INTRODUCTION
1.1. Background

*Aspergillus fumigatus* belongs to Trichocomaceae family of *Aspergillus* genus and ubiquitously present in the environment. In healthy peoples, innate immunity eliminates Aspergillus conidia and prevents fungal infections. Hence, only aspergilloma, allergic broncho-pulmonary aspergillosis (ABPA) and aspergillus sinusitis are commonly observed diseases in healthy peoples. However, in immunocompromised patients, it spreads and cause Invasive Aspergillosis (IA) which is usually fatal. IA has been reported for higher mortality rate of 55% (Denning and Stevens, 1990; Latge 2001). Mortality rate due to *Aspergillus* infection in bone marrow transplant was observed to be as high as 80% even after appropriate chemotherapy (Smalley et al., 2006; Okeke et al., 2005; Cuenca-Estrella et al., 2000). Cerebral aspergillosis signifies the symptoms of discriminating and always lethal. It is, therefore, important to diagnose and treat these fungal infections at the early stage to check severe the damage (Val et al., 2001; Giordani et al., 2001). Moreover, chemotherapies available for aspergillosis have serious drawbacks and often ineffective in invasive conditions (Lazar and Wilner 1990; Gearhart 1994; Goa and Barradell 1995). Hence, continuous efforts to discover novel antifungal agents and to understand underlying molecular mechanism are required.

1.2. Statement of the problem

*Aspergillus* is a saprotrophic filamentous fungi growing soil or organic debris. It primarily spreads through small asexually produced conidia and disseminated by the air in environment (Tillie and Tonnel 2005; Dagenais and Keller, 2009). It is usually not harmful but exposure to its conidia may lead to aspergilloma and ABPA in healthy individuals. It may disseminate to various organs including kidney and brain. Lungs are primary target during immunocompromised condition and form IA. It emits enormous peptidases or proteases that degrade host macromolecules to use them as nutrients and others are well replaced antigen that cause immune suppression. In all form of infections, it secret proteins, enzymes and toxins (Rementeria et al., 2005).
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*A. fumigatus* is a eukaryote; hence its most of the molecular processes are equivalent to the human beings. It makes difficult to identify molecular targets for drugs discovery. However, some advance or specific pathways i.e. cell wall synthesis process are the easy targets of *A. fumigatus*. Therefore, being limited choice as molecular targets so far a very few drugs have been developed to treat invasive conditions of *A. fumigatus*. Further, developments of resistance have been reported in *A. fumigatus* that make it more difficult to develop specific drugs.

At present, a number of antifungal drugs are present in the market including polyenes, azoles, echinocandins, allylamines and nucleosides which target the synthesis of cell wall molecules like ergosterol, β-1, 3 glucan, and chitin. Amphotericin B (amp B) is polyene drug, only drug of choice to treat IA. However, amp B is highly toxic and cause respiratory distress as well as nephrotoxicity (Gokhale et al, 1993). The interaction of present antifungal drugs with ergosterol has most important limitations due to structural homology of ergosterol with cholesterol; hence toxic to human being (Xu et al., 2007; Beauvais et al., 2001; Kontoyiannis et al., 2005). Nystatin, another polyene drug, was reported to have dose-limited toxicity. Further, occurrence of resistance development in fungal species makes this drug less useful (Bossche et al, 1994; Walsh et al., 1995). Azole antifungal drugs have been reported to induce liver toxicity and hypoglycemia. Azoles are also reported to decrease the secretion of stomach acids, hence decrease the intestinal absorption of important biomolecules (Denning et al, 1989; Bueid et al., 2010).

Overall, present drugs are not sufficiently enough to treat systemic fungal infections effectively. Moreover, limited numbers of drugs compel to put continuous and vigorous efforts to develop new antifungal drugs that can be effective or helpful to prevent fungal growth. To develop antifungal drugs large number of libraries containing compounds of diverse functional groups are required to screen. Plants extracts are natural chemical libraries contain thousand of compounds of different classes and functional groups. Furthermore, plant products being used in daily life and human systems are familiar with them, hence non toxic. A number of natural compounds have already been reported to
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have antifungal activity. However, due to various limitations, these compounds could not come or pass clinical trials. Hence, present study was undertaken to screen local plants for their antifungal activity with following objectives.

1.3. Objectives of the Study

Plant extracts are rich sources of diverse kinds of compounds having different functional groups. Hence, in the current study first objective was to screen the plant extract for the antifungal potential. However, focus was kept on the local plants grows in Haryana as there are limited studies conducted to explore the antifungal activity of these plants. Plants selection was random followed by antifungal assays. Plants were collected, identified by their local as well as botanical name and extracts prepared according to standard protocol (objective 1). Second objective of study was to screen extract for their antifungal potential using 96 well microbroth dilution and disc diffusion assay. Further, in order to identify active component or nature of fraction, the extracts were prepared in increasing polarity order successfully with different solvents. The active extracts were further subjected to column chromatography or chemical fractionation to identify or characterized active fraction. Isolated fraction or compound may be cytotoxic and cytotoxicity limits of a fraction (compound) should be known before use in vivo model. Hence active fraction/compound, were subjected to MTT (3-(4,5-dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay (objective 3). Characterization of antifungal compounds/ fraction having limited toxicity or no toxicity is essential in order to explore their mechanism of action and targeted pathways in fungi. Hence, the entire active fractions having limited toxicity were subjected to LC-MS for their characterization followed by chemical analysis (objective 4). After phytochemical analysis of active fraction, in order to explore its antifungal mechanism the metabolic profile of A. fumigatus grown under stress of active fraction were explored. Significantly affected metabolic pathways in fungus were explored using mass data analysis (objective 5).
1.4. Significance of the present study

During the earlier period of 10-15 years, interest in phyto-antimicrobials has been increased due to presence of effective phyto-compounds. Toxicity of present drugs in human beings with increased resistance towards fungal pathogens needs effective and non-toxic antifungal drug. Other reason for the prevalence of fungal infections is non-affordability of the recommended drugs and relapse after treatment.

Any lead toward identification of novel antifungal molecules or molecular targets, is important to develop or design antifungal drugs. In the present study, efforts to identify antifungal molecule(s) and to identify their mechanism of action have been made in order to develop new antifungal drugs. New antifungal compound(s) or extract(s) may lead toward the identification of novel targets. Hence, study aimed to develop or identify novel extracts or compounds having antifungal properties. Additionally, identification of their molecular mechanism may lead towards the identification of novel target. It also creates confidence toward natural products for their wellness and protective nature.