Chapter 2

Review of Literature
2. REVIEW OF LITERATURE

Dermatophytosis has been known for a long time and the dermatophytes were among the first pathogenic micro-organisms to be recognised and named (Matsumoto and Ajello, 1987). "Dermatophyte" literally means ‘skin plant’ and the earliest usage of the term goes back to as early as 1882-1885 (Tanaka et al., 1992).

Etiology

Dermatophytosis is clinically described as "Ringworm" or "Tinea" infections. The name ringworm was in use from the sixteenth century and was coined to describe the circular lesion produced by the dermatophytes on the skin or scalp.

The earliest written records of mankind contain numerous descriptions of various skin diseases. Because of its visibility, ringworm has been noted and described from the earlier times of recorded history. The Egyptian medical papyrus scrolls, the Torah, the Talmud and the new Testament, as well as Greek, Roman, Chinese and Islamic manuscripts all contain reports of dermatophyte infections (Marks, 1991).
Dermatophytoses clinically described as "ringworm" are capable of parasitizing keratinized tissue such as stratum corneum of the skin, hair and nails of human beings and animals.

Superficial fungal infections of the skin are common in developing countries such as India. Such infections are endemic among most of the population in developing countries, primarily due to low sanitary hygiene and low literacy levels (Caceres et al., 1991).

Dermatophytoses are very common throughout the world in both males and females. Over the past four decades, the incidence of dermatophytoses is reported to be on the increase especially due to the advent of HIV infection and due to the usage of cytotoxic and immunosuppressive drugs to treat malignancies and other conditions. The humid weather, over population and poor hygienic conditions are ideally suited for the growth of dermatophytes and these conditions are more prevalent in tropical countries such as India. Obesity, occlusion and maceration due to tight clothing and various other diseases / disorders such as diabetes mellitus, ichthyosis, atopy and immunosuppression were considered to be the major pre-disposing factors favouring the disease.
The effective control and prevention of the disease requires proper epidemiological characterisation, identification of the aetiological agents, its pathogenic role and mode of transmission and identification of reservoir of the disease.

Classification of Dermatophytes

Dermatophytes are classified into three anamorphic (asexual or imperfect) genera, *Epidermophyton*, *Microsporum* and *Trichophyton* of the Deuteromycota (Fungi imperfecti), essentially based on conidial morphology and formation. Those dermatophytes capable of reproducing sexually are classified in the Phylum Ascomycota, telomorphic genus Arthroderma (Weitzman et al., 1986). The genus Nanniza is the sexual genus of the Microsporum.

Historical Review

As early as 1834, Robert Remak had noted the presence of filaments resembling a mold in a specimen collected from a scalp disease. Perhaps, he was the first to study the fungal etiology of ringworm disease. The most remarkable work was by David Gruby, a Hungarian physician in the year 1841. He published a paper in which he has described the isolation of fungus
from favus and its growth on potato slices and production of the disease by inoculation of this fungus into normal skin. Thus, David Gruby was the first to establish that a microorganism was responsible for human disease. David Gruby also described the yeast, now called *Candida albicans* from thrush and named a dermatophyte isolated from tinea capitis *Microsporum audouinii* (Rippon, 1982).

In 1845, Malmstern created the genus *Trichophyton* and described *Trichophyton tonsurans*. In 1847, Charles Robin defined *Trichophyton mentagrophytes* and published a compilation of his early works on dermatophytes in the book "Historie Naturelle des Vegetaux Parasites" in 1853, which contained the first description of topical therapy for dermatophyte infection and the importance of epilation in tinea capitis (Emmons, 1974).

After a gap of 37 years between 1853 and 1890, Raymond Sabouraud, a French dermatologist began to publish his systematic and scientific studies on the dermatophytoses. He accumulated his studies in the volume "Les Teignes" in 1910. This book is considered as a classic in medical literature. Sabouraud classified dermatophytes based on clinical rather than botanical observation into four genera *Trichophyton, Microsporum, Epidermophyton*
and *Achorion*. Some of his species have been reduced to synonymy, and the genus *Achorion* has been dropped.

In 1935, Dodge published a book ‘Medical Mycology’ describing 118 dermatophytes isolated from all types of human disease. He also discussed the association of certain anthropophilic fungus with particular groups of races of man such as *Trichophyton violaceum* with Jewish people and *Microsporum ferrugenum* with northern Chinese, Koreans and Japanese.

Hopkins and Rhoda Benham (1920) from Columbia University were the first to study the fungi involved in a disease in a systematic way. Rhoda Benham is considered the founder of modern medical mycology. Followed by him, Chester Emmons in 1934, redefined dermatophytes according to botanical rules of nomenclature and taxonomy. He accepted the synonymy of *Achorion* with the genus *Trichophyton* as proposed by Langeron and Milochevitch in 1930. He included all the known dermatophytes in three genera.

Georg and Camp (1957), clarified the identity of several organisms by means of nutritional studies and thus she established the use of physiological characteristics and nutritional requirements in identification. Georg (1960)
reduced the number of dermatophyte species to sixteen with the descriptions of new, valid species of keratinophilic soil saprophytes and skin pathogens. Ajello (1962) described two species in the genus *Epidermophyton*, 16 in *Microsporum* and 21 in *Trichophyton*.

