Introduction
1.0 INTRODUCTION

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1. INTRODUCTION

Human mycoses can be categorised as superficial, localised or disseminated. Superficial mycoses are generally confined to the skin and nails, whereas localised mycoses include infections of the gastrointestinal, genitourinary and respiratory tracts. Disseminated or deep mycoses are characterised by fungal infections of the visceral organs, central nervous system and/or generalised fungal septicemia (Alice Clark 1992).

1.1 SUPERFICIAL MYCOSES

Superficial mycotic infections affecting skin, hair and nails are common among the people living in hot and humid climates (Bhutani 1993).

Superficial mycoses can be a source of great physical and in addition psychological discomfort which can be severe for esthetic reasons (Margarita silva - Hunter et al., 1981, Alice Clark, 1992). Since nail, hair and skin scrapings are readily obtained for direct microscopic examination and culture studies superficial mycoses can easily be identified (Tanaka et al., 1992).

Superficial fungi may be keratinolytic or non keratinolytic. Keratinolytic (ringworm fungi or dermatophytes) feed on keratin - the non viable part of the skin, hair and nails; unable to thrive in the living portions, hence necrophilic - fond of the dead.
Non keratinolytic fungi cannot penetrate the keratin; feed on the surface lipid film, hence live on and not in the skin. Common example *Malassezia furfur* (Bhutani 1993).

1.2 NON-KERATINOLYTIC FUNGI - EG. *MALASSEZIA FURFUR*

Petyriasis versicolor, a chronic superficial mycosis of the skin, caused by the fungus *Malassezia furfur*, affects the people world wide and is very common in tropics and subtropics. The disease, affecting mainly the stratum corneum is characterised by fawn to brown coloured scaly macules of various sizes with geographical patterns involving principally the trunk, axillae and even arms (Norman *et al*., 1971; Orlando canizares, 1975; Robert Berkow, 1982; Jan Faergemann, 1989).

The main complaint is often cosmetic disfiguration, but about one third of the patients also complain of moderate to severe pruritus. Itching is more pronounced when the patients are warm and sweating.

1.3 DIAGNOSIS

The greasy scale over the macules often loosens as a sheet in a characteristic way known as ‘Coup d’ angle’, when scratched with a wooden stick or fingernail. Under Wood’s light lesions show a yellow to yellowish blue florescence and lesions not visible to the naked eye are often found. An easy and reliable method is the direct microscopic examination. Scales are removed from lesions either by the use of a scotch tape or by finger nail, mounted on a
slide in 10% KOH solution and stained with methylene blue 1% for 1 minute and observed under the microscope. The round, budding cells and short hyphae "spaghetti and meat balls" are characteristic of the fungus *Malassezia furfur* (Jan Faergemann, 1989).

1.4 TREATMENT

Drugs currently used in the treatment of pityriasis versicolor are many with diversified chemical structures. Local application of solutions containing Selenium sulphide, Sodium hyposulphite, Tolnaftate, Alcoholic solution of iodine, Propylene glycol, Zinc pyrithione, Acrisorcin and Whitefield’s ointment is practiced for various lengths of time for the treatment of pityriasis versicolor. An oral antifungal agent Ketoconazole, is also prescribed for several months and the disease may recur in 6-12 months (Norman *et al*., 1971; Orlando canizares, 1975; Robert Berkow, 1982; Jan Faergemann, 1989).

1.5 KERATINOLYTIC FUNGI - DERMATOPHYTES

Dermatophytes are closely related fungi belonging to the imperfect genera *Microsporum*, *Trichophyton* and *Epidermophyton*. Both pathogenic and non pathogenic species of these genera typically have an affinity for keratin and can use this insoluble scleroprotein and hence keratinolytic. These fungi may exist as soil saprophytes (Geophilic) or as Zoopathogens (Zoophilic) some of which are exclusively pathogenic for man (Anthropophilic). Dermatophytes cause the diseases dermatophytoses (Margarita Silva - Hunter *et al*., 1981).
Anthropophilic dermatophytes

Anthropophilic dermatophytes are found in the genera Microsporum, Trichophyton and Epidermophyton. They infect humans almost exclusively. Infection in humans is attributed directly to close contact or indirectly to fomites (combs, brushes, chair backs, bed linens etc) or aerosols which may carry the infected scales and hair to others. M. audouinii, T. rubrum, T.tonsurans, T. mentagrophytes var. interdigitale and E. floccosum are examples of this category.

