1. Introduction

1.1. Fungi

Fungi are eukaryotic organisms. There are a total of approximately 8.7 million eukaryotic species on planet earth and fungi are approximately 7% of them (Mora et al., 2011). Out of these 7%, 300 species are pathogenic in nature (Microbiology, 2017). These pathogenic fungi cause severe infections with substantial mortality and morbidity. Emergence of pathogenic fungal infections is an important public health problem and has increased considerably in last two decades in immunocompromised patients and in patients suffering from serious diseases (Arendrup et al., 2005; Cantón et al., 2005). Fungal infections are studied mainly in two categories: (i) nosocomial and (ii) community acquired. Nosocomial infections are caused by the opportunistic fungus which remain harmless in healthy individual but causes life threatening infections in immunocompromised patients; however, community acquired infections are caused by opportunistic as well as by primary fungal pathogens which can cause disease in healthy individuals. Major pathogenic fungi are Candida, Aspergillus, Cryptococcus, Histoplasma, Pneumocystis and Stachybotrys. Different risk factors causing fungal infections are depicted in the figure 1.1 Fungal infections are mainly seen in immunocompromised patients or HIV patients. Exposure to ionizing radiation, less intake of fresh foods enriched with naturally occurring enzymes and long-term use of antibiotics are some other predisposing factors for fungal infections. Fungus can cause superficial infections to life threatening systemic infections. Some of the diseases caused by fungus are Pencilliosis, Dacryocysitis, Cutaneous Candidiasis, Cellulitis, Onychomycosis, corneal ulcer, sporotrichosis, Cutaneous Cryptococcus infection and Histoplasmosis.
1.2. *Candida* Species

*Candida* species are major human fungal pathogens which can cause mucosal to systemic infections. *Candida* species are opportunistic commensals which usually remain present in gastrointestinal tract, genitourinary tract and on skin without causing damage. However, in immunocompromised patients or ICU patients or in patients going through
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chemotherapy or when under antibiotic treatment, *Candida* species develop their colonies, invade host tissues and cause severe illness. Infections caused by *Candida* species are generally known as Candidiasis. *Candida* species cause wide range of diseases and can affect almost any organ or system in the body if pathogen gets entry into blood stream; however, HIV patients mostly suffer from oral candidiasis.

There are various kinds of *Candida* species and over 20 of them can cause disease in humans. Although 90% of *Candida* infections are caused by *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* and *Candida krusei*. (Pfaller et al., 2007). *Candida parapsilosis* and *Candida krusei* are less harmful and have lower frequency of occurrence in patients than *Candida albicans*, *Candida glabrata*, and *Candida tropicalis* (Arendrup et al., 2002). *Candida* infections are fourth most common cause of hospital acquired blood stream infections in ICU patients (Wisplinghoff et al., 2004). The occurrence of *Candida* species infections is also age related as incidence of infections are seen at higher rate in elderly patients. Increased use of Broad spectrum antibiotics, use of central vascular catheters, transplantation and use of chemotherapies in cancer patients are some of the factors responsible for higher occurrence of Candidiasis (Ortega et al., 2011).

1.2.1. Taxonomic position of *Candida* Species

The taxonomic position of *Candida* species is given below:

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Ascomycota</td>
</tr>
<tr>
<td>Subphylum</td>
<td>Saccharomycotina</td>
</tr>
</tbody>
</table>
Class : Saccharomycetes
Order : Saccharomycetales
Family : Saccharomycetacea
Genus : *Candida*

![Image of Candida albicans cells](image)

Figure 1.2. Scanning electron micrograph of *Candida albicans* cells viewed at 5.00 KX magnification

