

## INTRODUCTION

Medicinal plants continue to be an important resource material for therapeutic agents both in developed and developing countries. Plants are reservoir of potentially useful chemical compounds which serve as a drug. Finding healing power in plants is an ancient idea. People of all continents and civilization used plants in one form or other like poultice or decoction. History of herbal medicine is as old as human civilization. The documents, many of which are of great antiquity, revealed that plants were used medicinally in China, India, Egypt and Greece long before the beginning of Christian era. A large proportion of Indian population even today depends on Indian system of medicine –Ayurveda, an ancient science of life. India enjoys the privilege of having the time tested traditional system of medicines based on natural products. Medicinal and aromatic plants are one of the major natural resources of our country occurring in diverse ecosystem. Most of the drugs used in primitive medicine were obtained from the plants.

India is a rich meadow of medicinal plants and its wealth of medicinal and aromatic plants is well known world over. The variety and sheer number of plants with therapeutic properties is quite astonishing. India's rich heritage of plants is because of its wide diversity in soil and climatic conditions. Thus it has one of the oldest, richest and diverse folk tradition associated with the use of medicinal plants in healthcare system to promote, safeguard and relieve pain, heal wounds and refresh mind, muscles and nerves (Rajasekharan and Gneshan, 2002). The advent of Buddhism in India brought considerable changes in practice of Ayurveda. Some of the Buddhists rulers like Ashoka established several herbal gardens so that people could get drug conveniently for treatment of their diseases. Buddhist monks were encouraged to learn Ayurveda and practice it. Senior member of family were well acquainted with the use of herbs available in their vicinity. Only serious cases were brought to the physician,.

We in India have two (Eastern Himalaya & Western Ghats) of the 18 hot spots of plants diversity in the world, interestingly we are 7th among the 16 mega diverse

countries where 70 % of the world species occurs collectively. We are rich in our own flora. Macro analysis of the distribution of medicinal plants shows that they are distributed across diverse habitat. Around 70% of India's medicinal plants are found in tropical areas mostly in the various forest type spread across the Western and Eastern Ghats, the Vindhyas, Chotta Nagpur plateau, Arawali & Himalayas. Although less than 30 % of the medicinal plants are found in the temperate and alpine areas and higher altitudes they include species of high medicinal value. Macro studies show that a larger percentage of the known medicinal plant occur in the dry and moist deciduous vegetation as compared to the evergreen or temperate habitat. Analysis of habits of medicinal plants indicates that they are distributed across various habitats. One third are trees and equal portion shrubs and the remaining one third herbs, grasses and climbers. A very small portion of medicinal plants are lower plants like lichens, ferns and algae etc. Majority of medicinal plants are higher flowering plants. It is evident that Indian people have a tremendous passion for medicinal plants and use them for a wide range of health related applications from common cold to memory improvement and treatment of poisonous snake bite to a cure for muscular dystrophy and the enhancement of body's general immunity. In the oral traditions local communities in the every ecosystem from trans Himalayas down to the coastal plains have discovered the medical uses of thousands of plants found locally in their ecosystem. Indian system of medicine dwell heavily on medicinal plants, well developed pharmaceutical industry, rapidly growing phytochemical and herbal drug industry. India is thus in a vantage position to exploit this source both for meeting the domestic demand for drug and also for export. Cultivation of the medicinal plants in waste lands near villages and forest under their known identity is the need of the day. This will help reducing pressure on forests save their depletion. Also it would meet the requirement of the Drugs and Cosmetics Act 1940 which emphasises "The raw material used in preparation of drugs of ISM should be genuine. The demand of healthy medicinal plant material in India and International drug market is increasing continually, while many important medicinal plants have been declared endangered due to less seed viability, low germination percent and vegetative propagation also susceptible for disease. About 90 % of the medicinal plants used by the industries are collected from the wild. While over 800 species are used in the

production by industry, less than 20 species of plants are under commercial cultivation. Over 70 % Of the plant collections involve destructive harvesting because of the use of parts like roots, bark, wood, stem and whole plant in case of herbs. This poses definitive threat to the genetic stock and to the diversity of medicinal plants if biodiversity is not sustainably use

Apart from requirement of medicinal plants for internal consumption, India export crude drugs mainly to developed countries, viz. USA, Germany, France, Switzerland, UK and Japan, who share between them 70 to 80% of total export of crude drugs from India. The principal herbal drugs that have been finding good market in foreign countries are *Aconite*, *Aloe*, *Belladonna*, *Acorus*, *Cincona*, *Cassia tora*, *Dioscorea*, *Digitalis*, *Ephedra*, *Plantago*, *Cassia etc.* (Planning commission-Task force report, 2000).

