CHAPTER I

INTRODUCTION
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Diseases of man and animals caused by fungi are known as mycotic infections or mycosis. Of the thousands of fungi species named, well over 100 are potentially pathogenic in man (Emmons et al., 1977). These range from mild chronic infections to acute conditions which may affect only the superficial keratinized or mucosal parts of the body or involve the viscera and circulatory fluids.

A perusal of the literature reveals that the various aspects of medical mycology in parallel with other substances of medical microbiology are being studied throughout the world. Fungal diseases in general occur in all parts of the world and affect all ages, though a number of particular species are restricted geographically in their incidence.

Although some forms of mycosis can be attributed to modern medicine—systemic mycotic disease may develop following the use of immunosuppressive or cytotoxic drugs. Systemic mycoses are more common in temperate belts of the world, while superficial mycotic infection are more in tropical and subtropical parts of the world.

India being a tropical country with its varied topography is supposed to have a high incidence of mycotic infections. As a monsoon land, most of its regions are under the influence of sustained periods of combined heat and humidity recurring annually. These geographic features are therefore conducive to the acquisition and maintenance of these mycotic infections in the population. In spite of recent
advances in chemotherapy, treatment of most fungal diseases is far from satisfactory. Drugs currently available for the treatment of superficial or systemic mycoses leave much to be desired. Wide-spectrum activity, the possibility of oral administration with freedom from toxicity, and cheapness are all necessary in selecting an antifungal drug.

It is the purpose of this thesis to discuss the experimental approaches to the discovery of antifungal drugs and to survey and examine synthetic chemicals, natural product types of compound and traditional systems of medicine for antifungal activity, especially against the various strains of Candida albicans.

Drug design, discovery and development is an extremely costly procedure; many hundreds of compounds may need to be synthesised and many thousands of compounds screened before worthwhile activity is found. To discover a new drug today, costs $25,000,000 in research and development before that drug could be made available for use in man. Surveys indicate however that by far the most frequent mycological problem is Vaginal candidiasis, with dermatophyte infections being smaller, but still significant problem. Our ambition is to find a new antifungal agent which would control both systemic and vaginal candidiasis.

It would be intellectually very satisfying to study the biochemistry or physiology of the target organism and design a drug to combat it. This approach has very little chance of success, with the current status of knowledge on infectious diseases available to us. This pattern may be more
useful in the remote future. Nevertheless it would be wrong not to attempt any synthetic work, if inspiration can be derived from the currently available synthetic antifungal drugs. Chemical structure which have limited antifungal activity can be improved upon by the synthesis of analogs or more distantly related compounds with a view to increase potency, safety, and spectrum of activity. The another source for the compounds useful in treatment of infectious diseases are natural products and the discovery of such compounds is only by the empirical approach. The traditional systems of medicine in India have quite often provided the required lead in the discovery of useful drugs and natural compounds with remarkable biological activity.

a) CANDIDIASIS

Candidiasis is a primary or secondary infection involving a member of the genus Candida. Essentially, however, the disease is an infection caused by Candida albicans. The clinical manifestations of diseases are extremely varied, ranging from acute, subacute and chronic to episodic. Involvement is localised to mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs or the gastrointestinal tract or become systemic as in septicemia, endocarditis and meningitis.

The most common cutaneous manifestation are intertriginous regions of groin, the depths and folds under breasts, finger webs and toe-clefts. Multiple symptamatology is a rare but specific characteristic of candidiasis. Onychia
is regularly preceded and accompanied by paronychia. Folliculitis is seen around intertriginous areas of candidiasis. In all these clinical manifestation Candida albicans is considered as the etiological agent. The disease is very difficult to diagnose because members of the genus Candida are also commonly recovered from healthy people.