1.2.2. *Candida albicans*

*Candida albicans* is the most frequently isolated species in all the cases of *Candida* infections and is the major cause of invasive Candidiasis (Horn et al., 2009). *Candida albicans* cells are 2-5 µm in size and spherical to oval in shape Figure 1.2. It is a polymorphic fungus that mostly resides in gut, oral and genital mucosa, and remain as a commensal in approximately 30-70% healthy individuals and manifest their virulence
attributes in immunocompromised patients (Erdogan & Rao, 2015; Kauffman, 2006; Pappas, 2006; Pfaller & Diekema, 2007). *Candida albicans* shows morphological phenotypes and exhibits yeast to hyphae and white to opaque transitions. These transitions in *Candida albicans* are mostly induced by environmental factors. All morphological phenotypes help in survival and pathogenicity of *Candida albicans* in different environmental condition. Yeast form helps the pathogen to disseminate in blood stream while hyphae form helps to penetrate host tissues and provides virulence attribute. *Candida albicans* strain gives green color on chromogenic agar plates which helps to isolate it from other *Candida* strains.

1.2.3. *Candida glabrata*

In the last two decades the occurrence of non-*albicans* *Candida* species has increased in comparison to *Candida albicans* which is most occurring species. *Candida glabrata* is nondimorphic species and remains in bastoconidia form in all environmental conditions. Among non-*albicans* species, incidence of *Candida glabrata* occurrence has increased most as it is intrinsically fluconazole resistant and fluconazole is the drug most commonly used against *Candida* infections (Guinea, 2014; Maubon et al., 2014; Perlroth et al., 2007; Yapar, 2014). Patients having hematological or solid organ malignancies and neutropenia have more chances to develop *Candida glabrata* infections (Yapar, 2014). It is the second most common pathogen of causing Candidiasis after *Candida albicans*. As *Candida glabrata* is intrinsically fluconazole resistance, it is difficult to treat infected patients and hence they have high mortality rate. Very less is known about the virulence attributes of *Candida glabrata* and host defense mechanism against it. *Candida glabrata*
gives pink to purple color on chromogenic agar plates which helps to isolate it from other Candida species.

1.2.4. Candida tropicalis

Candida tropicalis is third most common Candidiasis causing species after Candida albicans and Candida glabrata. Patients having hematological or solid organ malignancies and neutropenia have more chances to develop Candida glabrata infections (Yapar, 2014). Candida tropicalis can form biofilms like Candida albicans. Candida tropicalis shows morphogenesis in which it changes from yeast from to hyphae form is and very closely related to Candida tropicalis. It is the most virulent species of Candida among non albicans Candida species as it is able to secrete hydrolytic enzymes and can adhere to epithelial and endothelial cells. (Moran, 2002). Candida tropicalis strains have the ability to survive in high salt concentrations and have an osmotolerant nature which help them to survive in saline environments and also helps it in virulence as well as confers resistance against drugs (Zuza-Alves et al., 2016). Candida tropicalis gives metallic blue color on chromogenic agar plates which helps to isolate it from other Candida species.

1.2.5 Candida Krusei

Candida krusei infections mostly occur in patients who have undergone hematopoietic stem cell transplantation (Yapar, 2014). This species has been found to be naturally resistant to fluconazole and itraconazole (Cartledge et al., 1999). Some strains of Candida krusei are also resistant to amphotericin B (Ellis, 2002). Frequent use of fluconazole is also responsible for increased occurrence of Candida krusei infections. Candida krusei shows rough morphology on agar plates that helps to identify it from
other *Candida* species. *Candida krusei* gives pink, fuzzy color on chromogenic agar plates.

### 1.3. Factors causing Invasive Candidiasis

As we know *Candida* species are opportunistic pathogens and live as a commensal in healthy individuals without any clinical manifestations. It turns to pathogenic forms in immunocompromised patients only. Predisposing factors that lead to invasive Candidiasis are shown in [figure 1.3](#).

![Figure 1.3. Predisposing factors of invasive Candidiasis](#)

**Figure 1.3. Predisposing factors of invasive Candidiasis** (Arendrup et al., 2011; Cleveland et al., 2015; Lortholary et al., 2014)
Patients suffering from critical illness like HIV/AIDS or Cancer or having malignant diseases related to blood or undergone organ transplantation or using antibiotics since long time are more prone to develop candidiasis.