A plant is said to be medicinal when at least one part posses therapeutic properties (Dhiman, 2003). With the recent advancement in research in the field of medicinal plants, it has become apparent that many of the species utilized by indigenous people as well as knowledge of traditional healers has began to make its mark on society as a possible avenue for curing to diseases, (Haggers *et al.*, 1993; Jager *et al.*, 1996; Fulton et al., 1990), in the world. Recently most of the research conducted in traditional medicine has shown some remedies obtained from traditional healers are very effective in spite of the fact that there is no scientific justification (Fox, 1999; Grierson, 1999). Currently the activities of many plant species have been reported (Gerison and Atolayan, 1999; Jager *et al.*, 1996; Kalmanson *et al.*, 2000). Scientific evidence has brought about the possibility of utilization of plants extract in treatment of fungal infection and development of antibacterial and antifungal products, (Farnsworth, 1994; Fox, 1999).

Pharmaceutical, Fragrance and flavor industries remained challenged and under immense consumers pressure to produce innovative and efficacious product for this lucrative industry. In the quest to satisfy consumer expectations and to produce products with some competitive edge these industries most often turn to nature for guidance,

inspiration and as a source of novel compounds for commercial development. Details including various names (botanical, vernaculars etc), with collection conditions and part of plant to be used should be documented to ensure proper identification (WHO, 2001).

Demand for medicinal plants is increasing in both developing and developed countries due to growing recognition of natural products, being non-narcotic, having no side effects, easily available at affordable prices and some time the only source of healthcare available to poor. Research efforts thus could be directed for a number of diseases for which suitable drugs are not available in modern system of medicine and where herbal drugs have a possibility of offering new drugs. About 3000 material from 2764 plant species have been screened for their pharmacological and chemotherapeutic properties (Anon, 1988, Rastogi & Mehrotra, 1990).

Bioactive principles are widely distributed among higher plants representing about 158 families (Tewari, 1999). Through many possible sources of extraction, synthesis of antimicrobial has been elaborately worked out, but work for better, safer & economic source is always necessitated (Avani & Neeta, 2005). Paradigm shift from synthetic to herbal drugs. This burgeoning worldwide interest in medicinal plants also reflect a recognition of validity of many traditional claim regarding value of natural products in health care..

In India there are many small manufacturing units using medicinal plants and thousands of vaidyas preparing their own drugs from various plants. The direct utilization of plant material is a feature of traditional medicines not only in developing world but also in Europe & U.S.A. for example herbal formulation on health food shops, preparation of decoctions, tinctures, galanicals and total extract of plant also form a part of many pharmacopoeias of the world. During past three and half decades bulk production of plant based modern drugs has become an important segment of Indian pharmaceuticals which are produced in India at present includes Morphine, Codein, Papaverine, Emetine, Quinine, Quinidine, Digoxine, Caffine, Hyoscine, Hyyoscyamine, Xanthotoxin, Psoralen, Colchicine, Rutine, Berbirine, Vinblastine, Nicotine, Strychnine, Brucine, Ergot, Alkaloid, Senna glycoside, Pyrethroids and

Podophyllotoxin resin etc. Medicinal plants based industry is progressing very fast in India but is beset with a number of problems. Most alarming problem the industry has started facing and will face in future is dwindling supply of plant material from natural resources.