Prognosis and Therapy:

The prognosis depends almost entirely on the type and severity of the predisposing conditions and the subsequent clinical form of candidiasis. Nystatin is effective in controlling thrush, cutaneous disease, paronychias, chronic esophageal disease, vaginitis and gastrointestinal infections. Amphotericin B can be used topically and is the drug of choice in treating systemic disease (i.e. endocarditis, meningitis, granuloma, chronic mucocutaneous or disseminated disease). Approximately 60% of Candida albicans isolates recovered from previously untreated patients are sensitive to 5-fluorocytosine. The drug has been useful in the treatment of systemic disease; susceptibility testing and monitoring of serum levels for 5-fluorocytosine are mandatory when this antimycotic agent is being utilised.

Histopathology:

The tissue reaction is initially acute suppurative inflammation followed by granulomatous inflammation. Microabcess formation is fairly common. Blastoconidia and psuedohyphae are both typically seen in tissue sections. These forms are better accentuated by special strains such as in the
PAS and GMS techniques for fungi. The blastoconidia are ovoid and usually 3-4μm in diameter. Under some circumstances, Candida species may produce true hyphae either separately or in conjunction with blastoconidia and pseudohyphae.

Candida albicans is a normal inhabitant of the alimentary tract and the mucocutaneous regions (Marples, 1965; Marples and Somerville, 1968). It is regularly present in small numbers in the mouths of normal healthy adults. The incidence of Candida albicans in the normal vagina of healthy nonpregnant women is about 5% (Marples, 1965) and can be as high as 30% in pregnant women or women on oral contraceptives (Linares de and Marin, 1978). The normal alimentary tract has a small but constant population of Candida albicans. It is common to the alimentary tract of almost all mammals and birds and all such species are susceptible to invasion by this opportunistic fungus.

Clinical Disease:

Candida albicans is perhaps the most protean infectious agent that afflicts man. All of the tissue and organ systems are subject to invasion, and the pathology evoked is as variable as are the clinical syndromes. In addition to active infection Candida albicans is also involved in several allergic conditions. The various clinical manifestations are:

1) Infectious disease:
   1) Mucocutaneous involvement:
      * Oral: thrush, glossitis, stomatitis, cheilitis perduche.
      * Vaginitis and balanitis
      * Bronchial and pulmonary
      * Alimentary: esophagitis, enteric, perianal disease
* Chronic mucocutaneous candidiasis

(i) Cutaneous involvement
* Intertriginous and generalised candidiasis
* Paroanychia and onychomycosis
* Diaper disease (napkin candidiasis)
* Candidal granuloma

(iii) Systemic involvement:
* Urinary tract
* Endocarditis
* Meningitis
* Septicemia
* Iatrogenic candidaemia

2) Allergic diseases:
* Candidids
* Eczema
* Asthma
* Gastritis

Laboratory Diagnosis:
Species of Candida (including Candida albicans) are present in the normal oral cavity, upper respiratory passages and intestinal tract. Their visual demonstration in and isolation in culture from, sputum therefore are common place.

1) Direct examination:
Scrapings from cutaneous and mucocutaneous lesions, sputum or pus are examined directly either in potassium hydroxide slide mounts or by gram stain.
Ovoid budding yeast cells approximately 3-7μm in diameter, pseudohyphae, true hyphae or both are usually seen in clinical specimens mounted in 10% KOH as described. At times it can be extremely difficult to differentiate such fungi as Aspergillus trichosporon and Geotrichum sp. from a *Candida* species in clinical specimens because they are all able to produce true hyphae.

2) Isolation by Culture:

For isolating by culture it is imperative that only freshly obtained specimens be examined. For this the clinical material is inoculated on to the Sabouraud's dextrose agar and incubated at 30°C. The growth is usually observed within 2 - 5 days. Blood and spinal fluid should be processed by filtration techniques.