1.6 ZOOPhilIC DERMATOPHYTEs

These fungi of the genera Microsporum and Trichophyton are primarily animal parasites. Human infections are acquired directly by contact with the animal or indirectly by contact with infected hair, feathers or scales from the animal. Commonly recognised zoophilic fungi include M. canis, T.mentagrophytes var mentagrophytes and T. verrucosum.

1.7 GEOPHILIC DERMATOPHYTEs

This group includes members of the genera Microsporum and Trichophyton and a new species of Epidermophyton, E. stockdaleae. These fungi generally inhabit the soil and are often associated with keratinaceous material acting as an enrichment medium. Exposure to soil is the main source of infection for humans and animals although direct and indirect contact with infected humans and animals are also a mode of transmission. Members of
*M. gypseum* complex are representatives of geophilic dermatophytes (Margarita Silva - Hunter *et al*., 1981).

1.8 CLINICAL MANIFESTATIONS OF DERMATOPHYTIC INFECTIONS

The clinical manifestations of dermatophytic infections may range from mild to severe depending on the virulence of the infecting agent, the anatomical location of the lesion(s) and host factors such as age, sex and immune status. A single fungal species can infect many anatomical locations and produce various types of lesions. Conversely, different species can produce clinically identical lesions (Margarita Silva - Hunter *et al*., 1981).

These fungi can cause erythema and edema with inflammation resulting in scaling of the stratum corneum and vesiculation. Microscopically there is marked hyperkeratosis, parakeratosis, acanthosis and dilation of the vessels of the papillary layer with plasma and cellular infiltration resulting in interstitial edema (Norman Conant, 1958).

1.9 CLASSIFICATION OF DERMATOPHYSES

Traditionally the diseases caused by dermatophytes have been named according to their anatomical location.

Tinea capitis (scalp), tinea barbae (beard), tinea corporis (face and trunk), tinea axillaris (armpits), tinea cruris (groin), tinea pedis (feet), tinea manuum (hands) and tinea unguium (nails) (Margarita Silva - Hunter *et al*., 1981; Bhutani, 1993).
1.10 DIAGNOSIS

Diagnosis of dermatophytoses is by microscopic observations of the skin, hair and the nails treated in 10% KOH. Dermatophytes appear as branching fragments of hyphae. The genus and the species of the invading fungus can be determined only by culture studies (Norman conant, 1958, Margarita Silva-Hunter et al., 1981, Bhutani, 1993).

1.11 MOST PREVALENT DERMATOPHYTOSES

Among the dermatophytoses tinea corporis, tinea cruris and tinea pedis are the most prevalent dermatophytoses affecting the mankind throughout the world (Norman conant, 1958, Bhutani, 1993).

1.12 TINEA CORPORIS

This term usually refers to dermatophyte infections of the glabrous skin, excluding nails or intertriginous areas. The lesions of tinea corporis vary depending on the patient's age and or the causative fungus. Lesions of this sort are usually circinate i.e., circular with a raised active border.

Chronic lesions of the glabrous skin are more often found throughout the trunk and extremities. The primary lesion is a small red macule which spreads peripherally while healing at the centre. The lesions are well defined, papulo-vesicular or scaly at the active borders. The presence of the fungus can be demonstrated by microscopic examination of scrapings taken from the

1.13 TINEA CRURIS

Tinea cruris is popularly called as Dhobie-itch, and found commonly in adult males. Acute, subacute or chronic eruption due to fungal infection spreading peripherally along the innersides of the thigh, the scrotal fold, the gluteal cleft, and the genitals. Occasionally the axillary region is also attacked where it presents a picture similar to that seen in groin.