1.4. Disease Spectrum of *Candida* Species

There is a wide spectrum of diseases caused by *Candida* species (figure 1.4). Infection caused by any *Candida* species is known as Candidiasis. Candidiasis may be divided into following types:

- **Mucosal Candidiasis**
  - Oropharyngeal Candidiasis
  - Vulvovaginal Candidiasis
  - Esophageal Candidiasis
  - Gastrointestinal Candidiasis
  - Respiratory Candidiasis

- **Cutaneous Candidiasis**
  - Nail infection or onchomycosis
  - Chronic mucocutaneous Candidiasis
  - Congenital cutaneous Candidiasis

- **Systemic Candidiasis**
  - Candidemia
  - Invasive Candidiasis
  - Chronic systemic Candidiasis
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Figure 1.4. Disease spectrum of *Candida* species

- Oropharyngeal Candidiasis
- Vulvovaginal Candidiasis
- Infection in nails
- Infection in skin
- Esophageal Candidiasis
- Mucocutaneous Candidiasis
1.5. In vivo challenges to Candida inside the host

Although infection by Candida is very common, the fungus has to deal with numerous challenges inside the host. It has to get past the microbial barriers which include competition for adhesion and nutrients with other microorganisms inside the host, and toxic metabolites, and also the quorum sensing molecules released by microorganisms that influence the growth of the Candida cells. Candida cells also encounter the hosts immune system cells like neutrophils, macrophages, dendritic cells and natural killer cells. Mucus, epithelial layers and fluid fluxes resist Candida and work as a mechanical barrier for the host. Reactive oxygen species (ROS), temperatures, pH and starvation pose yet another challenge.

1.6. Virulence attributes and survival strategies of Candida species

1.6.1. Morphogenesis

Candida albicans is a polymorphic fungus which can exist in three forms (i) Yeast form (ii) Pseudo hyphae form and (iii) Hyphae form (Sudbery et al., 2004). Yeast form is helpful to the fungus in dissemination through the blood stream while hyphal form is important for host tissue invasion (figure 1.5). Both forms are important for pathogenesis (Spiering et al., 2010); however it was earlier believed that hyphae form is more pathogenic than yeast form (Braun et al., 2000). Hyphae formation also depends on a number of virulence factors as well as on surrounding niche of pathogen. Enhanced filamentous growth protein 1 (EFG1) is major regulator of filamentation and occurrence of different forms of Candida depends on the expression level of EFG1 and helps in host dependent colonization (Pierce et al., 2012; Pierce & Kumamoto, 2012). Morphogenesis in Candida albicans requires two steps which are (i) hyphal initiation and (ii) hyphal
maintenance (Lu et al., 2011). Hyphal initiation requires various factors like presence of serum, elevated temperature, pH, presence of different nutrients and contact surface etc. (Inglis & Sherlock, 2013).

Figure 1.5. Different morphological forms of *Candida albicans* (Source. www.pinterest.co.uk)

Apart from yeast to hyphae transition *Candida albicans* cells also undergo phenotypic switching (Soll, 2014) which helps the pathogen in mating, commensalism and survival in host. Signal transduction pathway involved in yeast to hyphae transition is depicted in figure 1.6. White-to-opaque switching is example of phenotypic switching in *Candida albicans* (Xie et al., 2012). White cells are more virulent while opaque cells are better colonizer as White-to-opaque switching allows immune evasion (Sasse et al., 2012).
Figure 1.6. Signal transduction pathway involved in yeast to hyphae transition. (Sudbery, 2011)
1.6.2. Hydrolytic Enzyme Secretion

1.6.2.1. Proteinases

*Candida albicans* secrete proteinases which are called secreted aspartyl proteinases (Saps). There are 10 proteins in Sap family which are coded by genes SAP1 to SAP10 respectively. Out of these ten, Sap1-8 are secreted while Sap9-10 remain attached to cell surface (Naglik et al., 2003; Taylor et al., 2005). Secretion of proteinases by *Candida albicans* degrade the tissue barriers and hydrolyze many host proteins such as keratin, collagen, laminin, fibronectin, albumin, hemoglobin etc. This makes infection and colonization easier for *Candida*.

