



**COMPARATIVE ANTIDERMATOPHYTIC ACTIVITY EFFECTS OF SOME AMINO
ACIDS, TERBINAFIN AND *MYRTUS COMMUNIS* ON *TRICHOPHYTON
MENTAGROPHYTES* IN GUINEA PIG ANIMAL MODEL**

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ABSTRACT

Fungal infections in developing countries are a major problem. During recent years, increasing side effects for syntactic drugs have been motivated by more researchers for finding new compounds of the plant with antifungal activity. In this study, we compared effect of asparagine, methionin and tryptophan amino acid with terbinafin drug and *Myrtus Communis* on antidermatophytic activity by *Trichophyton mentagrophytes* in guinea pig animal model. 30 healthy male guinea pigs weighing 350-500 g and about 2 moth years old were used and for Cutaneous inoculate guinea pigs and make experimental disease, first the compound of *Trichophyton mentagrophytes* was prepared. Then, an area (dorsal surface of animal) of 3×3 cm square, shaved. So that determines the skin of animal. Then, scrape skin with a sterile scalpel in different directions. Scratched area was inoculated using a sterile spatula dipped in the pomade and finally the place with sterile gauze dressing. Dressing for treatment of patients with asparagines, methionine, tryptophan amino acid, terbinafine drug and *Myrtus Communis* in 2 steps daily for 23 days was carried out. Colony diameters were measured daily, and finally the data collected according to the normal distribution, all results were analyzed using one-way ANOVA (p<0.05). Result has shown that the diameter of colony in different concentration of

asparagines, methionine and tryptophan decreased in experimental fungi than control group ($p < 0.05$). Therapeutic effect of *Myrtus Communis* on *Trichophyton mentagrophytes* was concentration dependent. While *Myrtus Communis* in the concentration of 1.5, 1 and 0.75 mg/ml have effective and concentrations of *Myrtus Communis* in comparison with the control group was significantly reported ($p < 0.05$) and in the effect of terbinafine drug concentration on *Trichophyton mentagrophytes* was shown that this drug is used in concentrations ranging .005-.066 $\mu\text{g/ml}$ effectively inhibited the growth of *Trichophyton mentagrophytes* and the inhibition at all concentrations of the drug in comparison with the control group was significantly reported ($p < 0.05$).

Keywords: Antidermatophytic activity, Amino acids, *Trichophyton mentagrophytes*, *Myrtus Communis*, Terbinafin

INTRODUCTION

Dermatophytosis is a common contagious disease caused by fungi known as dermatophytes. Dermatophytes belong to a group of organisms that are able to break down the keratin in tissues such as the epidermis, hair, nails, feathers, horns and hooves. Most of these fungi reside in the soil and are involved in decomposition; however, the dermatophytes can infect living hosts. Several chemical factors such as hormones, fatty acids and amino acids in skin can be effective in dermatophytes growth. It is clear that human is continuously contact with dermatophytes, therefore, fortunately low amount show disease signs. For example, leg ringworm is one of the most prevalent types of tinea [1]. Some dermatophytes (anthropophilic species) are adapted to humans, and are usually transmitted from

person to person. Others (Zoophilic species) are adapted to animals. A few (Geophilic) species normally live in the environment, but occasionally act as parasites. The zoophilic and geophilic species are sometimes transmitted from animals to people. It is also possible for humans to transmit anthropophilic dermatophytes to animals, although this seems to be uncommon.

Dermatophytosis is caused by pathogenic, keratin-digesting fungi in the genera *Microsporum*, *Trichophyton* and *Epidermophyton*.

The genus *Trichophyton* contains a number of important species that are the principle causative agents of animal and human dermatophytoses [2]. Most rodents infected with *T. mentagrophytes* are asymptomatic or have few clinical signs. There may be areas of

partial or complete alopecia, erythema, scales, and crusts in symptomatic animals. In guinea pigs, the lesions tend to appear first on the face, and then spread to the back and limbs [3].