Microorganisms of medical interest include protozoa, fungi, bacteria and viruses. They are heterogeneous group of several distinct classes of living being. We live with bacteria and host a complex bacterial ecosystem. Ancient man had thought that the infectious diseases to be because of divine wrath and supernatural powers. The construction and use of compound microscope was an essential prerequisite to study the microbial forms. To Antony Van Leeuwenhoek (1683) must be ascribed the credit of placing the science of microbiology on the firm basis of direct observation. Antony Van Leeuwenhoek is known as father of bacteriology because it was he who first accurately described the different shapes of bacteria and pictured their arrangement in infected material. The first pathogenic bacterium to be observed under microscope was anthrax bacillus. Pollender (1849) and Davaine (1850) observed anthrax bacilli in the blood of animals dying of disease. Joseph Lister (1827-1912), an English surgeon and cotemporary of Pasteur, was among the first to appreciate the ramification of the emerging germ theory of disease. He established the guiding principle of antisepsis for good surgical practice upon which the present day specialists depend. For this work he is known as "Father of antiseptic surgery". Bacteria are prokaryotic microorganisms capable of performing all essential function of life. They are free living microscopic, unicellular organisms possess both DNA and RNA. Bacteria do not contain a membrane bound nucleus. Their DNA consist of single circular chromosome, which is attached to mesosome, a sac like structure in the cell membrane. Bacterial ribosomes are found free in the cytoplasm and attached to cytoplasmic membrane and are 70S in size. The cell envelope in bacteria consist of cytoplasmic membrane and cell wall. Some species also produce capsule and slime layer. They cause contamination, infection and decay. It thus becomes necessary to remove or destroy them from material or area by sterilization. Bacteria are the common cause of hospital borne infection. Excessive use of antimicrobial have been largely responsible for the sustenance of multidrug resistance bacteria in hospital following high prevalence of resistance for drugs like

Ampicillin, Tetracycline Ampicillin, Cotrimoxazole and Gentamycin drug like Quinolone, Cephalosporine and aminoglycosides like Amikacin and Netilmycin were induced. However last few years have witnessed resistance for these drugs as well.

The increased incidence of the fungal infection in past two decades has been overwhelming. Earlier, it was the pathogenic diamorphic fungi or the agents causing superficial fungal infections were known as pathogens for human. However, starting from 1980s, the opportunistic fungi started causing more infections specially in the compromised host. More recently newer and less common fungal agents are being increasingly associated with infection in immuno-suppressed host. Fungi are group of non motile eukaryotic organism because the genome is organized in a nucleus surrounded by membrane. They may be unicellular or multicellular, although there is a tendency for fungi to be multicellular and multinucleate. They exist as saprophytes, parasites and commensals. They are devoid of chlorophyll and are chemoheterotrophs with cell wall containing chitin and other cellulose and reproduce by means of spores and conidia. Morphological classification includes the yeast, yeast like, molds and diamorphic fungi. Fungi pathogenic to man can be conveniently separated in to two basic groups, moulds and yeasts. Moulds consist of those fungi that grow in a filamentous form, where as yeasts are characterized by unicellular morphology that reproduces by budding. The vegetative filament of a fungus is called a hypha. Hyphae are the actively growing assimilative phase of fungi. The diameter of the hyphae is a useful characteristic in distinguishing hyphae of Zygomycetes from other fungi. Fungi reproduce by asexual and sexual method by formation of spores. Asexual spores are sporangiospore and conidia. Sexual spore includes Zygosporangium, ascospores and basidiospore. *Saccharomyces cerevisiae* and *Cryptococcus neoformans* are example non pathogenic and pathogenic yeast respectively. *Candida* is yeast like fungus. Molds include *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*. Diamorphic fungi include *Histoplasma capsulatum*, *Sporothrix seuenckii*, *Blastomyces dermatitidis*, *Coccidioidis immitis* and *Paracoccidi-oides brasiliensis*.

Laboratory diagnosis depends upon the quality of specimen provided for laboratory analysis. Incidence of fungal infections has increased dramatically over past

decades due to increase in number of population susceptible to such infection. Newer antifungal have increased the therapeutic options and hence leading to demand for in vitro determination of antifungal susceptibility of some drugs of plants origin. Vegetative filament of fungus is called hypha. Fungi reproduce by asexual and sexual method by formation of spores. As eukaryotic fungi share more common with human cells than do bacteria. Therefore the development of safe antifungal agent is more difficult. The highly effective amphotericine B carries significant toxicities and problems in administration, Nystatin useful topically and in the digestive tract is not suitable for systemic use. The recent rise in fungal diseases has in turn spurred the development of additional agents, including oral with better safety profile from plants origin. The use of safe and effective oral option has greatly improved the quality of life for individuals with long term immune-suppression, notably AIDS patients and transplant recipients.

