CHAPTER I

INTRODUCTION

The fungal infections are very common in Thailand (Pruksaraj, 1978) since the country is located in tropical area where the hot and humid climate is very suitable for fungal growth.

Fungi

The fungi are eukaryotic organisms of the kingdom Fungi that usually grow in a filamentous, or yeastlike form or both (McGinnis, 1980 a, Chandler et al., 1980). There are about 100,000 species of fungi in the world but only about 175 species cause diseases in man (Rippon, 1982 a). Traditionally, the fungal diseases are divided into four broad categories :

Superfical mycoses : black piedra, tinea nigra, tinea versicolor and white piedra,

Cutaneous mycoses : dermatophytoses, cutaneous candidiasis, and cutaneous zygomycosis,

Subcutaneous mycoses : chromoblastomycosis, mycetomas, sporotrichosis and subcutaneous zygomycosis,

Systemic mycoses : systemic candidiasis, coccidioidomycosis and Histoplasmosis Duboisii (Chandler et al., 1980).

Among those pathogenic fungi dermatophytes, the keratinophilic fungi and *Candida albicans* are the commonest infectious agents in man and animal (Rippon, 1982 a, Rippon, 1982 b).

Dermatophytes and Dermatophytoses

Dermatophytes is a very similar and closely related group of fungi included 3 genera of moulds, Trichophyton, Microsporum and Epidermophyton. They are classified in the form class Hyphomycetes of the division Deuteromycota but their teleomorphic stages, Nannizzia (the teleomorph of Microsporum) and Arthroderma (the teleomorph of Trichophyton) are classified in class Plectomycetes of the division Ascomycota (Chandler, 1980).

Dermatophytoses is a colonization by a dermatophytic fungus of keratinized tissues, such as the nails, the hair and the stratum corneum of the skin. The disease is a consequence of the host's reaction to the metabolic products of the fungus rather than to the invasion of living tissue by the organism itself. The severity of the disease depends on the strain or species of the dermatophyte and the sensitivity of the host to that particular fungus, along with idiosyncrasies of the individual host.

Dermatophytoses includes several distinct clinical entities, depending on the anatomical site and the etiologic agent involved. The pathology induced in the host initially is an eczemaform response, followed by allergic and inflammatory manifestations. The lesions are creep in a circular or ring form and are called in various names depending on the location of lesion.

Disease	Location of lesion	Synonyms	Common Involved Organisms
Tinea Capitis	Scalp, eye brows, eye lashes	Ringworm of the scalp and hair, tinea tonsurans, herpes tonsurans	*Microsporum spp.* *Trichophyton spp.*
Tinea Favosa	Hair follicles	Farus, honeycomb ringworm	*Trichophyton schoenleinii* *T. violaceum* *M. gypseum*
Tinea Corporis	Glabrous skin	Ringworm of the body, tinea circinata, tinea glabrosa	*Trichophyton spp.* esp. *T. rubrum* and *T. mentagrophytes* *Microsporum spp.*
Tinea Imbricata	Glabrous skin	Tinea circinata tropical	*T. concentricum*
Tinea Cruris	Groin, perineum, and perianal region	Dhobie itch, Ringworm of the groin, eczema marginatum, jock itch, gym itch	*Epidermophyton floccosum* *T. rubrum* , *T. mentagrophytes* *M. gallinae*

Disease	Location of lesion	Synonyms	Common Involved Organisms
Tinea Unguium	Nail	Ringworm of the nail, dermatophytic onychomycosis	*T. rubrum* , *T. mentagrophytes*, *E. floccosum* , *T. tonsurans* , *T. violaceum* , *T. schoenleinii*, *T. concentricum* and all species
Tinea Barbae	Beard areas of the face and neck restricted to adult males	Tinea sycosis, barber's itch, ringworm of the beard	*T. mentagrophytes* *T. verrucosum*
Tinea Manuum	Hand (interdigitae areas and the palm or surfaces)	-	*T. rubrum* , *T. mentagrophytes*, *E. floccosum*
Tinea Pedis	Foot	Athlete's foot,Ringworm of the foot	*T. mentagrophytes* , *T. rubrum* , *E. floccosum*

Candida albicans and Candidiasis

Candida albicans is the yeast-like fungi in the form class Blastomycetes of the division Deuteromycota (Chandler, 1980, Rippon, 1982 b). It is the most virulent yeast and accounts for the vast majority of the diseases caused by the organisms. The clinical manifestations of *C. albicans* are protean. The diseases may be cutaneous, mucocutaneous, subcutaneous, or systemic, or it may involve all of the aforementioned anatomic areas. In addition to active infections, *C. albicans* is also involved in allergic conditions (Candidids, Eczema etc.) (Rippon, 1982 b). The infections caused by *C. albicans* is called candidiasis or candidosis. Normally, *C. albicans* does not cause diseases in man since it is a normal flora in the body but when the immune response of the host is decreased, such as in obese maturity-onset female diabetics, parenteral drug abusers or patient with long term steroid therapy or antibiotic therapy, *C. albicans* may infect the host and causes candidiasis.

