CHAPTER - I

Introduction
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Medicinal plants have been used as a source of drugs to combat diseases for several thousands of years. Medicinal plants of India found clinical applications from the time of *Atharva Veda* (1500 BC). The classical system of medicines such as *Ayurveda*, *Siddha* and *Unani* include mostly the drugs derived from medicinal plants. Out of these *Ayurveda* and *Siddha* originated in India, while *Unani* system came from Persia. Among *Ayurveda* and *Siddha*, the former is more popular in India. It is our traditional system of medicine which has been in vogue for many centuries. The word *ayurveda* is derived from *Ayu*, meaning life and *Veda*, meaning knowledge. Thus, *Ayurveda* literally means science of life. *Ayurveda* takes a holistic view of man, his health and illness. It aims at positive health, which has been defined as a well balanced metabolism coupled with a healthy state of being.

This ancient science is a rich repository of knowledge about herbal drugs. *Charaka Samhita* (~900 BC) is the first recorded treatise which deals with *Kayachikitsa* (Sharma, S.P.1981). The next landmark in *Ayurvedic* literature is *Sushruta Samhita* (~600 BC), which has special emphasis on *Shalyatantra* i.e. surgery (Krishnamurthy, 1991). *Charak Samhita* and *Sushrut Samhita* are the main classics that give detailed description of over 700 herbs. The study of medicinal plants continued to progress after the classic *samhita* period as evident from the advanced plant science of "*Sarangadhara Samhita*" (14th century). *Ashtanga Hridaya, Madhava Nidana, Bhava Prakash*, and *Nighantu Granthas* such as *Raj Nighantu* and *Madanpala Nighantu* are
some of the other monumental treatises, which give some glimpses of ancient wisdom (Majumdar, 1971).

Ayurveda was most popular before the advent of modern medicine and still remains dominant particularly for treatment of a variety of chronic diseases (Waxler-Morrison, 1988). Diseases, according to Ayurveda, can arise from body and/or mind due to external factors or intrinsic causes (Sharma, 1979). Basic principles of Ayurveda are based on concepts of Panchamahabhoota, Tridosha, Rasa and Guna. According to Ayurvedic approach, anyone who has developed an imbalance in their bodily elements, or "Doshas" which are 'Vata', 'Pitta' and 'Kapha' and has thereby weakened their immune system, may be subject to a microbial infection which is considered a symptom of the imbalance. Ayurveda generally does not recognize microbes as the primary cause of disease. However, the words like 'Jantughna', 'Kramighna' do appear in ayurvedic literature pointing towards the antimicrobial approach to some degree.

On the other hand, in western allopathic medicine, microbes are believed to be the primary cause of many diseases. Such diseases are skin diseases, diseases of respiratory tract such as cold, cough and throat infections, diseases of digestive tract such as diarrhoea and dysentery, diseases of urinary tract, leucorrhoea, conjunctivitis, otitis media, dental caries, venereal diseases, malarial, leishmaniasis, AIDS, etc. Bacteria, fungi, protozoans and viruses are the four major groups of microorganisms held responsible for these infectious diseases and a variety of chemotherapeutic agents are employed for their treatment.
For bacterial infections a wide range of antibiotics of microbial origin and some other chemotherapeutic drugs are commonly used. Discovery of Penicillin by Sir Alexander Fleming, had opened a new era of antibiotic therapy, which had revolutionized the field of medicine. Scientist had started believing that the infectious diseases would be soon eradicated from this world. However, nature has given every living organism, a remarkable capacity to fight for the survival under unfavourable conditions and "survival of the fittest...", as proposed by Darwin, holds true for microorganisms too. With the introduction of newer antibiotics in clinical practice, resistance to them also evolves in microorganisms. This problem of development of drug resistance has become more critical especially in bacteria. Since past two decades, there have been many reports indicating the emergence of resistance in bacteria not only to single but multiple antibiotics (Levy, 1998; Harrison, 1998; Deshmukh et.al., 1999; Joshi and Musadiq, 2003; Nanoti et al., 2003, Wise, 2004; Deshpande et al., 2004).