**Mycology (Principal Fungi) (Michael R. McGinnis):**

1) *Candida albicans*
2) *Candida guilliermondie*
3) *Candida krusei*
4) *Candida parapsilosis*
5) *Candida pseudotropicalis*
6) *Candida tropicalis*

The lesions of candidiasis are usually acute and inflamed with characteristically outlying satellite vesicles or pustules and often dry. Toe-clefts, when infected, may produce an itchy, macerated condition resembling tineapedis. A red itchy or slightly painful, well defined eruption of finger webs is known as 'erosio interdigitalis blastomycetica'. The paronychial skin is swollen, red and painful; the web of the
skin constituting the nail fold can easily by lifted off the plate with a cavity in the fold. Another distinctive but uncommon type of cutaneous candidiasis characterised by the development of purulent follicular papulopustules on sited susceptible to intertrigo. Constant wet-work by house-wives and by people whose occupation involves continuous wet work, and obesity are considered as predisposing conditions. Cutaneous candidiasis has been described as a biological contact dermatitis of the primary irritant type (Maibach and Kligman, 1962).

b) IMIDAZOLES AS ANTIFUNGALS (Wolfgang P.E. Raab, 1980)

As early as 1950, it was found that benzimidazole inhibits the growth of fungi and bacteria. The inhibitory action is abolished by the addition of the purine bases, adenine or guanine. This finding led to the following hypothesis regarding the mode of action of benzimidazoles. Benzimidazoles competitively inhibit the uptake of important metabolites into the cell and thus interfere with cell growth.

It was then observed, that substituted benzimidazoles have stronger antymycotic properties, particularly when the substitution is in the 2-position (Holt, 1976). Synthesis, research and extensive series of tests led to the development of chlormidazole, chemical name 1-p-chlorobenzyl-2-methyl-benzimidazole, an antifungal for local application (Raab and Windisch, 1971).

Chlormidazole was the first azole antymycotic used in medicine. The azole antymycotics are distinguished by two structural properties. They contain an unsubstituted imidazole or triazole ring, and the rest of the molecule is linked by a nitrogen-carbon bridge. The azole antymycotics are active
against all fungi pathogenic to man, they are broad spectrum antimycotics. The broad spectrum antimycotics are drugs whose activity spectrum covers all three groups of fungi pathogenic to man (dermatophytes, yeasts and moulds).

In the years that followed, numerous chemical modification and substitutions in the benzimidazole molecule led to the development of important new drugs for human use. The formulae of some imidazole derivatives for systemic therapy are shown in and their activity spectra given in Table 1.

The latest developments from the series of imidazole, isoconazole and econazole intended primarily for local application and ketoconazole, intended for oral use.

The family of imidazole derivatives has substantially enriched the area of local antimycotic therapy. According to initial results, they certainly bring significant improvements to the treatment of systemic mycoses as well. According to the data compiled until now, ketoconazole will be the drug of choice for the oral treatment of fungal infections in man. Another new imidazole antifungal N 148/76, which is still in the process of experimental and pharmacological evaluation, which seems quite promising for topical and systemic treatment too.