Situation of Cutaneous Mycotic Diseases in Thailand

The National Statistical Office of Thailand carried out a survey in conjunction with the Institute of Dermatology in 1981. A finding was that in the whole country, the percentage of skin patients is 31.25% of the population of Thailand. Five common skin diseases found it the Institute of Dermatology in 1980 are : Eczema, infectious skin diseases, acne, pigmentation, and dermatitis (psoriasis) (Kotrajaras, et al., 1984). Among the infectious skin diseases, the fungal infections are the most common incidence.

The more recent data came from Kotrajaras et al. (1984). They reported the 22 common skin diseases (5,793 cases) from 10,500 cases in 21 hospitals in 18 provinces of the seven regions of Thailand, Bangkok,central, north, south, east, west and northeast region and found that the predominant five skin diseases in each region are different.

The data showed that tinea versicolor, tinea corporis, tinea cruris, tinea pedis, tinea manuum and tinea facei were found in the 2^{nd}, 5^{th}, 10^{th}, 17^{th}, 19^{th} and 20^{th} rank of those 22 common skin diseases. (see Appendix A) But after frequency analysis of the five predominant skin diseases in the seven regions of Thailand, the eleven common skin diseases are ranked as follow :-

1. Contact dermatitis
2. Lichen simplex chronicus
3. Tinea versicolor
4. Tinea corporis
5. Acne vulgaris
6. Urticaria, acute
7. Contact dermatitis, irritation
8. Prurigo simplex
9. Tinea cruris
10. Seborrheic dermatitis
11. Melasma

It is interesting that mycocutaneous diseases such as tinea versicolor and dermatophytoses are twice common in male more than in female and most of the dermatomycoses occur in patients of age 15-24 years .

From such study, the incidence of all of the fungal infections are 17.048% (1,790 cases) of all skin diseases (10,500 cases). The highest incidence is tinea versicolor,5.048% (534 cases), followed by tinea corporis,3.592% (380 cases), tinea cruris,2.562% (271 cases), tinea pedis,1.012% (107 cases), tinea manuum,0.983% (104 cases), tinea facei,0.813% (86 cases), candidiasis,1.533% (161 cases) and other fungal infections,1.505% (147 cases).

Antifungal agents

The treatment of fungal infections is considerably more difficult than in the treatment of bacterial infections (Finch and Snyder, 1982). In the preantibiotic era, about the only treatment for systemic mycoses was supportive therapy for patient. Cinical cures can now be obtained for most of these mycotic diseases. Cutaneous infections were treated with various combinations of keratolytic and antimicrobial agents, such as Castellani's paint and Whitfield's ointment. Modifications of these preparations are still useful today in dermatologic practice (Gray, 1984). However, a variety of new antifungal drugs are now useful for treatment of these infections.

Antifungal drugs are now divided into two groups.

A. Nonsystemic or topical antifungal drugs

B. Systemic antifungal drugs

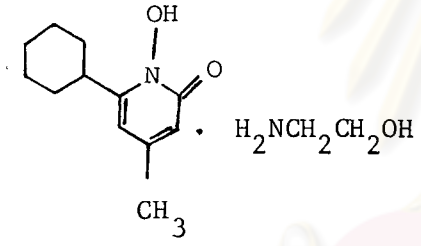
(Harvey, 1985).

A. Nonsystemic or Topical Antifungal Drugs are used to cure superficial and cutaneous fungal infections only, such as

dermatophytoses, candidal onychomycoses, etc. Drugs exert fungistatic and fungicidal actions by variety of mechanisms (Harvey, 1985). Some of their beneficial effects probably depend upon factors not related to any direct effect on fungi, such as keratolytic effect, astringent effect, etc.

1. Benzoic Acid and Salicylic Acid (or Whitfield's ointment) It combines the fungistatic action of benzoate with the keratolytic action of salicylate. It is used mainly in the treatment of tinea pedis.

2. Ciclopirox Olamine(Loprox®) is fungicidal to *C. albicans* and dermatophytes. It is available as a 1% cream for the treatment of cutaneous candidiasis and dermatomycoses with the cure rates of 81-94%. No topical toxicity has been noted (Harvey,1985) .

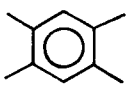


3. Propionic and Caprylic Acids They are fungistatic to dermatophytes and Candida. They may be used as sodium and calcium salt.