In recent years, a number of safe and highly effective antifungal agents have been introduced into clinical practice. Among them, terbinafine (TF), itraconazole (ITZ), fluconazole (FCZ), and more recently, voriconazole (VCZ) and the new triazole, still under clinical investigation, are probably the most promising. However, their activity against significant number of strains, representing a wide spectrum of dermatophyte species and following standard procedures, has not yet been investigated [4]. Recently terbinafine because of minimal side effects compared to other antifungal agents, as has been the drug of choice in the treatment of dermatophytosis [5, 6]. According to, increasing side effects for syntactic drugs have been motivated by more researchers for finding new compounds of the plant with antifungal activity. In surveys of scholars antifungal effects of a wide range of plants such as *Zataria multiflora*, *Myrtus Communis*, *Allium sativum*; Garlic, *Achillea clavennae* L., *Allium cepa*; Onion and *Juniperus phoenicea* on a diverse group of fungi has been reviewed and approved. *Myrtus*

Communis is a small genus belonging to the *Myrtaceae* family which includes approximately 100 genera and 3000 species growing in temperate, tropical and subtropical regions [7]. It is an evergreen shrub that grows to a height of about 1-5 meter. The oppositely arranged leaves are ovate-lanceolate, 2- 5 cm long, coriaceous, glabrous, punctuate-glandular and entire. When crushed, they have a delicate aromatic odor. White, star-like flowers, which have five petals, five sepals and a mass of tufted stamens, appear from June to September. The leaves of this plant are contained terpinolen, tannin, flavonoid and vitamine C [8].

According to studies by some researchers that amino acids have affected on lesions of dermatophytosis. The study that were done on *Microsporium gypseum* and *Trichophyton mentagrophytes* in India has shown that cysteine hydrochloride amino acids and aspartic acid have inhibitory effect and minimal inhibitory concentration of cysteine hydrochloride for *Microsporium gypseum* is 0.5 gr/dl and for *Trichophyton mentagrophytes* is 0.4 gr/dl were reported. Also, Acid aspartic with 1gr/dl concentration decreased the growth of *Microsporium gypseum* to 100 percent and growth of *Trichophyton mentagrophytes* to 48 percent [9]. So in current study we compared effect of

asparagine, methionin and tryptophan amino acid with terbinafin drug and *Myrtus Communis* on antidermatophytic activity by *Trichophyton mentagrophytes* in guinea pig animal model.

MATERIAL AND METHODS

In this study, 30 healthy male guinea pigs weighing 350-500 g and about 2 moth years old were used. Guinea pigs were controlled exactly that if there is any plaque or hair loss caused by bites or external factors are removed. Guinea pig independently in a cage thoroughly cleaned and disinfected at temperatures of 27-25 °C were deployed. The experimental animals were randomly divided into 4 groups such as: group 1 (Observed amino acid effect) that divided to tree subgroup, group 2 (Observed terbinafin effect), group 3 (Observed *Myrtus Communis* effect) and group 4 (Control group). Guinea pigs used of plate food as well as lettuce, carrots, spinach, and vitamin C were fed as a dietary supplement.

Trichophyton mentagrophytes provided from fungus collections and industrial and infectious bacterial dependent on Iran scientific and industrial researches organization. Then, a uniform suspension of *Trichophyton mentagrophytes* 14-day medium cultures grown on sabouraud dextrose agar produced by germany merck factory, using

sterile saline containing, 0.5 Percent Tween 80 was obtained. In order to eliminate excess particles and fragments of hyphae, the resulting suspension was passed through several sterile layers. Thus, fungal spores and isolation were collected [4]. Suspension containing spores collected in sterile tubes and the number of spores per ml was counted using neobar slide and finally concentration of 10³ cfu /ml to transfer a certain amount of suspension of the fungal culture on sabouraud dextrose agar and colony counts were made.