Tioconazole, a compound with a similar structure as miconazole and is 4 times more active against Candida species than miconazole in vitro. Thus more and more imidazole derivatives will be introduced as antifungals in medical therapy in the not too distant future. Further studies and observations with imidazoles other than those now available will perhaps solve the problems still associated with the treatment of systemic mycoses due to yeasts and molds.
<table>
<thead>
<tr>
<th>Substance</th>
<th>Bacteria</th>
<th>Activity against Fungi</th>
<th>Protozoa</th>
<th>Helminths</th>
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<tr>
<td>Metronidazole</td>
<td>Anaerobes</td>
<td>-</td>
<td>Trichomonads</td>
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<tr>
<td>Timorazole</td>
<td>Anaerobes</td>
<td>-</td>
<td>Giardia</td>
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<td>Tinidazole</td>
<td>Anaerobes</td>
<td>-</td>
<td>Trypanosomes</td>
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<td>Ronidazole</td>
<td>Dysenteric bacteria</td>
<td>-</td>
<td>Amoebas</td>
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<td>Thiabendazole</td>
<td>-</td>
<td>Trichophyton</td>
<td>-</td>
<td>Nematodes(Systemic)</td>
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<td></td>
<td></td>
<td>Microsporum</td>
<td>Aspergillus</td>
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<td></td>
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<td>(local application)</td>
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<tr>
<td>Mebendazole</td>
<td>-</td>
<td>Trichophyton</td>
<td>-</td>
<td>Nematodes</td>
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<td></td>
<td></td>
<td>Aspergillus</td>
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<tr>
<td>Tetramisole</td>
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<td>Nematodes</td>
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<td>Levamisole</td>
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<td>-</td>
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<td>Nematodes</td>
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<td>Nifurtimole</td>
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<td>Schistosomias</td>
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<td>Dracunculus</td>
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<td>Clofibrate</td>
<td>Gram-positive</td>
<td>Yeasts</td>
<td>Trichomonads</td>
<td>-</td>
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<tr>
<td>Miconazole</td>
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<td>Molds</td>
<td>Nematodes</td>
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<tr>
<td>Isoconazole</td>
<td>Trichophyton</td>
<td>-</td>
<td>Amoebas</td>
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<tr>
<td>Econazole</td>
<td>Microsporum</td>
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<tr>
<td>Ketoconazole</td>
<td>-</td>
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a Immune stimulant. b Nitrates are unsuitable for systemic administration (Wolfgang P.E. Raab, 1980)
Miconazole nitrate

Mebendazole

Clotrimazole

Miconazole nitrate
Isoconazole nitrate  Econazole nitrate  N-143/76  (German patent 2545793)

Isoconazole nitrate

Econazole nitrate

Fluconazole

Fluconazole  N-148/76  (German patent 2545793)
There are several proposed mechanisms for antifungal effects of the imidazole. Some studies have emphasized the rapidly induced membrane damage and loss of viability of fungi (Sud and Feingold, 1981). The selectivity of imidazole action on fungi and gram positive bacteria, that are sensitive to imidazoles, are rich in fatty acids, whereas mammalian cells and gram negative bacilli are resistant to imidazoles, since they have low levels of free fatty acids.

Several investigators have demonstrated that both miconazole and ketoconazole inhibit ergosterol synthesis in fungi by blocking C-14 demethylation. This results in decreased in ergosterol and an accumulation of C-14 methyl sterol intermediates, such as lanosterol. Since lanosterol cannot support the growth of yeast in the absence of ergosterol this may be the primary antifungal mechanism of imidazoles (Sud and Feingold, 1981).

c) NATURAL PRODUCTS AS ANTIFUNGALS

The use of corticosteroids, broad spectrum antibiotics and cytotoxic drugs has increased the absolute number of fungal infections in humans by several genera of yeast like fungi. Members of the genera Cryptococcus, Rhodotorula, Torulopsis and Trichosporon have all been implicated in disease, but members of the genus Candida are recognised as the most frequently encountered fungal opportunists.

They are resistant to most antibacterial agents and there are a few available antifungal agents useful in the
treatment of fungal disease. There is a need to develop a wider variety of antifungal compounds that are most effective and less toxic. Natural products of plant and animal origin are quite often known to possess medicinal properties.

*Allium sativum* L.,—garlic has been used as a folk medicine since ancient times for a variety of ills including snakebite, haemorrhoids, rheumatism, abdominal pains and parasitic infections. Garlic is employed today in several countries for many maladies (Stoll and Seeback, 1951). Medicinal, insecticidal, antibacterial, antiprotozoal and antifungal properties have been ascribed to garlic (Ribbon, 1974). Kabelik (1970), demonstrated that garlic extract was more effective against pathogenic yeasts, especially *Candida albicans* than was nystatin, gentian violet or methylene blue.

