Global Veterinaria 4 (2): 140-143, 2010
ISSN 1992-6197

Antifungal Effect of *Zataria multiflora*: An *In vitro* Evaluation

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Abstract: Due to the limitations of using food preservatives and antifungal drugs and due to the adverse effects associated with their use, research on antifungal compounds with fewer side effects seems necessary. In this study the antifungal effects of the essence of thyme (*Zataria muliflora*) was studied on five different saprophytes and dermatophytes. *Zataria multiflora* essence was extracted by the steam distillation technique with water using the clonger apparatus. Saboraud dextrose agar culture media was prepared with different concentrations of the essential oil and their effect on dermatophytes and saprophytes were studied by noting the mean diameter of fungal colony growth after seven and fourteen days. Data were analyzed using SPSS statistical software. Total (100%) fungal growth inhibition was brought about by *Zataria multiflora* essence concentration of >8% mg/ml for *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Epidermophyton floccosum* while a concentration of > 10% mg/ml totally prevented the growth of *Aspergillus fumigatus* and *Aspergillus flavus*. In conclusion the use of *Zataria multiflora* essence may be promoted as a suitable antifungal agent as well as an antiseptic and preservative in the food industry.

Key words: *Zataria multiflora* · *Trichophyton* · Essence

INTRODUCTION

The use of herbal medicine has widely increased during the past few decades and has been followed by a rapidly increasing demand throughout the world. The range of medicinal plants is very diverse and it has been estimated that around 70000 different plant species have been used at least once during the history of traditional medicine [1]. According to a WHO report, around four billion people (80% of the world's population) use herbal medicine [1] with eleven different bioclimatic regions and around 7500 different plant species. Iran is indeed an ideal bed for harvesting many valuable species of medicinal plants [1,2]. Regarding the limitations of using food preservatives and antifungal drugs and due to the side effects associated with their use, it seems necessary to find new antifungal compounds with minimum side effects. By using natural compounds present in plants, such as essential oils or essences, studying their antifungal effects and by identifying their effective agents, newer cheaper drugs with minimum side effects can be produced. *Zataria multiflora* is one of the plants which grow in the provinces of Fars, Isfahan, Lorestan, Kerman, Balouchestan, Khorasan and Yazd [3]. In this study the effects of *Zataria multiflora* essence was studied on five different fungi.

MATERIALS AND METHODS

In this experimental study, the process of preparing the plant essence was performed using the clonger apparatus by the steam distillation method with water. The essence derived from *Zataria multiflora* was colorless and soluble in ether, chloroform, alcohol and

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dimethyl sulfoxide (DMSO) [3]. The main components of this essence are Carvacrol, Thymol, Linalol and P-Cymene [4].

Preparation of Fungal Colonies: In order to prepare fresh fungal colonies, the fungi under study were primarily grown on carbodextrose agar culture medium (SDA) and subsequently incubated at 30°C for two weeks. The fresh cultures were then used to study the effects of essence.

Then, In order to obtain the desired dilutions of the Zataria multiflora essence,DMSO solvent was used to make dilutions of 1, 2, 3, 4, 6, 8, 0.1, 0.2, 0.5, 2.5, 5, 10,50,100 mg/ml in sterile Saboraud Dextrose Agar culture media. This medium was heated to 50°C in a water bath under absolute sterile conditions and then divided into several plates. Since this essence is soluble in DMSO and insoluble in water, the Saboraud Dextrose Agar culture medium was first prepared as above, autoclaved and then placed in a water bath at 50°C so that its solubility is not lost . Different dilutions of the essence were then prepared by pouring a specific volume of this mixture into similar sterile test tubes according to the volume of the plates. By calculating the amount of mixture required (essence+DMSO+SDA culture media), different dilutions of the essence were prepared and brought to equal volumes by adding as much SDA culture medium as required. The test tubes were filled by first adding the calculated amount of DMSO, then the calculated amount of essence and finally the SDA culture medium at 50°C, which is fluid in consistency.

The test tube components were uniformly mixed with vortex and subsequently transferred to sterile plates near the Bunsen flame. SDA solidifies in the plate. Each plate had a specific concentration of the essence. Clotrimazole was used as a control drug, Saboraud Dextrose Agar culture medium with DMSO as a soluble control medium without drug and Saboraud Dextrose Agar culture medium with DMSO or essence as control medium.

Preparation of the Dilutions and Culture Media: Overall in this study more than 500 culture media plates and 650 types of fungi were studied.

Test Method: Using a sterile puncher, 6mm diameter circles were cut from the fresh fungal colony plaques and subsequently transferred to the culture media using a sterile scoop-headed needle in the vicinity of a flame. In order to control the accuracy of the tests, two plaques were grown in each plate from the three series. The plates were incubated at 30°C for two weeks. The diameters of the colonies were measured after seven and fourteen days and the mean diameter of the colonies was considered as organism growth. The degree of colony growth inhibition was estimated by comparison with the mean diameters of the control colonies and reported as the percentage of growth inhibition according to the following formula:

Percentage of growth inhibition=Colony diameter in medium with essence-colony diameter in control colony×100 6-colony diameter in control colony Data derived by t-test in similar and independent groups were analyzed using SPSS,V. 13.

