Introduction
There are about two million kinds of living organisms on the earth, of which fungi constitute about a hundred thousand species and many more await discovery. Of those described, nearly 150 species are generally recognised as primary pathogens of man and animals. They may cause a variety of infections, ranging from systemic and potentially fatal diseases to localized cutaneous, subcutaneous or mucosal infections and the fungi causing these infections are termed as opportunistic pathogens. *Candida albicans* is an opportunistic, dimorphic fungus. Most of the time it exists as ovoid or spherical, single yeast cells, which reproduce by budding. Most yeasts do not produce mycelia, but *Candida* has a remarkable ability do so. Normal room temperature favours the yeast form of the organism, but under physiological conditions (body temperature, pH, and the presence of serum) it may develop hyphal forms and pseudohyphae (composed of chains of sausage shaped cells joined end to end) are also common (1).

*Candida albicans* and other species of *Candida* (*C. krusei, C. tropicalis, C. parapsilosis, C. stellatoidea*, etc) are yeasts that normally inhabit our digestive system: the mouth, throat, intestine and genitourinary tract. Multiplication of the organism is normally kept in check through physical barriers such as the skin, by competition with the endogenous microflora (*Lactobacillus acidophilus, L. bifidum, L bulgaricus, and L. salivarius*, etc), and through host defense mechanism. But under certain predisposing conditions an overgrowth of this yeast flora may take place which result in a disease known as candidiasis (2).

The incidence of opportunistic fungal infections has increased dramatically during the last two decades (3). *Candida albicans* is one such opportunistic pathogen that accounts for 8% of all nosocomial blood stream infections and has emerged as the fourth leading cause of such infections (4). The dramatic increase in *Candida* infections is due to the increase in immuno-compromised population, including victims of the acquired immunodeficiency syndrome (AIDS), cancer patients and other individuals treated with aggressive chemotherapy or cytotoxic drugs, as well as increasing medical intervention directed towards prolonging patient survival (e.g. Organ transplants, indwelling
catheter utilization, longer hospitalization or broad spectrum antibiotic use (5). Under such conditions, if the number of friendly bacteria is decreased in relation to the number of *Candida* cells, the immune system is weakened or if other conditions for yeast proliferation occur (diet high in sugar, improper pH in the digestive system), *Candida albicans* may shift from yeast to mycelial form and start invading the body. Candidiasis may range from asymptomatic colonization through superficial muco-cutaneous infections to disseminated systemic disease, often with multiple organ involvement (6, 7). Mortality due to hematogenously disseminated candidiasis is estimated to be approximately 38%-49% (4, 8).

Recent studies on *C. albicans* have indicated that the pathogen employs a number of mechanisms to escape the host immune system. Potential determinants include the ability to switch from an yeast form to a mycelia form and also between different colony morphologies (9, 10), the production of extracellular hydrolytic enzymes (11), synthesis of receptor-ligand molecules required for recognition and adhesion to the host tissues (12), antigenic variability, adhesion to inert and biological substratum and immuno-modulation of the host defense mechanisms (13). The transition from a commensal to a pathogenic lifestyle may also involve changes in the environmental conditions and dispersion within the host.

In addition to these virulence traits, *C. albicans* has also evolved various mechanisms to counter the front line antifungal drugs available for the treatment of candidiasis. Relatively few classes of antifungal drugs are available in the market and the development of resistance enlarges the problem. Main classes of antifungal drugs that are in clinical use or under advance stages of clinical evaluation are polyenes (amphotericin B, nystatin), azoles (clotrimazole, ketoconazole, fluconazole, itraconazole, voriconazole, posaconazole, oxiconazole and ravuconazole), 5-flucytosine, allylamines (terbinafine), echinocandins (caspofungin, anidulafungin, FK463) (14). Though resistance to polyenes is a rare phenomenon, the drug imposes significant side effects. *C. albicans* employs multiple mechanisms against azole drugs such as point mutations, over expression of plasma membrane drug expulsion
pumps and detoxifying enzymes, mechanism of resistance to echinocandins and allylamines are poorly understood and are still being investigated where possibilities include efflux pumps, degradation pathways, and echinocandin resistant β-1, 3-D-glucan synthase target. Some yeast strains are intrinsically resistant to flucytosine because of impaired cellular uptake. Defects in flucytosine metabolism may be the other reason (15, 16). Also most antifungal compounds are fungi-static giving rise to the possibility that the fungi are not cleared from the patients which may lead to the possibility of developing resistance against that drug. Thus, there is an urgent need for the development of new molecules with novel mode of action and identification of new targets that can be targeted to counteract the fungus effectively.