Mycotic diseases have become a great problem. There are several reasons for more frequent occurrence of mycosis. One of the most important being long term administration of some groups of medicines including broad spectrum antibiotics which suppress normal intestinal flora and there for support the growth of fungi. Species of genus *Candida* and *Aspergillus* are mainly included in the present study. *Candida* are small (4-6 micro meter), unicellular, thin walled, ovoid cells that reproduced by budding. They grow well on commonly used bacterial culture media and do not require special fungal media for cultivation.

There are more than 150 species of candida of these approximately 10 are considered important pathogen for human. *Candida* species are part of normal flora in human. They are found on skin and throughout the gastrointestinal tract.

*Candida* species are also commonly found in the expectorated sputum, female genital tract and urine of patients with indwelling foley catheters. Intact skin and mucus membrane are the most important defense mechanism in maintaining resistance to mucocutaneous or systemic candidiasis. Polymorphonuclear leucocytes play a major role fighting candidiasis. Interruption of normal defence mechanism is necessary for

Candida to become pathogen. The factors responsible for immune suppression fall in two categories: Naturally occurring and iatrogenic. Naturally occurring includes diabetes mellitus which predisposes to cutaneous Candidiasis. Iatrogenic factors are thought to be most important predisposing factors to Candida infections, especially disseminated infection. The most common iatrogenic factors involve the use of antibiotics and intravenous catheters. Antibiotics suppress the normal bacterial flora which then allows the yeasts to proliferate especially in gastrointestinal tract. Factors that provide a route for candida enter the blood includes the I/V drug abuse, parenteral nutrition, chemotherapy, cardiac catheters, prosthetic valves and other implanted materials. In general any immune suppression (Steroids, AIDS, organ transplantation) can potentially lead to disseminated candidiasis.

Species of genus *Aspergillus* are saprophytes. They decay organic remains and frequently occur in the humus and soil. From the view point of pathogenicity some species of genus aspergillus especially *A.fumigatus*, *A. flavus*, *A.niger* deserve special attention. *A. fumigatus* was isolated by Fresenius in 1850 from the lungs in aspergilosis of birds. It often occurs in the wipes from the acoustic meatus and sputum. It often causes human Aspergillosis, especially pulmonary and aural ones.

*A. niger* was described by Van Tiegh. This species is pathogenic for both man and animals. The surface of the colonies of *A. niger* is covered by dense aggregate of jet black conidia. The underside of colony is buff or yellow grey distinguishing *A. niger* from dermataceous fungi. Microscopically the vesicles are globose and measure up to 75  $\mu\text{m}$  in diameter. They bear a double row of sterigmata covering the entire surface. Conidiation is extremely profuse, to the extent that vesicle are obscured by dense aggregates of 3-5  $\mu\text{m}$  diameter, spherical black conidia that become rounded with maturity (Arora, 2006). *A. flavus* causes numerous diseases of man animals and insects. Treatment of all mycosis is very serious problem as these diseases are mostly long term and malign with frequent relapse. A search for new, more effective and little toxic antimycosis in which no resistance would develop is there fore necessary from plant origin.

Susceptibility testing for fungi is difficult and the evidence for correlation with in vivo activity is sparse. Both macro and micro dilution methods are routinely applied for sensitivity test. Direct microscopy is of immense important for mycological investigation of clinical specimens. Gram staining often gives the 1st evidence of infection with yeasts and direct smear gives more specific information concerning a molds infection. Other tests includes KOH preparation, KOH with Calcoflour white, India ink and tissue stain such as periodic acid Schiff (PAS) stain, Gamori methenamine silver nitrate (GMS) stain, Giemsa stain and Haemotoxillin and Eosin (H & E ) stain. Unlike bacteria, fungi may take pretty long time to grow in culture.

Moreover many fungi are saprophytic. Therefore, clinical situation should be correlated with direct microscopy findings and culture. The KOH (Potassium hydroxide) preparation is probably the most important microscopic test used by the mycologist. In this method 10-20 % KOH digests protein and mucinous material and dissolve the cement, which holds the keratinized cells together. Fungi withstand the digestion due to chitinous cell wall. Fungal hyphae can be seen under direct microscopy in wet smear .Culture is done on Subrouds dextrose agar medium .It is slanting medium. Three weeks incubation period is necessary for the declarion of negative result for fungal culture report. Medium should be kept at required temperature in BOD incubator. There is a need to integrate traditional medicine in to modern medicine practices. This requires clinical validation by conducting controlled clinical trials. The methods used for clinical validation for modern medicine must be applied to prove the safety and efficacy of finished herbal products. The design and scope of studies should be in accordance with the traditional use and in consultation with the traditional medical practiceners. Herbs are supposed to be safe but many unsafe and fatal side effects have recently been reported. There could be direct toxic effects, allergic reactions, effect from contamination and interactions with drugs and other herbs. The risk – benefit ratio of herbal drugs should be evaluated.

