BACKGROUND

Fungi which are eukaryotic organisms have approximately 300,000 different species, out of which little less than 200 are potential parasites few of which affect humans. Fungal diseases of mammals, mycoses, range from the common mild cutaneous or subcutaneous skin infections, such as athletes foot, to the potentially lethal acute or chronic infection of deep tissues that are typically caused by Candida species. Candida albicans will be the commonest yeast variety afflicting human beings. Candida albicans belongs to the class Ascomycetes and the family, Saccharomycetaceae. Almost half of the population carries this harmless yeast in many different body locations. However, in response to a change in the host environment, C. albicans can convert from a benign commensal into a disease-causing pathogen, causing infections in the oral, gastrointestinal and genital tracts. The infection caused by C. albicans can be defined in two broad categories, superficial mucocutaneous and systematic invasive, which involves the spread of the fungus to the blood stream (candidemia) and to the major organs.

2.1. Fungal infections

Around the world, both in developing and developed countries, fungi causing systemic infections have become a main public health trouble. Historically, the discovery with the etiologic purpose competed through fungi within ailment marked the beginning involving medical microbiology. In the past two decades systemic fungal infections prevalence has been overwhelming.
Previously, systemic infections were known to cause by pathogenic dimorphic fungi. However, preliminary from the 1960s, numbers of infections have increased mainly in the immunocompromised host by opportunistic fungi (Chakrabarti and Shivaprakash, 2005). In recent times, immunosuppressed hosts are being increasingly connected with newer and less common fungal infections. The etiology of systemic fungal infections can be broadly classified into two groups: endemic mycoses due to true pathogenic fungi and opportunistic fungal infections due to a vast array of saprophytic fungi.

2.1.1. True Pathogenic Fungi

Distinct forms of true pathogenic fungus exist throughout tissue on 37°C as compared to cultural mycelial variety on 25-30°C. A lot of this fungus usually is called dimorphic fungus including *Sporothrix schenckii, Blastomyces dermatitidis, Histoplasma capsulatum, Paracoccidioides brasiliensis, Penicillium marneffei, Histoplasma duboisii* and *Coccidioides immitis*. Dimorphic fungi are normally geographically minimal. *C. immitis* is usually a geophilic shape limited to "new world " and also used to reside especially within the desert-like landscape involving north, Central, and also South America (Viriyakosol *et al.*, 2013). *P. marneffei* is restricted to south-east of Asia probably leftover with it’s an environment bamboo mice (Chandler *et al.*, 2013; Ranjana *et al.*, 2002). *H. capsulatum* and also *B. dermatitidis* have a very world-wide distribution. There exists only one document involving systemic sporotrichosis caused by *S. schenckii var. luriei* and also represents the only report from an Asian country (Vaishampayan and Borde., 2013). Together with introduction connected with AIDS in India, histoplasmosis is actually significantly reported.
2.1.2. Opportunistic Fungi

Early in the 1960s, patients having cancer, sarcoidosis, diabetes and organ transplant were regularly connected with *Candida*, *Aspergillus*, *Cryptococcus*, and *Zygomycetes*. In comparison to all other fungi these 'big four' opportunists were gaining more pathology and more investigators' attention.

Nonetheless, within the last few 30-40 several years alterations include occurred, and more recent pathogens are now being identified particularly while using emergence involving AIDS. Often, it's not simply a single fungus, but alternatively a variety of fungi species below *Pneumocystis*, *Candida*, *Cryptococcus*, *Histoplasma*, *Coccidioides*, *Aspergillus*, and *zygomycetes*, which could produce concomitant and/or effective opportunistic systemic *Candida* transmissions. Apart from, a long list of a lesser amount of widespread yeast pathogens is being isolated routinely by clinical specimens. This specific result in problem in classifying as well as studying this kind of yeast infections.

2.2 General Introduction of *Candida*

*Candida* is a genus or sub-group of about 200 different species, which are naturally occurring yeasts (Arendrup, 2013). But out of them all disease causing species in man are limited (Table 2.1). There capability to cause infections in their human hosts, make these five species, among others the most clinically significant. *Candida* can also produce hyphae and pseudohyphae in tissues, but this behavior is a function of both species and the involved organ. Standard sugar assimilation and morphological techniques are the two techniques which based on the identification of the individual species.
2.2.1. Growth

*Candida albicans* grows on a simple defined medium containing a source of carbon (e.g. glucose), nitrogen (e.g. ammonia salts), and phosphate and has a requirement for biotin. The organism grows in the temperature and pH ranges of 20-40°C & 2-4 respectively. Growth rates on Synthetic medium at 30°C are generally in the range 0.3-0.4 h (Chander et al., 2013). This is due largely to the uptake of ammonia and the accumulation of protons & anions in the medium. *Candida albicans* has an intracellular pH of 6.7-6.8, except in mature hyphae where it is 6.4. It contains a variety of amines and trace metals. In *Candida albicans* most of the dehydrogenase enzymes involved in glucose metabolism require NADP rather than NAD. Yeast cells utilize glucose at a rate 0.9 µmol /min / 10^10 cells, and approximately 60% of the assimilated carbon is expired as CO₂. Glycogen is the principle store of intracellular carbohydrate within a concentration range of 0.8-1.5 µmol glucose equivalent per gram dry weight (Magee, 2010).

2.2.2. Morphology

The colonies of *Candida albicans* grown on Sabouraud dextrose agar at 25°C appear white to cream, soft, and smooth to wrinkle. The isolates grow at 35°C and it required cycloheximide media for growth. Abundant branched pseudohyphae and true hyphae with blastoconidia can be observed after 72 hours incubation at 25°C on cornmeal media (Figure 2.1).

2.2.3. Cellular Structure

*Candida albicans* is a eukaryotic cell having a membrane bound genetic material, and other membrane bound typical eukaryotic cellular organelles. It is a diploid organism with a chromosome count of 8 in haploid set. The receptors of *C. albicans* are mannoproteins (have acidic activity) that are needed for the adherence of the organism to the endothelial cells. The
exterior of the cell is made up of many types of polysaccharides such as, glucan, chitin, and mann. Fibrinogen and many extra-cellular matrix components are among the host proteins held in place by cell wall proteins.

Figure 2.1: Schematic representation of the three phenotypes adopted by *C. albicans*. Blue, nucleus; dark gray, septum (Source: Uchida et al., 2009)

*C. albicans* wall, mainly of the budding scars, ring around the constriction between mother cell, bud, and septa between independent cells compartments are made of Chitin which is present in small amount (0.6 to 9%) (Chaffin et al., 1998). *C. albicans* act as an opportunistic pathogen to humans, this biological ability is due to the morphological plasticity; that is the characteristic of other fungi too. Modifications inside cellular appearance need incorporation of the many cellular capabilities, in addition to be held inside reply to environmental improvements, mostly pH in addition to temperatures.
Table 2.1: Medically significant *Candida* spp

<table>
<thead>
<tr>
<th>Common Species</th>
<th>Less Common Species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td><em>C. guilliermondii</em></td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td><em>C. dubliniensis</em></td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td><em>C. kefyr (C. pseudotropicalis)</em></td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td><em>C. famata</em></td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td><em>C. haemulonii</em></td>
</tr>
<tr>
<td><em>C. lusitaniae</em></td>
<td><em>C. novegensis</em></td>
</tr>
<tr>
<td></td>
<td><em>C. viswanathii</em></td>
</tr>
</tbody>
</table>

2.3. Microbiology

The mode of reproduction in *Candida* is by budding. This yeast fungus has thin cell wall of 4-6 µm. Of note, *C. glabrata* is the one species that does not produce hyphae or pseudohyphae and it has variously been considered to belong to the genus *Candida* and the genus *Torulopsis* (Rodrigues *et al.*, 2013). Nevertheless, DNA-based information include positioned this living thing from the genus *Candida* fungus (Inglis *et al.*, 2012).
2.4. Epidemiology

There is lot of difference in terms of clinical associations, frequency and virulence in major pathogenic species of *Candida* (Table 2.2). In the Recent surveys the long-established pattern of *C. albicans* and its virulent nature are the main cause of different types of candidiasis (Colombo *et al.*, 2003; Pappas *et al.*, 2003; Pfaller *et al.*, 1998, 2002) and it has very high rate of fungus-related mortality (Pappas *et al.*, 2003). Vulvovaginal candidiasis is highly prone in women and out of more than 50% of them have had one incident of this (Ringdahl, 2000). The effects of hormonal imbalance mainly due to Pregnancy, contraception have negative effect on the normal acidic environment of the vagina that leads to increased susceptibility for infection. Diabetes as well as diet plans abundant with carbohydrates might also contribute to serious abolish infections (Leon *et al.*, 2002; de la Rosa-García *et al.*, 2013; Lanternier *et al.*, 2013; Sket *et al.*, 2013; Shibasaki *et al.*, 2013; Delaloye and Calandra., 2013; Rennert *et al.*, 2000; Hamad *et al.*, 2013; Schuetz, 2013 ). In hospitalized patients *Candida spp.* is just about the a few most common factors behind bloodstream attacks (Klatte *et al.*, 2013; Edmond *et al.*, 1999).