1.6.2.2. Phospholipases

*Candida albicans* also secrete phospholipases belonging to four different classes (A, B, C and D) based on the ester bond that they break (Niewerth & Korting, 2001). Phospholipase belonging to class B are extracellular and play an important role in pathogenicity through disruption of cell membrane (Mavor et al., 2005). Phospholipases secretion in *Candida albicans* helps to invade host tissue cell membrane by hydrolysis of ester linkage in glycophospholipids.

1.6.3. Biofilm formation

Biofilms are important virulence attribute of *Candida albicans* which are formed on abiotic or biotic surfaces in which a single cell changes to a specialized cell to perform specialized function. Biofilm formation is a sequential process in which cells first adhere to the surface, proliferate into hyphal cells from yeast form and accumulates extracellular matrix around themselves. Biofilms are highly resistant to antimicrobial agents and more problematic in clinical context since they reduce drug efficacy of established antifungal
drugs like fluconazole, amphotericin B and itraconazole many folds. The biofilm resistance mechanism is not known completely; however (i) less diffusion of drugs through biofilm matrix (ii) changes in the phenotypic forms of *Candida* cells, and (iii) higher expression of resistance genes, might be the reasons for higher resistance in *Candida* species. Biofilm formation by pathogen is seen to result in high pathogenicity and mortality. *Candida glabrata, Candida tropicalis* and some other species of *Candida* can also form biofilms but *Candida albicans* forms the most robust biofilms among all these strains. A picture of biofilm produced by *Candida albicans* is shown in figure 1.7

![Biofilm produced by Candida albicans](image)

**Figure 1.7.** Scanning electron micrograph of *Candida albicans* biofilm (Braga et al., 2008)
1.7. Antifungal Drugs

Antifungal drugs are classified on the basis of their target and are as follows;

1.7.1. Polyene Antifungal Agents

Polyene drugs act as by binding directly to ergosterol in fungal cell membrane. It creates pores in cell membrane which results in leakage of K$^+$ and Na$^+$ ions that ultimately lead to death of fungal cell. Amphotericin B and Nystatin (figure 1.8) belong to this group and are commonly used in fungal infection treatment. Amphotericin B is used to combat systemic infection while Nystatin is used for topical infections.

![Figure 1.8. Structure of (A) Amphotericin B and (B) Nystatin](image)

Polyenes can also react with other sterols like cholesterol but with less efficiency. Nephrotoxicity, nausea, chills and infusion related fever are common side effects of polyene antifungal drugs.

1.7.2. Azole Antifungals

Azoles are the most commonly used class of drugs against fungi. It inhibits the enzyme 14-α- demethylase which is responsible for demethylation of lanosterol. Lanosterol with
methyl group is accumulated and changes the shape and permeability of cell membrane. Inhibition of ergosterol biosynthesis or accumulation of demethylated sterols results in disruption of cell membrane integrity and lead to cell death. Azoles contains two categories of drugs (i) N-1 substituted imidazole and (ii) triazole. Fluconazole, ketoconazole, itraconazole, clotrimazole and voriconazole are some drugs of class Azoles (Figure 1.9). Liver toxicity, problems with vision and abdominal discomfort are some adverse effects ofazole drugs. Although azoles are most commonly used antifungal
drug, resistance against azole drugs is being reported in various fungal strains. Development of resistance in strains is because of alteration in drug target (14-α-demethylase), change in ergosterol biosynthetic pathway, decreased concentration of target enzyme inside the cell and overexpression of target enzyme.

