Chapter – 1

INTRODUCTION

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1.5 Aims and objectives
Plants are one of the most important sources of medicine. Plant derived compounds (phytochemicals) have been attracting much interest as natural alternatives to synthetic compounds. Extracts of plants were used for the treatment of various diseases and this forms the basis for all Indian systems of medicine. However, this area is not much developed when compared to modern system of medicine, mainly because of the lack of proper scientific substantiation in this field. Mostly the pharmacological activity of medicinal plants resides in its secondary metabolites which are comparatively smaller molecules in contrast to the primary molecules such as proteins, carbohydrates and lipids. These natural products provide clues to synthesize new structural types of anti-microbial and antifungal chemicals that are relatively safe to man and it can help to meet expensive and limited supply of synthetic chemicals. The main advantage of plant products over the synthetic compounds in the treatment of diseases is that it is seen in the eukaryotic system and so it will not have a deleterious effect in higher plants and animals including man (Krishankumar et al, 1997).

“A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs.” (Sofowora, 1993). This definition of medicinal plant has been formulated by WHO (World Health Organization). The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as “Medicinal Plants”. It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties.

India constitutes a unique and enormous diversity of flora and fauna within a relatively small geographical area due to variations in topography, altitude and
climate. In spite of being a diverse country and India is known to be veritable treasure trove of medicinal plants (Phoboo et al., 2008). Most of the wild floras of India are rich in medicinal and aromatic properties like antibacterial, antiviral, antihelminitic, anticancer, sedative, laxative, cardiotonic, diuretic and others. They are important sources of bio-molecules, with application for the manufacture of pharmaceuticals and cosmeceuticals (Heinrich and Gibbons, 2001).

People have used medicinal plants in health care since the time of earliest human evolution. These are the major sources of medication for a wide range of ailments for the rural people of India. More than 75% Indians still depends on the herbal plants as a local source of medicine. Local healers use various medicinal plants for primary health care (Devkota, 2001). Different type of bacterial infections such as dysentery, diarrhea, fever, cough, bleeding, burning etc is treated using plant extracts.

India is a land of rich biodiversity. The total number of lower and higher plants in India is about 45,000 species (Jain, 1992). The plants are potential source of medicines since ancient times. According to World Health Organization, 80% of the populations in the world depend on traditional medical practitioners for their medicinal needs. Many formulations of plants and their products such as medicines are said in the form of hymns in the Vedas (Kausik and Dhiman, 1997). Yet a scientific study of plant to determine their anti-microbial material is comparatively new. Numerous surveys on anti-microbial medicinal plants had been made in United States and in many countries throughout the world. Such study had demonstrated the wide occurrences of active compounds in higher plants (Hughes, Pates and Madsen, 1995).
As the plants represent an extraordinary reservoir for exploration of new drugs in the battle of disease, the present study has been planned to find out the antimicrobial potential in the extracts of five plants, viz. *Lallemantia royleana*, *Phyllanthus maderaspatensis*, *Plantago ovata*, *Rosa indica* and *Solanum nigrum* that are commonly found in India. Some drugs of plant origin in conventional medical practice are not pure compounds but direct extracts or plant materials that have been suitably prepared and standardized. Recently the World Health Organization (WHO) has recommended the use of Artemisinin derivatives derived from *Artemisia annua* (Composite), a Chinese herb, as a first line drug in the treatment of malaria. This is as a result of WHO’s recognition that 80% of world population use herbal medicine for some aspect of Primary Health Care.

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality. From 1980 to 1990, Montelli and Levy documented a high incidence of resistant microorganisms in clinical microbiology in Brazil. This fact has also been verified in other clinics around all over world.

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

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in Brazil. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency.

The use of plant extracts and phytochemicals, both with known anti-microbial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their anti-microbial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils, as well as in tannin. The anti-microbial properties of plants have been investigated by a number of researchers worldwide.

Research in herbal medicine and isolated drug discovery needs to be continued, considering the threat of new emerging disease such as SARS, bird flu, not to mention the killer HIV AIDS. Plants are a good source of herbal medicine and natural products/phytochemicals. Many synthetic drugs owe their discovery and potency as a result of a mimic of structures from natural products isolated from
plants rather than to the creativity and imagination of contemporary organic chemists. For example, the drug taxol (a diterpenoid), first isolated from the bark of the yew tree *Taxus brevifolia* has yielded two approved drugs for breast and ovarian cancer. In Guyana, there are many medicinal folklore practices but most are without scientific research. For example, drinking water extract of *Momordica charantia* is a good remedy for diabetes. Thus, there exists an urgent need to correlate folklore herbal practices with scientific evidences. With an increasing emphasis on scientific research, *Guyana* stands well in this area. Its our scientific endeavour, to correlate anti-microbial activity of *Momordica charantia* with its folklore practices.

