochemicals present in Colebrookea oppositifolia are carbohydrates, glycosides, volatile oil, phenols, tannins, flavonoids, ethanol, saponins, proteins, gums, mucilage and amino acids (Madhavan et al., 2011). The oil of Lamiaceae family has been studied extensively for anti-inflammatory and antimicrobial activities (Paula-Freire, et al., 2013; Novak et al., 2002), thus volatile oil of C. oppositifolia was extracted to analyze the synergism with erythromycin and amoxyclyvate against S. aureus, E. coli, K. pneumonia, S. pyogenes, S. enterica, S. sonnei and S. epidermidis. The major terpenoids present in volatile oil of C. oppositifolia were analyzed to see synergistic effect with erythromycin and amoxyclyvate. The structural and function group modifications of combinations (antibiotics and terpenoids) were analyzed by FTIR (Fourier transform infrared spectroscopy), HPLC (High-performance liquid chromatography), GCMS (Gas chromatography–mass spectrometry), UV-visible spectrometry and NMR (Nuclear magnetic resonance).
1.4 AIMS AND OBJECTIVES

The aim of the present study was to identify and characterize the plant derived compounds that exhibit synergism with commercial antibiotics against clinical pathogenic bacteria. To achieve the aim, following objectives were set:

i) Screening of traditional medicinal plants of District Shimla and Solan, Himachal Pradesh, which modulates the antimicrobial effect of erythromycin and amoxyclyave.

ii) Characterization of “lead phytochemical” which modulates the antimicrobial activity of erythromycin and amoxyclyave.

iii) Interaction of the “lead phytochemical compounds” and the antibiotics by Fourier transform infrared spectroscopy (FTIR), High Performance Liquid Chromatography (HPLC), UV spectrophotometry and Gas liquid Mass spectrometry (GCMS).