

Anti-Dermatophyte Activity of *Hedychium spicatum* Oil and Its Topical Formulation-Cream

Kavita Salkar*, Chetna Chotalia, Sachin Kundalwal, Rajiv Salvi

Phytomedicines Department, Piramal Enterprises Limited, Andheri, Mumbai, India

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*Corresponding author

Kavita Salkar

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Abstract: A skin infection due to Dermatophytes is the most prevalent skin disorder even in the todays world. Plant based products are being looked upon as an alternative to synthetic drug treatments due to the various side-effects reported for the later. *Hedychium spicatum* oil was tested for its Anti-dermatophyte potential and MIC in the range of 30 to 40 mcg/ml was obtained for it. Further a 5% cream developed using this oil also showed a potent activity of 0.5 mg/ml (MIC) against the dermatophyte species. The new Anti-dermatophyte formulation can be considered as an alternative option for fungal skin infections.

Keywords: Dermatophytes, *Hedychium spicatum*, MIC, Anti-dermatophyte

INTRODUCTION

Dermatophyte is a group of three types of fungus that commonly causes skin disease in animals and humans. These anamorphic (asexual or imperfect) genera are: *Microsporium*, *Epidermophyton* and *Trichophyton* [1].

Dermatophytes cause infections of the skin, hair and nails due to their ability to obtain nutrients from keratinized material. The organisms colonize the keratin tissues and inflammation is caused by host response to metabolic by-products. They are usually restricted to the nonliving cornified layer of the epidermis because of their inability to penetrate viable tissue of an immunocompetent host. Some of these infections are known as ringworm or tinea. Toe- and fingernail infection are referred to as onychomycosis [1-3].

In recent years the number of infections caused by these fungi has increased considerably and *Trichophyton rubrum* has been the most common dermatophyte since the fifties of last century, accounting for 80-90% of the strains. Dermatophytes are the most common agents of superficial fungal infections worldwide and widespread in the developing countries, especially in the tropical and subtropical countries like India, where the environmental temperature and relative humidity are high. Other factors such as increased urbanization including the use of occlusive footwear and tight fashioned clothes, has been linked to higher prevalence [1-3].

Current therapy generally uses steroids along with anti fungals to control the infection as well as inflammation. The most common therapy involves the use of drugs of the group azoles- Ketoconazole, Itraconazole, Fluconazole and Voriconazole, Allylamines like Terbinafine or others like Griseofulvin [3].

The major problem faced during the treatment with these agents is the resistance developed by the fungi and some adverse effects like local irritation,

allergic reactions exfoliative skin disorders, severe cutaneous reactions etc. [3-5]. Hence, there is a need to treat dermatophyte infection by natural phytopharmaceutical, which only possess a promising anti-dermatophyte potential but control inflammation and prevents the decolouration of skin.

Hedychium spicatum, commonly known as *shathi* or *kapurkachri*, is traditionally used as a stomachic, carminative, tonic and stimulant. It is considered useful in dyspepsia and diarrhoea when given in the form of a powder or decoction and is also said to promote hair growth. The rootstock is useful in liver complaints, as an expectorant in urinary disorders and for pains and inflammation. The roots are also useful in asthma and bronchitis. Paste of *Hedychium spicatum* is also used for wound healing and skin diseases [6-9].

It was conceived that the formulation from *Hedychium spicatum* could be useful in dermatophyte infections. The Ayurvedic line of treatment is to detoxify the body, balance Vata dosha and use rejuvenating herbs to repair the damage and restore strength and functional mobility to the area.

“*Hedychium spicatum*” has disease-modifying properties. Hence, it could be effective in the treatment of Dermatophyte infections after evaluation in the preliminary screening. In our approach to explore the anti-dermatophyte potential of this plant, we had initiated studies to evaluate the oils prepared from rhizomes and their active constituents for antidermatophyte activity by various microbial assays.

MATERIALS AND METHODS

Sample

Hedychium spicatum oil (solvent extracted) and Hespiderm cream comprising of 5% *Hedychium spicatum* oil was developed by us (described elsewhere).

In vitro susceptibility testing (Agar dilution method)

Organisms

Trichophyton mentagrophytes, *Trichophyton rubrum*, *Trichophyton violaceum*, *Trichophyton ajolai*, and *Microsporum gypseum* strains were used as test organisms.

Inoculum preparation

The cultures were grown on Potato Dextrose agar slants at 28°C for 4 days. The obtained growth was scraped using sterile saline and transferred to a sterile test tube. The heavy particles were allowed to settle and upper homogenous suspension was transferred to another tube. This suspension was adjusted to an optical density that ranged from 80 to 82% transmittance using a spectrophotometer at 530 nm which yields a stock solution of 1×10^6 to 5×10^6 cfu/ml. These stock suspensions were further diluted 1:10 with saline to obtain test inoculum (1×10^3 to 5×10^3 cfu/ml).

Sample preparation

Hedychium oil: A 1 mg/ml stock solution of oil was prepared in methanol. Calculated amount of stock solution was added to 20 ml of Sabarouds melted agar medium & poured into petri plates so as to get a series of serial two fold dilution of the oil in the medium. The final concentrations of oil in the plate medium ranged from 100 to 5 mcg/ml.