A rich heritage of knowledge to preventive and curative medicines was available in ancient scholastic works included in the Atharva veda, Charaka, Sushruta etc. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry (Stuffness and Douros, 1982; Baker *et al* 1995; Ripa *et al* 2009). Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. The characteristics of the plants that inhibit microorganisms and are important for human health have been researched in laboratories since 1926 (Ates, 2009).

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. In our country we are using crude plants as medicine since Vedic period. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources (Srivastava *et al*, 1996). Nowadays, multiple drug resistance has developed due to the indiscriminate use of commercial anti-microbial drugs commonly used in the treatment of infectious
disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune suppression and allergic reactions (Ahmad et al., 1998). This situation forced scientists to search for new anti-microbial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative anti-microbial drugs for the treatment of infectious diseases from medicinal plants. Several screening studies have been carried out in different parts of the world. There are several reports on the anti-microbial activity of different herbal extracts in different regions of the world.

Worldwide, infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing in developed countries, such as the United States. Herbal drugs have become increasingly popular and their use is widespread. Clear-cut proof of their efficacy in microorganisms inducing pathogenesis is yet to be explored. Various medicinal plants have been used for years in daily life to treat disease all over the world.

Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982) and natural products play an important role in drug development programs in the pharmaceutical industry (Baker et al., 1995). It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, anti-tumoral and anti-microbial agents. The use of plant extract for their anti-microbial action has been subjected of research in India.
by some workers. The plant extracts of *Aegle marmelos*, *Mormordica indica*, *Calotropis procera*, *Catharanthus roseus* and *Azadirachta indica*. *Tamarindus indica*, *Zingiber officinales*, *Curcuma longa*, *Allium sativum* (Rao and Satyanarayan, 1977) have shown profound antibacterial activities.

In less developed states of India and particularly in West Bengal, low income people such as farmers, people of small isolate villages and native communities use herbal medicine for the treatment of common infections. It is necessary to evaluate, in a scientific base, the potential use of herbal medicine for the treatment of infectious diseases produced by common pathogens. In the present study we have chosen some plants used in herbal medicine to determine their antibacterial property. Evidently, there are not sufficient scientific studies that confirm the antimicrobial activity of these plants. This study looks into the *in vitro* anti-microbial activity of these plants against some gram positive and gram negative pathogenic microorganisms.

Plants naturally synthesize several carbon compounds, basically for physiologic functions or for use as chemical weapons against disease organisms, insects and predators. The investigation of plants for bioactive secondary metabolites is an area which most plant scientists have recently focused with an aim of discovering new clinically useful and commercially important plant products. It is estimated that 70-80% of all over the world largely depend on traditional herbal medicine to meet their primary health care needs (Hamayun *et al.*, 2006). The global demand for herbal medicine is growing. While plant species used in traditional medicines continue to be reliable sources for discovery of useful compounds, screening of plants growing under various environmental conditions could provide another source for compounds with anti-microbial activities. Biological and pharmacological activities of phytochemical compounds take into account different
parameters and factors such as species, ecological factors and environmental conditions. Thus, each plant species will present a profile which it will express differently among these factors. Phenological age of the plant, percent humidity of the harvested material and method of extraction are possible sources of variation for the chemical composition, toxicity and bioactivity of the extracts (Rajakaruna et al., 2002). There is a wide variation in the susceptibility of organisms to toxic compounds. It is probable that a large number of plants with biological activities remain untested.

Aspilia plant (Aspilia mossambicensis) is widely spread in south, south west and west of Kenya from coast to Lake Victoria. Due to diversity of ecological conditions, the chemical composition of the plants is known to vary and due to this reason plants have been used to treat different diseases in different places (Njoroge and Newton, 1994).