Another important issue is the diagnosis of systemic candidiasis. Rapid diagnosis of fungi may be helpful in reducing the use of inappropriate antifungal compounds to treat Candida spp. that are resistant to a particular agent (16). Diagnosis of invasive Candida infections may be difficult due to the variability and lack of specificity of clinical presentations and also the symptoms (eg. fever) of Candida infections are not very specific (17). A definitive diagnosis is not reached until late in the infection, with subsequent delays in the initiation of therapy that may result in substantial morbidity and mortality (18). Laboratory diagnosis of Candida infections includes microscopic examination of smear from cutaneous, mucosal, oesophageal and vaginal lesions, culture of sputum, bronchoalveolar lavage, oesophageal brushings, urine, stool and surgical drains. These methods are time consuming, tedious and are not fool proof. Biopsy may be required in some cases of deep-seated candidiasis. Novel methods of diagnosis include PCR based amplification of the infectious agents DNA. This method is sensitive, rapid and less cumbersome but is limited due to false positive results arising from the non-specific contamination with other microorganisms sharing the same ecological niche and also the technique is not available commercially (19). Antibody based detection techniques for immuno-diagnosis of systemic candidiasis include latex agglutination (20), counter immuno-electrophoresis, indirect immuno-fluorescence and enzyme linked immunoassay (21, 22).
antibody-based approaches for diagnosis of Candida infections are rapid and sensitive but often the sensitivity decreases considerably when it comes to discriminating between superficial and disseminated candidiasis (23). This is because of the poly-specific nature of the serum antibodies that sometimes may also lead to false positive results. Thus there is an urgent need to develop new techniques for rapid and accurate diagnosis of these infections. Monoclonal antibodies (MAbs) are antibody molecules produced by the cells resulting from a single clone and thus are specific to a single epitope of a protein. Diagnostic techniques based on MAbs are rapid, sensitive and very specific (24). The technique can be pathogen specific, species specific and even stage specific for the same pathogen. Identification of stage specific molecules and development of monoclonal antibodies against them is a promising method of diagnosis (25). Many protein molecules are constitutively expressed by C. albicans. These molecules are attractive targets for diagnosis as they are expressed at all times and thus offer a reliable diagnostic method decreasing the percentage of false positive results (26). Many monoclonal antibodies have been produced against C. albicans that have therapeutic value (27, 28). Thus identification of new target molecules that are stage specific or constitutively expressed can open new avenues for the development of diagnostic as well as therapeutic monoclonal antibodies. Also many molecules identified may be targeted for the development of new antifungal compounds.

Initially, the cell wall was considered an almost inert structure that supplies rigidity and protection to the protoplast. Today, the cell wall is well established as being essential to almost every aspect of the biology and pathogenicity of C. albicans (29). The cell wall acts as a permeability barrier and is the structure that maintains the characteristic shape of the fungus. Also, as the most external part of the cell, the wall mediates the initial physical interaction between the microorganism and the environment, including the host. Cell wall harbours many virulence factors that are required for survival, adherence and tissue penetration. For these reasons, the cell wall of C. albicans is the focus of study by numerous research groups. Proteins that are found in the in vitro
growth medium are often called secreted or extracellular proteins. To reach this location, these proteins travel through the cell wall, where they coexist with cell wall-bound moieties and contribute the total cell wall proteinaceous component (30).

There are many proteins that are either cell associated or secreted depending on growth condition. Many enzymes on the other hand are not present in the cell wall but are recovered from the culture supernatants (31). Regenerating protoplast offer a novel approach to study cell wall as well as secretory proteins that are released into the medium while regeneration (32). Several workers utilized protoplast to study the cell wall and secretory proteins of *C. albicans* yeast form and there are well-established proteome maps available for yeast form (33, 34). Recovery of proteins released into the medium by hyphal form of *C. albicans* is difficult, as the fungus needs serum for its morphological transition. Serum itself contains innumerable number of proteins and interferes with the recovery and identification of proteins secreted into the medium. A serum free protein free media has to be formulated for the preparation of mycelial spheroplast and its regeneration under conditions promoting mycelial form. This approach might lead to the identification of new virulence factors of *C. albicans* and might establish the mycelial form as the virulent form. Availability of novel genomic and proteomic tools such as 2D gel electrophoresis, MALDI-MS, N-terminal sequencing of proteins has added to the specificity and rapidity with which novel target molecules are being identified. Genome data bank, protein data bank are also available which could be utilized for the faster validation of novel targets.

The main objective of the present work was to identify potential target molecule(s) of *C. albicans* with therapeutic and/or diagnostic value for candidiasis. In this piece of work, the isolation of secretory proteins produced by the regenerating protoplast of both the morphologies (yeast and mycelia) was done, while maintaining the culture conditions required for the growth of these morphologies. Proteomic approach has been employed to study the differences in protein profile of cell wall and secreted proteins in the two morphologies separately using immobilized pH gradient 2D gel
electrophoresis. Monoclonal antibody producing hybridoma was generated by fusion technique using proteins secreted by the regenerating protoplast of the virulent form (mycelial form) of C. albicans. The monoclonal antibody was evaluated for its therapeutic and diagnostic potential. Also, affinity columns of this monoclonal antibody was employed to purify the proteins recognized by it and identify them through N terminal sequencing followed by bioinformatics' data base search. Finally evaluation of Th1/Th2 responses triggered by these proteins was done by studying the cytokine profile secreted by the splenocytes against them.