Plants have various chemical compounds that have medicinal role called as phytochemicals. These phytochemicals are used to cure the diseases in herbal and homeopathic medicines. There are hundreds of medicinal plants that have a long history

of curative properties against various ailments. The medicinal value of the plant is due to the presence of some chemicals present in them. The identification of a particular compound against a particular disease is a challenging long process. Thus importance of any plant lies in their biological active principles. There are two types of plant chemicals, primary metabolites and secondary metabolites. Primary metabolites includes sugars, proteins, amino acids, chlorophylls etc. The secondary metabolites includes alkaloids, terpenoids, saponin and phenolic compounds. These chemicals exert a significant physiological effect on mammalian system. Plant extract and essential oils, as well as their constituents are used in food, pharmaceutical industries. Many essential oils and ingredients have been shown to possess diverse biological activities, including antibacterial and antifungal activities.

The state of Haryana was carved out of the territories of composite state of Punjab Recognition act 1966. It is one of the north-western state of India adjoining Delhi, the capital of the country. It is bounded by the Himachal Pradesh in North, Uttar pardesh and Delhi in the east, by Rajasthan in the south and Punjab and Chandigrah In the north west. Haryana has a plain area excepting few hills of Shivalik system in the north and of Arawali system in the south. Eastern Haryana plains covering districts of Panchkula, Ambala, Yamunanagar Kurukshetra, Karnal, Panipat, Sonapat, Rohtak, and Jhajjar. Western Haryana plains covering districts of Sirsa, Fatehabad, Hissar, and Bhiwani. Southern Haryana plains covering districts of Mohindergrah, Gurgeon, Mewat, Faridabad, Rewari and Delhi - union territory. The climate of Haryana is semi arid in the south west and of gangetic type in the rest of the state, due to its continental location on the outer margin of monsoon region between Thar desert and Himalaya in the north west of Indian subcontinent. Rainy season is from July to September brought by Monsoon. The weather is dry except from few showers received from western cyclones from October to June. The rainfall is a low rainfall in the south and south-western Haryana and is gradually increases in the north east. In the northern half, the rainfall gradually increases towards Shivalik hills. The climate of Haryana is attributed to short wet months or weeks and long spelt of dry months or weeks but there are considerable variation in the monthly or weekly regime of rainfall from place to place

depending upon the distance from mountains, topographical alignments and location in respect of Thar desert.

The systemic screening of the plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories (Jain et al., 1998). The search for components with antimicrobial activity has gained increasing importance in recent time due to growing world wide concern about the alarming increase in rate of infection by antibiotic resistance microorganisms. Hence there is constant need for new and effective therapeutic agents. In Haryana many plant species have been utilized as traditional medicines. It is necessary to establish the scientific basis for the therapeutic action of such traditional medicine as these may serve as the source for development of more effective drugs.

In the present study we have selected 18 medicinal plants from Haryana to be screened against nine bacterial strains including *Staphylococcus aureus*, *Escherichia coli*, *klebsiella pneumoniae*, *Salmonella typhi*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Vibrio cholera*, *Serratia marcescens* and two fungal strains *Candida albicans* and *Aspergillus niger*. The selection of medicinal plants is based on their traditional uses.

## **Aims and objectives**

- 1) To collect and identify medicinal plants, specially those plants which are known in folklore for antibacterial and antifungal properties against human pathogens.
- 2) To study in vitro antibacterial susceptibility of different parts of some selected medicinal plants and to determine their minimum inhibitory concentration (MIC).
- 3) To study in Vitro antifungal susceptibility of some selected medicinal plants and to determine their minimum fungicidal concentration (MFC).
- 4) To study the bioactive compounds of medicinal plants which showed antibacterial and antifungal properties.