The plant belongs to the family of Compositae (Asteraceae) and genus Aspilia. There are reports that the plant possess antimalarial activity against Plasmodium falciparum, galactogogue activity and is used to alleviate menstrual cramps (Offulla et al., 1996). Literature search indicate that this plant has been used traditionally by many African communities to treat several diseases (Kokwaro, 1976; Johns et al., 1990), such as cystitis and gonorrhea, abdominal pains, backache and the root decoction is normally given to breast feeding mothers to increase milk production. Pounded leaves are rubbed on the skin and freshly cut wounds for faster healing of wounds and ringworms. Pounded leaves decoction is drunk in order to treat intestinal worms including hookworms. Among the Kamba community of Kenya, pounded leaves decoction is applied to circumcised young men for faster healing of the wounds (Musyimi, personal communication). Some
phytochemical constituents of this plant have been reported to have medicinal or antimalarial properties (Kokwaro, 1976; Offulla et al., 1996).

1.1 Anti-microbials
The use of anti-microbial agents is critical to successful treatment of infectious diseases. Although there are numerous classes of drugs that are routinely used to treat infections in humans, there are several reasons why the discovery and development of new anti-microbial agents are important. Over the past decade there has been an increased development of resistance in organisms that are typical pathogens in humans. These include methicillin/oxacillin-resistant *Staphylococcus aureus*, vancomycin-resistant and intermediate *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, gram-negative bacilli that produce extended spectrum betalactamases, carbapenem-resistant *Klebsiella pneumonia*, and *Pseudomonas* and *Acinetobacter* strains that are resistant to all antibiotics that are typically used for treatment. The increased resistance has limited the selection of anti-microbials that may be used to treat specific organisms. New anti-microbials are also needed for certain groups of organisms. Very limited numbers of anti-microbials are available to treat infections caused by fungi and mycobacteria. Infections with these organisms continue to be a major concern.

Chemotherapy for cancer treatment, immunosuppressive drugs for treatment of autoimmune diseases and organ transplant recipients, and infections (such as AIDS) that alter the effectiveness of the host immune system render individuals at high risk for fungal infections and certain mycobacterial infections. Often these infections are caused by environmental organisms that would not typically cause disease in a normal host. There are increased numbers of reports of multi-drug resistant mycobacteria in the United States and throughout the world. Another complicating factor that reduces the effectiveness of treatment of these organisms
is non-compliance in completing treatment regimens. Extended length of treatment (sometimes up to one year) and adverse side effects of the drugs used for treatment contribute to the lack of compliance. Although many infectious diseases have been known for thousands of years, over the past 30 years a number of new infectious diseases have been discovered. Some examples include Lyme Disease caused by *Borrelia burgdorferi*, Legionnaires’ Disease caused by *Legionella pneumophila*, peptic ulcers caused by *Helicobacter pylori*, antibiotic associated diarrhea caused by *Clostridium difficile*, and AIDS caused by Human Immunodeficiency Virus. In addition, microorganisms are constantly changing, finding new places to live and new ways to survive, and adapting to new situations.

During this process, harmless organisms may turn deadly or deadly strains may move from their normal host to humans. With the continuing discovery of new infectious diseases and the development of new disease processes of existing pathogens (i.e., necrotizing fasciitis caused by *Streptococcus pyogenes*), it is important to continue to find anti-infective agents that can be used to treat these infections. Development of novel classes of drugs, drugs with fewer side effects, and drugs with shorter lengths of treatment are key in continuing the fight against infectious disease.

Plants produce substances that help protect them from microorganisms, herbivores, competing plants, and aide in reproduction. They also produce chemicals for offensive chemical warfare targeting cell proliferation of pathogens. These chemicals may have general or specific activity against key target sites in bacteria, fungi, viruses. The general belief that the advent of antibiotics will bring end to the occurrence of infectious diseases was cut short with the occurrence of resistance to anti-microbial drug. The incidence and increasing frequency of microorganisms that are resistant to common and generally accepted effective first choice drugs is
on the increase. The development of resistance to the newer antibiotics by the microbes causing most of the infectious diseases with debilitating effects made the case worse. Resistance to penicillin by *S. aureus* was first reported in 1942 and by 1960, more than 80% of both community – and hospital- acquired staphylococcal isolates were resistant to penicillin. The rate of resistance to these drugs is higher in developing countries when compared with developed countries. This may be due to the indiscriminate use of antibiotics and also self medications without prescription by physician. Furthermore, the use of antibiotics in animal feeds may induce resistance. As this resistivity increases, the need for newer and/or alternative therapy becomes very necessary.