*C. tropicalis* is the main non-*albicans* and the most virulent species. It infects gastrointestinal mucosa during colonization that leads to altered bacterial flora (Lackey *et al.*, 2013; da Costa *et al.*, 2012; Silva *et al.*, 2012), chemotherapy-induced direct damage, and neutropenia which can cause insidious diseases (Goldani and Santos, 2010; Dey and Maiti, 2013; Juyal *et al.*, 2013). The most prevalent non-*albicans* types obtained in the actual blood can be *C. glabrata* (Table 2.2). Severely immunocompromised patients are infected with *C. glabrata* that have moderately low virulence (Jackel and Lai, 2013; Tietz, 2012). The infections caused by this non-*albicans* species have high mortality rate (Arroyo-Heluerga *et al.*, 2012; Ferrari *et al.*, 2011).
Table 2.2: Characteristics of the major *Candida* spp

<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency (%)</th>
<th>Virulence</th>
<th>Clinical Associations</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>42-65</td>
<td>High</td>
<td>Most common in all settings</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>11-25</td>
<td>High</td>
<td>Cancer</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>7-15</td>
<td>Low</td>
<td>Cancer</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>7-18</td>
<td>Variable</td>
<td>Plastic devices, hyperalimentation</td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>1-4</td>
<td>Low</td>
<td>Cancer</td>
</tr>
<tr>
<td><em>C. lusitaniae</em></td>
<td>1-2</td>
<td>Low</td>
<td>Cancer</td>
</tr>
</tbody>
</table>

In the bloodstream the fourth most widespread *Candida* sp. is *C. parapsilosis*. The infections caused by *C. parapsilosis* are powerfully associated with the presence of intravenous catheters and other prosthetic devices (Bonfietti et al., 2012; Garcia-Effron et al., 2012; Pammi et al., 2013; Tosun et al., 2013; Wingard, 1991).

*C. parapsilosis* considered to be much less virulent than *C. albicans* (Tóth et al., 2013; Meunier-Carpentier et al., 1981; Pfaller et al., 1995; Plouffe et al., 1977; Weems, 1992; Wingard, 1995 Huang et al., 2000). According to the study on fungemia *C. parapsilosis* was less frequently linked with septic shock than *C. albicans* (Alonso-Valle et al., 2003; Branchini et al., 1994; Hawser and Douglas, 1994; Pfaller et al., 1995, Shin et al., 2002), and it has lesser mortality rate in cancer patients (Moris et al., 2012). However, isolates involving *C. parapsilosis* prove considerable inter-isolate anatomical dissimilarities (Singaravelu et al., 2013; Horváth et al., 2012) and a broad range of pathogenicity (Silva et al., 2012; Borghi et al., 2011; Girmenia et al.,...
1996). This cause(s) with the broad range regarding pathogenicity on this kinds will not be thoroughly understood, nevertheless the genetic heterogeneity involving isolates will be so that two brand new kinds, *Candida orthopsilosis* and *Candida metapsilosis*, have recently been proposed for less common two of the three major variants (Cantón E *et al.*, 2011; Tavanti *et al.*, 2005). According to the proposal, *Candida parapsilosis* is the most common DNA variant. The relationship between the patterns of disease and there new species names is completely unclear. Also, isolates colonizing the bloodstream and skin are variance in respect to mechanisms for invasion and relative pathogenicity (De Bernardis *et al.*, 1999).

In immunocompromised patients the fifth most common species is *C. krusei* (Hager *et al.*, 2010; Yadav *et al.*, 2012) and the sixth most common species is *C. lusitaniae* (Werner *et al.*, 2011).

In 1995 separate species *C. dubliniensis* (Sullivan *et al.*, 1995) is identified using the specialized techniques and its phenotypic resemble is found with *C. albicans* (Koga-Ito *et al.*, 2011). Standard agents are typically vulnerable by the isolates of this species (Moran *et al.*, 1997; Odds *et al.*, 1998; Sebti *et al.*, 2001). In the blood stream of oropharynx of HIV-infected individual the rate is normally ≤ 2% (Sancak *et al.*, 2004; Sebti *et al.*, 2001; Sullivan *et al.*, 2004). Even it is seen in adults and in pediatric patients that this species has less virulence than *C. albicans* (Kim *et al.*, 2003).

### 2.5. Clinical Manifestations

The clinical manifestations of candidiasis are diverse. First, there are a number of localized syndromes that are widely appreciated. Depending upon the area infected, candidiasis is of following types:
2.5.1. Oropharyngeal candidiasis: including thrush, glossitis, stomatitis and angular cheilitis

Figure 2.2: The figure shows Oral candidiasis in a new born (left) and in an immunosuppressed patient (right)
(Source:www.mycology.adelaide.edu.au/Mycoses/Cutaneous/Candidiasis)

Up to 5% of newborn infants and 10% on the seniors suffer from acute oral candidiasis and it is hardly ever seen in healthy adults. On the other hand, it is linked to critical immunological impairment as a result of diabetes mellitus, leukemia, lymphoma, malignancy, neutropenia as well as HIV infection in which it reveals as being a predictor regarding clinical progression to AIDS. Clinically, whitened plaques which form around the buccal mucosa appear like milk curd and less commonly on the gums, the pharynx tongue or the palate.
2.5.2. Cutaneous candidiasis: including intertrigo, paronychia and onychomycosis

Figure 2.3: The figure shows Interdigital candidiasis (left) and Candidiasis between the toes mimicking tinea (right)

(Source: www.mycology.adelaide.edu.au/Mycoses/Cutaneous/Candidiasis)

Intertriginous candidiasis will be most often observed in the particular axillae, groin, inter- and also sub-mammary folds, intergluteal folds, interdigital spots, and also umbilicus. Humidity, heat, friction and also maceration of the skin are classified as the basic principle predisposing aspects in the normal patient.

Figure 2.4: "Nappy rash" candidiasis in an infant which spread to the mouth area (Source: www.mycology.adelaide.edu.au/Mycoses/Cutaneous/Candidiasis)
Diaper candidiasis is usually common within babies beneath unclean ailments associated with chronic moisture and local skin maceration related to ammonitic irritability due to irregularly changed grubby diapers.

Figure 2.5: *Candida* onychomycosis in an immunosuppressed patient (Source: www.mycology.adelaide.edu.au/Mycoses/Cutaneous/Candidiasis)

Chronic *Candida* onychomycosis typically causes total damage associated with nail cells and is also affecting individuals together with chronic mucocutaneous yeast infection or perhaps additional actual variables that impact either this hormonal or perhaps immunologic position from the host. For instance, diabetes mellitus, hypoparathyroidism, Addison's disease, disorder from the thyroid, malnutrition, malabsorption in addition to numerous malignancies.

2.5.3. Vulvovaginal candidiasis

Vulvovaginal candidiasis is usually a frequent problem throughout women of all ages, generally connected by using broad-spectrum antibiotics, the third trimester of pregnancy, lower vaginal pH along with diabetes mellitus.
Sexual Intercourse and oral contraceptive can also be adding to aspects in addition to microbe infections may well expand to add the perineum, the vulva along with the whole inguinal region. Chronic refractory vaginal candidiasis, associated with oral candidiasis, can also be some sort of demonstration associated with HIV contamination or AIDS.

2.5.4. Chronic mucocutaneous candidiasis

The famous example of persistent candidiasis is chronic mucocutaneous candidiasis. It is generally originated from *C. albicans*. The infection is normally occurred in the pores and skin, nails and also mucous walls involving patients that result in varied metabolic agitations to cell-mediated immunity. This specific trigger defects within endocrine problems like disorder with the thyroid, hypothyroidism, Addison's sickness, diabetes, hypoparathyroidism along with polyglandular autoimmune sickness.

2.5.5. Neonatal and congenital candidiasis

A systemic infection throughout neonates typically arises due to the Low birth weight and age, extended intravascular catheterization and using antibiotic drug treatments. The cultures of blood are found to be positive. This type of candidiasis has high prevalence of meningitis. The formation of fungal ball may also occur in the ureters or renal pelvis that leads to renal difficulty. Congenital candidiasis received in utero is normally confined on the skin by means of a generalized erythematous vesicular rash. Abortion sometime leads to intrauterine candidiasis.
2.5.6. Oesophageal candidiasis

AIDS is highly related with oesophageal candidiasis. Concomitant oral candidiasis is frequently present. Disseminated Candidiasis and septicemia may also occur from Oesophagitis.

2.5.7. Gastrointestinal candidiasis

The stomach of patients and less commonly the duodenum and intestine those having hematological malignancies or acute leukemia may found to have several ulcerations. Perforation can lead to peritonitis and also hematogenous multiply to the liver, spleen along with other parts. Colonization and invasion of the stomach or maybe abdominal mucosa can often be associated with the actual removal connected with more and more yeasts that could be diagnosed inside stools.

2.5.8. Pulmonary candidiasis

Pulmonary infections can be had through possibly hematogenous dissemination causing any diffuse pneumonia or even through bronchial extension in individuals having oropharyngeal infections.

2.5.9. Candida Peritonitis

*Candida* fungus peritonitis can easily result of colonization connected with indwelling catheters used for peritoneal dialysis or gastrointestinal perforation caused by ulcers, diverticular colitis, surgical procedures or intra-abdominal neoplasm.
2.5.10. Urinary tract candidiasis

Transient asymptomatic candiduria may occur throughout antibiotic or maybe corticosteroid therapy which stimulates the growth of *Candida*, over the gastrointestinal, sexually transmitted disease tracts, and also lower urinary tract infections results from local spread of yeasts from these sites. This disorder is most frequent throughout women. *Candida* cystitis or bladder colonization might be caused by continuous catheterization along with concomitant antibiotic therapy, diabetes and also glycosuria, anatomical uropathy, earlier bladder endoscopy or surgery, diabetic neurogenic bladder from prostatic hypertrophy, or pelvic irradiation regarding cervical melanoma.

2.5.11. *Candida* Meningitis

*Candida* meningitis is an extraordinary entity, mainly diagnosed in low birth weight neonates with septicemia and also in patients with hematological malignancies, complicated neurosurgery or intracerebral prosthetic devices such as ventriculoperitoneal shunts.