Amino acids that used in this study were asparagine, methionine and tryptophan produced by germany merck factory with 4 concentrations such as 0.1, 0.4, 0.7 and 1 percent. For the preparation of terbinafine solution, One mg of terbinafine powder (Novartiz) dissolved in one ml dimethyl sulfide (DMSO) and was maintained for 30 min in Laboratory temperature. Drug solution divided in 100 µl sterile vials and until use at - 70 ° C was maintained. For the preparation of *Myrtus Communis*, The plant materials were washed with water and 2% sodium hypochlorite for 30 minutes was disinfected, then to remove residual hypochlorite, washed with sterile distilled water and dried in the shade and the dried plant material was powdered. 50 g of dried powder was soaked in 500 ml of methanol and 48 hours was

shaken by shaker. Then by two layers of sterile linen filtered after that centrifuged for 10 min at 9000 rpm and filtered whatman paper number 41 again. The extract of Plant filled in separate vials in concentrations of 0.5, 0.75, 1 and 1.5 mg/ml.

For inoculate guinea pigs and make experimental disease, the compound was prepared. First of all *Trichophyton mentagrophytes* Cultured on sabouraud dextrose agar after that from 2 to 3 weeks of medium culture, pomade was prepared. About 10 to 15 sq cm of medium culture with fungal colonies grown beside the flame was cut with sterile forceps and mixed with 20 ml of honey in sterile Porcelain mortar. Medium with *Trichophyton mentagrophytes* Cultured in honey and under biological hood were mixed. So, uniform pomade makes.

In order to inoculation guinea pigs, firstly an area (dorsal surface of animal) of 3×3 cm square, shaved. So that determines the skin of animal. Then, scrape skin with a sterile scalpel in different directions. Scratched area was inoculated using a sterile spatula dipped in the pomade and finally the place with sterile gauze dressing. After ensuring that the guinea pigs infected to *Trichophyton mentagrophytes*, infected animals were classified in separate cages and then were treated. Dressing for treatment of patients

with asparagine, methionine, tryptophan amino acid, terbinafine drug and *Myrtus Communis* in 2 steps daily for 23 days was carried out. Colony diameters were measured daily, and finally the data collected according to the normal distribution, all results were analyzed using one-way ANOVA ($p < 0.05$).

RESULTS

Almost all guinea pigs after 10 to 14 days showed infection with *Trichophyton mentagrophytes*. Because the recovery times of amino acids, terbinafin and Myrtus Communis in this study were different, thus samples (guinea pigs) followed 23 days after infection with *Trichophyton mentagrophyte*.

In the effect of terbinafine drug concentration on *Trichophyton mentagrophytes* was shown that this drug is used in concentrations ranging .005-.066 $\mu\text{g/ml}$ effectively inhibited the growth of *Trichophyton mentagrophytes* and the inhibition at all concentrations of the drug in comparison with the control group was significantly reported ($p < 0.05$). Since the fastest treatment period related to terbinafin, so that in day 15 showed complete recovery (**Table 1**).

According to results, the lesions diameter in different concentrations of asparagine, methionin and tryptophan amino acids has significant decreased than control group ($p < 0.05$). In amino acids, all concentrations

rather than each other have significant differences while the minimum average is related to Concentration of 0.1% and maximum average is related to concentration of 1% (Table 2).

Therapeutic effect of *Myrtus Communis* on *Trichophyton mentagrophytes* was concentration dependent. While *Myrtus Communis* in the concentration of 1.5, 1 and 0.75 mg/ml have effective and concentrations

of *Myrtus Communis* in comparison with the control group was significantly reported(p<0.05), but in the concentration of 0.5 mg/ml Didn't show any significant effect(p<0.05). Maximum recovery time by *Myrtus Communis* was about day 20 (Table 3) and this indicates that *Myrtus Communis* in the long time effective on lesion by *Trichophyton mentagrophytes*.

