CHAPTER 1

INTRODUCTION
At the beginning of the 20th century, bacterial epidemics were a global and important cause of mortality. In contrast, fungal infections were almost not taken into account. Since the late 1960s when antibiotic therapies were developed, a drastic rise in fungal infections was observed, and they currently represent a global health threat. This increasing incidence of infection is influenced by the growing number of immunodeficient cases related to AIDS, cancer, old age, diabetes, cystic fibrosis, and organ transplants and other invasive surgical procedures (Vandeputte et al., 2012). It is estimated that invasive fungal infections develop in 10% to 25% of patients with acute leukaemia and those receiving bone marrow transplants (Sahin and Akova, 2005). Invasive fungal infections are generally fatal with mortality rate up to 90% particularly in patients with persistent neutropenia (Gorschlüter et al., 2006). The pathogens that most frequently cause fatal conditions have been the species of *Candida* and *Aspergillus*. So, fungal infections are emerging as major cause of morbidity and mortality in immunocompromised patients (Bierman and Bubalo, 2006). Still, fungi are one of the most neglected pathogens, as demonstrated by the fact that the amphotericin B, a polyene antibiotic discovered as long ago as 1956, is still used as a “gold standard” for antifungal therapy (Spellberg et al., 2004).

1.1. Fungal infections

The incidence of disseminated candidiasis has increased dramatically over the past several decades. In invasive form, *Candida* can produce large mycelia which can penetrate mucosa or intestinal walls, making way for toxins, undigested food particles, bacteria and yeasts to enter the bloodstream. The blood poisoning known as candidal sepsicaemia may become fatal in persons with severely compromised immune system. Invasive disease may affect major organs, such as kidneys, spleen, liver, lungs, eyes, brain and heart. If the infection is not treated appropriately, the pathogen may invade the central nervous system leading to lethal conditions. Although *C. albicans* remains the most frequent pathogen in oropharyngeal and cutaneous candidiasis, reports on infections due to non-*albicans* species such as *C. tropicalis, C. glabrata, C. parapsilosis, C. dubliniensis, C. krusei* and *C. lusitaniae* have also been documented (Godoy et al., 2003; Sutton, 2008). Prolonged uses of antibiotics, central venous catheterization and extended intensive care unit stay have been shown to be associated with an increased risk of invasive candidiasism (Pfaller and Diekema, 2007).

Although, *A. fumigatus* is the most common etiologic agent responsible for approximately 90% of *Aspergillus* induced disorders in humans, the infections due to other species such as *A. flavus, A. niger, A. terreus* and *A. nidulans* have also been reported (Walsh et al., 2008). Aspergillosis is the most common cause of infectious pneumatic mortality in
recipients of lung, bone marrow, heart, pancreas and renal transplants (Denning, 1998). At present, invasive aspergillosis is the leading cause of infection-related mortality in patients with bone marrow transplant recipients and for patients with central nervous system or disseminated aspergillosis (Chhillar et al., 2008). Although invasive fungal diseases are now more frequent than during the first half of the century, they are still difficult to diagnose clinically. During the latter half of the century, particularly during the past two decades, a number of different classes of antifungal agents have been discovered. Although, since the discovery of amphotericin B, there has been much progress in this field, there is still a critical need for new antifungal agents to treat life threatening invasive mycoses. Therefore, the diagnosis and therapy of invasive aspergillosis remains a clinical challenge. Moreover, Aspergillus has been found to be of very special concern because its clinical symptoms overlap so much with tuberculosis that it is often misdiagnosed and treated wrongly.

The Blastomyces, Coccidioides and Cryptococcus are other important fungi which have been found to be of clinical importance (Dodds et al., 2000). Recent epidemiological reports have indicated that fungi such as Fusarium, Trichosporon, Rhizopus and Rhizomucor also may cause health hazards (Sahin and Akova, 2005). Moreover, opportunistic fungal infections are increasing at an alarming rate, whereas; allergic reactions of the skin are also increasing day by day.

1.2. Antifungal Chemotherapy

The natural and synthetic drugs have been developed over the years to treat mycotic infections. Despite extensive research dedicated to the development of new therapeutic strategies, there are only a limited number of available drugs to fight against invasive mycoses. During the latter half of the century, particularly during the past two decades, a number of different classes of antifungal agents have been discovered. The antifungal agents which have been or are currently being evaluated for use in treating invasive mycoses are classified by their site of action in fungal cells (Dismukes, 2000). Indeed, only four molecular classes that target three distinct fungal metabolic pathways are currently used in clinical practice to treat essentially systemic fungal infections: fluoropyrimidine analogs, polyenes, azoles, and echinocandins. Several other classes, such as morpholines and allylamines are only used as topical agents due to either poor efficacy, or severe adverse effects when administered systemically.