Despite the remarkable progress made in chemistry, pharmacology, molecular biology, genome research and high throughput screening, the new chemical entities (NCE) pipeline of pharmaceutical companies are at historically low. This is majorly due to the development of microbial resistance against antibiotics. Modern medicine provides an impressive array of antibiotics and other drugs that ensure cure but none of which are completely non-toxic and safe for human consumption. A 40% increase in the research and development spending in pharmaceutical research from 1996-2001 did not overcome this problem. However many experts believe that the majority of plant derived natural products possibly valued at billions of dollars–remain undiscovered or unexplored for their novel pharmacological activities (Cragg and Newmann, 2005). Some other experts believe that medicinal plants are losing importance in areas like traditional drug discovery process but making comeback in several other areas of human health (*i.e.*, functional foods, dietary supplements and recombinant protein manufacturing). The growth in popularity and use of neutraceuticals and medicinal products from plants has taken a very large share of health care market.
Most microbiologists distinguish two groups of anti-microbial agents used in the treatment of infectious disease, one is antibiotics, natural substances produced by certain groups of microorganisms, and other is chemotherapeutic agents that are chemically synthesized. Some anti-microbial compounds, originally discovered as products of microorganisms, can be synthesized entirely by chemical method. In the medical and pharmaceutical worlds, all these anti-microbial agents used in the treatment of disease are referred to as antibiotics, which can be sub-distinguished into antibacterial and antifungal agents, interpreting the word literally.

**Anti-bacterial agents**

The antibiotic agents that kills or inhibits the growth of bacteria is said to be antibacterial. The antibacterials can be classified into two categories based on their action:

- Bacteriocidal agents act primarily by killing bacteria.
- Bacteriostatic agents act primarily by arresting bacterial growth.

The action of antibacterial agent is dependent on selective toxicity ie the drug is toxic but only to target organisms. It exploits the differences between human cells and those of bacteria. The most striking difference is that bacteria have cell walls as well as a cell membrane while human cells only have a cell membrane. The cell wall is the principal target of b lactam antibiotics. The other principal targets are intracellular. As a result the effectiveness of those antibiotics which act at these sites is dependent on their ability to penetrate the cell. Aminoglycosides, for example, have to be actively transported across the bacterial cell membrane. Glycoproteins (eg vancomycin, teicoplanin) are unable to penetrate the outer membrane of gram-negative organisms and thus have restricted activity against these organisms.
**Anti-fungal agents**

Antifungal agents are used to treat fungal infections. The antifungal agents owe their fungicidal activities to inhibition of synthesis of or direct interaction with ergosterol. Ergosterol is the predominant component of the fungal cell membrane (Parks, L. W., and W. M. Casey. 1996). Unlike bacteria, both fungi and humans are eukaryotes. Thus fungal and human cells are similar at the molecular level. This makes it more difficult to find or design drugs that target fungi without affecting human cells. Consequently, many antifungal drugs cause side-effects. Some of these side-effects can be life-threatening if the drugs are not used properly.

**Non-pharmaceutical anti-microbials**

A wide range of chemical and natural compounds are used as anti-microbials. Organic acids are used widely as anti-microbials in food products, e.g. lactic acid, citric acid, acetic acid, and their salts, either as ingredients, or as disinfectants. For example, beef carcasses often are sprayed with acids, and then rinsed or steamed, to reduce the prevalence of E. coli O157:H7.

Traditional healers long have used plants to prevent or cure infectious disease. Many of these plants have been investigated scientifically for anti-microbial activity, and a large number of plant products have been shown to inhibit the growth of pathogenic microorganisms. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal. So, it is worthwhile to study plants and plant products for activity against resistant bacteria.
1.2 Brief History of Anti-microbials

The traditional definition of medicinal plants is given in *Ashtaanga Hrdaya* (600 AD) *Sutra sthana* Ch. 9 - verse 10 as:

\[
\text{jagatyevam anoushadham} \\
\text{na kinchit vidyate dravyam} \\
\text{vashaanmaarthayagayoh'}
\]

"There is nothing in this universe, which is non-medicinal, which cannot be made use of for many purposes and by many modes."

The use of plants for medicinal purposes is as old as human civilization. The first known written record of curative plants was of Sumerian herbal of 2200 BC. In the 5th century BC, The Greek doctor Hippocrates list out some 400 herbs in common use. Dioscorides, in the 1st century AD, wrote a herbal by using 600 plants which ultimately became the base for many later works.

The study of herbs dates back over 5,000 years to the Sumerians, who described well-established medicinal uses for such plants as laurel, caraway, and thyme. Ancient Egyptian medicine of 1000 B.C. are known to have used garlic, opium, castor oil, coriander, mint, indigo, and other herbs for medicine and the Old Testament also mentions herb use and cultivation, including mandrake, vetch, caraway, wheat, barley, and rye.