Table1: Diameter of Experimental Lesion by *Trichophyton mentagrophytes* and Treatment Period in Concentrations Ranging .005-.066 µg/ml with Terbinafin

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Day
Diameter of lesion (mm)																							Treatment
30	30	29	29	28	24	20	18	15	10	7	5	3	2	*	*	*	*	*	*	*	*	*	Terbinafin

NOTE: *Healthy

Table2: Diameter of Experimental Lesion by *Trichophyton mentagrophytes* and Treatment Period in Different Concentrations of Asparagine, Methionin and Tryptophan Amino Acids

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Day
Diameter of lesion(mm)																							Treatment
30	30	30	29	27	27	25	20	20	20	19	18	18	18	16	16	16	15	15	15	14	13	13	Asparagin (1×10 ⁻¹)
30	30	30	29	26	26	25	21	21	19	18	18	17	16	16	16	15	15	15	14	13	13	12	Asparagin (4×10 ⁻¹)
30	30	30	28	25	24	24	22	22	19	19	18	17	17	15	15	15	14	14	13	12	12	12	Asparagin (7×10 ⁻¹)
30	30	29	28	25	24	23	22	21	21	19	17	16	16	14	14	14	13	13	13	12	12	12	Asparagin (1)
30	30	30	29	28	27	25	21	20	19	18	17	15	13	13	10	10	9	9	8	7	5	5	Methionin (1×10 ⁻¹)
30	30	30	29	27	27	24	21	20	20	19	19	18	16	14	13	11	9	7	5	4	4	3	Methionin (4×10 ⁻¹)
30	30	29	28	26	25	23	17	17	16	15	15	14	13	12	11	10	9	8	6	5	4	3	Methionin (7×10 ⁻¹)
30	30	29	27	26	24	20	17	16	15	14	13	13	12	12	11	9	9	7	7	5	4	2	Methionin (1)
30	30	30	29	27	27	26	21	19	19	18	17	17	15	15	14	11	10	8	8	7	6	4	Tryptophan (1×10 ⁻¹)
30	30	30	29	28	27	24	22	20	20	19	17	16	15	14	12	10	8	6	5	5	4	2	Tryptophan (4×10 ⁻¹)
30	30	30	28	27	26	23	19	17	15	15	13	12	11	11	10	9	7	7	6	4	3	2	Tryptophan (7×10 ⁻¹)
30	30	29	28	26	26	21	16	15	15	15	14	14	12	12	10	8	8	7	5	3	3	1	Tryptophan (1)

Table3: Diameter of Experimental Lesion by *Trichophyton mentagrophytes* and Treatment Period in Different Concentrations of *Myrtus Communis*

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Day
Diameter of lesion(mm)																							Treatment
30	30	30	30	30	30	30	29	29	28	28	28	28	28	27	27	27	27	27	27	27	27	27	<i>M.Communis</i> 0.5 mg/ml
30	30	29	29	27	25	23	22	19	17	15	11	9	6	6	5	3	2	2	*	*	*	*	<i>M.Communis</i> 0.75 mg/ml
30	30	29	27	25	23	18	16	13	12	12	10	9	7	5	3	2	2	*	*	*	*	*	<i>M.Communis</i> 1 mg/ml
30	30	29	26	24	21	17	15	12	9	8	6	5	5	4	4	2	2	*	*	*	*	*	<i>M.Communis</i> 1.5 mg/ml

*Healthy

DISCUSSION

The use of herbs to treat various diseases, such as cutaneous fungal disease has long been of interest to human review. Prevalence of Refractory dermatophytes infections in recent years has greatly increased. And that the choice of effective and appropriate treatment protocols for a specific period of time makes it unavoidable. Since dermatophytes as one of the most important causes of cutaneous fungal infections in humans and animals are raised [10, 11]. Side effects of antifungal compounds that are used to treat patients with dermatophytosis, researchers toward finding effective antifungal agents with minimal side effects have forced [12, 13]. Plants have long been considering the use of human attention and applications in the sciences, especially medicine, many plants have been observed. Use herbs such as *Zataria multiflora*, *Myrtus Communis*, *Allium sativum*; Garlic, *Achillea clavennae* L., *Allium cepa*; Onion and *Guniperus phoceani* Compounds as antimicrobial, anti-parasitic and anti-fungal

examined in several studies and has proven positive effects in this regard [14].