**Ecology and Etiology**

The dermatophytes may be grouped into three categories based on host preference and natural habitat (Kaplan et al., 1958). Thus based on the ecological habitat dermatophytes are divided into three groups anthropophilic, zoophilic and geophilic. This grouping is significant epidemiologically and may be helpful in determining the source of an infection.

The primary reservoirs of the anthropophilic species are man, whereas the primary reservoirs of the zoophilic and geophilic species are animals and soil respectively. Fungi in all the three categories, however can cause human infections.

*T.mentagrophytes var. interdigitale*, *E.floccosum*, *T.rubrum*, *T.tonsurans* and *T.violaceum* are some examples of anthropophilic dermatophytes. *M.canis*, *T.simii* and *T.verrucosum* belong to the zoophilic type. *M.gypseum*, *M.nanum*, *M.fulvum* and *M.cookei* are some of the
geophilic species. The pathogenic potential of dermatophytes considerably vary from species to species (Wilson, 1959) and from host to host, the most virulent organism being, *Trichophyton rubrum* which is an important aetiological agent of the tinea cruris infection in most cases.

Many of the geophilic dermatophytes are known to have less or no virulence at all. Transmission may be direct, human to human, (Stiller *et al.*, 1992) or via fomites (Kane *et al.*, 1988; Mackenzie, 1961). Geophilic dermatophytes live in the soil, primarily associated with the keratinous material, which serves as a source of infection for humans and lower animals (Alsop and Prior, 1967). Some geophilic species belonging to the three anamorphic genera exist as soil saprobes and have not been reported to cause infection.

The three major genera *Epidermophyton*, *Microsporum* and *Trichophyton* which produces the dermatophytic infections comprises of 39 species and 4 varieties. Five species account for most of the infections worldwide, while the other species cause low level infections. *Microsporum* and *Trichophyton* contain multiple species, many of which are pathogenic (Weitzman and Summerbell, 1995).
The genus *Trichophyton* is extremely complex, apart from the existence of at least 15 recognised species in the genus (Weitzman and Summerbell, 1995), there are several different variants within the species of *T. mentagrophytes*, occurring in both man and animals (Rippon, 1988). While *T. mentagrophytes var. interdigitale* is anthropophilic and frequently found in man, *T. mentagrophytes var. mentagrophytes* is zoophilic and isolated from animals. Interestingly, some authors refer to the different varieties of *T. mentagrophytes* as separate species, e.g., *T. interdigitale* rather than *T. mentagrophytes var. interdigitale*. Dermatophytes are the only group of human-infecting fungi which have evolved into obligate infectious agents, although anthropophilic, zoophilic and geophilic species are known (Rippon, 1985).

*Tinea cruris* is the most common infection followed by *tinea corporis*. The common aetiological agents are *T. rubrum, T. mentagrophytes*, and *E. floccosum*. These infections tend to be chronic and often recur or relapse after treatment. Invasion of deeper tissues by dermatophytes are known to be rare. Symptoms vary from mild itching, inflammatory lesion to recurrent or chronic disease and in some cases granulomatous disease. Although the aetiological agents in different geographic areas vary considerably, the most frequently encountered species is *T. rubrum* followed by *T. mentagrophytes*. 
*T. rubrum* causes infection of the glabrous skin, groin and nail. There may be a genetic predisposition for the high prevalence of *T. rubrum* in families (English, 1957; Many *et al.*, 1960; Zaias *et al.*, 1996). Almost all chronic dermatophyte infections of the skin involves the anthropophilic fungus *T. rubrum*. Most infections with the dermatophytes are self-limited, or are cured easily with the topical or systemic antifungal agents. Occasionally, some of these infections become chronic, especially those which are caused by *T. rubrum*. It is an anthropophilic fungus capable of invading stratum corneum and nails, but incapable of invading hair. Infections typically are largely asymptomatic and may be chronic or recurrent. It can infect any area of the skin, but the groin area and toe areas are especially predisposed.

**Ecology and Host Preference**

Although some zoophilic varieties of *T. mentagrophytes* produce only minor or subclinical infections in animals, they can initiate a severe inflammatory response in man (Rippon, 1988). Therefore from an epidemiological point of view, it is important to determine the variety of *T. mentagrophytes* and its origin in order to prevent the spread of infection.
The geophilic dermatophytes, *M. cookei*, *M. racemosum*, and *M. vanbreuseghemii* seldom cause an infection. Zoophilic dermatophytes

**Tinea capitis in India**

A study conducted by Kamalam and Thambiah (1980) in Madras, showed a gradual increase in the incidence of tinea capitis from 3.56% to 6.25% during a 3 year period. Male children were more commonly affected than female children, and the chief age groups affected, were between 5 & 10 years. *Trichophyton violaceum* was the commonest (73.94%), followed by *T. tonsurans* (13.16%). Another study conducted by Kumar and Lakshmi (1990) in Tirupati, showed an higher incidence of tinea capitis infection in males (58%), than in females (42%) and the incidence was found to be more in children aged between 6-15 years. *Trichophyton violaceum* (63.15%) was the predominant species isolated.

Dasgupta *et al.* (1975), reported the presence of *T. violaceum* (84%) and *T. tonsurans* (16%) in cases of tinea capitis in Pondicherry.

higher in the third decade (between 21-30 years of age). Their study showed
that *Tricophyton rubrum* was the most common etiological agent which accounted for 55.18% of the infection.