Extensive and inappropriate use of antibiotics has augmented the emergence of drug resistance giving rise to alarming situations. In case of pneumonia, the number one killer among infectious diseases, as many as 70% of chest samples were shown resistant to first line antimicrobials. Since the newer treatments are unaffordable to the vast majority of those living in poor developing nations, the mortality rates due to this disease are very high. In 1998, 3.5 million people died as a result of this disease (WHO, 2000).

Multidrug resistance is also occuring in microbes that cause diarrhoeal diseases. Shigella dysenteriae, a highly resistant microbe, is becoming resistant to almost every available drug. Few years ago, Shigella
epidemic could be easily controlled with co-trimoxazole. Today, nearly all Shigella are non responsive to the drug and showing increasing resistance to Ciprofloxacin, the medication of choice. Vibrio cholerae and Salmonella typhi are also revealing acquired drug resistance. In case of typhoid, until, 1972 Chloramphenicol was the treatment of choice throughout much of the Indian subcontinent. By 1992 two third reported cases were resistant to Chloramphenicol. New strains of Salmonella typhi have emerged showing resistance to first line, second line and also third line drugs (WHO, 2000). In 1990, all cholera isolates from New Delhi were sensitive to cheap first line drugs Furazalidone, Ampicillin, Co-trimoxazole and Nalidixic acid. By 2000, these drugs became almost useless to contain cholera epidemics.

Tuberculosis is yet another ancient killer which is becoming increasingly resistant to anti-TB drugs. Although percentage of multidrug resistant TB (MDR-TB) cases is low (1.60% - 2%), the picture is of great concern considering the respiratory mode of transmission of the disease and the ability of HIV to accelerate the onset of acute MDR-TB.

Drug resistance is an especially difficult problem for hospitals since the critically ill admitted patients are more vulnerable to infections. At the same time, organisms from hospital get constant exposure to various antibiotics which is a factor for selection and proliferation of drug resistant strains. Methicillin resistant Staphylococcus aureus and Vancomycin resistant Enterococcus are wreaking havoc in hospital wards around the world. In U. S. alone, there are 90,000 deaths a year due to hospital acquired infections (NIAID Fact Sheet, 2004).
Besides bacterial infections, infections induced by pathogenic fungi are increasingly recognized as emerging threat to public health (Wu, 1994; Walsh et al., 1996). The incidence of opportunistic fungal infections in immunocompromised hosts, including AIDS patients, has increased dramatically in recent years (Anaissie, 1992; Sharma et al., 1997). Opportunistic yeasts including *Candida* and *Cryptococcus* and dermatophytes are some of the more common fungal pathogens. Antimycotic drugs commonly employed for the management of mycoses include polyene antibiotics such as Amphotersin B, and Nystatin, azole derivatives such as Fluconazole and Ketoconazole, allylamines such as Terbinafine and Naftifine and fluoropyrimidines such as Flucytosine.

Like drug resistance in bacteria, a number of reports indicate the development of resistance to many antifungal agents. Fatal disseminated candidiasis due to Amphotericin-B-resistant *Candida guilliermondii* (Dick, 1985), fatal septicemia due to Amphotericin-B-resistant *Candida lusitaniae* (Guinet et al., 1983), emergence of 5-fluorocytocine resistance in *Cryptococcus neoformans* and *Candida albicans* (Shadomy, 1997), development of resistance to azoles in *Candida species* (Odds, 1993; Rex et al., 1995) etc. are some of the examples emphasizing the need to find out the ways to manage the drug resistance in fungi.

Drug resistance is also developing in malerial parasites. Resistance to chloroquine is widespread in 80% of the countries where malaria continues to be a major killer. Similar is the picture with drug resistance in *Leishmania* to heavy metal based, highly toxic antimonials (WHO, 2000).
The continuously developing drug resistance in bacteria, fungi and other microorganisms to the currently available drugs has necessitated search for new antimicrobial compounds having novel mechanisms of action.