2.5.12. Hepatic and hepatosplenic candidiasis

Patients suffering neutropenia, usually acute leukemia are prone to have Hepatosplenic candidiasis. Histopathology demonstrates diffuse hepatic and/or splenic necrotic lesions on your skin or maybe abscesses that contain tiny variety of pseudohyphae. The definitive medical diagnosis is often tough because of the incapability to thoroughly biopsy these types of individuals.
2.5.13. Endocarditis, myocarditis and pericarditis

Most general form of cardiac candidiasis is Endocarditis. Elements for example Pre-existing valvular ailment using concomitant intravenous catheterization as well as antibiotic treatment, intravenous substance abuse, cardiovascular system surgical treatment as well as control device prosthesis are generally the most prevalent.

2.5.14. Candidemia (Candida septicemia) and disseminated candidiasis

Candidemia has been defined as the presence of yeasts in the blood with or without visceral involvement. Hematogenous dissemination may then happen to more than one other body organ programs while using development for many microabscesses. Candida species have been reported to cause up to 15% of cases of septicemia seen in hospital patients.

2.5.15. Ocular candidiasis

![Figure 2.6: Endophthalmitis due to Candida](Source: www.mycology.adelaide.edu.au/Mycoses/Cutaneous/Candidiasis)
Candida endophthalmitis is frequently related to candidemia, throughout property catheters or perhaps substance abuse, nevertheless it is really rare throughout affected individuals having tough neutropenia. Patients complain of cloudy vision due to Lesions formation near the macula. Exogenous Candida endophthalmitis is very uncommon, but some cases do happen following ocular trauma or surgery. Similarly, conjunctival and corneal infections are also reported following trauma.

2.5.16. Osteoarticular candidiasis

Arthritis is often an overdue progress regarding Candidemia within neonates or maybe neutropenic affected individuals. Yeast causes disease often through hematogenous spread as well as direct inoculation throughout surgical procedures as well as intra-articular corticosteroid injection throughout Prosthetic as well as rheumatoid joints.

2.6. Laboratory Diagnosis

For the diagnosis of all forms of candidiasis, Culture is the gold standard. Any time cultures of non-sterile strains will be concerned, it should understand that Candida spp. can also be surface colonizers. Thus, the problem of progress of Candida spp. via skin as well as mucosa is just critical when there is similar proof of condition based either on physical findings as well as microscopic examination of tissues.

Growth of Candida from a sterile specimen should never be ignored and is almost always clinically significant. Growth from the blood is the most common such report. Especially for Candidemia, it is significant to understand the part of intravascular catheters within the pathogenesis connected with Candidemia (Nucci and Anaissie, 2002; Walsh and Rex, 2002). The most assaults of Candida bloodstream infection begin by having an affected person that
penetrates blood by means of the gut (Huang et al., 1999; Levin et al., 1998; Lupetti et al., 2002b; Sanchez et al., 1993). In either occurrence, especially *C. parapsilosis* or *Candida* spp, has a significant capability to adhere to plastic surfaces. The ability to adhere and form these biofilms does vary between species and between isolates of a given species (Kojic and Darouiche, 2004; Shin et al., 2002), but *C. parapsilosis* in particular appears to have a significant ability to form biofilms. So, the catheter might function as the nidus for a true endovascular source of the affected individual. As a result, removal of intravascular catheters may be a helpful adjunct in therapy of *Candida* bloodstream infections.

There are no consistent non-culture-based serodiagnostic tests available for routinely diagnosis of invasive candidiasis. Preferred assessments including assays intended for 1-3-D-beta-glucan (Obayashi et al., 1995; Odabasi et al., 2004), PCR for *Candida* DNA (Walsh et al., 1995), and detection of *Candida* metabolites (Walsh et al., 1994) have resulted in positive response but have not gained well-known and widespread recognition due to lack of commercial licensure and limited availability of supportive data.

2.7. *Areas generally affected by Candida*

2.7.1. Genitals

Most common form of infections caused by *Candida albicans* are Vulvovaginal infections. Almost every females encounter some type of genital *Candida* contamination eventually of their lifetimes (Edwards, 2004) in addition to about seventy-five percentage of those attacks take place through the reproductive years (Mitchell, 2004).

Itchiness, often together with irritation or burning is probably the most popular signs and symptoms of any vulvovaginal illness. Nevertheless *C. albicans* is probably the widespread
factors behind vulvovaginal infections; they can also be caused by bacteria (Edwards, 2004). At times more symptoms might include unpleasant intercourse or urination in addition to inflammation in the vulva and inside legs. Fungus microbe infections that are attributable to *C. albicans*, accomplish mainly while pH changes occur because of hormonal variations, before as well as immediately after menstruation, while in perimenopause, or even while taking common oral contraceptives (Edwards, 2004).

2.7.2. Skin

Primarily *Candida* infection in the skin takes place between the fingers, toes, throughout the anus (*Candida albicans* is commonly present in faeces), and on the penis. A rash on the inner thighs can accompany infection in the rectal and genital areas. A skin fungal infection generally occurs at the site of an abrasion or where skin is continuously moist. Cutaneous infection also occurs under skin-folds such as under pendulous breasts or genital skin folds (Bennett, 2004a, 2004b).

2.7.3. Mouth and Throat

Oral candidiasis, or yeast infection, arises in the mouth. Apparently it appears as white areas or patches on the lips, tongue, internal cheeks, or roof in the jaws. These lesions are usually painless unless they occur at the corners of the mouth. Perleche is a *Candida* fungus characterized by cracks as well as tiny cuts at the corners of the jaws, a disorder frequently brought on by ill-fitting dentures (Braunwald et al., 2001). Oral thrush occurs most commonly in neonates and immunocompromised individuals, especially people infected with HIV (Bennett, 2004a, 2004b).
2.7.4. Systemic Infection

Among People having low immunity Candida infections can take place in any part of the body but under normal conditions gut and some other parts are most prone to traces of Candida. Systemic, or deep, Candida infections are extreme health conditions which usually require immediate medical therapy.

Characteristics of Invasive Candida infections are low blood pressure, elevated heart rate, respiratory distress, multiorgan distress, systemic rash or skin peeling and fever and shock. All the above are very dangerous, even potentially fatal and there is no such thing as a low-grade systemic Candida infection with mild symptoms (CDC (Centers for Disease Control), 2003).

If candidiasis is associated with diabetes or malignancy, the underlying disease must be treated in order to discourage yeast growth. Invasive disease (deep candidiasis) may affect major organs, such as the kidneys, spleen, liver, lungs, eyes, brain, and heart. Organ involvement can lead to organ failure if infection is not treated quickly and effectively.

2.8. Pathogenesis

The actual understanding of Candida virulence variables is generally limited. Aspartyl proteinases (De Bernardis et al., 2001), phospholipases (Ibrahim et al., 1995), phenotypic switching (Soll, 1992), and adherence and biofilm formation (Chandra et al., 2001) are among the prospective mechanisms used by these organisms (Fidel et al., 1999; Perfect, 1996; Alonso-Monge et al., 1999; Mishra et al., 2007a).

2.8.1. Aspartyl proteinases

The Candida albicans Saps are encoded by 10 SAP genes (Felk et al., 2000). SAPs are not limited to C. albicans and their presence has been demonstrated in other Candida species such as
Candida tropicalis, and Candida parapsilosis (Cerikcioglu and Kotiloglu, 1995; De Bernardis et al., 1996; Kuriyama et al., 2003; Ollert et al., 1995; Wu et al., 1996). Unique SAP family genes seem to be important for mucosal (SAP1–SAP3) and also systemic (SAP4–SAP6) attacks and in addition get excited about C. albicans adherence, injury, and also evasion of host immune responses (Cassone et al., 1999; Ibrahim et al., 1998; Sanglard et al., 1997). The proteinases have distinct variations with pH optima, using SAP1–SAP3 (yeast associated) having optimum activity at lower pH values, as well as SAP4–SAP6 (hyphal associated) having optimum activity at higher pH values, with a pH array in between 2.0 as well as 7.0 (Hube & Naglik, 2002; Hube, 1996).

Hemolysin is another putative virulence factor which aides to Candida pathogenesis. Particularly, the secretion of hemolysin, followed by iron acquisition, facilitates hyphal invasion in disseminated candidiasis (Odds, 1988; De Bernardis et al., 1999; Hube B, 1996; Leidich et al., 1998; Sanglard et al., 1997; Watts et al., 1998).
2.8.2. Phospholipases

The secreted aspartyl proteinases (SAP) along with phospholipases (PL) usually are two rather huge families of *C. albicans* enzymes, many of which have been associated with virulence (Mohandas and Ballal, 2008). Just like proteinase, phospholipase activity also acts as avirulence factor in *Candida* species. There are four types of phospholipases recognized so far; PLA, PLB, PLC and PLD (Figure 2.8). In an animal model of candidiasis mainly PLB1 has been shown to be necessary for virulence (Ghannoum, 2000). In recent times the experience regarding Plb1p continues to be diagnosed with hyphal ideas throughout tissues intrusion (Ghannoum, 2000). Plb1p is a glycoprotein of 84 kilodalton having both a lysophospholipase-transacylase and hydrolase activity (Theiss et al., 2006), and is possibly secreted. Many investigators has found phospholipase enzyme in microbial adherence to host cells. Since phospholipases lead to cell lysis via targeting the membrane phospholipids and digests these components (Salyers and Witt,
It has been anticipated the most important mechanism contributing to microbial virulence by direct host cell damage and lysis. Given the lipid degradation products and the substrate specificity of the enzyme, *Candida* PLB could also be involved in a yet unidentified signal transduction pathway potentiated at the lysophospholipid levels (Sugimoto and Yamashita, 1994). The important different clinical fungal genera are found to secret the enzyme phospholipases and evidence that they share the identical class of phospholipases, namely, PLB. Phospholipases are the hydrolytic enzymes, they act as a universal virulence factor for pathogenic fungi (Ghannoum, 1998, Singh et al., 2010).