Gupta *et al.*, (1993) studied the mycology of dermatophytosis from Ludhiana, Punjab. Their study showed that *Tricophyton rubrum* was the most common etiological agent of the disease. Selvi (1995) studied the clinical aspects of chronic dermatophytosis in Madras. She reported that, atopy, ichthyosis, diabetes mellitus, long term steroid therapy were the major predisposing factors of the disease. The study also showed that *Tricophyton rubrum* was the most common etiologic agent of the disease and tinea cruris, the most common type of infection.

**Management Strategy of the disease**

Though, the treatment is available in many cases recurrent and chronic dermatophytoses has been reported. Management of these infections depends upon a random selection of an antifungal agent out of a large number of topical agents belonging to different groups e.g., polyenes, tolnaftate, imidazole derivatives and other antifungal agents (Jones, 1982). Response to these topical agents is also variable depending upon the causative species of dermatophytes. All dermatophytes were thought to be uniformly sensitive to
Griseofulvin, but this has been demonstrated to be incorrect (Artis et al., 1981). Occasionally, they may appear less sensitive, when there are problems such as bad vascularisation, which prevents the drug from reacting at the site of infection.

Eventhough chemical control strategies have been developed since the mid-1880's with agents such as the 'Bordeaux mixture', it is only with recent times the more selective and potent antifungals have been developed in response to the requirements of the modern medicine. One such example of control agents are the azole antifungals. This group of antifungals, with activity against most yeasts and filamentous fungi (but not phycomycetes) consist of imidazole and triazoles and have been commercially available since the mid-1960's. Currently this constitute one quarter of the world's market for systemic fungicides (Horne and Hollomon, 1997).

The antifungal agents used today in the treatment of systemic mycosis fall into three structural classes: Polyenes, Flucytosine and synthetic azoles. The polyenes are Amphotericin-B and nystatin which binds sterols in the fungal cell membranes resulting in disruption of cellular integrity. Flucytosine is a synthetic nucleoside that is converted intracellularly to 5-fluoro uracil which interferes with protein synthesis (Polak and Scholer, 1975).
Before 1950's no reliable treatment existed for deep fungal infections and treatment of superficial infections depend on empirical topical preparations. During the 1950's Nystatin was introduced for the topical treatment of candidiasis. Griseofulvin was first used for oral treatment of dermatophytosis and Amphotericin-B was used for deep fungal infections. With the introduction of 5-fluorocytosine for the treatment of candidiasis and cryptococcosis, drug resistance was encountered with the antifungals for the first time. During the 1970's and 1980's a large number of broad spectrum antifungal agents that are N-substituted imidazole or triazole compounds were introduced. The earliest members of this group, clotrimazole and miconazole were used for topical therapy while some of the more recentazole compounds eg. Ketoconazole, Itraconazole and Fluconazole are effective after oral administration.

Since the 1950's, amphotericin B has been the drug of choice for most fungal infections (Gallis et al., 1990). In spite of the introduction of the triazoles, amphotericin B is used for treating most of the patients presenting with infection caused by Aspergillus spp. and other filamentous fungi. In the late 1950's griseofulvin became the first oral agent for superficial fungal infections. It is effective against dermatophytes but not active against
*Candida albicans*. Nevertheless, it has proved to be an effective and safe therapy for dermatophyte infections (Odum, 1997).

The main classes of antifungals currently employed in the topical treatment of superficial fungal infections are the polyenes, the imidazoles, and the allylamines / benzylamine drugs (Brennan and Leyden, 1997). Imidazoles are broad spectrum agents being active against fungi, bacteria and protozoa. They have recently become available for use as antifungal agents and it includes clotrimazole, miconazole, econazole, ketoconazole, itraconazole, fluconazole, thiabendazole.

Among the earliest of the "modern" topical antifungal is the polyene nystatin, an antibiotic isolated from the *Streptomyces noursei*. The polyenes act by binding irreversibly to ergosterol, an essential component of fungal cell membranes. The polyenes are not active against dermatophytes, and the clinical use is limited to the treatment of infections caused by *C. albicans* and other *Candida* species (Medoff and Kobayashi, 1980).
Azole antifungals

The azole group of antifungals have in common an imidazole or triazole ring with N-carbon substitution which is responsible for their interaction with target sites within the fungal cell. The fungistatic effects of these drugs result from inhibition of membrane sterol synthesis by inhibition of cytochrome P450 sterol demethylase (Smith, 1986).

Ketoconazole

The first systemic azole, ketoconazole, was introduced in the early 1980's and was approved for the treatment of severe recalcitrant cutaneous dermatophyte infections. It has also been used for the treatment of tinea versicolor and mucosal candidiasis.

Ketoconazole, the first orally available imidazole antifungal, was introduced in US in 1981. It has a number of dose limiting adverse effects, drug interactions and absorption problems, including poor absorption at elevated gastric pH, variable absorption with food and poor availability in bone marrow transplant and AIDS patients (Kauffman and Carver, 1997).
Fluconazole

Fluconazole, introduced in the US in 1990 and slightly earlier in Europe, is frequently used for a number of fungal infections, including cryptococcosis and mucocutaneous and disseminated candidiasis. The high oral bioavailability and minimal drug interactions have contributed to its wide spread use (Kauffman, 1996; Kauffman and Carver, 1997; Como and Dismukes, 1994).