The infection is characterised by an elevated, papular, marginated erythematous and scaly patches with curved well defined borders studded with vesicles and vesico pustules. The disease is usually bilateral.

As a result of the warmth and moisture of the affected region, the scaly lesions may become eczematized and resemble intertriginous eczema. In the obese patients, where the affected areas may become macerated due to perspiration and friction. Secondary bacterial infection is also common.

On microscopic examination, the scales from the lesions will reveal the presence of the branching, septate hyphae (Herbert Mackey, 1968; Margarita Silva-Hunter et al., 1981; Bhutani, 1993).
1.14 TINEA PEDIS

Tinea pedis is popularly known as athlete’s foot. Fungal infection of the feet invading particularly the toe webs and soles. The disease is characterised by white, macerated and sodden appearance accompanied by scaling and fissures in the interdigital clefts. The cleft between the fourth and fifth toes of foot is the commonest site of infection. When the epidermis is rubbed off, a reddened area is exposed. Vesicles may form and the eruption may extend on to the dorsum of the feet and on to the soles, Hyperkeratotic patches, sometimes thickened, occur on the soles, heels and sides of the feet. Microscopic examination of scrapings from the lesions treated in 10% KOH will reveal the presence of the fungus. (Herbert Mackey, 1968; Margarita Silva-Hunter et al., 1981; Albert Kligman and James leyden, 1981; Bhutani, 1993).

1.15 DRUG THERAPY

Drugs currently used in the treatment of dermatophytoses are many with diversified chemical structures. Local application in the form of creams or gels or lotions or solutions or powders containing Potassium permanganate, Gentian violet, Diiodohydroxy quinoline with hydro cortisone, Calcium or Sodium propionate, Thymol iodide, Sulphur, Salicylic acid, Ammoniated mercury, Tolnaftate, Ciclopirox olamine or Haloprogin or Naftifine hydrochloride or Undecylinic acid and its salts or Whitefield ointment or Castellani’s paint, Benzimidazoles like clornidazole, clotrimazole, miconazole
and econazole either alone or in combination are prescribed for various lengths of time.

Oral antifungal agents like Terbinafine or Benzimidazoles like Ketoconazole or Fluconazole or Griseofulvin (Fig. 1.1) are also prescribed.

The treatment is continued for a couple of weeks after clinical subsidence. Recurrences are common (Herbert Mackey, 1968; Margarita Silva-Hunter, 1981; Bhutani, 1993).

Infections by fungi are often chronic and require prolonged treatment by antimycotic drugs that are expensive and sometimes non effective (Doctor’s desk reference, 1991).

Reports of low sensitivity and even resistance to the antimycotics such as Griseofulvin and Benzimidazoles by the dermatophytes and the consequent problems in current therapy are forthcoming (Cabafles et al., 1989). The process of discovering and developing new drug is continuous, expensive, risky and time consuming. Estimates for the cost of bringing a new drug molecule from the test tube to the market, today range, conservatively from 150 to 359 million U.S. dollars covering a period of 10 years (Rondel, 1991; Robert Borris, 1996). Thus drug development today involves phenomenal costs, which developing countries can ill afford.

The chemotherapy of fungal diseases has progressed far less than that of bacterial diseases. Inspite of intensive efforts by many pharmaceutical
FIG. 1.1
SOME IMPORTANT SYNTHETIC ANTIFUNGAL AGENTS
IN THE TREATMENT OF SUPERFICIAL MYCOSES.

CLORMIDAZOLE.

CLOTRIMAZOLE.
KETOCONAZOLE

FLUCONAZOLE
GRISEOFULVIN.
companies, progress beyond the major antifungal agents, griseofulvin, and the azoles has not yet been achieved. The main reason for the lack of new drugs against fungal infections is the eukaryotic nature of fungi making selective therapy a more difficult task (Prabhavathi, 1992).