4. Undecylenic Acid or Undecanoic Acid (Desenex®) It is primarily fungistatic, although fungicidal activity may be observed with long exposure to high concentrations of drug. It inhibits respiration, carbohydrate metabolism, phosphate uptake and phospholipid metabolism of the fungi(Das and Banejaree, 1982). The preparations are useful in the treatment of various dermatomycoses, especially tinea pedis with the criteria cure rates of 50% (Harvey,

1985). It is used in both acid and the zinc or calcium salt form.

5. Haloprogin(Halotex®) is a halogenated phenolic ether. It is fungicidal to dermatophytes and Candida. During treatment of this drug, irritation, pruritus, burning sensations, vesiculization, increased maceration, and "sensitization" occasionally occur. The systemic toxicity from topical application appears to be low (Harvey,1985). Its principal use is against tinea pedis, for which the cure rate is about 80%. It is also used in tinea cruris, tinea corporis, tinea manuum, and tinea versicolor (Harvey, 1985), but not in tinea capitis and tinea unguium (Finch and Snyder, 1982).

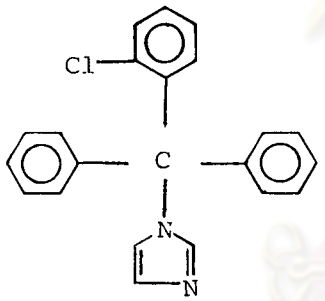


6. Imidazoles There are a number of related imidazoles that have broad-spectrum antifungal activity in vitro and which are effective topically against nearly all of the fungi of clinical interest. In addition, they are active against certain bacteria and protozoa. Four of the six imidazoles currently available are used topically in the treatment of the superficial mycoses, Clotrimazole, Econazole, Miconazole and Isoconazole. All the members of the group has essentially the same spectrum of activity, although there are differences in relative activities against specific microorganisms. They are fungicidal if the concentration is sufficiently high.

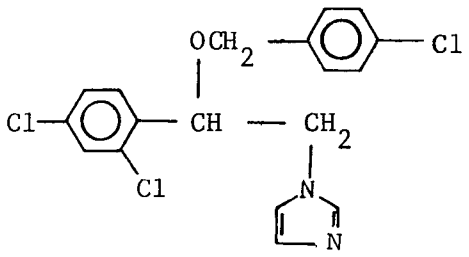
By electronmicroscopy, according to most investigators in this field, the primary site of interaction of the imidazole derivatives

is in the cytoplasmic membrane, whereas Cope (1980) claims that it is the cell wall, but Bastide et al. (1982) stated that clotrimazole interacts with the cytoplasmic membrane but only when put in direct contact with spheroplasts, and that the other imidazole derivatives tested (ketoconazole, miconazole, isoconazole and econazole) interact mainly with the yeast cell wall. Clotrimazole caused the formation of numerous circular opening in the cytoplasmic membrane: Ketoconazole, miconazole, isoconazole and econazole cause convolutions and wrinkles in the cell wall(Bastide et al., 1982).

6.1 Clotrimazole (Canesten®, Lotrimin®) has been reported to cure dermatophytoses in 60-100% of cases and the cure rates in cutaneous candidiasis are 80-100%. It is used primarily for topical treatment of vulvovaginal candidiasis with the cure rates as high as above 90%. The 10-mg troche has been used successfully for the treatment of oral candidiasis and for prophylaxis of oral candidiasis in certain immunocompromised hosts, recipients of renal transplant, and patients with solid tumors (Sande and Mandell, 1985). The drug is available as a 1% cream, lotion or solution, 1% vaginal cream or 100-mg vaginal tablets (Gyne-Lotrimin®, Mycelex-G®), and 10-mg troches (Mycelex®) (Sande and Mandell, 1985).



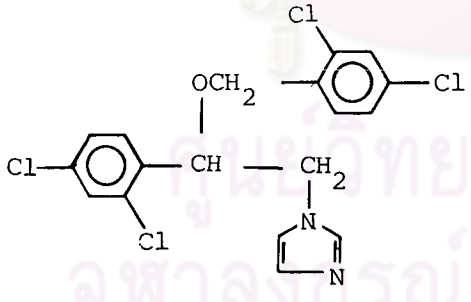
6.2 Econazole (Pevaryl®, Spectazole®) is the deschloro derivative of miconazole. It has been variously reported on the one hand to be less active than miconazole against the yeasts and on the other hand to be the most active of topical imidazoles. It is two to eight times more active than miconazole to filamentous



Econazole

fungi. Econazole is especially active against the mycelial forms. The drug is available as a water-miscible cream (1%). It is approved for the treatment of tinea pedis, tinea cruris, tinea corporis, tinea versicolor, and cutaneous candidiasis, Econozole has been used successfully in the treatment of various mycoses (mostly candidiasis) of the ear, nose, and throat. It is also effective in oculomycosis. In the treatment of vaginal candidiasis, it appears to be slightly less effective than or equieffective with clotrimazole.