The Shennong Bencao Jing, the first Chinese herbal book, was compiled during the Han Dynasty but dating back to a much earlier date, possibly 2700 B.C., where 365 medicinal plants and their uses are given. These plants include ma-Huang, a shrub that introduced the drug ephedrine to modern medicine.
The ancient Greeks and Romans also used medicinal plants. Greek and Roman use of medicinal plants are as preserved in the writings of Hippocrates and Galen which also provides the pattern for later western medicine. Hippocrates advocated the use of a few simple herbal drugs - along with fresh air, rest, and proper diet, while Galen, recommended large doses of drug mixtures - including plant, animal, and mineral ingredients. The Greek physician compiled the first European treatise on the properties and uses of medicinal plants, De Materia Medica. In the first century AD, Dioscorides wrote a compendium of more than 500 plants that remained an authoritative reference into the late 17th century. Similarly important for herbalists and botanists of later centuries was the Greek book that founded the science of botany, Theophrastus’ Historia Plantarum, written in the fourth century B.C. Thyme from Project Gutenberg EBook of Culinary Herbs: Their Cultivation Harvesting Curing and Uses, by M. G. Kains

The uses of plants for medicine and other purposes changed little in early medieval Europe. Many Greek and Roman writings on medicine, as on other subjects, were preserved by hand copying of manuscripts in monasteries. The monasteries thus tended to become local centers of medical knowledge, and their herb gardens provided the raw materials for simple treatment of common disorders. At the same time, folk medicine in the home and village continues uninterrupted, supporting numerous wandering and settled herbalists. Among these were the “wise-women,” who prescribed herbal remedies often along with spells and enchantments. It was not until the late Middle Ages that women who were knowledgeable in herb lore became the targets of the witch hysteria. One of the most famous women in the herbal tradition was Hildegard of Bingen. A twelfth century Benedictine nun, she wrote a medical text called Causes and Cures.
Medical schools known as Bimaristan began to appear from the 9th century in the medieval Islamic world, which was generally more advanced than medieval Europe at the time. The Arabs venerated Greco-Roman culture and learning, and translated tens of thousands of texts into Arabic for further study. As a trading culture, the Arab travellers had access to plant material from distant places such as China and India. Herbals, medical texts and translations of the classics of antiquity filtered in from east and west (Manual of Pharmaceutics and Alchemy by Ibn al-Baytar). Muslim botanists and Muslim physicians significantly expanded on the earlier knowledge of materia medica. For example, al-Dinawari described more than 637 plant drugs in the 9th century (Morelon & Rashed, 1996) and Ibn al-Baitar described more than 1,400 different plants, foods and drugs, over 300 of which were his own original discoveries, in the 13th century (Diane Boulanger, 2002). The experimental scientific method was introduced into the field of materia medica in the 13th century by the Andalusian-Arab botanist Abu al-Abbas al-Nabati, the teacher of Ibn al-Baitar. Al-Nabati introduced empirical techniques in the testing, description and identification of numerous materia medica, and he separated unverified reports from those supported by actual tests and observations. This allowed the study of materia medica to evolve into the science of pharmacology (Huff Toby, 2003).

Avicenna's The Canon of Medicine (1025) is considered the first pharmacopoeia and lists 800 tested drugs, plants and minerals. Book Two is devoted to a discussion of the healing properties of herbs, including nutmeg, senna, sandalwood, rhubarb, myrrh, cinammon, and rosewater. Baghdad was an important center for Arab herbalism, as was Al-Andalus between 800 and 1400. Abulcasis (936-1013) of Cordoba authored The Book of Simples, an important source for later European herbals, while Ibn al-Baitar (1197–1248) of Malaga
authored the Corpus of Simples, the most complete Arab herbal which introduced 200 new healing herbs, including tamarind, aconite, and nux vomica (Castleman, Michael, 2001 and Kasem Ajram, 1992). Other pharmacopoeia books include that written by Abu-Rayhan Biruni in the 11th century and Ibn Zuhr (Avenzoar) in the 12th century (and printed in 1491). The origins of clinical pharmacology also date back to the Middle Ages in Avicenna's The Canon of Medicine, Peter of Spain's Commentary on Isaac, and John of St Amand's Commentary on the Antedotary of Nicholas. In particular, the Canon introduced clinical trials, randomized controlled trials, and efficacy tests (D. Craig Brater and Walter J. Daly, 2000).