1.16 IMPORTANCE OF PHYTOCHEMICALS IN ANTIMICROBIAL THERAPY

Plants are important sources of various pharmaceutical agents and useful pharmacological activity has been widely exploited in phytochemicals. Plants synthesise various antimicrobial phytochemicals as a self defense system against microbial infections.

Much attention is increasingly paid to these phytochemicals as these compounds are promising sources of therapeutic agents in antimicrobial therapy where the existing therapeutic agents fail or against which the microbes develop resistance (Hussain Qadri et al., 1995).

In addition to a reliable antimicrobial effect derived from their natural functions these natural products have fewer side effects and the microbes have less tendency to acquire resistance compared with conventional synthetic or semi synthetic antimicrobials (Munekazu et al., 1994). The World Health Organisation has long been aware of the vital role of herbal medicines in the health delivery systems and has taken initiative in strengthening its contribution to health care. Herbal medicines should be subjected to systematic and scientific evaluation, both in the preclinical and clinical
settings, with the use of modern methods of assessment of safety and efficacy (Menon and Nair, 1991).

There are many plants that have a potential role in the treatment of fungal infections, since *invitro* screening methods showed that some plants have activity against the most common fungal pathogens. Several studies are presently undertaken to find out these plants and to determine the best solvent system for the extraction of clinically effective antifungal compounds. These studies are also aimed at evaluating the chemical structures of the active principles, the pharmacological activity of the extracts and the possible application in experimental and clinical studies. Eventually when no toxicity in humans is demonstrated these plants could be used as an accessible and safe alternative to synthetic antimycotics (Caceres *et al.*, 1993).

1.17 THE PHARMACOGNOSY, PHYTOCHEMISTRY AND MORPHOLOGY OF CASSIA ALATA, LINN.

*Cassia alata*, Linn., is one such a plant used in the Indian system of medicine namely Ayurveda, siddha and unani. *Cassia alata*, Linn., belongs to the family Caesalpiniaceae, R.Br. and is distributed mainly in tropics and subtropics.


1.18 PROPERTIES AND USES

Leaves bruised into a paste with an equal weight of simple ointment or borax is a specific for ringworm and similar other skin afflictions. Leaves in decoction is considered as a cure for herpes and other skin diseases, even venereal affections and all poisonous insect bites, and also as a general tonic. Decoction of the leaves and flowers, is used as expectorant in bronchitis and dyspnoea and as astringent it is used as mouth-wash in stomatitis. Tincture of the dried leaves or an extract from the leaves acts as a purgative like that of senna or colocynth. Strong decoction of the leaves and flowers is a good wash for eczema. The drug is used in snake-bite also (Kirtikar and Basu, 1975).

_Cassia alata_, Linn., is a shrub, grows to a height of about eight feet. The leaves are 1-3 feet long in which fourteen to sixteen pairs of broad-oblong-obtuse leaflets are present. The leaflets are usually 2-7 inches long and the terminal two leaflets are always broader than the remaining leaflets. The flowers are golden yellow in colour - one inch across and are produced in large spike like raceme inflorescence. The buds are enclosed by yellow, short lived bracts. Stamens are unequal in size. Of the ten stamens present seven stamens are fertile and three posterior stamens are without anthers. The fruit is four to six inches long with four broad crenulate wings (Bailey, 1973) (Fig 1.2).
Rhein (Hauptmann et al., 1950) Aloe-emodine, β-sitosterol, kaempferol (Seshagiri Rao et al., 1975), Quinones and sterols (Mulchandani and Hassarajani, 1975), Chrysophanol and emodin (Villaroya and Maria, 1976) and anthraquinone glycosides (Harrison Jack and Garro Virginia, 1977) were isolated from the leaves of Cassia alata, Linn. (Fig 1.3). The constituents of the leaves have been investigated for their laxative (Rai, 1978) antibacterial (Fuzellier et al., 1981) antifungal (Fuzellier et al., invitro 1982) and antiinflammatory and analgesic effects (Palanichamy and Nagarajan, 1990a,b).