6.3 Miconazole nitrate (Daktârin®) Miconazole is a very close chemical congener of econazole. The cure rates are



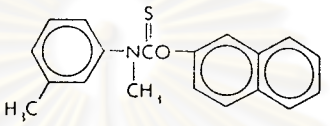
Miconazole nitrate

almost identical to that of clotrimazole (Harvey, 1985). It is about as effective as tolnaftate for the treatment of dermatophytoses and more effective than topical nystatin for cutaneous candidiasis. It is also very effective in vaginal in fections caused by *C. albicans* (Rippon, 1982 c).

Miconazole can be used systemically too. De Nollin and Borgers (1974) stated that miconazole exerts its effect primarily on the plasmalemma and the cell wall.

6.4 Isoconazole (Travogen®) is a recently introduced topical antifungal drug. It is available as cream for topical application and a vaginal suppositories for vulvovaginal candidiasis.

7. Tolnaftate (Ezon-T®, Tinaderm®) is a thiocarbamate with the following structure :

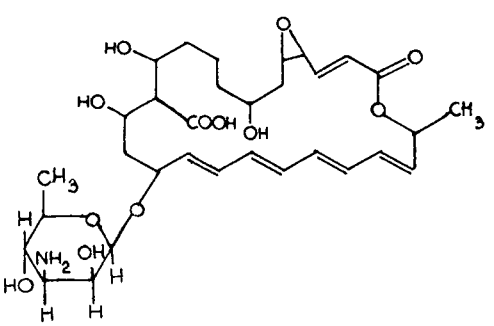


Tolnaftate

It is one of the first synthetic chemical agents to have topical antifungal activity (Rippon, 1982 c). It is effective in the tratment of dermatophytoses but not candidiasis (Harvey, 1985; Martinadale, The Extrapharma-copocia, 1982; Rippon, 1982 c)。 Its mechanism of action is unknown (Finch and Snyder, 1982).

8. Polyenes The basic structure of all of them is a macrolide ring containing a number of conjugated double bonds The drugs in this group available as topical antifungal drugs are Pimaricin and Candicidin (Finch and Snyde, 1982; Rippon, 1982 c).

8.1 Pimaricin or Natamycin is produced from *Streptomyces natalensis*. It is the drug of choice in mycotic keratitis.



Pimaricin

8.2 Candicidin NF (Candeptin®) is produced from *Streptomyces griseus*. It is used in vaginal candidiasis.

9. Miscellaneous Topical Antifungal Agents :

- Acrisorcin
- Carbol-fuchsin
- Sulfur
- Selenium sulfide
- Aminacrine hydrochloride

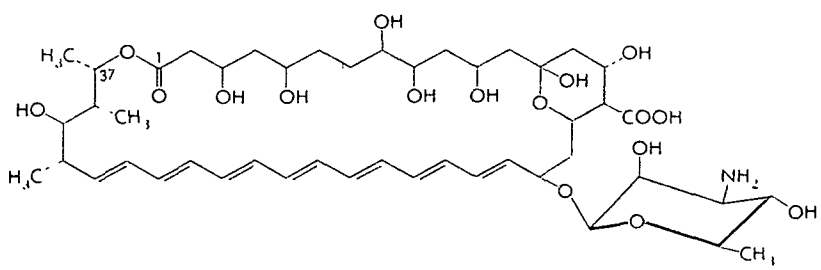
etc.

(Harvey, 1985).

B. Systemic Antifungal Agents. are the drugs used to treat systemic mycoses or the orally administered antifungal drugs to treat cutaneous or subcutaneous mycoses.

1. Polyenes

1.1 Amphotericin B (Fungilin®, Fungizone®) is a polyene antibiotic from *Streptomyces nodosus*, a soil Actinomycete. It is either fungistatic or fungicidal depending on the concentration of the drug and the sensitivity of the fungus. Its fungicidal activity

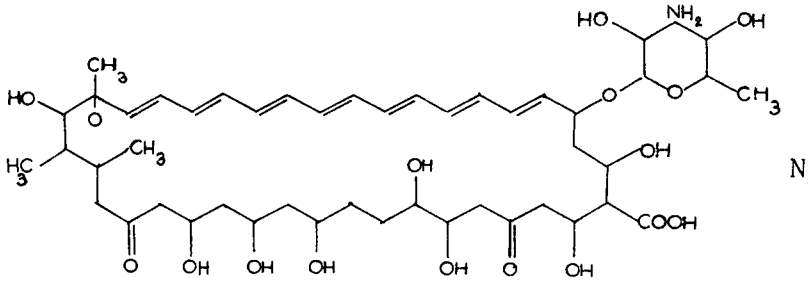


Amphotericin B

is at least in part dependent on its binding to a sterol moiety, primarily ergosterol, present in the membrane of sensitive fungi causing leakage of potassium and other metabolites (Sande and Mandell, 1985; Gray, 1984: Rippon, 1982 c; Bastide et al., 1982). However, effects of amphotericin B on the permeability of sterol free membranes indicate that additional mechanism may also be involved. Recent data indicate that low concentrations of amphotericin B have paradoxical stimulatory effects on cell proliferation, including that of fungal cells and animal cells in culture (Sande and Mandell, 1985).