Hespiderm cream: A 10 mg/ml stock solution of cream was prepared in DMW+0.01% Tween 20. Calculated amount of stock solution was added to 20 ml of Sabarouds melted agar medium & poured into petri plates so as to get a series of serial two fold dilution of the cream in the medium. The final concentrations of cream in the plate medium ranged from 1 to 0.1 mg/ml. A solvent control plate and growth control plate without any oil or formulation was included in the study.

Assay

The culture suspensions prepared by above method were spotted in 10µl amount on the solidified plates. Spots were allowed to dry at room temperature & then the plates were incubated at 28°C/48 – 72 hours.

Endpoint criteria

The MIC was defined as the lowest concentration of oil or formulation giving no visible growth or causing almost complete inhibition of growth in the plates.

RESULTS AND DISCUSSIONS

Table-1: Anti-dermatophyte activity of *Hedychium spicatum* oil

Sr.No.	Dermatophytes tested	MIC (µg/ml) ^a				
		Hedychium oil				Std.
		Batch I	Batch II	Batch III	Batch IV	
1	<i>Trichophyton mentagrophytes</i>	40	40	40	40	5
2	<i>Trichophyton mentagrophytes-1*</i>	40	40	40	40	5
3	<i>Trichophyton rubrum-1*</i>	40	39	38	38	5
4	<i>Trichophyton rubrum-2*</i>	40	42	38	40	4
5	<i>Trichophyton ajolai*</i>	40	42	44	42	4
6	<i>Trichophyton violaceum*</i>	38	40	40	36	3
7	<i>Microsporum gypseum-1</i>	40	40	40	40	5
8	<i>Microsporum gypseum-2*</i>	40	40	38	38	4

Note: * clinical isolates, std.-Ketoconazole

^a Results are representative of at least two independent experiments, each performed in triplicate.

Table-2: Anti-dermatophyte activity of Hespiderm cream

Sr. No.	Dermatophytes tested	5% Hespiderm cream	Std. Nizoral (2% Ketoconazole) cream
1	<i>Trichophyton mentagrophytes</i>	0.5	0.5
2	<i>Trichophyton mentagrophytes-1*</i>	0.5	0.5
3	<i>Trichophyton rubrum-1*</i>	0.5	0.5
4	<i>Trichophyton rubrum-2*</i>	0.5	0.4
5	<i>Trichophyton ajolai*</i>	0.6	0.4
6	<i>Trichophyton violaceum*</i>	0.5	0.3
7	<i>Microsporum gypseum-1</i>	0.6	0.6
8	<i>Microsporum gypseum-2*</i>	0.6	0.6

Note: * clinical isolates

^aResults are representative of at least two independent experiments, each performed in triplicate.

A topical cream was developed using natural oil obtained from the plant *Hedychium spicatum*. The plant *Hedychium* is known for its skin benefitting and antimicrobial properties since ancient times. The solvent extracted oil of *Hedychium spicatum* showed potent anti-dermatophyte activity as tested in the lab models. The oil was tested against a panel of dermatophyte species many of which were clinical isolate samples. The oil showed MIC value in the range of 30 to 40 mcg/ml which can be considered as a potent activity.

A cream developed using this oil was also tested for anti-dermatophyte activity. The formulation retained the activity and MIC value of 0.5 mg/ml was observed for the formulation against the tested species of dermatophytes. The MIC value of Hespiderm cream was comparable with the marketed sample of Ketoconazole cream.

We have used *Trichophyton* species in our study, it is the common causative agent for most of the superficial tinea infections. *Microsporum* is the second most encountered species. *Hedychium* oil and Hespiderm cream was active against both these species of dermatophyte infection group.

Some plants are being studied for their Anti-dermatophyte potential. AA. Andrade *et al* have reported anti-dermatophyte activity of *Piper aduncum* leaves extracts with MIC value of 310 mcg/ml against *Trichophyton* species [11]. Biasi-Garbin RP *et al* have also reported Brazilian plants with anti-dermatophyte activity [13, 14]. Some Indian medicinal plants have also been reported for anti-dermatophyte activity by J. Vajjayanthimala *et al* and Kavitha Sagar *et al* in two different studies [15,16]. However in all these studies the MIC obtained by the researchers was in mg/ml, none of these plants had shown MIC in the mcg/ml range. In such case *Hedychium* oil proves to be highly potent Anti-dermatophyte oil as we have obtained MIC in the range of 30 to 40 mcg/ml.

Topical therapy is used for most dermatophyte infections. Treatment requires attention to exacerbating factors such as skin moisture and choosing an appropriate antifungal agent. Azole formulations are the most commonly used treatment for dermatophyte infections. However with extensive use of these agents, resistance to the treatment is increasing highly. Secondly many allergic reactions have also been reported with the use of these antifungals. Severe hypersensitivity reactions to these drugs can occur and systemic allergic reaction to fluconazole have also been documented, specifically, causing clinicians to be very cautious in prescribing another azole as an antifungal agent [10- 12].

In such scenarios antimicrobial potentials of plants can be used as an alternative option to synthetic drugs. Essential oils from plants can be considered as the most promising group among the other chemical groups isolated from plant species to be used in formulations.

Thus *Hedychium spicatum* oil based Hespiderm cream has potential as a new anti-infective agent for dermatophyte infections in humans.

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