1.19 BACKGROUND INFORMATION OF DRUG THERAPY IN SUPERFICIAL MYCOSES AND SCOPE OF THE PRESENT INVESTIGATION

It is understood that the existing systems of medicine could not deliver the assured therapy as a result of which superficial mycotic patients are chronic sufferers.

India is perhaps the richest repository of traditional knowledge on the medicinal uses of plants. The country possesses an ancient system of health care based chiefly on medicinal plants and their therapeutic products have been used for 6000-7000 years (Mehrotra, 1996).

Indeed, the World Health Organisation is taking an official interest in indigenous systems of medicines, particularly plant medicines. This is because about 80 per cent of the world’s population use herbal medicines and by the proper development of such systems, the organisation’s aim of making health
FIG. I.3
PHYTOCHEMICALS ISOLATED FROM THE LEAVES OF CASSIA ALATA.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure" /></td>
<td>ANTHRAQUINONE</td>
</tr>
<tr>
<td><img src="image2" alt="Structure" /></td>
<td>RHEIN (1,8-DIHYDROXY ANTHRAQUINONE-3-CARBOXYLIC ACID)</td>
</tr>
<tr>
<td><img src="image3" alt="Structure" /></td>
<td>EMODIN (1,6,8-TRIHYDROXY-3-METHYL ANTHRAQUINONE)</td>
</tr>
<tr>
<td><img src="image4" alt="Structure" /></td>
<td>ALOE-EMODIN (1,8-DIHYDROXY-3-HYDROXY METHYL ANTHRAQUINONE)</td>
</tr>
</tbody>
</table>
CHRYSOPEANOL
(1,8-DIHYDROXY-3-METHYL ANTHRAQUINONE)

PHYSICION
(1,8-DIHYDROXY-6-METHOXY-3-METHYL ANTHRAQUINONE)

SENNSIDE A COOH TRANS.
SENNSIDE B COOH MESO.
SENNSIDE C CH2OH TRANS
SENNSIDE D CH2OH MESO.

SITOSTEROL (R = Et)
care available to all by the year 2000 A.D. will be fulfilled (George Edward Trease and William Charles Evans, 1983).

Inspite of the rapid progress in medical field during the past 30 years, the progress of antifungal chemotherapy is far from satisfactory.

Oral Ketoconazole (Fig 1.1) widely used in pityriasis versicolor and dermatophytoses is now known to cause gynecomastia, presumably as a result of its non selective action on mammalian sterol biosynthesis. Other therapy related problems include the most common side effects of gastro intestinal distress (nausea, vomiting, abdominal pain) as well as headache and hepatotoxicity (Terrell and Hermans, 1987; Jan Faergemann, 1988; Bhutani, 1993; Kastrup and Boyd, 1980) and antithyroid activity (Francis comby et al., 1994). All the azoles (Fig 1.1) are fungistatic in their action which will eventually result in resistance (Alice Clark, 1992). Above all, azole are expensive (Bhutani, 1993).

Oral griseofulvin (Fig 1.1) prescribed for 4 to 8 weeks for dermatophytoses causes severe hepatotoxicity (Doctor's desk reference 1991).

Inspite of these antifungals in market and existence of medicinal plants in nature, the prevalence of superficial mycoses is high in developing countries (Chetty et al., 1979; Imwidhaya et al., 1989; Belec et al., 1991; Das et al., 1995). Superficial mycotic infections need long term therapy (Norman et al., 1971; Orlando canizares, 1975; Robert Berkow, 1982; Jan Faergemann, 1989; Doctor's desk reference, 1991; Bhutani, 1993). People in developing countries
can not afford either the expensive azoles or the Griseofulvin for long term therapy.

Taking into account of all the above facts, the present study is aimed to devise a simple, cheap and reliable method for the extraction of the clinically effective antifungal principle(s) from the leaves of *Cassia alata*, Linn., its mode of application and to critically evaluate the clinical efficacy and safety in the treatment of superficial mycoses of humans. Such a study is the first of its kind and has not been carried out so far.