Moreover, the demonstration that phospholipase secretion is not limited to one fungal genus increases the potential of fungal phospholipases as a therapeutic target. A number of drug development approaches could be pursued which may lead to the discovery of novel agents targeting fungal phospholipases. Price et al described a plate method for the detection of phospholipase activity in *C. albicans* (Price et al., 1982). Since egg yolk contains large amounts of phospholipids, predominantly phosphotidylcholine and phosphatidylethanolamine, it was incorporated into a Sabouraud dextrose agar-based medium. When grown on this medium, phospholipase-positive *candida* isolates produce a distinct, well-defined, dense white zone of precipitation around the colony. This white zone is probably due to the formation of calcium complex with the fatty acids released by the action of phospholipase on the phospholipids present in the egg yolk (Macfarlane and Knight, 1941). In this assay, phospholipase activity (expressed as a Pz value) is defined as the ratio of colony diameter to the diameter of the dense white zone of precipitation around phospholipase positive colonies. This easy plate method became the traditional screening method for phospholipase activity for *Candida* species (Lane and Garcia, 1991; Samaranayake et al., 1984; Williamson et al., 1986).
Figure 2.8: Sites of action of various phospholipases-(a) A1 and A2, PLA1 and PLA2, respectively; B, PLB; C, PLC; D, PLD. (b) Lyso-PL and Lyso-PL transacylase. (Source: Ghannoum et al., 2000)

2.8.3 Yeast-Hyphae transition

*C. albicans* biology remarkably can assume a variety of cell morphologies (Figure 2.9). Morphogenesis refers to the transition between the unicellular yeast cells and the filamentous growth form. *C. albicans* reversibly converts unicellular yeast cells to either pseudohyphal or hyphal growth.
Figure 2.9: Early events in the pathogenesis of candidiasis are portrayed on a mucosal surface. A yeast cell of *Candida albicans* is shown either budding (right) or germinating (left). At the mucosal surface, germination of yeast cells (left) and penetration of the mucosa is shown. However, persorption of yeast cells (center) also results in the uptake of budding cells into the submucosa. On the far right, induced phagocytosis of yeast by a mucosal cell is shown. These events are promoted by adhesins [Als1p, Als5p (Ala1p), Hwp1p and Int1p] and enzymes (Saps and Plb1p). The germ tube is pictured as expressing different antigens compared with the yeast cell (Source: Calderone and Fonzi, 2001).

An interesting feature of *C. albicans* is its ability to grow in two different ways; reproduction by budding, forming an ellipsoid bud, and in hyphal form, which can periodically fragment and give rise to new mycelia, or yeast-like forms. Transitions between the two phenotypes can be induced *in vitro* in response to several environmental cues such as pH (Kaur *et al.*, 1988) or temperature, or different compounds such as N-acetyl glucosamine or proline. Induction of the mycelial form by serum or macrophages is one of the most critical criterions for pathogenicity (Liu *et al.*, 1994; Malathi *et al.*, 1994; Saporito-Irwin *et al.*, 1995). *Candida albicans* indicates the power to switch between the yeast as well as the hyphal manner associated with pathogenicity. *C. albicans* is a
significant opportunistic pathogen which tends to cause both superficial and serious invasive infections. It has an ability to transit reversibly its morphology from round budding cells to elongated hyphae or filamentous growth forms (Mishra et al., 1992).

Of all *Candida* spp., only *C. albicans* and *C. dubliniensis* form both types of filamentous growth. The most common form in which it can be found in laboratory is yeast form and its transition to elongated growth is attributed by an ample of environmental conditions (Table 2.3).
Table 2.3: Morphogenesis-inducing conditions in *C. albicans*

<table>
<thead>
<tr>
<th>Morphogenesis-inducing conditions</th>
<th>Yeast cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Cell density &gt;10^6 cells ml^{-1}</td>
<td></td>
</tr>
<tr>
<td>• Growth below 30 °C</td>
<td></td>
</tr>
<tr>
<td>• pH 4.0</td>
<td></td>
</tr>
<tr>
<td>Pseudohyphae</td>
<td></td>
</tr>
<tr>
<td>• pH 6.0, 35 °C</td>
<td></td>
</tr>
<tr>
<td>• Nitrogen-limited growth on solid medium (SLAD)</td>
<td></td>
</tr>
<tr>
<td>Hyphae</td>
<td></td>
</tr>
<tr>
<td>• Serum, &gt;34 °C</td>
<td></td>
</tr>
<tr>
<td>• Lees medium, 37 °C</td>
<td></td>
</tr>
<tr>
<td>• pH 7.0, 37 °C</td>
<td></td>
</tr>
<tr>
<td>Other filament-inducing conditions</td>
<td></td>
</tr>
<tr>
<td>• Spider medium</td>
<td></td>
</tr>
<tr>
<td>• Engulfment by macrophages</td>
<td></td>
</tr>
<tr>
<td>• Mouse kidneys</td>
<td></td>
</tr>
<tr>
<td>• Growth in agar matrix</td>
<td></td>
</tr>
<tr>
<td>• Iron deprivation</td>
<td></td>
</tr>
<tr>
<td>• Anoxia</td>
<td></td>
</tr>
<tr>
<td>• <em>n</em>-acetyl glucosamine</td>
<td></td>
</tr>
</tbody>
</table>

(Source: Berman and Sudbery, 2002)
The progression of morphogenesis is extremely regulated under transcriptional control. The expression of both types of genes—the inhibitory and inductive is required in the process of germ tubes formation by *Candida* (Table 2.4). Concomitantly, two signal pathways associated with pseudohyphae phenotype were identified not only in *S. cerevisiae* but also in *C. albicans* (Lo et al., 1997; Brown and Gow, 1999; Lengeler et al., 2000). The initial process within *C. albicans* includes homologs with the STE12 (Lo et al., 1997) mating along with pseudohyphae pathway involving *S. cerevisiae*.

### Table 2.4: Summary of *Candida albicans* genes involved in the regulation of morphogenesis

<table>
<thead>
<tr>
<th>Candida gene</th>
<th>Mutant phenotype</th>
<th>Putative function</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>CLA4</em></td>
<td>Unable to form hyphae under all conditions tested; avirulent</td>
<td>Protein kinase in MAP kinase cascade</td>
</tr>
<tr>
<td><em>CST20</em></td>
<td>Partial defect in hyphae formation; reduced virulence</td>
<td>Protein kinase in MAP kinase cascade</td>
</tr>
<tr>
<td><em>HST7</em></td>
<td>Partial defect in hyphae formation; reduced virulence</td>
<td>Protein kinase in MAP kinase cascade</td>
</tr>
<tr>
<td><em>CPH1</em></td>
<td>Partial defect in hyphae formation; reduced virulence</td>
<td>Transcription factor regulated by MAP kinase cascade</td>
</tr>
<tr>
<td><em>TUP1</em></td>
<td>Constitutive hyphal formation</td>
<td>Transcriptional repressor</td>
</tr>
<tr>
<td><em>EFG1</em></td>
<td>Forms pseudohyphae, unable to form true hyphae, reduced virulence</td>
<td>Transcriptional activator and repressor</td>
</tr>
</tbody>
</table>
A second morphogenesis pathway in *C. albicans* is identified by the transcription factor Efg1p, which is a member of the family of basic helix-loop-helix transcription factors (Lo *et al.*, 1997; Stoldt *et al.*, 1997; Brown and Gow, 1999; Lengeler *et al.*, 2000; Hameed *et al.*, 2008). This particular process can be self-regulating although similar to the Cph1p pathway. The Efg1p pathway includes homologs of Ras, adenyl cyclase and protein kinase A (*TPK2*). Ras might activate both the Cph1p and Efg1p pathways (Brown and Gow, 1999). Another protein kinase (*CRK1*) that is in the Cdc2 subfamily of kinases has been shown to be required for conversion of yeasts to a filamentous growth on serum-containing agar media (Chen *et al.*, 2000).

The proteins discussed above are in some measure needed for morphogenesis, however, many different proteins, such as Tup1p and Rbf1p are suppressors of morphogenesis (Braun and Johnson, 1997; Ishii *et al.*, 1997).

It appears that other pathways are required for morphogenesis, including the cell wall integrity [protein kinase C (PKC)] and the osmoregulation [two-component high osmolarity glycerol (Hog1)] pathways (Navarro-Garcia *et al.* 1998; Alonso-Monge *et al.*, 1999; Calera *et al.*, 2000; Calera and Calderone, 1999; 2001; Calera *et al.*, 1999). Additional identified histidine kinase proteins (Chk1p and Cos1/Nik1) are required pertaining to morphogenesis (Calera and Calderone, 2001) and, including PHR1; CHK1 is vital pertaining to invasive condition but not vaginal infection (Calera *et al.*, 1999).

### 2.9. Plasma membrane H⁺-ATPase in *Candida*:

The fungal plasma membrane H⁺-ATPase is an electrogenic proton pump with a Mw of 100 kDa that maintains ion balance, regulates intracellular pH and generates the electrochemical proton gradient required for nutrient uptake (Portillo *et al.*, 1989; Serrano, 1988). It is delivered to plasma membrane through secretory pathway and is made in rough endoplasmic reticulum.
Figure 2.10: Topological model of fungal plasma membrane H⁺-ATPase (Source: Ambesi et al., 2000)

It has 3 major portions of polypeptide which are embedded in to the cytosol: an N-terminal tail of about 115 amino acids and 2 large loops of approximately 130 and 300 amino acids. The larger loop contains the site which is phosphorylated during the reaction cycle and therefore is thought to be involved in ATP binding and hydrolysis (Figure 2.10).