Fluconazole remains the drug of choice for treatment of mucocutaneous candidiasis in both neutropenic and AIDS patients (Sangeorzan et al., 1994; Koletar et al., 1990; Walsh et al., 1994). Fluconazole remains the drug of choice for maintenance therapy of cryptococcal meningitis in AIDS patients (Powderly et al., 1992; Saag et al., 1995).

However, fluconazole has been associated with birth defects (Lee et al., 1992; Pursley et al., 1996) and even single dose therapy should not be used in pregnant women (Inman et al., 1994).
With the introduction of new triazole compounds, antifungal therapy has gained a new momentum. The imidazoles, discovered in the late 1960's relatively have broad spectrum activity against dermatophytes and yeasts and are primarily fungistatic. They act by inhibiting fungal ergosterol synthesis, causing defects in the fungal cell membrane. Azole antifungals, e.g., clotrimazole, sulconazole and miconazole interfere with the ability of the cytochrome P - 450 (CYP) enzyme lanosterol 14 demethylase to catalyse the conversion of lanosterol to ergosterol (Saag and Dismukes, 1988).

**Itraconazole**

Itraconazole is a triazole and is ideal for the treatment of dermatophytes, *Candida* and *Aspergillus* infections (Jacobs, 1992). Itraconazole was approved for use in the US in 1992.

Itraconazole is more specific for fungal (vs mammalian) cytochromes P 450, resulting in less toxicity and greater efficacy than ketoconazole. Serious drug interactions can occur when itraconazole is used with certain other drugs metabolised by CYP (Kauffman and Carver, 1997; Como and Dismukes, 1994).
Allylamine and Benzylamine Derivatives

Allylamine / benzylamine drugs are fungicidal against dermatophytes and fungistatic against *C. albicans* at therapeutically achievable drug concentrations. No resistance patterns have yet been detected for this class of drugs. The allylamine drugs are well tolerated, and adverse effects include itching, burning or redness at the application site in 2% to 3% patients (Villars and Jones, 1989; Fukushiro *et al.*, 1992).

In the late 1980’s naftifine, an allylamine was introduced for the treatment of dermatophyte infections. The allylamines naftifine and terbinafine, and the allylamine-like benzylamine derivative, butenafine, suppress the biosynthesis of ergosterol at an earlier stage of the metabolic pathway than the azoles, independent of cytochrome P-450 enzymes, by inhibiting the activity of squalene epoxidase. The resulting ergosterol deficiency is accompanied by an accumulation of squalene in the fungal cell that leads to cell death (Maeda *et al.*, 1991; Petranyi *et al.*, 1984).
Terbinafine

Terbinafine is a new agent approved for treating onychomycosis and cutaneous tinea infections (Balfour et al., 1992). Terbinafine became available in the mid-1990's. Topical naftifine and terbinafine are fungicidal against dermatophytes and fungistatic against \textit{C. albicans}. The first allylamine, terbinafine, was approved in 1996 for the treatment of onychomycosis. The drug has been available both as a cream and as an oral formulation in Europe for several years and the tablet formulation has just been released for the treatment of onychomycosis and tinea infections in the US.

Terbinafine acts by the inhibition of squalene epoxidase, is fungicidal for most of the filamentous organisms including \textit{Aspergillus} spp., and concentrates in nails and stratum corneum (Schmitt et al., 1988; Ryder, 1992). Although more active against dermatophytes than against \textit{Candida} spp., recent data showed that terbinafine also may be beneficial for \textit{Candida} skin infections (Jung et al., 1994). Adverse effects appear to be minimal and include, most commonly, taste perversion and gastrointestinal disturbances and rarely hepatitis and rash.
Problems posed with the present antifungal agents

Though a variety of antifungal drugs are available for the treatment of dermatophytoses, the disease has a tendency to recur at the same site or at different sites of the body with the cessation of treatment. Severity of the infection produced by the dermatophytes also varies from host to host and from species to species. Moreover, the existing antifungal drugs has one or more of the following shortcomings such as toxicity, fungistatic mechanism of action, development of resistance, suboptimal pharmokinetics, unfavourable route of administration and undesirable drug-drug interaction.

Thus the ideal antifungal agent should have broad spectrum of activity, be effective at low concentrations, be fungicidal in activity rather than fungistatic, orally absorbed and parenterally effective, well distributed, metabolically stable, water soluble, must have high affinity for stratum corneum, high mycologic and clinical cure rates, lack of development of fungal resistance, low relapse rates, low incidence of adverse effects and low cost.

The variability in efficiency of these antifungal compounds in recurrent and chronic dermatophytic infection and in immuno-deficient patients, as
well as the prohibitive cost in developing countries have necessitated the search for newer drugs.

Therefore, the use of medicinal plants in treatment regimen is gaining importance. Herbal medicines have been known to man for many centuries. The therapeutic efficacy of many indigenous plants for a variety of diseases / disorders have been widely documented in traditional medicinal literature (Satyavathi and Gupta, 1987). Herbs are known to have medicinal properties and the need for research is felt to find out efficacious, broad spectrum activity, cheaper and safer natural product. Medicinal plants are natural resources yielding valuable herbal products which are often used in the treatment of various ailments. Many Indian medicinal plants were widely used in the treatment of various skin diseases by the siddha and ayurveda physicians (Kirtikar and Basu, 1935). Ayurveda, the traditional system of medicine practised in India can be traced back to 6000 B.C. (Samhita, 1949).