Alongside the university system, folk medicine continued to thrive. The continuing importance of herbs for the centuries following the Middle Ages is indicated by the hundreds of herbals published after the invention of printing in the fifteenth century. Theophrastus’ Historia Plantarum was one of the first books to be printed, but Dioscorides’ De Materia Medica, Avicenna's Canon of Medicine and Avenzoar's pharmacopoeia were not far behind. Marjoram from Project Gutenberg EBook of Culinary Herbs: Their Cultivation Harvesting Curing and Uses, by M. G. Kains.

The fifteenth, sixteenth, and seventeenth centuries were the great age of herbals, many of them available for the first time in English and other languages rather than Latin or Greek. The first herbal to be published in English was the anonymous Grete Herball of 1526. The two best-known herbals in English were The Herball or General History of Plants (1597) by John Gerard and The English Physician Enlarged (1653) by Nicholas Culpeper. Gerard’s text was basically a pirated translation of a book by the Belgian herbalist Dodoens and his illustrations came from a German botanical work. The original edition contained many errors due to faulty matching of the two parts. Culpeper’s blend of traditional medicine with
astrology, magic, and folklore was ridiculed by the physicians of his day yet his book - like Gerard’s and other herbals - enjoyed phenomenal popularity. The Age of Exploration and the Columbian Exchange introduced new medicinal plants to Europe. The Badianus Manuscript was an illustrated Aztec herbal translated into Latin in the 16th century.

The second millennium, however, also saw the beginning of a slow erosion of the pre-eminent position held by plants as sources of therapeutic effects. This began with the Black Death, which the then dominant Four Element medical system proved powerless to stop. A century later, Paracelsus introduced the use of active chemical drugs (like arsenic, copper sulfate, iron, mercury, and sulfur). These were accepted even though they had toxic effects because of the urgent need to treat Syphilis. The rapid development of chemistry and the other physical sciences, led increasingly to the dominance of chemotherapy - chemical medicine - as the orthodox system of the twentieth century.

1.3 Current status of Medicinal plants

International Status

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. Recent estimates suggest that over 9,000 plants have known medicinal applications in various cultures and countries, and this is without having conducted comprehensive research amongst several indigenous and other communities (Farnsworth and Soejarto, 1991).

Medicinal plants are used at the household level by women taking care of their families, at the village level by medicine men or tribal shamans, and by the practitioners of classical traditional systems of medicine such as Ayurveda, Chinese medicine, or the Japanese Kampo system. According to the World Health
Organization, over 80% of the world’s population, or 4.3 billion people, rely upon such traditional plant-based systems of medicine to provide them with primary health care (Bannerman et. al., 1983).

Allopathic medicine too owes a tremendous debt to medicinal plants: one in four prescriptions filled in a country like the United States are either a synthesized form of or derived from plant materials (Srivastava, et. al., 1995). Even from the earliest trade data available, it is clear that the global market for medicinal plants has always been very large. According to the International Trade Centre, as far back as 1967, the total value of imports of starting materials of plant origin for the pharmaceutical and cosmetics industry was of the order of USD 52.9 million. From this amount, the total values grew to USD 71.2 million in 1971, and then showed a steady annual growth rate of approximately 5-7% through to the mid-1980s.

To give an example of the extent of trade volumes even at that time, according to one report commissioned by the World Wide Fund for Nature, the total import in 1980 of "vegetable materials used in pharmacy" by the European Economic Community was 80,738 tons. India was the largest supplier by far, with 10,055 tons of plants and 14 tons of vegetable alkaloids and their derivatives.

However, it is only during the last decade that the real significance of the medicinal plants sector has begun to be realized. Interest in natural materials by the dominant economic powers had waned from the late 1960s to the early 1980s as new possibilities in biotechnology and the synthesization of drugs beckoned. But by the mid-1980s, there was a renewed interest in natural materials and approaches to health care, coupled with a recognition that technology alone could not solve the pressing health care needs of the world’s population (Tempesta and King, 1994).
This new drive for natural and plant-based medicines made itself felt in the market from the mid-1980s onwards. As Table 1.1 illustrates, growth in the market in various regions is now on average 3 to 4 times the average growth rates of the national economies in the same regions. Some of these phenomenal rates, in some cases nearly 20%, imply that the market is now doubling in size every 4-5 years.

The participation of various companies in the market also attests to its new strength and importance. By 1990, some 223 major companies worldwide (of which about half were in the United States) were reportedly screening plants for new leads; the figure had been zero in 1980. Also in 1990, more than 2000 companies in Europe alone were marketing herbal medicinals, with 30% having a turnover in excess of $20 million- expenditure in the United States on "unconventional, alternative, or unorthodox" therapies reached $13.7 billion dollars during the same year (Tiwari, 1998).