RESULTS

In case of *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Epidermophyton floccosum*, growth was totally (100%) inhibited at essence concentration > 8% mg/ml (Table 1). As seen in Table 1, mean fungal (*Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Epidermophyton floccosum*) growth inhibition reached maximum value (100%) at a concentration of 8% ($p<0.01$). with regard to saprophytes (*Aspergillus fumigatus* and *Aspergillus flavus*) mean of fungal growth inhibition reached maximum value (100%) at a concentration of 10% ($p<0.05$) (Table 2).

Table 1: Mean and 95% confidence interval of fungal growth inhibition of Trichophyton mentagrophytes, Epidermophyton floccosum and *Trichophyton* rubrum *(Dermatophyt)* based on essence concentrations in the first and second weeks

		First week		Second week	
Fungi	Essence Concentration (mg/ml)	Mean (SD)	95% CI	Mean(SD)	95%CI
Trichophyton mentagrophytes	0.08	100 (0)	100	100(0)	100
Epidermophyton floccosum	0.08	100(0)	100	100(0)	100
Trichophyton rubrum	0.08	100(0)	100	100(0)	100

Table 2: Mean and 95% confidence interval of fungal growth inhibition of *Aspergillus fumigatus* and *Aspergillus flavus* (Saprophytes) based on essence concentrations in the first and second weeks

		First week		Second week	
Fungi	Essence Concentration (mg/ml)	Mean (SD)	95% CI	Mean(SD)	95% CI
Aspergillus fumigatus	0.1	100(0)	100	100!0)	100
Aspergillus flavus	0.1	100(0)	100	100(0)	100

Due to the large number of concentrations used, all concentrations and values have not been stated here. Absolute (100%) fungal growth inhibition was seen for all fungi in case of the clotrimazole 1mg/ml culture medium (control drug). However there was no difference between fungal growth in plates containing the essence solvent (DMSD1%) and those grown in saboraud dextrose agar culture medium.

DISCUSSION

During the past few years, the increasing resistance towards azoles compounds and antifungal drugs on one hand and the increasing side effects related to these drugs on the other has resulted in the focus of researchers in pharmacology as well as fungal specialists on studying the antifungal effects of medicinal plants as well as encouraging their use. The aim of this study was to assess the antifungal effects of one of these traditional Iranian plants, thyme (*Zataria multiflora*), on five different dermatophyte and saprophyte species. The antifungal action of *Zataria multiflora* essence was studied for the first time in Iran on different dermatophytis and saprophytic fungi. It was found that the growth of the dermatophytes: *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Epidermophyton floccosum* were 100% inhibited by an essence concentration of 8%. Also the growth of saprophytes: *Aspergillus fumigatus* and *Aspergillus flavus* were100% inhibited by an essence concentration of 10%. As compared to dermatophytes, saprophytes require higher essence concentrations for absolute (100%) fungal growth inhibition. In comparison with the minimum concentrations of other plant essences required for absolute growth inhibition of the fungi studied in the current study, 8% was the lowest concentration required; this can be considered as a unique advantage of thyme essence. Although the antifungal effects of this plant on different dermatophytes and saprophytes have been studied for the first time in this study, but the results of the current study are in accordance with previous studies in which the effect of thyme was studied on a limited number of fungi and microorganisms. Yazdi *et al.* [5] studied the *in vitro* antimicrobial effects of a number of medicinal plants including the thyme from Shiraz and it was seen that this plant can inhibit the growth of three organisms, namely, *Streptococcus pneumonia*, *Hemophilus influenza* and *Moraxella catarrhalis*, all of which are important organisms in sinusitis and bronchitis [5]. Abbasifar [6] in his study reported that a concentration of 30 ppm thyme essence in feta cheese had the highest inhibitory effect on the growth of *Staphylococcus aureus*. Also, Gandomi Nasrabadi [7] stated that 400-1000ppm thyme essence prevents scarification and causes morphological changes in *Aspergillus flavus*. Mahmoodabadi *et al.* [9] in a study on the anti *candida* effect of thyme reported that a concentration of 1127 mg/ml methanol thyme essence has antifungal effect on *Candida albicans*. A study performed by Amanloo [10] with the aim to assess and compare the effect of 2% miconazole gel with 1% thyme gel, showed that thyme gel is more effective in treating infection of tooth prosthesis. Mahboobi *et al.* [9] showed that in comparison to rosemary and lavendulla essences, thyme essence has more antifungal effects on *Candida albicans*. Considering that thyme is a traditional Iranian herb and that essence has many uses. We hope that this study could provide a suitable ground for future research in the field of essential oil extraction. Also, it is hoped that the antifungal effect of this plant be studied by performing *invivo* studies on laboratory animals and finally to help increase the production of medicinal drugs.

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