A large proportion of the world population especially in developing countries depends on the traditional system of medicine for a variety of diseases. Several hundred genera are used medicinally mainly as herbal preparations in the indigenous system of medicine in different countries and
were sources of potent and powerful drugs which have stood the test of time and modern chemistry has not been able to replace most of them.

Many pharmaceuticals we use today are of botanical origin and are based on herbal remedies from the folk medicine of native peoples (Tyler et al., 1988). Schultes (1986) suggests that the most important drugs of the past 50 years or so, were first isolated from plants used ethnomedically. In fact, 74% of the 119 biologically active plant derived compounds at present used were discovered as a result of research on species first identified on ethnobotanical surveys (Farnsworth and Soejarto, 1985; Farnsworth, 1988). The phytochemical screening of plant species of ethnopharmacological use will provide valuable baseline information in the search of new pharmaceuticals. Yet fewer than 10% of tropical plant species have been examined for the presence of bio-active compounds (Myers, 1984).

Medicinal plants form the principle component of traditional medicine. This means that in the order of 3300 million people use medicinal plants on a regular basis. Medicinal plants used in traditional medicine should therefore be studied for safety and efficacy (Farnsworth, 1994).
Medicinal components from plants also play an important role in conventional western medicine. In 1984, at least 25% of the prescription drugs issued in the USA and Canada are derived from or modelled after plant natural products (Farnsworth, 1984). In 1985, Farnsworth et al. identified 119 secondary plant metabolites that are used globally as drugs. It has been estimated that 14-28% of higher plant species are used medicinally, that only 15% of all angiosperms have been investigated chemically and that 74% of pharmacologically-active plant derived compounds were discovered after following upon ethnomedical use of plants (Farnsworth and Soejarto, 1991).

Extracts of medicinal plants have mainly been superseeded in the pharmaceutical preparations of the developed countries. However natural products from higher plants continue to be used in pharmaceutical preparations either as pure compounds or as crude extracts. 25% of prescriptions dispensed in community pharmacies in USA during 1980 contained an active ingredient that was plant derived. The WHO estimates that around 20,000 species of higher plants are used medicinally throughout the world (Philipson, 1994) but over 85% of the plants await scientific investigations for their biological activity and chemical constituents (Houghton, 1995).
The developed countries are endowed with rich flora as they are mostly situated on the tropical belt and between them, cover a wide range of geographic and climatic conditions. Of about 15,000 species of higher plants in India, medicinal uses are attributed to at least 1,500 plant species (Hussain, 1992).

In India, herbal medicines have been the basis of treatment and cure for various diseases / physiological abnormalities in traditional methods under practice such as ayurveda, siddha and unani. Indian folk medicine comprises numerous prescriptions for therapeutic purposes which may be as varied as healing wounds, treating inflammation due to infection, skin infections, leprosy, diarrhoea, scabies, venereal diseases, ulcers, snake bite etc.

The present study was aimed to screen some of the Indian medicinal plants for their antidermatophytic activity in vitro and to determine the therapeutic efficacy in a suitable animal model and also to compare the results obtained with the commercial antifungal agents.
Pharmacological Properties of Important Medicinal Plants Used in the Present Study

Many kinds of diseases have been treated with herbal medications throughout the history of mankind. It is necessary from the scientific point of view to establish the relationship between chemical composition, biology and therapeutic activity. The search for biologically active compound from natural source has always been of great interest to scientists looking for new sources of drugs that are useful in infectious diseases. In recent years a number of studies have been reported dealing with extraction and separation of important components / constituents from the medicinal plants.

*Aegles marmelos* (L.) Corr. (Rutaceae), locally known as ‘Bael’ or ‘shripala’ is found growing wild and cultivated throughout the Indian sub-continent. Various parts of the plant are used in ayurveda the traditional Indian medicine, as well as in unani medicine for treatment of various diseases. All parts of the plants are used for medicinal purposes (Kirtikar and Basu, 1933). The bael fruit possesses important medicinal property to treat dyspepsia, diarrhoea and dysentery (Jauhari *et al.*, 1969; Satyavathi *et al.*, 1976). The fruit is also used as an important dietary supplement (Barthakur and Arnold, 1989). The root bark extract of this plant has been reported to
be beneficial to cure intermittent fever, mental diseases, pericarditis and angina pectoris (Nadkarni, 1976). The constituents of bael are administered for the treatment of heart ailments (Kakiuchi et al., 1991).

Several plants of the family Euphorbiaceae are employed for the treatment of infectious diseases (Morton, 1981) and some experimental studies have confirmed their anti-microbial effects (Mensah et al., 1990; Cruz et al., 1994).