Besides the problem of drug resistance, a major problem related with the currently available drugs is serious adverse side effects of many of these drugs. To mention a few, Bacitracin, Gentamicin, Methicillin, Polymyxin B, Cephalosporin, Streptomycin, Sulfonamides, Tetracyclin, Trimethoprim and Amphotericin B. causes renal damage; Erythromycin, Isoniazid, Rifampicin, Tetracyclin and Sulfonamides cause hepatic injury; Trimethoprim and Flurocytosine induce leucopenia; Chloramphenicol adversely affects bone marrow function; Amphotericin B cause convulsions and cardiac arrest and Polymyxin B is neurotoxic. Gastrointestinal upset, allergic response, nausea and vomiting are some of the more common side effects associated with many of the drugs (Modi et. al., 1995).

Many of the currently available drugs, especially the newer ones, are associated with one more major drawback and that is their unaffordable cost particularly for the economically poor class. The importance of cost factor can be highlightened by example of tuberculosis treatment. Currently, a single treatment course of six months for regular tuberculosis costs as little as US$ 20. With multidrug resistant TB (MDR-TB) the costs shoot upward to US$ 2000 or even more (WHO, 2000). Inadequate treatment owing to this cost factor corresponds to growing transmission rates of resistant TB organisms. This has serious implications for the humanity in the light of the fact that HIV can accelerate the onset of acute MDR-TB.
In view of all the above factors associated with the currently available antimicrobial drugs, development of new antimicrobial agents is urgently required along with some other measures to contain developing antimicrobial resistance and spread of infectious diseases. Otherwise it is being predicted that infectious diseases may become one of the main scourges of mankind in the near future (Sukh Dev, 1997).

Mainly three approaches are being used for the discovery of new therapeutic agents: pharmacological screening of compounds of natural or synthetic origin, probing human (and mammalian) biochemistry and physiology and rational drug design. Pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents and many of the currently available antibiotics are the products of such screening programmes (Kroschwitz et al., 1992). The second approach i.e. of probing human / mammalian biochemistry has led to the discovery of vitamins and hormones including insulin which have proved valuable in correcting several deficiency diseases. The third approach that is rational drug design or the structure based drug design requires the knowledge of molecular biology and techniques such as X-ray crystallography and high field multidimensional NMR spectroscopy. With these techniques, three dimensional structure of the complexes of enzyme, receptor or antibodies with their ligands is studied and with the help of computational and graphical tools novel design of the drug is suggested suiting the needs (Gupta, 1994). This approach has been used to design small molecules likely to bind specific receptor proteins or inhibit a particular enzymatic transformation involved in the pathogenesis of disease (Erickson and Fesic, 1992). Recently this approach has been utilized in
designing effective drugs against AIDS and certain types of cancers (Sukh Dev, 1997).

Thus out of the above three approaches, the first approach of screening is very important, as far as the discovery of antimicrobial agents is concerned. Many of the antimicrobial compounds have been discovered utilizing random screening. Novel compounds with structural complexities can only be discovered by natural product screening and are unlikely to be rationally designed (Singh et al., 2001). Moreover natural products screening and rational drug design are not mutually exclusive. The later can be used for second generation of drugs, where powerful analogues of the natural active compounds can be created based on structure activity information.

For biological screening of the natural compounds, plants represent an unparalleled source of molecular diversity. Nature has engineered plants to produce innumerable secondary metabolites with unimaginable features. Hence it is worthwhile to explore the potential of plants for the development of new therapeutic agents. In this context, the contribution of higher plants for the introduction of new therapeutic agents is worth mentioning. Globally, at least 130 drugs, all single chemical entities extracted from higher plants or modified further synthetically are currently in use (Singh et al., 2001).