Gray S. Moore and Robin D. Atkins (1977) in their studies with garlic extracts have shown that the garlic extract consistently inhibits the growth of *Candida albicans*. Their study has provided experimental evidence that garlic extract has inhibitory and cidal properties against those fungi commonly associated with vaginitis.

The increasing incidence of opportunistic infections from the yeast like fungi combined with the apparent lack of effective antifungal agents gives meaning to the investigation of alternate antifungal compounds. Enough positive evidence exists to encourage further investigations of natural products in combating mycotic disease. In yet another study Abraham et al., (1986) have screened about 295 plants extracts from 267 plant species, of which 64 plants species showed various
biological activities and two of them showed antifungal activity.

d) ALTERNATIVE SYSTEMS OF MEDICINE AS A SOURCE OF ANTIFUNGALS
(SIDDHA SYSTEM)

There has been a worldwide interest in scientifically validating the therapeutic efficacy of traditional medicines. There are inherent problems in the scientific validation of clinically acclaimed effectiveness of plant products which are further accentuated by the lack of availability of suitable experimental and clinical models. However, that should not deter the development and quest for researching for new drugs from these alternative systems of medicine.

Of the three indigenous systems of medicine, Ayurveda, the traditional Hindu system of medicine based on the Vedic scriptures, is the one that is practised in all parts of the country. Siddha is extensively practised in the Southern state of Tamilnadu and in the neighbouring states. Unani, also known as the Greek/Arab system of medicine was brought into India by Muslim conquerors and has been practised for several hundred years, predominantly in areas of Muslim culture.

Siddha, is an ancient system of medicine. In treatment it uses minerals and metals mainly, but some products of vegetable and/or animal origin as well. Works relative to Siddha of which there are at least 500, plus 3000 formula were written in Tamil initially on palm leaves. The exact number of Siddha practitioners is uncertain, but it is known that there are thousands in the state of Tamilnadu. An analysis of the
practice of traditional medicine in Madras, showed that Siddha practitioners, numbering 635, dominated the scene.

Siddha medicine is essentially a psychosomatic system of medicine. Unlike Ayurveda importance is given more to minerals and metals rather than herbs in pharmaceutics. Herbs are used only to triturate and calcinate the metals into their basmam and sidooram.

Skin diseases are more common in tropical countries like India. Due to inadequate health facilities, inadequate health education, poverty and illiteracy the disease is more common. In Siddha system of medicine, skin diseases are classified into 18 types. This classification is based on the symptoms and the parts affected (Yugimunivar, 1959) classifications based on the vitiation of humor is also seen in Siddha literature (Kuppuswamy Mudaliar, 1953).

When going in for treatment, such a classification alone will be useful to assess the therapeutic efficacy of certain herbal drugs. Some researchers have already elucidated the validity of humoral classification (Anandan, 1983). Fungal infections of skin (Dermatomycosis) are dealt under "Padarthamarai Perunkuttam" (Kuppuswamy Mudaliar, 1953). The word "padarthamarai" refers to the circular (ring like) lesions that expand peripherally.

Though there are many clinically effective antibiotics for bacterial diseases only few are effective antifungal agents against systemic mycoses and superficial fungal infections. Search for naturally occurring compounds with antifungal activity has become quite intense due to the side effects of the synthetic fungicides and the development of
A pilot study with three Siddha drugs Sivanaramirtham, Akasakaruden kizhangu churanam and Sanguparpam tried in different humoral types of dermatomycosis showed significant activity (Anandan et al., 1991). Numerous essential oils have been found to possess varied degree of fungi toxicity against different pathogens. "Chirattitallam", which is prepared by extracting the oily ingredients from coconut shells by the application of excessive heat (destructive distillation) is used in the Siddha system of medicine as a reputed cure for several skin diseases like eczema, dhobe's itch and ringworm (Mudaliar, 1969). In combination with Nigella sativa it is claimed to cure leucoderma and other skin affections.

The research and further study for the development of newer antifungal drugs from this indigenous system of medicine would definitely be worthwhile.