*Myristica fragrans* (Myristicaceae) is an evergreen aromatic tree cultivated in many tropical countries. Nutmeg is the dried kernel of *M. fragrans*. It has been claimed to possess medicinal properties (digestive, carminative and expectorant) in the traditional system of medicine (Pharmacopoeia of India, 1955). It is also mentioned in modern scientific literature as a medicinal plant (Merck index, 1989). The plant material has been reported to contain pectin (0.5%) which has hypolipidaemic action. It also contains 26-33% fixed oils and 5-13% volatile oils, myristic acid 11.8%, palmitic acid 14.3%, stearic acid 1.2%, oleic acid 5.2%, linoleic acid 1.5% and lauric acid 0.4%) and chemical substances such as myricitin, elimicin and myristic acid (Nadkarni, 1976; Merck Index, 1989).
Many volatile oils are known to possess antifungal properties and there is potential application of such oils as antifungal agents (Deans et al., 1989). A large number of studies concerning the antimicrobial activity of essential oils have already been reported (Janssen et al., 1987). It has been established scientifically that about 60% of the essential oils possess antifungal properties and 35% were found to exhibit antibacterial activity also (Chaurasia and Vyas, 1977). Essential oils are a potent source of natural pesticides (Singh and Upadhyay, 1993). The plant material has been reported to contain pectin (0.5%) which has hypolipidaemic action. It also contains 26 - 33% fixed oils and 5 - 13% volatile oils, myristic acid 11.8%, palmitic acid 14.3%, stearic acid 1.2%, oleic acid 5.2%, linoleic acid 1.5% and lauric acid 0.4%) and chemical substances such as myricitin, elimicin and myristic acid (Nadkarni, 1976; Merck Index, 1989).

Essential oils are the odourous, volatile products of the plant secondary metabolism, normally formed in group of cells or as glandular hairs, found on many leaves and stems. They may be present in glandular cells or ducts in any or all organs of the plant including roots, stem, buds, leaves, flowers and fruits. The habitat of the plants is known to play an important role in the formation of secondary metabolites. However, oils are concentrated in one particular region such as leaves, bark or fruit, and when occurring in various
organs in one plant may possess different individual chemical components (Bonner, 1991).

_Cassia alata_ L. (family: Leguminosae) employed in traditional medicine in many parts of India and West Indies for the treatment of various ailments (Dymock, 1980). Decoctions of the leaves, flowers, bark and wood are used in skin diseases such as eczema, pruritis, itching and in constipation (Kirtikar and Basu, 1975). The flowers are also used in bronchitis and asthma (Chopra _et al._, 1956). The leaves has been reported to have laxative effect and are also used against ringworm, scabies, ulcers and other skin diseases. (Seafourth, 1962).

_Curcuma longa_ L. Turmeric has long been used as a common household medicine and as a spice in Southeast Asia. Turmeric contains essential oil, yellow pigments (curcuminoids), starch and oleoresin (Leung, 1980). The rhizome of _C.aromatica_ is used for bruises, contusions, and sprains. They are also used as a substitute for turmeric (_Curcuma domestica_ valeton) but not as a condiment (Ambasta, 1994). Hajji _et al._ (1993) and Gundidza _et al._ (1993) have reported the antibacterial and antifungal activities of the essential oils of _Eucalyptus_ spp.
The family Lamiaceae comprises many essential oil bearing species. The essential oil of the *Ocimum canum, O.gratissimum, O.trichoden* and *O.urticifolium* are reported to have antimicrobial properties. Furthermore, the essential oils of *Ocimum* spp. grown in Rwanda is also shown to possess antimicrobial activities (Janssen *et al.*, 1989). The plant *Ocimum sanctum* Linn. consist of two types: the green type (“Sri Thulsi”) and purple type (“Krishna Thulsi”). Leaves yield an essential oil with appreciable note of cloves. It possesses insecticidal, pesticidal, antibacterial and mosquito repellent properties. The juices of leaves of *O.sanctum* are stimulant, used as expectorant, diaphoretic used in catarrh and bronchitis, ringworm and other cutaneous diseases. The roots are given in the form of decoction in malarial fever, seeds are used in treating the disorders of genito-urinary system (Chopra *et al.*, 1992).

*O americanum* Linn. yields a volatile oil used in soaps and cosmetics. The fragrant leaves of *O.americanum* are used in sauces, soaps and salads. *O.basilicum* yields a volatile oil, used both as a flavouring agent and perfume. The plant has antipyretic, expectorant, carminative, stimulant and anthelmintic properties. Juices of leaves are used as nasal douche and for ringworm infection. *O.gratissimum* is more strongly scented than other species. It yields a volatile oil which show marked antibacterial activity and
it acts as a mosquito repellent. It is also used for relief from tooth-ache, ear-ache and abdominal colic in children (Ambasta, 1994).

*Pongamia pinnata* is widely reported to have various therapeutic properties in the ayurvedic system of medicine. The plant is applied as a medicine in scabies, herpes and other cutaneous diseases. The seeds were used as an external application in the case of skin diseases. The oil obtained from the seeds have been used in rheumatism, herpes and scabies. The fresh bark was used to cure bleeding piles. The plant material of *Acalypha indica* is used in bronchitis, pneumonia and asthma. The leaves were employed for cutaneous troubles and in snake bite (Ambasta, 1994 and Chopra *et al.*, 1992).

The leaves of *Lawsonia alba* in the form of a paste with or without lime juice is regarded as an excellent topical remedy for ringworm in indigenous medicine (Kirtikar and Basu, 1935). The aqueous leaf extract of *Lalba* has been reported to have antidermatophytic property in *vitro* (Venugopal *et al.*, 1993) and similar findings were reported by earlier workers (Radhakrishnan *et al.*, 1976). Dixit *et al.* (1980) have isolated an antifungal compound lawsone from the leaves of *Lawsonia inermis* which is fungicidal with broad spectrum activity.
The alcoholic extract of *P. zeylanica* showed greater activity than the aqueous and hexane extracts (Ahmad *et al.* 1998). They also assayed the crude alcoholic extract in sheep erythrocytes and found no cellular toxicity.