Fluoropyrimidines, of which only 5-fluorocytosine (5-FC) and 5-fluourouracil (5-FU) are used in human medicine, are synthetic structural analogs of the DNA nucleotide cytosine. Despite its numerous pharmacological advantages, the use of 5-FC in clinical practice is
decreasing because of the frequent occurrence of innate or acquired resistance to this drug in fungal pathogens. More than 200 molecules belonging to the chemical class of polyenes have an antifungal activity, most of them being produced by *Streptomyces* bacteria. However, only three possess a toxicity allowing their use in clinical practice: amphotericin B, nystatin, and natamycine. Polyene drugs target ergosterol, the main sterol component of fungal membranes. Their amphiphilic structure allows them to bind the lipid bilayer and form pores. For these reasons, amphotericin B is the most used polyene antifungal for systemic infections. Due to its high hydrophobicity and poor absorption through the gastrointestinal tract, it is necessary to administer amphotericin B intravenously (Lemke et al., 2005). However, amphotericin B administration is accompanied with adverse effects, mostly at the level of kidneys and liver. New formulations, such as liposomal amphotericin B or lipid amphotericin B complexes, minimize such side effects (Barrett et al., 2003). Azoles are by far the most commonly used antifungals in clinical practice, and consequently, they are also the mostly studied by the scientific community regarding their mode of action, their pharmacological properties, and the resistance mechanisms developed by microorganisms. Azole drugs target the ergosterol biosynthetic pathway by inhibition of a key enzyme, the lanosterol 14-alpha demethylase, encoded be the *ERG11* gene. This inhibition occurs through the binding of the free nitrogen atom of the azole ring to the iron atom of the heme group of the enzyme. The resulting accumulation and metabolism of 14-alpha methylated sterol species leads to the synthesis of toxic compounds, which are unable to successfully replace ergosterol (Carrillo-Munoz et al., 2006). Echinocandins constitute the only new class of antifungals made available for clinicians to fight invasive fungal infections within the past 15 years (Denning 2002). Echinocandins are synthetic derivatives of lipopeptides. These lipopeptides are naturally produced by several fungal species: *Aspergillus rugulovalvus* synthesizes caspofungin B, *Zalerion arboricola* synthesizes pneumocandin B, and *Papularia sphaerosperma* synthesizes papulacandin. Echinocandins are noncompetitive inhibitors of β (1-3) - glucan synthase, an enzyme that catalyzes the polymerization of uridine diphosphate-glucose into β (1-3) glucan, one of the structural components responsible for the maintenance of fungal cell-wall integrity and rigidity (Marco et al., 1998). Inhibition of β (1-3) - glucan synthase leads to cell wall destabilization and to the leakage of intracellular components, resulting in fungal cell lysis (Stone et al., 2002). However, echinocandins are the latest group of anti fungal polypeptides introduced but they too had to face serious toxicity issues in phase II clinical trials.
It is, therefore, evident that currently available antifungal drugs are not sufficiently broad in their spectrum and consistently effective against fungi. Most of these drugs are highly toxic and immunosuppressive in nature. Many of these antifungal drugs have been found to act on targets also found in mammalian cells, which may result in toxicity or adverse drug interaction. In view of these limitations of currently used drugs for antifungal therapy, many researchers, both from academic centres and pharmaceutical companies, are looking for new formulations developed based on novel compounds and new targets identified in the pathogens. Therefore, the exploitation of natural bio resource for isolating lead molecules for development of new antifungal formulations has been emphasized in recent years.

1.3. Plants as the Alternate Source of Antifungal Agents

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in treatment of various infectious / microbes borne diseases. Plants have been used throughout the world as drugs and remedies for various diseases since time immemorial and their extracts have been used for centuries as a popular method for treating several health disorders. Traditional medicines based on herbal remedies have always played a key role in the health system of many countries. According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance. Natural products are the most consistent and successful source of drugs. In India, Ayurveda remains one of the most ancient and living traditions, which is practiced for the treatment of various diseases and disorders. India has many number of plant species and medicinal properties have been assigned to several thousands. In Haryana region, there is a long tradition of using herbal products for skin and other problems by healers and old peoples (Tomar and Singh, 2006). They are using these herbal remedies because they are cheap and fast healing. Healers and few old people acquire such knowledge which is verbalized and is limited only to their knowledge; it may get lost in near future (Rahman et al., 2006). Therefore such plants should be investigated to understand their properties, safety and efficacy for a search of new potent antifungal compounds or fractions. From now, scientific efforts to discover new potential antifungal drugs are principally leaned towards synthetic and natural products of plant origin (Ismail et al., 2008). The search for bioactive natural products isolated from higher plants, are attracting considerable attention.
from researchers worldwide, as indicated by the increase of work and publications on therapeutic potential during recent years. Because the plants are a good reservoir of bioactive compounds which provide the drugs for effective treatment of various diseases (Evans, 2006). The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds (Fleming et al., 2002) which can prove to be an important source of lead compounds in the development of new antifungal drugs. Therefore, the present research work reveals the antifungal activity of medicinal plants used in Ayurveda and traditional medicinal system for treatment of manifestations caused by Aspergilli pathogens.

1.4. Rationale for Plants Selection

It is important to understand the methods and rationale used to provide the best opportunities to isolate novel antifungal metabolites from number of plant species. The selection of suitable plants for investigation is a very important and decisive step, to avoid wasting unnecessary time. There are several ways in which this could be done, including traditional use; chemical content; toxicity; randomised selection or a combination of several criteria. The most common method used is ethno-pharmacology or ethno-botany. The method involves a careful observation of the use of natural resources in folk medicine in different cultures. Here also, the reasonable plants selection has been done on the bases that have been exploited for human use as traditional medicines in some place. In Haryana region (State of India) there is a great biodiversity of medicinal plants and there is a long tradition of using herbal products for skin and other problems by healers and old peoples. A number of medicinal plants described in Ayurveda still need to be testifying according to the modern parameters to ensure their activity and efficacy. Therefore, *Aegle marmelos*, *Capparis aphylla*, *Callistemon lanceolatus*, *Commelina bengalensis*, *Justicia adhatoda*, *Argemona mexicana*, *Achyranthes aspera*, *Catharanthus roseus* and *Syzygium cumini* were selected based on their use in respiratory and other disorders in traditional systems of medicine, which have been used for thousands of years in India. Moreover, the use of these plants in the traditional medicine systems of many other cultures has been extensively documented.

The extensive and cost-effective medicinal importance of selected medicinal plants demands a systematic detailed investigation of their antifungal and other potentials as well as to facilitate their effective use to improve the total economic values. The present study was undertaken with the aim to “Identification and toxicological studies of antifungal molecule from natural sources.”