The so-called "nutraceuticals" sector--consisting of herbal medicines which are dubbed food or dietary supplements in order to pass FDA criteria more easily--is now estimated to be valued at USD 27 billion. The use of such alternative medicines has become increasingly popular in the developed world. For example, 1 in 3 Americans have at some time used unconventional medical therapies according to a national telephone survey published in the New England Journal of Medicine in 1993. In another survey conducted in 1994, it was found that 60% of doctors had at some time referred patients to practitioners of alternative medicine. In response to the overwhelming interest in alternative therapies, many of the prestigious allopathic medical institutions have also recognized their importance: an example is the National Institute of Health which created the Office of Alternative Medicine in 1991 to provide the public with information on alternative
treatments and to assess those therapies which have proven successful. (Kolata, 1996).

According to one account, in 1992 significant amounts of at least 74 species of medicinal plants were being commercially traded in the global market. In addition to these major species, hundreds of others are bought and sold in lesser quantities across national boundaries, sometimes illegally.

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**Table1.1. Natural Medicines Market: Regional Growth Rates 1991-98**

(in %)

A comparison of the volumes of traded materials with those of the previous decade also provides dramatic evidence of the market’s growth. During the last 3 years, approximately 40,000 tons of plant drug materials (valued at DM 160 million) were imported into Germany, annually, implying that one country’s imports in the mid 1990s equaled half the number of plants imported by the entire continent in the mid 1980s.

**Status in India**
The global context briefly sketched above suggests several tremendous opportunities for India, a country unrivaled in terms of diversity of medical systems and practices, in addition to being a major storehouse of biological diversity, with 2 of the 14 mega-biodiversity areas of the world located within its borders. The global market would appear to be more receptive than ever to the mounting of a concentrated Indian effort at supplying it with medical materials and know-how. Such an effort would also appear to be increasingly remunerative for the country. India is of course already an active participant in the global medicinal plants market having been for some time the world’s largest supplier of raw materials (though an insignificant supplier of finished products) (Patnaik, 1994). Of the 74 species accounted for in one of the studies mentioned above, India was known to be exporting 22 and importing 8, while the German study quoted earlier, which is now underway, has found India to be Germany's largest trading partner by far. Moreover, medicinal plants are one of the most important components of the non-wood forest products sector which supplies over 80% of India’s net forest annual export earnings (Jain 1996).

Despite these promising indications, however, it is unclear whether India is truly exploiting her comparative advantage in the medicinal plants sector and tapping the full potential of an expanding consumer base. In addition, several concerns arise in relation to the current consequences of participation in the market, with regard to the sustainability and equitability of prevailing practices in the sector.

Although only micro-studies are currently available in this regard, most of these indicate that current practices are both unsustainable, as they rapidly deplete the natural supplies of the country’s plant base, and inequitable, perpetuating impoverishment for those charged with stewarding and harvesting the resource, while a few profit in dramatic disproportion to their inputs. Negative impacts on
local primary health care, as plants become diverted to national and international markets have also been cited in some cases. To add to all of these negative aspects, the market in India has been shown to be highly inefficient and imperfect.

The need of the hour, then, is to re-plan India’s participation in the expanding global market, in light of the interests of all the stakeholders who are affected and who play a role in this sector. There is a need to collate all the available information regarding medicinal plants development in the country in order to obtain a comprehensive overview which will provide the necessary insight for coordinated and effective action. Such an overview could form the basis of a renewed development of India’s medicinal plants sector, and a strategic exploitation of her comparative advantage in the global market on a sustainable and equitable basis.

India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. These systems have rightfully existed side-by-side with Allopathy and are not in ‘the domain of obscurity’, as stated by (Venkat Subramanian, 2003). Millions of Indians use herbal drugs regularly, as spices, home-remedies, health foods as well as over-the-counter (OTC) as self-medication or also as drugs prescribed in the non-allopathic systems (Gautam, V., Raman, R.M.V., and Ashish, K., 2003). The more than 500,000 non-allopathic practitioners are trained in the medical colleges (>400) of their respective systems of health and are registered with the official councils which monitor professionalism. Hence, these systems are not folklore or traditional herbal practices. There are basic axioms of these systems leading to a logical and systematic structure of pathogenesis and diagnosis, which serves also as a determinant for therapy (Vaidya, A.D.B, 1992). The developer of potent natural
product penicillin, Nobel-laureate Ernst Boris Chain wrote an inspiring article entitled “The quest for new biodynamic substances”. In 1967, he wrote, “In China and India there has been an extensive drive aimed at the systemic study of medicinal plants traditionally used in these countries in folklore medicine; this has failed, so far, to bring to light new classes of compounds with interesting pharmacologic activities. As far as drug research is concerned, therefore, we cannot expect many major surprises to come from the study of plant constituents” (Chain, E.B., 1967). The current overview would disprove the infallibility of this Nobel laureate, by giving examples of novel activities of Indian medicinal plants.