Amphotericin B is effective against deep mycoses (Sande and Mandell, 1985; Rippon, 1982 c, Finch and Shyder, 1982; Laurence and Bennett, 1980), but its usefulness is limited by toxicity because it binds to the surface sterols of human erythrocytes too (Rippon , 1982 c,Gray, 1984). All patients requiring amphotericin B must be hospitalized, at least for the initiation of therapy and they must be under close observation throughout a course of systemic administration of the drug because of its hematopoietic side effects. The drug is availabe as lyophilized powder for injection. (Sande and Mandell, 1985).

1.2 Nystatin ((Mycostatin®, Fungicidin®) is a polyene antibiotic from *Streptomyces noursei*. It is both fungistatic and fungicidal (Sande and Mandell, 1985). It is used in the topical



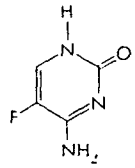
Nystatin

treatment of cutaneous and mucocutaneous infections especially those caused by *Candida spp.* eg. thrush, intestinal, esophageal or vaginal candidiasis.

It is occasionally used in keratitis and otomycosis of other origin but not in deep mycoses or dermatophytoses (Rippon,1982 c). The mechanism of action is similar to that of amphotericin B.

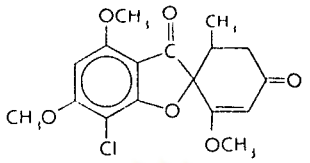
Nystatin is available as oral tablets, oral suspension vaginal suppositories and aerosol. It can be used topically or administered orally.

2. Flucytosine (5-fluorocytosine)(Ancobon®) Flucytosine a fluorinated pyrimidine, is a fungistatic (Finch and Snyder, 1982). It is converted in fungal cells to Fluorouracil by the enzyme cytosine deaminase. Fluorouracil is then metabolized to 5-fluorodeoxyuridylic acid, an inhibitor of thymidylate synthetase. Synthesis of 5-fluorouridine triphosphate and RNA that contains this analog may also take place. Mammalian cells do not convert large amounts of flucytosine to fluorouracil. This is crucial for the selective action of this compound (Sande and Mandell, 1985). It is used predominantly in combination with amphotericin B (Sande and Mandell, 1985). It is now considered the treatment of choice in chromoblastomycosis and cryptococcal meningitis (Rippon,1982 c). It is less toxic than amphotericin B and it can be administered orally (Sande and Mandell, 1985; Rippon,1982 c; Finch and Snyder, 1982; Gray, 1984). The drug is available as oral tablets and capsules



Flucytosine

3. Griseofulvin (Fulcin®, Grisovin®) is an antibiotic from *Penicillium griseofulvum* and a number of *Penicillium spp.*



Griseofulvin

(Rippon,1982 c). It is fungistatic in vitro for various species of the dermatophytes (Sande and Mandell, 1985, Rippon,1982 c; Finch and Snyder, 1982), but has no effect on other fungi, yeasts, actinomycete or nocardia (Sande and Mandell, 1985). Young, actively metabolizing cells may be killed by the drug, but older, more dormant elements or static organisms are only inhibited (Sande and Mandell, 1985; Gray, 1984).

A prominent morphological manifestation of the action of griseofulvin is the production of multinucleated cells as the drug inhibits fungal mitosis (Sande and Mandell 1985), by binding to the fungal microtubules (Finch and Snydex, 1982). It causes abnormal multiple branching of the fungus and interferes with protein and nucllic acid synthesis. It is bound to lipids within the cell but not to RNA or DNA. DNA metabolism is enhanced, and there is a greater content of this substance in drug treated cells than in normal cells (Rippon, 1982 c).

Grisefulvin is an effective chemotherapeutic agent in dermatophytoses but not in deep mycoses. (Sande and Mandell, 1985; Gery, 1984; Finch and Snyder, 1982; Rippon, 1982 c). It may be applied topically but is of questionable efficacy (Sande and Mandell, 1985). It is administered orally. A preparation of fine particles (micronized and ultramicronized) of the drug and fatty meals

facilitates absorption, from the GI tract (Sande and Mandell, 1985 ; Gray, 1984; Finch and Snyder, 1982).

