



## Research Article

### ANTIFUNGAL POTENTIAL OF *ROSMARINUS OFFICINALIS* AGAINST *MICROSPORUM GYPSEUM* AND *TRICHOPHYTON RUBRUM*

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#### ABSTRACT

Dermatophytoses is a serious contagious fungal infection mainly infests skin; nail and hair caused by dermatophytes and is threat to human health. In recent years, the infections caused by these fungi have been increasing at an alarming rate. We have undertaken this research since no scientific data and valid proofs were available regarding the antifungal potential of the hydroalcoholic extract of the *Rosmarinus officinalis*. In this study, hydroalcoholic extract of *Rosmarinus officinalis* was investigated for antifungal activity against *Trichophyton rubrum* and *Microsporum gypseum*. The antifungal activity was determined using the agar well diffusion method. The diameter of the clear zone of inhibition surrounding the well was measured. The hydroalcoholic extract of *Rosmarinus officinalis* had antifungal activity against all the test organisms. It can be concluded from results that hydroalcoholic extract of whole plant of *Rosmarinus officinalis* may be considered as a potent antifungal agent and can be used as a potent source for possible treatment of fungal infections.

**Key words:** *Rosmarinus officinalis*, hydroalcoholic, *Trichophyton rubrum*, *Microsporum gypseum*.

#### INTRODUCTION

Dermatophytes are considered as class of fungi which mainly cause superficial infections, called dermatophytoses, by invading the keratinized tissues like hair, skin and nails in human and animals. Dermatophytes consist of three genera including *Microsporum*, *Trichophyton* and *Epidermophyton*. In recent decades, infections caused by these fungi have been developing at an alarming rate which is a considerably important in patients with immune deficiency and chronic diseases. The treatment of dermatophyte is a combination of topical and systematic treatments. The most preferred treatments of dermatophytoses for human are Terbinafine and Griseofulvin. However, recent studies have attempted to find natural antifungal compounds with lesser complications to the common treatments due to their multiple side effects, high costs, low efficiency and high drug resistance properties<sup>1</sup>. *Trichophyton rubrum* is a dermatophytic fungus in the phylum Ascomycota, class Eucaryota. It is an exclusively clonal, anthropophilic saprotroph that mainly colonizes in the upper layers of dead skin, and is one of main reason of athlete's foot, fungal infection of nail, jock itch, and ringworm. It is also a reason for Tinea Endothrix (hair), Tinea Corporis (body) and Tinea Unguium (nails). The species is keratinophilic and is known for causing infections and many skin diseases<sup>2</sup>. The genus *Microsporum* contains a number of pathogens to both humans and animals<sup>2</sup>. The disease it causes is classified as tinea or ringworm, with an adjective prescribing to the afflicted body part. Infection usually occurs as tinea capitis with suppurative and kerion formation<sup>3</sup>. Recent research says that natural plants and their herbal extracts are being utilized for the treatment of variety of infectious diseases mainly caused by bacteria, fungi, protozoa and viruses. So, nowadays importance has been given to the application of natural herbal compounds against dermatophytoses causing

organisms<sup>4,5</sup>. Rosemary is a perennial, aromatic and beautiful plant with fragrant evergreen leaves; the leaves and flowering branches of rosemary are used for medicinal applications. It is an herb native to Mediterranean Sea which is currently being cultivated all over the world. Rosemary contains phenolic compounds (Carnosol, Rosmarinic acid and Caffeic acid), Flavonoids (Diosmin and Luteolin) and Menotropins (Camphor, Cineol and Borneol) <sup>6</sup>. Researches related to the antifungal properties of this plant are minimal in the Indian scenario. Therefore, the current research work intends to screen hydroalcoholic extract *Rosmarinus officinalis* for antifungal activity.

#### LITERATURE REVIEW

A brief literature survey was done before starting this research, which is summarized in table 1.

#### MATERIALS AND METHODS

In the present study, *Rosmarinus officinalis* plant with easy accessibility have been used to evaluate their antifungal potential against the keratinophilic fungus *Trichophyton rubrum* and *Microsporum gypseum*. *Rosmarinus officinalis* was obtained from local areas of Chandigarh, identified and herbarium sheets with voucher no CCP/RO-2018 are submitted at Pharmacognosy Department, Chandigarh College of Pharmacy-Landran (Mohali). Fungal strain *Trichophyton rubrum* with MTCC no. 3272, *Microsporum gypseum* with MTCC no. 2829 were procured from IMTECH Chandigarh. To carry out extraction, initially plant material was washed; shade dried and was powdered with the help of grinder. 10 grams of powdered drug was kept for maceration for 48 hours with 60 ml water and 40 ml ethanol (hydroalcoholic extract 60:40). The crude material was then filtered through

double layered muslin cloth and filter paper. The filtrate thus obtained was used for evaluation of antifungal studies.

**Evaluation of anti fungal activity**

The antifungal activity of hydroalcoholic extract of *Rosmarinus officinalis* on mycelial growth was evaluated in, in-vitro condition on Sabouraud Glucose Agar medium. The medium supplemented with desired concentrations of plant extracts was poured in petriplates. These petriplates were inoculated with 5mm diameter mycelial disc taken from the margins of 8-10 day old colony raised on Sabouraud Glucose Agar medium. Sabouraud Glucose Agar medium without plant extract served as control. Three replicates of each concentration were maintained. The inoculated plates were incubated at 28±20C for seven days. The diameter of the colony was recorded on 3rd, 5th and 7th days<sup>7</sup>.