1.7.3. Allylamines

Allylamines is another class of antifungal drugs which target squalene epoxidase, a key enzyme of ergosterol biosynthetic pathway. Squalene epoxidase oxidizes squalene to 2,3-oxidosqualene which is the first oxygenation step of ergosterol biosynthesis and a rate determining step. Inhibition of this step results in accumulation of squalene and deficiency of ergosterol. Accumulation of squalene increases membrane permeability and malfunction of membrane bound enzymes. Terbinafine, naftifine (figure 1.10) and amorolfine

![Figure 1.10. Structure of some allylamines antifungal drugs.](image)
butenafine are examples of allylamines. Common side effects of allylamines are allergic reactions (like rashes, itching and swelling), leukopenia, agranulocytosis, headache, dizziness and erythroderma etc.

1.7.4. Echinocandins

Echinocandins class of drugs target the cell wall of fungal pathogens. Some of the drugs belongs to this class are shown in the (figure 1.11). Drugs of this class inhibit the synthesis

![Figure 1.11. Structure of some of the echinocandins antifungal drugs](image-url)
of 1, 3-\(\beta\)-D-glucan by targeting the enzyme 1, 3-\(\beta\)-D-glucan synthase. 1, 3-\(\beta\)-D-glucan and chitin together form strong fibrils which provide shape, strength and maintain osmotic integrity of cell wall. Caspofungin, micafungin and anidulafungin are examples of echinocandins antifungals. Liver toxicity and headache are common side effects of echinocandins.

1.7.4. Pyrimidine analogue

5- flucytosine (figure 1.12) is member of this group and commonly used with amphotericin B for effective treatment against fungal infections. Flucytosine causes

![flucytosine](image1.png)

**Figure 1.12. Structure of 5-flucytosine**

miscoding by incorporation in RNA as fluorouracil and inhibit the synthesis of essential proteins. It also inhibits the synthesis of DNA by inhibition of thymidine synthase.
Flucytosine is very less toxic to mammalian host. Loss of appetite, diarrhea and vomiting are common side effects of flucytosine.

The target of each class of antifungal drugs on a fungal cell is sown in Figure 1.13.

Figure 1.13. Mode of action of various antifungal drugs
1.8. Requirement of new antifungal agents

In last two decades fungal infections are on the rise. Fungal pathogens are increasingly getting resistant against azoles, the most commonly used class of antifungal drugs, and other classes of antifungals day by day due to use of broad-spectrum drugs. At present we have limited options to treat serious systemic fungal infections; therefore, we are in need to develop new and better antifungal drugs with newer targets inside the fungal pathogens.

Several drugs (azoles, polyenes, pyrimidines) are available to treat serious systemic infections but *Candida albicans* is showing increased resistance to these traditional antifungal drugs. Also, the problem posed by high cost, adulteration and increasing toxic side effects of these synthetic drugs coupled with their inadequacy in disease treatment (found especially in developing countries) cannot be overlooked. In the last ten years there has been a noticeable increase of mycoses, localized on the skin or mucous and systematic caused by yeast species from the genus *Candida*, as well as by moulds from the genera Aspergillus, Fusarium and Zygomyces, especially in immunocompromised patients. The development of resistance in known fungal pathogens and emergence of new fungal pathogens intrinsically resistant to the currently available antibiotics demonstrate the urgent importance of identifying novel antifungal drugs.

1.9. Nanotechnology

Nanotechnology is the field in which materials are being synthesized in nanoscale range i.e. 1 to 100 nm. These nanosized materials are in range of biological molecules and can be further engineered for various applications. **Figure 1.14** shows the comparative size analysis of the nanomaterials with biological molecules and cells.
Figure 1.14. Comparative size analysis of nanomaterials (Kim et al., 2010)
Liposome, dendrimer and gold nanoparticles are some of the FDA approved nanomaterials. Nanoparticles possess unique physical and optical properties (Allen et al., 2002; Anderson & Shive, 1997; Anshup et al., 2005; Bergen et al., 2006; Bindhani et al., 2013; Butterworth et al., 2010; J. Chen et al., 2005; Malugin & Ghandehari, 2010) which are being used remarkably well to develop customized nanoparticles for various applications. Nanomaterial have low melting point and phase transition temperature. They also possess different optical properties like semiconductor blue shift and metallic nanoparticles color change significantly different from their bulk crystal form. All nanoparticles have large surface area to volume ratio property which expose the surface million times and this surface can be used to coat them with different molecules for various drug delivery and other applications. Nanomaterials are being synthesized with ease and have biocompatibility to develop them for clinical purpose in therapeutics, diagnostic kits and imaging techniques.