1.4 Antibiotic resistance
Resistance to anti-microbial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem.

Appropriate anti-microbial drug use has unquestionable benefit, but physicians and the public frequently use these agents inappropriately. Inappropriate use results from physicians providing anti-microbial drugs to treat viral infections, using inadequate criteria for diagnosis of infections that potentially have a bacterial aetiolog, unnecessarily prescribing expensive, broad-spectrum agents, and not following established recommendations for using chemo prophylaxis. The availability of antibiotics over the counter, despite regulations to the contrary, also fuel inappropriate usage of anti-microbial drugs in India. The easy availability of anti-microbial drugs leads to their incorporation into herbal or "folk" remedies, which also increases inappropriate use of these agents.
Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of anti-microbial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs.

Resistance factors, particularly those carried on mobile elements, can spread rapidly within human and animal populations. Multidrug-resistant pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts. Antibiotic resistance patterns may vary locally and regionally, so surveillance data needs to be collected from selected sentinel sources. Patterns can change rapidly and they need to be monitored closely because of their implications for public health and as an indicator of appropriate or inappropriate antibiotic usage by physicians in that area.

The results of in-vitro antibiotic susceptibility testing, guide clinicians in the appropriate selection of initial empiric regimens and, drugs used for individual patients in specific situations. The selection of an antibiotic panel for susceptibility testing is based on the commonly observed susceptibility patterns, and is revised periodically.

The principles of determining the effectivity of a noxious agent to a bacterium were well enumerated by Rideal, Walker and others at the turn of the century, the discovery of antibiotics made these tests (or their modification) too cumbersome for the large numbers of tests necessary to be put up as a routine. The ditch plate method of agar diffusion used by Alexander Fleming was the forerunner of a variety of agar diffusion methods devised by workers in this field. The Oxford
group used these methods initially to assay the antibiotic contained in blood by allowing the antibiotics to diffuse out of reservoirs in the medium in containers placed on the surface.

With the introduction of a variety of anti-microbials it became necessary to perform the anti-microbial susceptibility test as a routine. For this, the anti-microbial contained in a reservoir was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Even now a variety of anti-microbial containing reservoirs are used but the anti-microbial impregnated absorbent paper disc is by far the commonest type used. The disc diffusion method of AST is the most practical method and is still the method of choice for the average laboratory. Automation may force the method out of the diagnostic laboratory but in this country as well as in the smaller laboratories of even advanced countries, it will certainly be the most commonly carried out microbiological test for many years to come. It is, therefore, imperative that microbiologists understand the principles of the test well and keep updating the information as and when necessary. All techniques involve either diffusion of anti-microbial agent in agar or dilution of antibiotic in agar or broth. Even automated techniques are variations of the above methods.
1.5 Aims and Objectives

1. Preparation of different plant extracts using organic and inorganic solvents.
2. Screening of Indian medicinal plants for their broad spectrum antibacterial and antifungal activities.
3. \textit{In-vitro} efficacy of selected promising plant extracts and their fractions against different drug resistant enteric bacteria.
4. Phytochemical screening of biologically most active plant extracts and fractions.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of Plant</th>
<th>Vernacular Name</th>
<th>Plant part used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>\textit{Lallemantia royleana}</td>
<td>Tukhm-balanga</td>
<td>Seeds</td>
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<tr>
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<td>Kanocha</td>
<td>Seeds</td>
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<tr>
<td>3</td>
<td>\textit{Plantago ovata}</td>
<td>Isabghol</td>
<td>Seeds</td>
</tr>
<tr>
<td>4</td>
<td>\textit{Rosa indica}</td>
<td>Gul-e-surkh</td>
<td>Petals</td>
</tr>
<tr>
<td>5</td>
<td>\textit{Solanum nigrum}</td>
<td>Makoy</td>
<td>Fruit</td>
</tr>
</tbody>
</table>

Table 1.2. List of plants undertaken study