However the number of new chemical entities (NCE) emerging as therapeutic agents from higher plants has been rather low. During 1971 to 1990 period over 600 NCEs were launched world wide but the number of plant based drugs was not even 2% and out of 200 NCEs launched globally in the next five years, the plant based NCEs were not more than 2% (Sukh Dev, 1997). This indicates that efforts have to be intensified for new lead generation
For drug discovery from plants, four general strategies are employed: random biological screening, screening based on chemotaxonomic considerations, screening based on ethnotherapeutics and screening based on traditional systems of medicine. Random biological screening involves random search for biological activity and subsequent isolation of bioactive agents. This strategy has been followed by National Cancer Institute, USA, Central Drug Research Institute, Lucknow and CIBA-GIEGY Research Centre, Mumbai, but has not been very productive. Furthermore it is labour intensive. The second strategy that is chemotaxonomic consideration is useful once the occurrence of a bioactive agent is identified in a plant of a particular family. The third strategy involves the prospection of plant resources based on the knowledge of plants by tribals. Recently more importance is being given to screen the plants on the basis of accumulated information obtained from 'Ethnobiology'. A number of organisations such as Foundation of Ethnobiology (FEB) in UK, International Society of Ethnobiology (HQ America), the All India Co-ordinated Research Project on Ethnobiology (AICRPE) have been set up for the investigations concerned with this resource (Singh et. al., 2001). The fourth strategy of screening the plants based on their medicinal usage in traditional system of medicine is being utilized not only in India but all over the world. In this context, along with Ayurveda, Chinese traditional system deserves special mention. It is called as Zhong Yau in Chinese and Kampo in Japanese and has been in practice since some 2000 years ago in China, Japan and other far Eastern countries. Intense modern scrutiny of the Chinese drugs have yielded outstanding results confirming many claims of ancients (Sukh Dev, 1997).
Ayurveda, our traditional system of medicine, which was scientifically organised discipline in its prime time, is very much alive system widely practised even today. In recent years it has been attracting much attention in the economically developed countries such as Europe, USA and Japan (Harfzell and Zysk, 1995). Ayurvedic materia medica holds a great potential for modern drug development. There are a few artilals throwing light on the role of Ayurveda in discovery of novel drugs (Patwardhan and Hooper 1992; Sukh Dev, 1997; Valiathan, 1998; Patwardhan, 2000; Mallavarapu, 2001; Patwardhan et. al., 2004). Especially the importance of this subject is beautifully highlighted in the artical by Sukh Dev. Ayurvedic experiential data base has been the starting point for the discovery of numerous molecules which include rauwolfia alkaloids for hypertension, psoralens in vitiligo, holarrhena alkaloids in amoebiasis, guggulsterors as hypolipidimic agents, curcumin in inflammation etc. (Patwardhan, 2000). Moreover many leads have been obtained which are in the late stages of their clinical trials.

Thus the strength of knowledge data base of Ayurveda combined with the dramatic power of technological advances in modern science could be explored for developing novel therapeutic agents. Since ayurvedic medicines have been effectively tested for thousands of years on people, their non-toxic character comes as a presumption. Moreover ayurvedic materia medica clearly mentions the medicinal and poisonous properties of plants. This has the advantage that right in the initial stages, drug development can be started from safe and time tested materials. Secondly if ayurvedic medicines (even in their crude form) gain support from modern scientific investigations, they could be introduced in regular clinical practice of western medicine thereby allowing
to capitalize this resource. This factor is extremely important especially for our country where an enormous wealth of medicinal plants is present.

Considering all the above factors intensive efforts are being focused for the development of therapeutic agents from plants including antimicrobials. Expectations from plants for the development of antimicrobial agents are based on the assumption that plants, having co-evolved with bacteria and fungi by parasitic and symbiotic relationships, must possess chemicals active against a myriad of microorganisms (Sushil Kumar, 1997). This assumption is valid since plants do possess a variety of phytochemicals such as alkaloids, flavons, terpenes, tannins and phenolic derivatives accounting for their constitutive antimicrobial activity (Mansfield, 1983). Plants also produce antimicrobial phytochemicals in response to stimuli from microbial infections (Dixon et al., 1983). These factors have triggered the search of antimicrobial agents from plants and consequently there have been innumerable reports demonstrating antimicrobial activity in plant extracts (Choudhari, 1996; Shrivastava et al., 2000). However, in spite of tremendous efforts in this direction presently there is no plant derived compound which is at par with the currently available and clinically used antibiotics (Shrivastava et al., 2000).