Griseofulvin is deposited in keratin precursor cells and persist in keratin and makes this substance resistant to fungal invasion. Only small fraction of a dose of the drug is present in body fluids and tissues. (Sande and Mandell, 1985; Finch and Snyder, 1982).

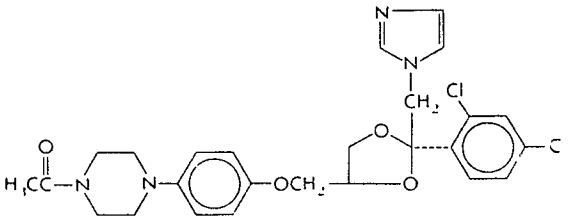
Usually there are no serious side effects to therapy (Rippon, 1982;Gray, 1984) but very high doses of griseofulvin are carcinogenic and teratogenic in laboratory animals, so the drug should not be used to treat trivial infections that response to topical therapy (Sande and Mandell, 1985; Laurence and Bennett, 1980). It is available as an oral tablets.

4. Hydroxystilbamidine Isothionate an aromatic diamidine, is active against fungi and protozoa (Sande and Mandell, 1985; Rippon, 1982 c) . It produces favorable results in cutaneous and pulmonary human North American blastomycosis, however, the incidence of relapse has been high (Sande and Mandell, 1985).

5. Imidazoles There are two of them used systemically, Miconazole and Ketoconazole. Testing in vitro has shown these agents to be highty active against a broad spectrum of fungi, including dermatophytes, yeasts,dimorphic fungi, eumycetes, etc. However, activity is greatly dependent upon factors such as culture medium, pH, and the size of the inoculum. The data are thus difficult to interprete, and there are no firm indications for performance of sensitivity testing in vitro (Sande and Mandell, 1985).

The drugs alter membrane permeability of the fungi and they also block ergosterol biosynthesis by inhibiting the demethylation of lanosterol, a precursor of ergosterol. Both miconazole and ketoconazole have profound effects on the cytochrome p-450 of yeasts which may explain many of the effects of those drugs including the inhibition of lanosterol demethylase (Sande and Mandell, 1985).

5.1 Ketoconazole (Nizoral®), administered orally, has broad therapcutic potential for the treatment of a number of superficial and systemic fungal infections. The drug is particularly useful in the treatment of histoplasmosis involving the lungs, bones and joints or skin or soft tissues as well as the progressive disseminated disease. It can be used in both systemic and cutaneous mycoses with the exception of cryptococcal meningitis. (Sande and Mandell, 1985). Ketoconazole has several limitations. It is administered orally, and absorption may be erratic. The response to treatment is typically slow, and this agent is thus less useful in acute, severe fungal infections; it is desirable for chronic suppressive therapy. Testing of the susceptibility of isolates <u>in vitro</u> is often not useful for predicting the clinical response (Medical letter, 1984). It should not be used in combination with amphotericin B. The drug is available as an oral tables.



Ketoconazole

5.2 Miconazole (Monistat® IV)

Miconazole is used primarily as a topical agent (described before). It is also used parenterally for the treatment of systemic fungal infections (Sande and Mandell, 1985), Finch and Snyder, 1982). However, because of its toxicity and limited efficary, indications for such use are few. (Sande and Mandell, 1985).

Many imidazole and triazole derivatives are now understudy, such as bifonazole, butoconazole, fenticonazole itraconazole, oxiconazole, sulconazole, terconazole, tioconazole and vibunazole, etc. (Sande and Mandell, 1985 ; Rippon, 1982 c).

6. Potassium Iodide (KI) is an inorganic, water soluble salt. Its mode of action is unknown but may involve iodination of proteins in the fungus cell wall and membrane. KI is used only for the treatment of cutaneous lymphatic forms of infections caused by *S. schenckii*. It is readily absorbed from the intestinal tract. It is well tolerated and available as a saturated solution (1mg/ml) for the oral treatment of cutaneous lymphatic sporotrichosis (Finch and Snyder, 1982).

A great deal of research is presently underway to develop more effective and less toxic antifungal agents. The properties that a new antimycotic drug requires in order to represent a real break through in the combat against systemic and cutaneous mycoses are

1. broad-spectrum activity
2. easy mode of administration

3. wide spread tissue distribution
4. low degree of inactivation
5. good patient tolerance

and 6. low toxicity profile (Borger et al., 1983).

TEA SEED CAKE

Tea seed cake [Ch'a-tzū-ping (Shiu-ying Hu, 1980), Tae kow] was obtained from seeds of *Camellia oleifera* Abel [*C. drupifera* lour., *C. oleosa* (Lour.) Rehd; *Thea oleifera* (Abel) Rehd. & Wils.] and *C. sasanqua* (Perry, 1980) of the family Camelliaceae after extracting the tea seed oil (Ch'a yu). These species of camellia is wide spread in China and Indochina (Perry, 1980).