So, in the present study antidermatophytic activity effects of asparagine, tryptophan, methionine amino acids, terbinafine and *Myrtus Communis* on *Trichophyton mentagrophytes* were compared with each other. Because of the prevalence of dermatophytes infections in fairly high in Iran Therefore, studies to find effective antifungal agents with minimal side effects is important in improving patients with dermatophytosis.

In inoculated subcutaneously at guinea pigs only behind the animal was infected. Select the area due to the inability of the animal to lick or rub the lesion and the lack of contact with hands and feet, and also due to the visible surface to assess the infection. Arika *et al.*, Also in one research selected dorsal surface of male guinea pigs to study the effects of antifungal plant and have noted that the incidence of secondary bacterial infections in contact with the animal to lesion area due to Treatment period will be longer [15].

Achieve results have shown that asparagine also in concentration of 1 and 0.1 percent have inhibitory effects but were not causes complete growth inhibition even in concentration of 1 gr/dl. In current study the inhibitory effect of tryptophan on *Trichophyton mentagrophytes* were assessed and shown that concentration of 1% tryptophan causes maximum decrease in *Trichophyton mentagrophytes* growth. The colony diameter in different concentrations of tryptophan in experimental fungi than control group was decreased. This appears that tryptophan causes growth decreasing in *Trichophyton mentagrophytes*. Therefore tryptophan amino acid probably has inhibitory effect on growth of *Trichophyton mentagrophytes*. Comparison between differences concentrations of methionine were shown that increasing of methionine concentration causes decreasing *Trichophyton mentagrophytes* growth. The colony diameter in difference concentrations of methionine in experimental fungus than testifier group were decreased ($p < 0.05$). This appears that methionine causes growth decreasing in *Trichophyton mentagrophytes*. In one other study that was done by Garachorlou A. reveled that asparagin and methionine amino acids causes decrease in the *Trichophyton rubrum* and *Trichophyton verrucosum* growth

[16, 17]. Acidic amino acids also either was shown inhibitory effect on two dermatophytes that the acid aspartic inhibitory effects on *Microsporium gypseum* growth were determined in Pansy's study [9].

Therefore mentioned amino acid probably has inhibitory effect on growth of *Trichophyton mentagrophytes*.

Effects of antifungal agent terbinafine on *Trichophyton mentagrophytes* showed that the drug MIC range of .005-.066 $\mu\text{g/ml}$ and at concentrations upper than 0.165 $\mu\text{g/ml}$ completely inhibited the growth of *Trichophyton mentagrophytes*. Since the fastest treatment period related to terbinafin, so that in day 15 showed complete recovery. Many researchers in their studies have proven the effectiveness of terbinafine antidermatophytal [4, 18, 19, 20].

The results of current study showed that *Myrtus Communis* by concentration dependent are able to inhibit the growth of dermatophytes. Bonjar G.H et al., to investigate the antibacterial effects of *Myrtus Communis* were used of this plant and the positive effects of *Myrtus Communis* on micro organism were reported [21]. While *Myrtus Communis* in the concentration of 1.5, 1 and 0.75 mg/ml have effective and concentrations of *Myrtus Communis* in comparison with the control group was

significantly reported ($p < 0.05$) and in day 20 causes complete recovery.

In conclusion the result indicate that terbinafin in all concentrations have suppressive effect on *Trichophyton mentagrophytes* and in 2 weeks will make a full recovery. We observed concentration-dependent effects by *Myrtus Communis* on *Trichophyton mentagrophytes* and amino acids have inhibitory effect on growth of *Trichophyton mentagrophytes*.

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