Parimala and Sachdanandam (1993) investigated the changes in the rate of glycolysis and glyconeogenesis in tumour-bearing rats and the effects of treatment with plumbagin. The investigations revealed the molecular basis of the biological behaviour and the anticarcinogenic property of plumbagin against hepatoma studied in rats.

The LD$_{50}$ of plumbagin by oral route has been found to be 16mg/kg body weight and the ED$_{50}$ of plumbagin for fibrosarcoma in rats by oral route is found to be 0.75 mg/kg body weight.

Durga *et al.* (1990) studied the effect of plumbagin in antibiotic resistance in *Escherichia coli* and *Staphylococcus aureus*. A delayed growth was seen when the organism were inoculated with the antibiotic. However, complete prevention of bacterial growth was observed in the medium containing antibiotic and plumbagin together, and this was attributed to the prevention of development of antibiotic resistant cells.
The bark of *Wrightia tinctoria* was dried, ground and rubbed over the body in dropsy. The seeds were used in the case of fever, diarrhoea, dysentery and it has anthelmintic properties. The dried and powdered plant material of *Enicostemma littorale* along with honey is used as a blood purifier. It has also been used in rheumatism, abdominal ulcer, hernia and insect poisoning. Seeds possess expectorant properties and are used as antimalarial (Chopra *et al.*, 1992).

*Plumbago zeylanica* Linn. belongs to the family Plumbaginaceae. It is found throughout India, in the W.Peninsula and in Bengal. The root is an appetizer and used in skin diseases and piles etc. The extract of the plant made into a paste with vinegar, milk or salt and water has been used for external application in leprosy and skin diseases (Chopra *et al.*, 1956). Panchcole - an ayurvedic formulation containing *P.zeylanica* as one of its chief ingredients has been known to produce hypolipidaemic effect (Sharma *et al.*, 1990).

Plumbagin (5-hydroxy-2-methyl-1,4-napthaquinone), a compound derived from roots of *P.zeylanica* has been reported to have antibacterial activity (Satyavathi *et al.*, 1987). The root of *P.zeylanica* is used in dyspepsia, piles, diarrhoea and skin diseases. The infusion of the root is used
in influenza and black water fever and the root bark contains the active principle (Ambasta, 1994). Plumbagin has been reported to have antimicrobial, antitumour and anticancer properties (Krishnaswamy and Purushothman, 1980).

Peach and Tracey (1955) have published volumes of modern methods of plant analysis. They have described at length, the preliminary methods of drying, powdering and extracting plant products with several solvents beginning from highly polar to non polar solvents. Harborne (1973) in his phytochemical methods of plant analysis, described various simple methods of extraction by percolation and soxhlet extraction. Another study has described the preparation of plant extracts using three different solvents wherein, the extract was subjected to filtration using Whatmann filter paper no. 1 followed by concentration in vacuo (Alade and Irobi, 1993).

Samy et al. (1998) described that the excised plant parts (i.e. bark, leaf, root, seed and whole plant) should be shade dried at room temperature and then should be powdered using electric blender. Each of the powdered plant material was fractionated sequentially using hexane, diethyl ether, dichloromethane, ethyl acetate, methanol and water for preferential selection of soluble compounds based on the polarity of the solvent.
Ibrahim and Osman (1995) in their study reported the extraction of plant products using soxhlet apparatus with 95% ethanol as solvent. The resultant extract was then concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 40°C.

**In vitro Studies**

Research on the antimicrobial activity of medicinal plants has encountered some problems because of the diversity of criteria and the techniques that are employed. Rios *et al.* (1988) have prepared a bibliography on the different techniques and methods employed in the antimicrobial study of medicinal plants and the principles obtained from them. While the methods can be classified into three groups (diffusion, dilution and bioautographic methods), a great number of facts can influence the results: the extraction methods (Nadir *et al.*, 1986, inocula volume (Bauer *et al.*, 1966; Hamburger and Cordell, 1987), culture medium composition (Bauer *et al.*, 1966; WHO, 1977), pH (Leven *et al.*, 1979; Gutkind *et al.*, 1981) and incubation temperatures (Emeruva, 1982). Moreover experimental factors such as the microorganisms used and the volume of the assayed samples can interfere with the *in vitro* antimicrobial testing procedure (Janssen *et al.*, 1987).
1. **Principal diffusion methods**

   A technique that does not require homogenous dispersion in water is the agar-overlay method using a disk, hole or cylinder as reservoir. The reservoir containing the sample to be tested is brought into contact with an inoculated medium and, after incubation, the diameter of the clear zone around reservoir (inhibiting diameter) is measured. This method was originally designed to monitor the amount of antibiotic substances in crude extracts. In order to lower the detection limit, the inoculated system can be kept at a low temperature before incubation, which favours diffusion through the culture medium and this increases the inhibition diameter. This technique can also be used for obtaining biograms.

a. **Disc method**

   The filter paper discs containing the antibiotics was placed on the surface of the agar, immediately after inoculating the plate with the organism to be tested.
b. **Hole-plate method**

This method depends upon the diffusion of the antibiotic from a vertical hole through the solidified agar layer of a petriplate to such an extent that growth of the added microorganism is prevented in a circular area or zone around the hole containing a solution of the antibiotic.

c. **Cylinder method**

This method is similar to the hole-plate method. Stainless steel or porcelain cylinders were used for the assay. After incubation the cylinders are removed, and the average diameter of each zone is measured and recorded.