In China, the tea seed cake is used as a toxic agent to insect larvae and deters their development, it is employed in some places as a fish poison and has a fertilizing properties too (Perry, 1980). It is also used as a shampoo and an antifungal agent for tinea unguium (Thongvichai, 1983).

Lohakhajornpan (1979) reported that ethanolic extract and water extract of tea seed cake had good antifungal activity against *Aspergillus niger*.

Laohapaibul and Tosukhovong (1981) reported that tea seed cake extract had antifungal activity against *Trichophyton mentagrophytes*, *T. tonsurans*, *Microsporum gypseum*, *M. audouinii*, *M. canis* and *Epidermophyton floccosum*, and Thongvichai (1983) reported that it had the antifungal activity against *Saccharomyces cerevisiae* too.

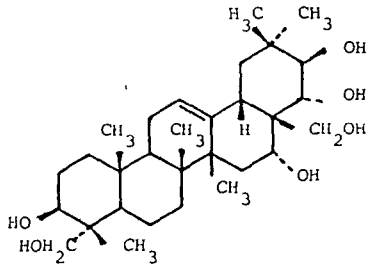
Thongvichai (1983) stated that the antifungal activity of tea seed cake extract retained at a wide range of pH, acid to mild alkali and was found to increase at the pH of human skin. Its activity was abolished in strong alkali, particularly at the pH above 11.6. She also developed the antifungal preperations of tea seed cake.

Yoshioka et al. (1967) found that the sapogenin constituents of *C. sasanqua* seeds are sapogenols, barringtogenols, terpene sapogenols, steroid sapogenols and theasapogenols (A,B,C,D). Other components of *C. sasanqua* seeds are sasanqua saponin, sasanquaprosapogenin A (Yamada et al., 1970 a), A_1 barrigenol or theafolisaponin (Ogino et al.,1968), primeverosides, xylopyranosyl, sasanquin (Yamada et al., 1967) and eugenol (Yamada et al.,1967 and Angel' Skii, 1974).

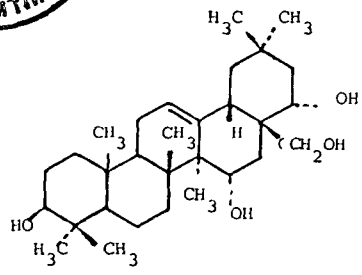
Sokol Skii et al. (1975) reported that, there are triterpenoid glycosides in *Camellia oleifera* and *Camellia sasanqua* and Tosukhovong (1985) (unpublished) found that there are steroid moiety and glucose molecules in tea seed cake extract (*C. oleifera*).

The literature surveys show that the major constituents of the tea seed and tea seed cake are "Saponins"

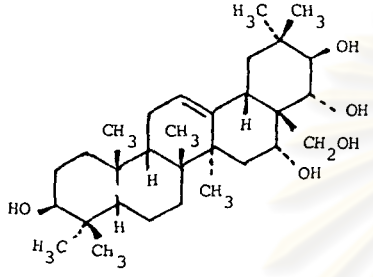
Sapoins are a kind of glycosides, the plant chemicals consisted of genin or aglycone part and sugar molecules or glycone. The most important characteristic of saponins are bubble forming property (detergent property) and hemolytic property.



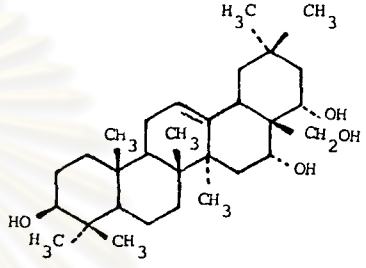
Theasapogenol A



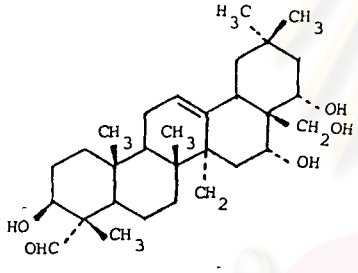
A_1-Barrigenol



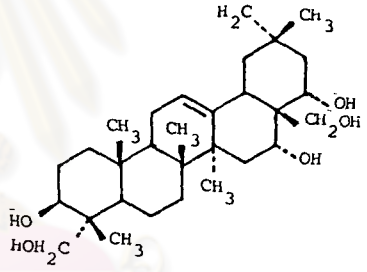
Barringtogenol C
(Theasapogenol B)