The H⁺-ATPase is encoded by the PMA1 gene (Serrano et al., 1986) and is a member of the extended family of the P-type ATPases that mediate cation transport. The PMA genes from several fungal species are highly conserved at both the DNA and amino acid levels, despite the long evolutionary history of the group (Ghislain et al., 1987; Hager et al., 1986; Serrano et al., 1986). Intracellular pH transients have been observed during morphogenesis of the human pathogen Candida albicans, and ATPase inhibitors have been used to block germ tube
formation (Stewart et al., 1988; Bushra et al., 2007). The factors which determine morphogenic choice are important aspects in the pathogenic mechanism for *C. albicans*, since the blastoconidial morphology characterizes topical colonization, the mycelia form of the organism is required for tissue penetration, and both morphologies are generally observed in disseminated infections (Odds, 1987). The diploid opportunistic fungal pathogen *C. albicans* is of increasing importance to modern medicine (Odds, 1987; Odds et al., 1985). Those at greatest risk from life-threatening infection include immunocompromised individuals, such as victims of AIDS, cancer patients, transplant recipients, and neonates, while others with a predisposition to infection include patients undergoing antibiotic therapy, pregnant women, infants, intensive care patients with permanently implanted catheters, and burn victims. *Candida* infections are frequently acquired in hospital environments and significantly extend the length of hospitalization (Crislip and Edwards, 1989). The genetic diversity of the extracytoplasmic faces of P-type ATPases and the physiological role of fungal plasma membrane ATPase suggest *C. albicans* plasma membrane ATPase as a target for the biologically based rational design of soluble and highly specific antifungal agents. Drugs targeted at this enzyme can be expected to limit both fungal growth and morphogenic choice and therefore have potential as prophylactic agents. The plasma membrane contain an ATPase which is maximally expressed at pH 5.3 while the mitochondrial membranes exhibit optimal activity at pH equal or higher than 9.0.

### 2.10. Ergosterol in *Candida albicans*

Primary sterol found in plasma membrane of *Candida albicans* is Ergosterol. It contributes to a variety of cellular functions, including fluidity and integrity of the membrane and the proper function of membrane bound enzymes such as proteins associated with nutrient transport and chitin synthesis (Joseph-Horne and Hollomon, 1997; Lupetti et al., 2002a; Mishra et al., 2007b)
In the ergosterol biosynthesis pathway, steps prior to squalene formation are important for the pathway regulation and early intermediates are metabolized to produce other essential cellular components (Fryberg et al., 1973; Mukhopadhyay et al., 2004). The functional deletion of the genes from squalene to lanosterol is essential in *S. cerevisiae*, since it completely prevents ergosterol production (Lees et al., 1995). However, reaction sequences for ergosterol biosynthesis downstream in the pathway from lanosterol are specific to fungal taxa. For instance, in *S. cerevisiae* multiple routes have been reported for the sequence of steps leading from lanosterol to ergosterol depending on growth conditions (Ruan et al., 2002). Ergosterol is the target of polyene antifungal agents, and the enzymes involved in its biosynthesis pathway are the targets of allylamines, azoles, and morpholines (Ghannoum and Rice, 1999). In the pathway of ergosterol biosynthesis, squalene and lanosterol are the main precursors. Besides serving as a bioregulator of membrane fluidity, asymmetry and integrity, it contributes to proper function of membrane integrity. Azoles target ergosterol biosynthetic enzyme lanosterol 14 α-demethylase and are a widely applied class of antifungal agents. Ergosterol is a constituent of membranes in mycelia, spores, and vegetative cells (Newell, 1992;1994). Ergosterol content has been widely used as an estimate of fungal biomass in various environments, e.g., in soil and aquatic systems, because a strong correlation has been found between ergosterol content and fungal dry mass (Matcham et al., 1985; Newell 1992; 1994; Schnurer, 1993; Suberkropp et al., 1993). However, the amount of ergosterol in fungal tissue is not constant. There are interactions between the amount of ergosterol and fungal species, age of the culture, developmental stage (growth phase, hyphal formation, and sporulation), and growth conditions (growth media, pH, and temperature), although no clear trend for the ergosterol content in any of these factors has yet been detected (Gessner and Chauvet, 1993; Newell, 1994; Schnurer, 1993). Ergosterol has also been suggested
for use in quantifying fungal growth in solid substrates because of a good correlation between
the ergosterol content and hyphal length (Schnurer, 1993). Recently, ergosterol measurements
were proposed as a new method for determination of total fungal biomass in investigations of
indoor environments (Flannigan, 1997; Miller and Young, 1997).
2.11. Antifungal drugs available in market

![Figure 2.11: Structures of antifungal agents in clinical use. The structures of the many imidazoles used for treatment of superficial mycoses have been omitted, and those structures shown here are examples of the diversity that has been explored in this class (Source: Odds et al., 2003)](image-url)
2.12. Mechanisms of antifungal action

The antifungal drugs which are mostly used against *Candida* are shown in figure 2.11.

Figure 2.12: Generalized cartoon showing target areas for antifungal agents. The cross-section through fungal hyphae shows the intracellular sites of action of antifungal agents. The callouts show details for each site. The cell envelope structure illustrated is based on data for *Candida albicans*. Other fungal species differ in the details of their cell wall composition. The steps illustrated for ergosterol synthesis are the major steps found in all fungi; species can differ in having additional steps that bypass or parallel those shown (Source: Odds *et al.*, 2003)
2.12.1. Griseofulvin

Initially Griseofulvin was specific inhibitory agent to fungal species (Figure 2.12). It's advocated the drug interferes with microtubule construction but its detail mechanism of action is still not known (Develoux, 2001). The particular array connected with motion with Griseofulvin can be particular mainly on the dermatophyte fungi which cause athlete’s foot or so in addition to ringworm and it are moderately toxic for fungi (toxicity in liver is mainly recognized as an occasional hazard). Ingredients apart from Griseofulvin hinder the actual microtubule set up along with functionality within pathogenic fungus, such as *C. neoformans* (Woyke et al., 2002). Initially compounds like benzimidazole were used and there antifungal activity is explained by their effect on microtubules. However, as compared to fungicide research against plant pathogens very little attention have been paid to microtubules seeing that antifungal targets with regard to professional medical employ.

2.12.2. Flucytosine

Flucytosine (5-fluorocytosine) when converted to the 5-fluorouracil within target cells works as an antifungal agent. Fluorouracil becomes incorporated into RNA, causing premature chain termination, and it inhibits DNA synthesis through effects on thymidylate synthase. For this mechanism of action, the target cells must possess cytosine permease to internalise the flucytosine molecule, cytosine deaminase to convert it to 5-fluorouracil, and uracil phosphoribosyl transferase to convert 5-fluorouracil into a substrate for nucleic acid synthesis. Most filamentous fungi lack these enzymes and hence the useful spectrum of flucytosine is restricted to pathogenic yeasts (*Candida* species and *C. neoformans*). Flucytosine is used as adjunctive, rather than primary therapy, in the clinic, because primary and secondary resistance
(resulting from defects in the permease, deaminase and/or phosphoribosyl transferase enzymes) was thought to occur at a high frequency. However, recent large-scale susceptibility testing of Candida species by current reference methodology found surprisingly few primary resistant isolates (Pfaller et al., 2002b), in contrast to the results of non-standardised tests done in the 1970s. Therefore, flucytosine might deserve more use in the clinic than it has previously enjoyed.

2.12.3 Polyene antifungal agents

For last so many years Amphotericin B (Figure 2.12) is needed since simply the antifungal polyene which can be administered systemically to treat the visceral contamination. Its mode of action is typical for an antimicrobial molecule: instead of inhibiting an enzyme, it binds to ergosterol, the principal sterol in fungal membranes, thereby perturbing membrane function to the point of causing leakage of cellular contents. The exact fungicidal consequence of this drug is still unclear. Cylindrical three-dimensional structure of the fungi’s ergosterol molecule makes it different from the chief sterol throughout mammalian walls, cholesterol that includes a sigmoid shape (Figure 2.13). This conformational dissimilarity is nearly undoubtedly sufficient to go into detail the more presenting affinity of amphotericin B regarding ergosterol above cholesterol. The inspiration with the antifungal selectivity of amphotericin B is a result of this specific difference in addition to better relation of ergosterol: phospholipids in fungi (Kotler-Brajtburg et al., 1974). Even so, this selectivity is low and suggests that amphotericin B will have potential toxicity for mammalian cells. Binding to a sterol leaves the amphotericin B molecule with its hydrophilic edge unbalanced relative to the larger hydrophobic portion of the complex. Almost certainly, such a complex when formed in a membrane will migrate to align the hydrophilic faces within aggregations of complexes, thus creating areas of local tension within the membrane. The
mechanism of action correctly predicts that amphotericin B will act on a broad spectrum of fungal species. Despite considerable toxicity problems, this breadth of action of this molecule has made it clinically popular.

Figure 2.13: The polyene antifungal agent, amphotericin B, ergosterol and cholesterol, visualised in three dimensions. Ergosterol, the sterol found in fungal cell membranes, retains a cylindrical shape in all rotations and binds better to the hydrophobic (right-hand) side of the amphotericin B molecule than does cholesterol, with its sigmoid structure (Source: Odds et al., 2003)

Cholesterol is the membrane layer sterol present in mammalian cell; the differential binding affinity of amphotericin B for the two sterols is the basis of its selective antifungal action.