2. **Dilution Methods**

Dilution techniques are those which require a homogenous dispersion of the sample in water. They are used principally to determine the minimum inhibitory concentration (MIC) values of an extract / pure substance. They are used in the preliminary screening of antimicrobial activity.
In the liquid dilution method, the transparency of the medium was taken as the growth index. When no growth takes place, the medium remains clear. If there is growth of the organism in the medium, it becomes turbid. The inhibition is related to the turbidity of the medium and measured by spectrophotometry.

In the agar dilution method, known amount of antibiotic is mixed with nutrient agar and allowed to set. The advantages of this method are its simplicity, speed and the ability to study both water soluble and insoluble samples such as essential oils.

3. **Bioautographic methods**

Bioautography is the most important detection method for new or unidentified antimicrobial compounds (Betina, 1973). The typical bioautography procedure is based on the so called agar-diffusion technique, whereby the antimicrobial compound is transferred from the chromatographic layer to an inoculated agar plate. The inhibition zones are visualised by dehydrogenase activity detecting reagents (Begit and Kline, 1972).
Though diffusion methods are more widely employed in research, the credibility of the test becomes less especially when dealing with samples having low diffusion power and contradictory results were obtained with that of dilution methods against the similar microorganisms (Pellecuer et al., 1976).

Many authors use inhibition zones for comparison (Leven et al., 1979; Singh et al., 1983; Emeruva, 1982) and other researchers relate MIC values with inhibition zones (Ayafor et al., 1982). The application of regression lines in relating the use of dilution method for MIC determination of pure samples and inhibition zones has been proposed by the WHO committee (Ericsson and Sherris, 1971). Diffusion methods should not be employed against lipophilic samples or to determine the MIC of a sample, but holds good for preliminary screening of pure substances (alkaloids, flavanoids, terpenoids, etc.) under optimal conditions (Bauer et al., 1966; Mitscher et al., 1972; WHO, 1977).

Dilution methods are the widely accepted and most recommended technique to assay lipophilic samples and to determine the MIC of compounds (Clark et al., 1984; Miski et al., 1983; Adeoye et al., 1986). Comparative studies on the solid dilution methods and liquid dilution
methods showed similar sensitivities, and the former method being more quick and time saving (Mitscher et al., 1972; Baron and Bruckner, 1984; Gabrielyen et al., 1985). The agar dilution method is applicable to both polar and non-polar samples, and the use of emulsifying agents with lipophilic samples yielded good results (Allegrini et al., 1973; Yousef and Tawil, 1980; Pellecuer et al., 1976).

In vivo Studies

The in vitro assessment of antifungal activity offers an entirely different set of advantages and limitations than do in vivo models. Although in vitro susceptibility testing is performed virtually for all new agents, these tests have distinct limitations. An important approach in the modern day evaluation of the medicinal plants is to study the toxicology of these plants by conducting safety studies in animal models in order to ascertain the safety nature and validity of using the plant for human use. Hence, all new antifungal agents must be studied in vivo with use of experimental animal infection models. The guinea pig is the model of choice for studying dermatophyte infections (Maggon et al., 1991).
Several *in vivo* models of mycotic diseases have been devised and studied, many of which have been used in assessing antifungal activity (Hare and Loebenberg, 1988; Rinaldi, 1987). Experimental dermatophyte infections have been produced with varying degrees of success on the skin of man and lower animals for many years (Chittasobhon and Smith, 1979). The inhibitory activity of turmeric oil in experimentally induced dermatophytosis on Guinea pigs is also reported (Apisariyakul *et al.*, 1995). Guinea pig is also used as an animal model in testing the controlling effect of santolin oil against superficial cutaneous candidiasis (Suresh *et al.*, 1997). In an another study, guinea pig was used to study experimentally induced shigellosis (Vijaya and Ananthan, 1996).

Attempts to correlate results of *in vivo* therapeutic response with *in vitro* data are laudable, and any effort towards standardization procedures will involve such correlation. In addition animal models must be used for studies of drug toxicity and pharmokinetics. An important concept is that drugs may act similarly in animals and humans and that laboratory animals can thus serve as "model analogs" of man. In general, animal models correlate better with clinical efficacy than do *in vitro* methods (Brennan and Leyden, 1997).
Cytotoxicity Assay

It is a fundamental concept in drug screening that each drug action involves alterations in the tissue which are reflected in functional changes (Irwin, 1962). Before proceeding for in vivo trials, it is important to ascertain the cytotoxicity of any natural product in an established cell line like the Vero cell line.

Several studies have been previously reported on the use of cell culture methods in the determination of cytotoxicity testing, antibacterial effect (Ramachandran et al., 1983; Vijaya et al., 1995) and antiviral properties of plant extracts in vitro (Elanchezhian et al., 1993; Padma et al., 1998).

Recent studies on the cellular toxicity testing of plant extracts have been reported using the larvae of brine shrimp (Ajaiyeoba et al., 1998) and using fresh sheep erythrocytes (Ahmad et al., 1998).

The present study was aimed to screen some of the commonly available Indian medicinal plants for their antidermatophytic activity in vitro to determine the therapeutic efficacy in a suitable animal model and to compare the results with the commercial antifungal agents. The study would evolve with newer strategies of plant based compounds in the treatment of various dermatophytic infections.