**RESULTS**

Hydroalcoholic extract of *Rosmarinus officinalis* at two different concentrations (5% and 10%) were evaluated for their antifungal properties against *Trichophyton rubrum* and *Microsporum gypseum*. The results were recorded on 3rd, 5th and 7th days and are presented in the Tables (2-4). Table -2 gives an account of the

effect of plant extracts on the growth of *Trichophyton rubrum* and *Microsporum gypseum* recorded on 3rd day. It was recorded that maximum fungal growth inhibition was noticed with the hydroalcoholic extract of *Rosmarinus officinalis* and no fungal growth was recorded at 10% concentration and a minimum growth was recorded at 5% concentration. It was seen that, the inhibitory effect became more noticeable with the increase in concentration of the hydroalcoholic extract. The effect of hydroalcoholic extract on the growth of *Trichophyton rubrum* and *Microsporum gypseum* was recorded on 5<sup>th</sup> day and is shown in the Table -3. As per results recorded, maximum fungal growth inhibition was achieved on 3<sup>rd</sup> day, since a minimum amount of fungal growth was observed at 5% and 10% concentrations. It has been observed that effect of the hydroalcoholic extract of *Rosmarinus officinalis* on the growth *Trichophyton rubrum* and *Microsporum gypseum* were more or less similar with a little difference. Table -4 gives an account of the effect of hydroalcoholic extract of *Rosmarinus officinalis* on the growth of *Trichophyton rubrum* and *Microsporum gypseum* which was recorded on 7th day. The results were quite similar to those which were recorded on 3rd and 5th days. The effect of hydroalcoholic extract *Rosmarinus officinalis* was quite promising and can be used to formulate an antifungal formulation in future.

**Table 1: Literature survey of *Rosmarinus officinalis***

Plant	Part of Plant	Extract	Results
Rosemary <i>Rosmarinus officinalis</i> (Lamiaceae)	Leaves	Essential oil	ZOI ranging from (10 to 27 mm). The most active effect observed against <i>Trichophyton interdigitale</i> 27 mm <sup>8</sup> .
Rosemary <i>Rosmarinus officinalis</i> (Lamiaceae)	Leaves	Essential oil	<i>Bacillus cereus</i> , <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> , <i>Microsporum canis</i> showed the most susceptibility with 70 mm <sup>9</sup> .
Rosemary <i>Rosmarinus officinalis</i> (Lamiaceae)	Leaves	Essential oil	MIC against <i>Trichophyton simii</i> , <i>Trichophyton interdigitale</i> , <i>T. tonsurans</i> , <i>T. violaceum</i> , <i>T. Floccosum</i> , <i>Mentagrophytes</i> with 60% was 20mg/ml <sup>10</sup> .
Rosemary <i>Rosmarinus officinalis</i> (Lamiaceae)	Leaves	Ethanol	Against <i>Pseudomonas aeruginosa</i> , <i>Bacillus cereus</i> , <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> . Minimum inhibitory concentration from 6.25 mg/ml to 100 mg/ml <sup>11</sup> .
Rosemary <i>Rosmarinus officinalis</i> (Lamiaceae)	Leaves	n-butanol, chloroform, ethanol, water	Hydro-alcoholic extract, N-hexane fraction of decantation, and Chloroform %100 fraction of SPE had anti-dermatophyte index; however, their potency was not comparable with Terbinafine <sup>12</sup> .

**Table 2: Effect of 5% and 10% plant extracts on the growth of strains in cm (3rd day)**

S No	Fungal Strains	Hydroalcoholic Extract-5%	Hydroalcoholic Extract-10%
1	<i>Microsporum gypseum</i>	0.64	0.30
2	<i>Trichophyton rubrum</i>	0.81	0.52
3	Control	2.16	2.16

**Table 3: Effect of 5% and 10% plant extracts on the growth of strains in cm (5th day)**

S No	Fungal Strains	Hydroalcoholic Extract-5%	Hydroalcoholic Extract-10%
1	<i>Microsporum gypseum</i>	1.10	0.91
2	<i>Trichophyton rubrum</i>	1.27	1.01
3	Control	3.21	3.21

**Table 4: Effect of 5% and 10% plant extracts on the growth of strains in cm (7th day)**

S No	Fungal Strains	Hydroalcoholic Extract-5%	Hydroalcoholic Extract-10%
1	<i>Microsporum gypseum</i>	1.04	0.78
2	<i>Trichophyton rubrum</i>	1.39	0.94
3	Control	2.46	2.46

**DISCUSSION**

The present study revealed that hydroalcoholic extract of *Rosmarinus officinalis* was found to be effective almost completely checking the mycelial growth at 10% concentration showing 86.09% inhibition. The results revealed that the hydroalcoholic extract of *Rosmarinus officinalis* were inhibitory to the mycelial growth. As the concentration of extract increased

in the medium, maximum growth inhibition of the test fungus was recorded. From this it can be clearly seen that herbal plants are untouched vital and beneficial reservoirs of various valuable phytochemicals with different pharmacological actions. These antifungal, antipathogenic and antimicrobial phytochemicals can be obtained from various medicinal and traditional plants belonging to different families, genera and species. These beneficial phytochemicals are widely distributed throughout the

herbal plant or may be in certain parts of a plant or also in its special tissues (like volatile oil in vittae). So, at the end of this research, we can conclude that wide range of plants is still untouched and unexplored for their antimicrobial and antifungal activity. It needs to be demonstrated that the phytochemicals obtained from medicinal plants with antifungal potential may be effectively and beneficially exploited in the control of keratinophilic infections like tinea and onychomycosis.

## CONCLUSION

Hydroalcoholic extract of *Rosmarinus officinalis* shown great potential as antifungal herbal plant against *Trichophyton rubrum* and *Microsporum gypseum*. Further studies can be performed on isolation studies by isolating bioactive compounds and evaluate their extracts as antimicrobial agents and alternatives to synthetic antibiotics which generally produce resistance and adverse effects in patients with multi drug therapy.

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