As predicted on the basis of its mechanism of action, amphotericin B is toxic to mammalian cells, particularly causing nephrotoxicity. This was observed from the earliest days of clinical use of the drug. To overcome amphotericin B toxicity, a variety of reformulated versions of the agent have been introduced. They probably reduce nephrotoxicity by slowing the rate at which
amphotericin B is delivered to the kidneys. The most successful, clinically proven versions of novel formulations are based on lipid combinations with amphotericin B, encapsulated in liposomes or in ribbon-like and disc-like lipid complexes (Dupont, 2002). Other formulations like amphotericin B–cochleate preparation (Zarif and Mannino, 2000) and an arabinogalactan complex (Falk et al., 1999) are under investigation. The antifungal polyene nystatin (Figure 2.12) is also being developed in a liposomal formulation for systemic use (Anonymous, 2001).

2.12.4 Antifungal azoles

Imidazoles and triazoles (azoles) are the largest class of antifungal agents in clinical use (Figure 2.11). Their main effect is to inhibit 14α-demethylation of lanosterol in the ergosterol biosynthetic pathway (Vanden Bossche et al., 1995), but in some fungal species, they can also inhibit the subsequent Δ22-desaturase step (Kelly et al., 1997). With ergosterol depleted and replaced with unusual sterols, the normal permeability and fluidity of the fungal membrane is altered, with secondary consequences for membrane-bound enzymes, such as those involved in cell wall synthesis (Marichal et al., 1985).

As outlined by diverse gene-based nomenclatures, the key molecular goal associated with azole antifungals can be a cytochrome P450–Erg11p or even Cyp51p, that catalyses this oxidative removal in the 14α-methyl group of lanosterol and/or eburicol with fungi by way of a common P450 mono-oxygenase activity. This protein contains an iron protoporphyrin moiety located at the active site, and the antifungal azoles bind to the iron atom via a nitrogen atom in the imidazole or triazole ring (Figure 2.14). The remainder of the azole molecule binds to the apoprotein in a very dependent on the individual azoles structure. Amongst the many mammalian
P450 mono-oxygenases and different fungal species there is a variation in exact conformation of active site (Vanden Bossche et al., 1995).

The precise nature of the interaction between each azole molecule and each kind of P450 therefore determines the extent of the azoles inhibitory effect in different fungal species. So far, the only crystal structure of a Cyp51p molecule to have been published is for the one from *Mycobacterium tuberculosis*, and studies with this enzyme should be consulted for details of the precise interactions between sterols, azoles and the active site protoporphyrin moiety (Podust et al., 2001).
Figure 2.14: Cartoon giving an approximate impression of the protoporphyrin moiety located at the active site of Erg11p (Cyp51p), the cytochrome P450 enzyme target for imidazole and triazole antifungals. Three triazole antifungals, itraconazole (top), fluconazole (centre) and voriconazole (bottom) are shown in comparable orientations. Arrows link the azole nitrogen atom to the iron atom where the azoles bind to block the active site of the enzyme (Source: Odds et al., 2003)
2.12.5. New triazoles

Of the three most up-to-date triazole antifungals, posaconazole demonstrates a detailed similarity in order to itraconazole, although when using the dioxolane ring altered to the tetrahydrofuran (Figure 2.11). Although voriconazole as well as ravuconazole usually are structurally in connection with fluconazole. The structural differences might seem small, but they dictate antifungal potency and spectrum, bioavailability and drug interaction and toxic potential – very important considerations for compounds that bind to heme groups in P450s (Espinel-Ingroff et al., 2001).

Posaconazole also acts against a broad spectrum of susceptible fungi, and shows interestingly promising efficacy against coccidioidomycosis in preclinical studies (Gonzalez et al., 2001). Ravuconazole, with an identical pharmacophore but a longer side-chain than voriconazole, stands out for its unusually long plasma half-life in humans (Olsen et al., 2000).

2.12.6. Other sterol synthesis inhibitors: allylamines and morpholines

These two other classes of antifungal agents target the ergosterol biosynthetic pathway. The squalene epoxidase is hindered by the allylamines, notably terbinafine (Figure 2.11), which is an initial step in visceral mycoses, and much more strangely enough the opportunity of combining terbinafine having various other paths is actually envisioned, having fungicidal implications inside vulnerable kinds. Few pathogenic types of yeast and many filamentous fungi are included in this. However acceptance terbinafine being a treatment associated with ergosterol synthesis inhibitors to realize synergistic inhibitory outcomes is not naturally. For human medicine of the phenylmorpholine class, amorolfine (Figure 2.11) could be the lone consultant that influences 2 locates later inside ergosterol pathway: Erg2p, the particular Δ8-Δ7isomerase enzyme in addition
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to Erg24p, the particular Δ14 reductase enzyme. Amorolfine can be used only for topical treatment of superficial mycoses, and neither of its targets has attracted recent research interest.

2.12.7. Echinocandins

The echinocandins are fungal secondary metabolites comprising a cyclic hexapeptide core with a lipid side-chain responsible for antifungal activity (Figure 2.11). Antifungal activity in the prototypes, echinocandin B and aculeacin A was discovered by random screening in the 1970s. A modified form of echinocandin B, cilofungin, was developed to the point of phase 2 trials, but was abandoned when its formulation showed toxicity to patients. In the late 1990s, three echinocandin-class compounds, anidulafungin, caspofungin and micafungin (Figure 2.11 and 2.15), all entered clinical development (Van den Bossche, 2002). The three-dimensional configuration of all three molecules is similar. A central, common core bears a long, ‘gun-barrel’-like side chain known to be a determinant of the spectrum of susceptible species (Figure 2.15), and a hydroxylated side chain that appears opposite the ‘gun barrel’ in flat structural representations (Figure 2.11) but is adjacent in energy-minimised 3-D structures (Figure 2.15) (Van den Bossche, 2002).

The target for the echinocandins is the complex of proteins responsible for synthesis of cell wall β-1, 3 glucan polysaccharides (Figure 2.15). In S. cerevisiae, where the enzyme complex has been best studied, two proteins (Fks1p and Fks2p) are regulated by a GTP-binding peptide, Rho1p, and by elements of the calcineurin pathway (Douglas, 2001). Homologues for all three gene products have been found in C. albicans, but it seems that the Fks2p homologue is not expressed in growing cells (Douglas, 2001; Vanden Bossche, 2002). Mechanistic details of glucan synthesis and its inhibition by echinocandins still remain obscure, largely because a
membrane-associated protein complex is involved. There is no doubt that the component to which echinocandins bind is Fks1p, but their non-competitive inhibitory effects on glucan synthesis do not necessarily imply that Fks1p itself is the catalytic subunit, nor is it clear whether the echinocandin-binding site on Fks1p is external or internal to the cell membrane.

Figure 2.15: Cartoon depicting the transmembrane complex of two proteins. Fks1p and Fks2p involved in synthesis of β-1:3 glucan in cell walls of *Saccharomyces cerevisiae* (Source: Odds *et al.*, 2003)

Among common fungal pathogens, only *Cryptococcus neoformans* is excluded from the echinocandin spectrum; they also lack activity against emerging pathogens, such as *Fusarium spp.* and *Scedosporium spp.* However, they are active against *Pneumocystis jiroveci* (Letscher-Bru and Herbrecht, 2003). All the three new echinocandins can only be administered by intravenous injection, which also places constraints on their use. Nevertheless, the echinocandin
class represents a particularly interesting advance in antifungal therapeutics because their target is a new one. Already, there are clear signs of interest from clinicians to explore combination therapy with echinocandins and azoles.

2.12.8. Sordarins

The sordarin antifungal class, although not developed for clinical use, merits mention among the new mechanisms of action. Sordarins (two examples of structures are shown in Figure 2.11) inhibit protein synthesis by blocking the function of fungal translation Elongation Factor 2 (EF2). The class was discovered by routine screening but was abandoned in the early 1970s (Odds, 2001). Interest in sordarins was re-awakened as a result of a prospective screen for inhibitors of *C. albicans* protein synthesis *in vitro*, which pinpointed the nature of the sordarin antifungal effect. When refined experimentation revealed EF2 as the specific target of the sordarins (Dominguez and Martin, 1998), the result engendered surprise because *C. albicans* EF2 displays more than 85% amino acid sequence identity to the human equivalent, and EF2 would never have emerged as a potential target from genomics-based screening.

2.13. Antifungal agents and the future

Over the 50 or more years in which specific antifungal agents have been discovered, the clinical needs for the agents have altered considerably and continuously. Superficial mycoses remain relatively easy to treat, with a large range of antimycotic products now available over the counter for the purpose. The spectrum of disseminated mycoses in highly immunocompromised individuals has undergone many changes, with the advent and subsequent therapeutic suppression of HIV-associated problems, and with changes in the nature of management of all types of serious illness. It is obvious that, at present, the prime requirement is for agents with a
broad spectrum of susceptible species. The six newest agents in development go a long way to meet this requirement, particularly the three new triazoles. A consequence of the huge investment in genome-centred approaches is that antifungal research is currently rich in validated targets for new agents, but it is likely to be many years before clinically useful inhibitors of these targets are discovered and developed. Well-understood, fungal-specific targets, such as chitin synthesis, have so far eluded chemical exploitation, emphasizing the gulf between target discovery and drug development. As with all antimicrobial agents, the spectra of emergence of resistance is a real one, and appropriate vigilance in the arms race between fungi and humans means that new targets and new inhibitors will continue to be required for effective antifungal therapy in the future.