1.9.1. Silver nanoparticles and their properties

Silver nanoparticles possess various unique properties other than bulk silver (Sharma et al., 2009). Some of the properties are optical properties, electrical and thermal conductivity properties, size dependent properties, mechanical properties, physical and chemical properties, bulk properties, dielectric properties etc. (Krutyakov et al., 2008). These properties of silver nanoparticles are used in microelectronics, imaging techniques and catalysis (Monteiro et al., 2009; Shiraishi & Toshima, 1999). Silver nanoparticles have also been reported for their antibacterial and antifungal properties and therefore are also being used in many products like soaps, plastics, textiles and in
many other consumer products (Dallas et al., 2011; Fabrega et al., 2011; García-Barrasa et al., 2011)

1.9.2. Synthesis of silver nanoparticles

There are four commonly used methods to synthesize silver nanoparticles each having their merits and demerits.

1.9.2.1. Chemical methods.

Chemical method is most commonly used method to synthesize nanoparticles of any kind. In this method a nanoparticle precursor reactant, a reducing agent and a stabilizer is used for the synthesis of silver nanoparticles. Size, shape and nanoparticle properties can be controlled by changing the reaction parameters like temperature, concentrations of reactants used and ratio of reducing agent with substrate etc. Monodisperse nanocubes of silver can be synthesized by polyol process on large scale in which silver nitrate is reduced by ethylene glycol in the presence of polyvinylpyrrolidone (PVP) (Sun & Xia, 2002). Spherical nanoparticles can be synthesized by injection technique in which injection rate and temperature plays important role in controlling size and shape of silver nanoparticles (Kim et al., 2006). Other than these methods oleylamine- liquid paraffin system is also used for monodispersed silver nanoparticles synthesis (Chen et al., 2007). Chemical method is an easy method for nanoparticle synthesis, but it has drawbacks of having toxic byproducts that are not suitable for developing nanoparticles to be used as therapeutic agents.
1.9.2.2. Physical method.

In physical method of synthesizing silver nanoparticles, various physical forces are used to synthesize nanoparticles of various size and shape; this is the best method to synthesize nanoparticles in powder form. Thermal decomposition method (Lee & Kang, 2004), ceramic heater method (Jung et al., 2006), arc discharge method (Tien et al., 2008) and direct metal sputtering method (Siegel et al., 2012) are some of the reported physical methods for synthesis of silver nanoparticle synthesis. Physical methods of synthesis are not cost effective as costly instruments are required to provide reaction conditions.

1.9.2.3. Photochemical method

In this method silver nanoparticles are synthesized by the reduction of precursor molecules in the presence of different light sources at normal room temperature (Sato-Berrú et al., 2009). Light sources either break bulk in smaller parts or in ionic forms from which nanoparticles are synthesized by photophysical and photochemical methods (Christy & Umadevi, 2012; Sakamoto et al., 2009), respectively. Direct photo reduction method in presence of sodium citrate (Sato-Berrú et al., 2009) and UV- photo activation method in aqueous TritonX-100 (Ghosh et al., 2003) or in an alkaline solution of carboxymethylated chitosan are some of the reported photochemical method of silver nanoparticle synthesis (Huang et al., 2008).

1.9.2.4. Biological methods

In biological method of synthesis, precursor molecules are reduced by the enzymes or cytosolic extract obtained from different plants or from different microorganism (Sintubin, Verstraete, & Boon, 2012). In this method biological components work as reducing agent and capping agent in the reaction. Synthesis by bacterium Shewanella
oneidensis (Mallik et al., 2001), Lactobacilus spp. (Sintubin et al., 2009) and by the fungus Trichoderma viride (Fayaz et al., 2010) are some of the examples of biological synthesis of silver nanoparticles. Biological synthesis is environment friendly and cost-effective method without toxic byproducts for silver nanoparticle synthesis.