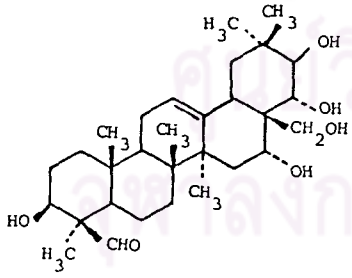
Camelliagenin A
(Camellia sapogenol I)
(Theasapogenol D)



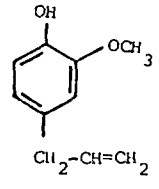
Camelliagenin B
(Camellia sapogenol II)



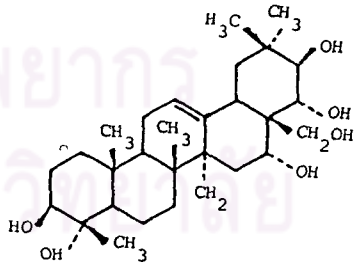
Camelliagenin C
(Theasapogenol C)



Camelliagenin D



Eugenol



Camelliagenin E
(Theasapogenol E)

Figure 1.1 Chemical structures of constituents of *Camella oleifera* and *C. sasanqua* seed (Ruangrungsi , 1986).

There are some saponins that have antimicrobial and antifungal activity (Wongratanasathit, 1980). Although they have hemolytic property, there are no harm of oral intake of saponin in Mammal and Aves, because the saponin form cannot be absorbed from the GI tract but it is first hydrolysed by gastric juice to sapogenin which has no hemolytic activity before being absorbed. Toxic effect, however, is observed if it is administered in the body by injection.

Saponins also have gum paralytic activity in fishes so they are used as a fish poison (Wongratanasathit, 1980). Large amount of saponin can cause GI irritation and vomitting and in addition it also irritates nasal mucosa.

There are two kinds of saponins, depending on glycone parts :

"Steroidal saponin" which has sterol structure with spiroketal side chain and "Triterpenoid saponin".

Although the cutaneous and mucocutaneous mycoses are not the virulent diseases that cause the patient death, but they are bothersome and very common in Thailand especially in young and working people. The treatment is usually a long term and considerably expensive. In addition, the symptom persists and is mostly very difficult to treat. The data of the fungal infections obtained from the Institute of Dermatology of Thailand showed only the number of patients who came to register in 21 hospitals during a certain period, where the actual number of those with fungal infections would be certainly far exceed the total number shown in such reports.

Some of them went to the private clinics, some did not tried any treatment since they could tolerate such annoyance, some had self-medication by taking antifungal drugs from drug stores, and some went to see traditional doctors. Furthermore, the incidence of some of the diseases was higher in some conditions, such as tinea pedis was more common in the flood area and all of the fungal diseases are observed more frequently in the low hygienic people. In addition, the diseases relapsed frequently especially when the last treatment was not complete.

According to the statistics from the National Statistical Office of Thailand and the Institute of Dermatology of Thailand, 31.25% of the population got skin diseases and 17.048% of those skin diseases are fungal infections. If there are 51 million people in Thailand, there will be approximately at least 2,717,025 patients who acquired cutaneous fungal diseases.

The costs of treatment of fungal diseases are very high because most of the antifungal agents, except Whitfield's ointment, are very expensive and it took a minimum of 2 weeks to treat such the diseases completely. Almost all of the antifungal agents were imported. The Technical Division, Food and Drug Administration of Thailand have been reported in the last complete data that in 1982, the value of the antifungal agents imported (both raw materials and finished products) was 86,905,253 baht; of this amount 24,364,326 baht was for systemic antifungal agents and 62,540,927 baht was for nonsystemic antifungal agents. The recent data of the antifungal drugs imported (only finished products) from January to September of 1985 was 24,860,836 baht (Department of Custom, 1985).

These showed that we paid a lot of money for the antifungal drugs from the foreign countries. If we can explore the medicinal plants in our own country that can be used in place of those antifungal drugs, it would be beneficial to the Thai people and to Thai economics.

Tea seed cake is one of the medicinal plants that may be used as an antifungal agent. Few studies on tea seed cake as an antifungal agent have been published, but there is no study about its mode of action or even the determination of the minimal inhibitory concentration (MIC) and the minimal fungicidal concentration (MFC) of this agent.

The purpose of this study is to evaluate the antifungal action of the tea seed cake extract by :

1. To determine whether its antifungal activity is fungistatic or fungicidal, and to determine its MIC and MFC.

2. To study the effect of tea seed cake extract on morphological changes of the tested organisms by light microscopy and electron microscopy.

3. To study the biochemical changes (macromolecular synthesis) in the tested organisms when treated with tea seed cake extract.

The results obtained would clarify how and the site by which this agent exerts its antifungal effect on the fungi in order that we can further use the tea seed cake extract as an antifungal drug. With the hope that tea seed cake extract or clinically active ingredients of tea seed cake could be used in place of the antifungal drugs imported from abroad in the future.