2.14. Resistance developed by antifungal drugs

Several lines of evidence implicate a modification in the quantity or quality of 14α-demethylase in the expression of resistance to azole antifungal agents (figure 2.16). A recent study examined the biochemical mechanisms for resistance to fluconazole by comparing sterol composition, fluconazole accumulation, and inhibition of 14α-demethylase by fluconazole in two clinical C. krusei strains (expressing intrinsic resistance to fluconazole) and a susceptible C. albicans isolate (Orozco et al., 1998). Other studies have implicated altered 14α-demethylase in resistance to azoles. Over expression of 14α-demethylase has also been implicated as a mechanism of resistance to azole antifungals (Vanden Bossche et al., 1992). Candida albicans has also become resistant to antifungal agents, in particular triazole compounds, by expression of efflux pumps that reduce drug accumulation.
Figure 2.16: Mechanisms by which microbial cells might develop resistance. 1. The target enzyme is overproduced, so that the drug does not inhibit the biochemical reaction completely. 2. The drug target is altered so that the drug cannot bind to the target. 3. The drug is pumped out by an efflux pump. 4. The entry of the drug is prevented at the cell membrane/cell wall level. 5. The cell has a bypass pathway that compensates for the loss-of-function inhibition due to the drug activity. 6. Some fungal "enzymes" that convert an inactive drug to its active form are inhibited. 7. The cell secretes some enzymes to the extracellular medium, which degrade the drugs (Source: Ghannoum and Rice, 1999)
2.15. PLANT PRODUCTS AS ANTIMICROBIALS

Drug resistant microorganisms and up till now unknown disease-causing microbes show an enormous threat to public safety and health by causing sporadic incidents of epidemics. Therefore global initiative and inventiveness for the development of fresh strategies is required for the prevention and treatment of infectious disease. Over the last century large numbers of clinically proven drugs which have been chemically isolated from medicinal plants are now being re-assessed as antimicrobial agents (Mahady, 2005). Plant extracts, oils and other preparations have been used from time immemorial in ancient system of Indian medicine (Ayurveda). Plant extracts and oils from Aloe vera, Turmeric, Neem, Clove, Thyme, Ocimum, Mentha, Lavandula, Cinnamon and Coriandrum possess potent antimicrobial activities (Charak Samhita), however among those plant products, essential oils (Oregano, Clove, Thyme, Malaleuca, Ocimum, Mentha, Lavandula, Cinnamon and Coriandrum) are the most effective antimicrobials (Hammer et al., 1999; Salgueiro et al., 2003; Pinto et al., 2006; Fu et al., 2007; Reichling et al., 2009; Pinto et al., 2009; Zuzarte et al., 2011). A number of research possess (Knoblock et al., 1989; Arras and Usai, 2001; Vishnu et al., 2008; Edris and Farrag, 2003) explained the antifungal influence regarding necessary natural essential oils of countless aromatic plant life. Definite anti-candidal activity shown by several of these oils and their component molecules is also well established (Nenoff et al., 1996; Suresh et al., 1997; Khan et al., 2010a; Sharma et al., 2009; Khan et al., 2011). Plant extracts and oils have been used in traditional medicines for their antimicrobial activities since antiquity (Baris, 2006; Nair et al., 2007; Mkaddem et al., 2009; Sindhu et al., 2011; Mariita et al., 2011; Pandey and Mishra, 2010; Sharma et al., 2009; Soković et al., 2010., Matasyoh et al., 2009). Some of the most commonly
available important Indian medicinal plants valued in day to day antimicrobial preparations are listed below.

2.15.1. Aloe vera: *Aloe vera Linn.* or *Aloe barbadensis Miller* is a tropical plant and its extract which is moist, juicy and succulent from the *Aloe* family (400 different species) which is effortlessly grown in hot and dry climates and broadly distributed in Asia, Africa and other tropical areas. The use of aloe vera is being promoted for a large variety of conditions. *Aloe vera* plant has been long used in cosmetics and sanitary preparations (Pandey et al., 2010). As per the reports this leaf has antifungal action against mycelial growth of *Botrytis gladiolorum, Fusarium oxysporum f.sp. gladioli, Heterosporium pruneti* and *Penicillium gladioli* (Rosca-Casian et al., 2007). Even after the promising and potential results of the use of aloe vera for diverse dermatologic conditions, there is a great room for exploration of clinical efficiency of oral and topical aloe vera (Feily and Namazi, 2009).

2.15.2. Turmeric: Petroleum ether, chloroform, hexane, acetone and ethanol extracts of *Curcuma zedoaria* and *Curcuma malabarica* tubers were shown to exhibit antibacterial activity including that against *Staphylococcus aureus* (Gram positive) as well as antifungal activity (Wilson et al., 2005; Sindhu et al., 2011). Good Antifungal activities possessed by The ethanolic extract of *Curcuma longa* against *Trichophyton longijus* (Khattak et al., 2005), turmeric oil has also found to have antifungal activity against *Aspergillus flavus, A. parasiticus, Fusarium moniliforme* and *Penicillium digitatum* (Jayaprakasha et al., 2001; Apisariyakul et al., 1995).

2.15.3. Neem: *Azadirachta indica* which is commonly known as Neem is a home-grown plant widely found in India. Numerous workers have studied the medicinal properties of the plant and its effect such as the antipyretic effect, antimalarial effect, antitumour effect, antiulcer effect,
antidiabetic effect, antifertility effect, effect on the central nervous system, and cardiovascular effect, have been studied (Subapriya and Nagini, 2005). Several authors have also studied Antimicrobial properties of *Azadirachta indica* and its antifungal effects were against *Trichophyton mentagrophytes* (Subapriya and Nagini, 2005). Antifungal effects of neem leave and seed extracts obtained by ethanol, hexane and petroleum ether were also found evident against *Fusarium oxysporum*, *Rhizoctonia solani*, *Alternaria solani* and *Sclerotinia sclerotiorum* (Moslem and El-Kholie, 2009; Natarajan et al., 2002). Aqueous extracts of neem have been found effective against the virulence attributes of *C. albicans* like biofilm formation, hydrophobicity, adhesion, and biofilm formation (Polaquini et al., 2006).

2.15.4. **Tulsi**: *Ocimum sanctum* the sacred 'Tulsi' finds diverse uses in the indigenous system of medicine. The leaves of the plant have been used as an expectorant, diaphoretic, anticancer, antihelminthic, antiseptic, analgesic and tonic rejuvenator (Kiritikar and Basu, 1975; Chopra et al., 1958). Indian material medica describes the use the plant in a verity of ailments by traditional medicinal practitioners, as expectorant, analgesic, anticancer, antiasthamatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive and antistress agent. Antiplasmodial (Bagavan et al., 2011) and leishmanicidal (Suzuki et al., 2009) activities of *Ocimum sanctum* are also reported. However it also has antimicrobial characteristics (Mondal et al., 2009).

To treat fungal infections dry leaves are used and for cure of bronchitis, otitis media, and skin diseases the fresh juice of the leaves are used (Nadakarni, 1954). Antibacterial activity of ether extract of the leaves against *Staphylococcus aureus* and *Mycobacterium tuberculosis* has been reported elsewhere (Joshi and Magar, 1952; Gupta and Vishwanathan, 1955). Good antibacterial activity against *Staphylococcus aureus*, *Bacillus pumilus* and *Pseudomonas aeruginosa* is shown
by *Ocimum sanctum* fixed oil. (Singh and Majumdar, 1999). Aqueous and alcoholic extracts of *Ocimum sanctum* show considerable inhibitory activity against the enteric pathogens including *E. coli* and *Clostridium difficile* (Geeta *et al.*, 2001). Ethanolic extract of Tulsi has potent antimicrobial activity against *Streptococcus mutans* (Agarwal *et al.*, 2010). Aqueous extracts of *Ocimum sanctum* have been shown to possess quorum-sensing (QS) inhibitory activity against N-acyl-homoserine lactone (AHL)-mediated violacein production in *Chromobacterium violaceum* and virulence factor expression in *Pseudomonas aeruginosa* PA01 (Musthafa *et al.*, 2010). Ethanolic extracts of leaves of *Ocimum sanctum* with *Cassia alata* combined together shows considerable anti-*Cryptococcus* activity, and this activity has been proven to be heat-stable and worked at acidic pH (Ranganathan and Balajee, 2000).

Holy basil, *Ocimum sanctum* (L.) will be age-old identified for the therapeutic components; alternatively it is antimicrobial features along with qualities are used merely inside ‘Ayurvedic medicines’. Attention has been drawn to antifungal activity and a possible synergistic antifungal effect of *Ocimum sanctum* essential oil and established azole antifungicals (Khan *et al.*, 2010b). The mechanism of their fungicidal action was assessed by studying their effect on the plasma membrane using flow cytometry, confocal imaging and determination of the levels of ergosterol, a fungal-specific sterol (Khan *et al.*, 2010c). Proton pumps are important for growth and metabolism of *Candida* species and so H⁺ extrusion studies were performed to explore the possible mechanism of the tulsi test compounds (Eugenol, methyl eugenol, linalool and 1,8-cineole). Linalool was the most active constituent of tulsi essential oil, whereas inhibition of H⁺ extrusion appeared to be a synergistic function of the lead molecules (Khan *et al.*, 2010c).


2.15.5. **Peppermint**: *Mentha piperita* (Lamiaceae), the peppermint (mint) plant is a very useful and important medicinal plant which is mainly found in North America, Asia and Europe. Oil is extracted from the leaves of the flowering plant for which it is primarily cultivated (Scavroni *et al.*, 2005). Main uses of Peppermint oil are flavoring pharmaceuticals and oral preparations such as tooth pastes, dental creams and mouth washes. Higher and aromatic plants have traditionally used to increase the shelf life of food and in folk medicines, showing inhibition against fungi, yeast and bacteria. Most of their properties are due to essential oils produced by their secondary metabolites (Saeed *et al.*, 2006; Fabio *et al.*, 2007; Bansod and Rai, 2008).