1.9.3. Therapeutic potential of silver nanoparticles

AgNPs have been reported for their antimicrobial properties. They have antibacterial, antifungal and antiviral properties, and are found to be effective in inhibiting the growth of different bacterial strains. AgNPs have been reported to inhibit the growth of Eschericia coli, ampicillin resistant Eschericia coli, Vibrio cholera, Pseudomonas aeruginosa, Salmonella typhus and Staphylococcus aureus at concentrations ranges from 1 µg/ml to 100 µg/ml (Kim et al., 2007; Morones et al., 2005; Shrivastava et al., 2007; Sondi & Salopek-Sondi, 2004).

AgNPs have shown their antifungal properties against different species of Candida, Trichophyton rubrum and against Trichophyton mentagrophytes and inhibitory concentration ranges from 0.3 µg/ml to 10 µg/ml (J. S. Kim et al., 2007; Morones et al., 2005; Shrivastava et al., 2007; Sondi & Salopek-Sondi, 2004).

AgNPs have also shown antiviral properties against HIV1, HBV, MPV and H1N1 influenza virus (Kim et al., 2007; Morones et al., 2005; Shrivastava et al., 2007; Sondi & Salopek-Sondi, 2004).
1.10. Aim and objective of the study

The opportunistic human pathogen *Candida* is a fungus associated with a range of clinical conditions. The organism most commonly disseminates from vaginal and oral mucosal infections to a more complex and life-threatening systemic condition and particularly manifest in immunocompromised patients, such as those suffering from AIDS or undergoing chemotherapy (Cutler, 1991; Odds, 1988).

Although most *Candida* infections occur in patients who are immunocompromised or debilitated in some other way, *Candida* species are commonly responsible for fungal infections and express several virulence factors that contribute to pathogenesis. These factors include host recognition biomolecules (adhesins), morphogenesis (the reversible transition between unicellular yeast cells and filamentous growth forms), secreted aspartyl proteases and phospholipases. Additionally, 'phenotypic switching' is accompanied by changes in antigen expression, colony morphology and tissue affinities in *C. albicans* and several other *Candida* spp. Switching might provide cells with a flexibility that results in the adaptation of the organism to the hostile conditions imposed not only by the host but also by the physician treating the infection.

A limited range of anti-fungal treatments is available, but these are often associated with unpleasant side effects. The increasing resistance to favored treatments using fluconazole or amphotericin necessitates investigations into new treatments for fungal infection (Lemar et al., 2007). Nanotechnology is the field in which materials are being synthesized in nanoscale range i.e. 1 to 100 nm. These nanosized materials are in range of biological molecules and can be further engineered for various applications. AgNPs have
been reported for their antimicrobial properties. AgNPs have antibacterial, antifungal and antiviral properties.

Aim and objective of this study was biological synthesis of silver nanoparticles from cytosolic extract of fungus *Candida tropicalis* and to determine their efficacy against *Candida* species. This study was performed under the following objectives;

i. To evaluate the effect of silver nanoparticles on growth and viability of *Candida* species (MIC, MFC, Time kill studies, Disc Diffusion assay)

ii. To study the effect of silver nanoparticles on adhesion and biofilm formation.

iii. To study the effect of silver nanoparticles on plasma membrane H\(^+\)-ATPase activity & sterol quantitation.

iv. To study yeast dimorphism and related pathogenicity markers like proteinase and phospholipase secretion by *Candida* cells in the presence of silver nanoparticles.

v. To study mode of cell death in the presence of silver nanoparticles by analyzing generation of oxidative stress in *C. albicans* through determination of ROS generation.

vi. To study morphological alterations in the presence of silver nanoparticles.

vii. To study the synergistic effect of silver nanoparticles along with conventional antifungal drugs.