However research in this direction can generate interesting outcomes that can be utilized in different ways for the treatment of diseases due to drug resistant microorganisms and for containment of ever increasing drug resistance in these organisms. Firstly if new molecules with broad spectrum antimicrobial activity are discovered, they could be directly employed for the treatment of infections due to multidrug resistant refractory strains. Secondly if ayurvedic drugs, with single or multiple components (and not necessarily
pure compounds), gain support from investigations of modern science, they could be utilized commonly to reduce unnecessary use of currently effective antibiotics. This would reserve these antibiotics for emergency. One more approach would be to exploit the active phytocompounds for the combination therapy of infectious diseases as a measure to control development of antibiotic resistance. Thus plant based antimicrobial agents can provide a solution to the existing problem of drug resistance. Their additional advantages would be non-toxicity for host tissues and affordable cost.

With all this background the present work was undertaken to study the antimicrobial activities of medicinal plants. The subject became more fascinating on considering the distribution of forest area in and around the Akola district. Akola district is the northern district of Maharashtra situated in the western part of Vidarbha region. It is surrounded by Amravati, Buldhana and Washim districts. Its area is 5429 Sq.Km. Forest region of this district covers 396.59 Sq.Km. area. The majority of the important forests fall under the type "Southern Tropical Dry Deciduous Forests". Forests of Alegaon range covering Alegaon, Malsood, Chikhhalwal, and Chondi rounds, Akola range covering Bordi (Akot) and Lohgadha rounds and Patur range which includes Malrajura and Patur rounds (out of 4 rounds) come under Akola Forest Division. Part of the Melghat area of Amravati district is present adjoining the boundary of Akot round, which includes Khatkali, Dargadh, Gullarghat and Somthana. A large number of medicinal plants belonging to more than fifty different families inhabit these forests. A concise information booklet (Technical notification No. 5, for private circulation) on medicinal plants of Melghat by R. B. Giri, Range Forest Officer, Chikhaldara, is available in forest department
which deals with the medicinal uses of many of the medicinal plants found in this region.

Studies on the medicinal properties of these plants are both of commercial and social significance. If proper avenues are found to utilize this botanical resource without harming the nature, this could be harnessed to commercial benefits leading to development of tribal regions. Thus concentrated efforts are required to reap its commercial and social advantage.

As an initial step towards these long term goals the present work was undertaken to study the antimicrobial activities of the medicinal plants found in and around Akola District with following aims and objectives.

1) Considering the increasing emergence of drug resistance in bacteria to currently available antibiotics coupled with the problems of their side effects and high cost, development of novel antibacterial drugs is urgently required. As a preliminary step towards this direction, the present work was undertaken with the objective to screen higher plants for the presence of antibacterial activity.

2) Plant extracts showing significant antibacterial activity would have prospects as future candidates for development of antibacterial agents. For deciding the possibility of their future use the assessment of their antibacterial potential is required. For this purpose the next objective was to assay the antibacterial activity of the extracts. On this basis extracts showing potent antibacterial actions could be selected for further investigations.
3) Presently the development of resistance in fungal pathogens to currently available antifungal drugs and high toxicity of some of the effective drugs are the two major problems associated with the chemotherapy of fungal infections. Therefore development of new antifungal agents is also a present day need. The present studies were, therefore, undertaken as a primary step for evaluation of plants for their antifungal potential.

4) Phytochemical analysis of the bioactive extracts is the basic requirement for the study of medicinal properties of plants. Hence phytochemical studies were proposed to be carried out to lay down the basis for further detailed investigations in future.

5) Ayurvedic materia medica is a treasure of knowledge about herbal drugs and it holds a great potential for modern drug development. The approach of the present work was taken with an objective to check the validity of the alleged claims of Ayurveda as per the principles of modern science.

6) Although there have been few studies dealing with the botanical aspects of the medicinal plants of Melghat region, extensive antimicrobial studies have not been reported so far. For this reason, it was decided to carry out the investigations on the plants that are commonly found in and around Akola district. This area covers some part of Melghat area adjoining Bordi (Akot) round of Akola Forest Division.