Mint (*Mentha piperita*) is generally used as antiulcer, anticholic and as a cure to gastrointestinal ailments. Its major constituent menthol is commercially used as a soothing agent in ointments, toothpaste and oral care products. It is a perennial flowering member of the mint family. The herb has medicinal properties and has been used since antiquity as a digestive aid, for management of gallbladder and respiratory diseases (Ronald, 2003; Reichling *et al.*, 2009; Kligler and Chaudhary, 2007; Lei *et al.*, 2011) Extracts of peppermint are used in many cosmetic products and in over the counter medicines (Derwich *et al.*, 2011; Marta Cristina Teixeira Duarte *et al.*, 2005). Essential oil of aerial parts of *Mentha piperita* obtained with hydrodistillation was found to be fungi toxic against three pathogenic fungi - *Rhizopus stolonifer*, *Botrytis cinerea* and *Aspergillus niger in vitro* (Behnam *et al.*, 2006). Essential oil of *Mentha piperata* is also effective against *Candida albicans* at very low concentrations (Tampieri *et al.*, 2005). *Mentha* essential oils are also found inhibitory against *Escherichia coli* and *Trichophyton spp.* (Mimica-Dukić *et al.*, 2003). Essential oil of *Mentha* is inhibitory against 17 micromycetal food poisoning, plant, animal and human pathogens (Soković *et al.*, 2010).
2.16. ANTIFUNGAL ACTIVITIES OF *MENTHA PIPERITA* ESSENTIAL OIL

Peppermint oil main features are that it is chemo-preventive and anti-mutagenic. It is very beneficial in symptomatic relief of common cold and as an analgesic and to treat headache. It can also be used for treating many ailments of skin, respiratory system, circulatory system, digestive system, nervous system and immune system. The principal active constituents of peppermint are essential oils which comprises about 1% of the herb. Monoterpenes like menthone, menthol and their derivatives (e.g; isomenthone, neomenthone, acetyl menthol, pulegone, menthofuran) mainly dominate the constituency of Essential oil. These essential oils dilate blood vessels and inhibit bacteria; especially menthol has a broad spectrum antibacterial activity (Pattnaik *et al.*, 1997). Peppermint oil is also found to have antiviral and fungicidal activity (Mohsenzadeh *et al.*, 2007). It is virucidal against influenza, herpes and other viruses. Antifungal activity of the essential oil of *M. piperita* was also reported (Barrera-Necha *et al.*, 2009).

Although the inhibitory activity of Mint EO and its major constituents against various pathogenic yeasts has been demonstrated earlier (Sardi *et al.*, 2013; Carretto *et al.*, 2010; Saharkhiz *et al.*, 2012; Li *et al.*, 2011; Pramila *et al.*, 2012), their effect on certain crucial virulence factors and the effect on membrane has not been verified. *Mentha piperita* essential oil has been extensively studied for its antifungal activity against filamentous fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus flavus*, *Aspergillus glaucus*, *Aspergillus ochraceous* (Jeyakumar, 2011), *Aspergillus fumigatus*, *Vasin factum* (Kazemi *et al.*, 2012), *Alternaria alternate* and *Aspergillus versicolor* (Soković *et al.*, 2009, 2010, 2012).
Other rare but clinically important filamentous fungi like *Fusarium solani*, *Sclerotium rolfsii* (Gokalp et al., 2002) *Rhizopus stolonifer*, and *Botrytis cinerea* are also susceptible to *Mentha piperita* essential oil (Behnam et al., 2006).

*Mentha piperita* essential oil is reported to be fungicidal to *Candida* species also (Carreto et al., 2010; Tampieri et al., 2005). *Mentha piperita* essential oil reduces biofilm formation, an important virulence attribute, in *C. albicans* (Vishnu et al., 2008). Fungicidal activity is considered as a desirable quality for antifungal agents, since it could totally eliminate the fungus from tissues. Mint (*Mentha piperita*) oil has been reported to be fungicidal at 0.5% concentration, shows inhibition (MIC) even at 2% (Kazemi et al., 2012). The disc diffusion method was used to study the antimicrobial activities indicated that all the bacterial and fungal strains tested showed growth of inhibition toward the plant extract. *C. albicans* showed a higher inhibition zone of diameter 16mm (Pramila et al., 2012). The antimicrobial activity of the *Mentha piperita L.* essential oil was determined by the agar diffusion test (Carreto et al., 2010).
2.17. ACTIVE CONSTITUENTS OF MENTHA PIPERITA ESSENTIAL OIL

Figure 2.17: Chemical structures of the lead molecules present in Mint essential oil

2.17.1. Carvone: Carvone is a member of a family of chemicals called terpenoids. Carvone with the IUPAC name as 2-Methyl-5-(1-methylethenyl)-2-cyclohexenone has molar mass of 150.22 g mol\(^{-1}\) and density of 0.96 g/cm\(^3\). Carvone is used in the food and flavor industry (De Carvalho and Da Fonseca, 2006). The biosynthesis of carvone is by oxidation of limonene. Carvone occurs naturally in kuromoji oil as well as in \textit{Ocimum species} oils. Some oils, like gingergrass oil, contain a mixture of both enantiomers. Many other natural oils, for example peppermint oil, contain lower concentrations of carvones.

The essential oils from different species of \textit{Lippia}, (genus of Colombian plants) constituting limonene, and carvone as one of major components were reported to be strongly potent against \textit{Leishmania chagasi} and \textit{Trypanosoma cruzi} (Escobar et al., 2010). \textit{Lippia alba} (Mill.) essential oils constitute Carvone and citral chemotypes, possessing cytotoxicity against HeLa cells and antifungal activity against \textit{Candida parapsilosis}, \textit{Candida krusei}, \textit{Aspergillus flavus} and...
Aspergillus fumigates (Mesa-Arango et al., 2009). The antifungal activities of Carvone was observed in vitro on Fusarium subglutinans, Fusarium cerealis, Fusarium verticillioides, Fusarium proliferatum, Fusarium oxysporum, Fusarium sporotrichioides, Aspergillus tubingensis, Aspergillus carbonarius, Alternaria alternata, Penicillium. The naturally occurring compounds tested showed toxic effects on in vitro mycelium growth of all these fungal strains (C. Morcia et al., 2012)

2.17.2. Menthol: Menthol with IUPAC name as 2-isopropyl-5-methylcyclohexanol is an organic compound made synthetically or obtained from commint, peppermint or other mint oils. It has molar weight of 156.27 g mol⁻¹ and density of 0.890 g cm⁻³. Menthol is a crystalline substance, clear or white in color and waxy in nature which is solid at room temperature and melts slightly above. Its natural form is (−) menthol, which is assigned the (1R, 2S, 5R) configuration. Menthol shows local anesthetic and counterirritant properties due to which, it is widely used to relieve minor throat irritation. Menthol also acts as a weak kappa opioid receptor agonist.

The essential oils from different species of Mentha spicata, Ocimum basilicum, Lavandula angustifolia, Salvia officinalis, Citrus limon and C. aurantium constituting menthol as one of major components were reported to be strongly potent against dermatomycetes, Trichophyton mentagrophytes, T. rubrum, and T. tonsurans. (Soković et al., 2010; Baris et al., 2006). Menthol in essential oils of Lavandula officinalis and Melissa officinalis were shown to possess antibacterial and antioxidant activity against Gram-positive (Staphylococcus aureus, Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Sarcina lutea and Streptococcus-β-haemolyticus) and nine Gram-negative (Salmonella typhi, Shigella dysenteriae, Shigella shiga, Shigella sonnei, Shigella boydii, Escherichia coli, Klebsiella sp., Pseudomonas aeruginosa and Proteus sp.
(Rostami et al., 2012) and Mentha longifolia L. (Lamiaceae) essential oil, menthol achieved considerable antifungal activity against the yeast *C. albicans* (Firas A, 2009).

### 2.17.3. Menthone:

Menthone occurs naturally and is an organic compound with a molecular formula C\(_{10}\)H\(_{18}\)O. \(l\)-Menthone (or \((2S, 5R)\)-trans-2-isopropyl-5-methylcyclohexanone), having a density of 0.895 g/cm\(^3\) and molar mass of 154.25 g mol\(^{-1}\). Menthone is a constituent of the essential oils of pennyroyal, peppermint, *Pelargonium* geraniums, and others. Due to its aromatic character and minty odor Menthone is also used in making perfumery and cosmetics. Menthone is the main component of *Mentha piperita* and *Mentha spicata* essential oil (Rostami et al., 2012) and were reported strongly potent agent *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhi*, *Shigella dysenteriae* (Bacteria) and *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* (Fungi).

### 2.18. COMBINATIONAL THERAPY

Fungi have higher number of chromosomes and complex nuclear membrane, cell organelles and cell wall composition. Since the last three decades, the rate of death every year due to fungal infections has risen significantly. With the increased use of antifungal agents there is an increase in the number and variety of fungal strains resistant to the drugs. Also the present antifungal therapeutics is often toxic. Alternative therapy needs to be developed to suppress the emergence of antifungal resistance. This can be achieved by the use of combinations of existing agents or the development of new, safer and effective agents primarily from plant sources which can exhibit synergy with drugs. The accurate prediction of synergy between commercial drugs or between a drug and a natural product based upon the results of *in vitro* testing is very crucial. A number of methods are used to detect synergy. However, the checkerboard and time-kill curve
methods are the two most widely used techniques and the former is a relatively easy test to perform (White et al., 1996). The checkerboard is prepared in microtiter plate for multiple combinations of two antimicrobial agents in concentrations equal to, above, and below their minimal inhibitory concentrations for the microorganism that is being tested. The standardization of these techniques for routine laboratory testing is the need because of the common use of combination therapies against the growing numbers of multiple drug-resistant strains.