BIHAREAN BIOLOGIST 9 (2): 105-107
Article No.: 151401
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Comparison of antifungal activity of different extracts from *Satureja khuzistanica* Jamzad against dermatophytes

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Received: 31. August 2014 / Accepted: 24. November 2014 / Available online: 15. November 2015 / Printed: December 2015

Abstract. Dermatophytosis, commonly known as ringworms can cause superficial infections in the skin, hair and nails. Due to the ability of dermatophytes to acquire the resistance to available antifungal agents and their human toxicities, we compared the antifungal activity of different extracts of *Satureja khuzistanica* (essential oil, ethanol and aqueous extracts) against dermatophyte fungi by disc diffusion and micro broth dilution assays. The antifungal activity of *S. khuzistanica* essential oil (MIC =40-190 µg/ml) was higher than its ethanol extract (MIC =40-770 µg/ml); while *S. khuzistanica* aqueous extract had no antifungal activity against dermatophytes (MIC=1550->3100 µg/ml). Their anti-elastase activity evaluations confirmed that *S. khuzistanica* aqueous and ethanol extracts had the higher anti-elastase activity than that of essential oil. *S. khuzistanica* essential oil or its ethanol extract can be further extended to explore more for their antifungal activities.

Key words: *Satureja khuzistanica*, essential oil, ethanol extract, dermatophyte fungi.

Introduction

The frequency of fungal infections and rates of morbidity and mortality due to fungal infections have increased greatly over the last three decades. The increasing in prevalence of fungal infections is as a result of change in diseases management. The use of immunosuppressive agents, multiple antibiotics have been increased the population at risk for fungal infections (Fleming et al. 2002, Wisplinghoff et al. 2004).

Dermatophytosis or ring worm infections are problematic fungal infections that can cause the superficial infections in the skin, hair and nails of humans and animals (Hirschmann 2001). There are only limited numbers of drugs against fungal infections; furthermore, these available limited antifungal agents can cause toxicity in humans. Also, the fungi have ability to acquire resistance to current antifungal therapies (Vandeputte et al. 2012).

The tendency to finding new antifungal agents brings about the investigation on *Satureja khuzistanica*. *S. khuzistanica* is member of *Satureja* genus (Labiatae family) that this genus has 12 annual herbaceous species in Iran (Mozaffarian 1996). In Iranian traditional medicine, *S. khuzistanica* is famous for its analgesic and antiseptic effects (Zargari 1990); the antifungal activities of *S. khuzistanica* were confirmed against *Cryptococcus neoformans* (Amirrajab et al. 2010), and *Candida albicans* (Amanlou et al. 2004), some filamentous fungi such as *Aspergillus flavus*, *A. niger*, *Penicillium* sp., *Fusarium* sp., *Alternaria* sp., *Rhizopus* sp. and *Mucor* sp (Sadeghi-Nejad et al. 2010). Our literature surveys showed there is no study that evaluates the anti-dermatophyte activity of *S. khuzistanica* Jamzad against dermatophytes. The aim of this study was to evaluate the antifungal activity of *S. khuzistanica* ethanol, aqueous extracts and essential oil against dermatophyte fungi. This study also evaluated the anti-elastase activity of *S. khuzistanica* extracts and essential oil.

Materials and methods

Plant Materials and Extraction

Dried full flowering aerial parts of *S. khuzistanica* Jamzad were collected from Lorestan Province in June 2014 and authenticated under Number.168-1. The essential oil was extracted by hydrodistillation from grinded aerial parts in Clevenger type apparatus for 3 hrs. The essential oil kept in a dark vial in a cold place until the analysis. The GC apparatus was Agilent technology (HP) 6890 system, capillary column of HP-1MS (30 m × 0.25mm, film thickness 0.25 µm). The oven temperature program was initiated at 40 °C, held for 1 min then raised up to 230 °C at a rate of 3 °C /min held for 10 min. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The detector and injector temperatures were 250 and 230 °C, respectively. For ethanol and aqueous extraction, the dried powder of grinded plant was separately mixed with water, and ethanol water (70:30, v/v) for 24 h at ambient temperature in percolator. The extracts was separated and filtered. The filtered solutions were dried and were kept in cold place until the analysis.

Dermatophytes

Trichophyton rubrum PTCC 5143, *Trichophyton mentagrophytes* PTCC 5054, *Microsporum canis* PTCC 5069, *Microsporum gypseum* PTCC 5070, *Trichophyton schoenleinii* PTCC 5221, *Trichophyton verrucosum* var. *album* PTCC 5056 were used as dermatophytes for screening the antifungal activities.

Antifungal activity evaluation

The antifungal activities of extracts and essential oil were screened by disc diffusion method, micro broth dilution assay and food poisoning method. The turbidity of fungal suspensions in normal saline containing 0.01% Tween-80 was adjusted to 10^5–10^6 CFU/ml. Then, the suspensions were separately distributed uniformly on Sabouraud dextrose agar medium. Sterile paper discs were impregnated different concentrations of diluted extracts or essential oil and placed on the cultured plates. The diameters of inhibition zones (mm) around the disks were measured after cultivation at 25±2 °C for 5 days. The values recorded as the means ± SD. All experiments were conducted in triplicate. Ketoconazole (15 µg/disc) were used as positive control (CLSI 2009).

Minimal inhibitory concentration (MIC) and Minimal fungicidal concentration (MFC) of extracts and essential oil were determined by the broth micro dilution assay. The stock solution of extracts and oil were prepared in their solvents. Then, they were serially diluted with distilled water (3.1-0.02 mg/ml). Ketoconazole was used in the ranges of 128-0.125 µg/ml. After shaking, 100 µl of diluted solutions

were added to the wells of 96-well plates. The suspension of each organism was adjusted to 10^3–10^4 CFU/ml in RPMI 1640 (Sigma) and was added to each wells, and cultivated at 25±2 °C. The MIC was defined as the lowest concentration that completely inhibited visible fungal growth in the wells after 72 h of incubation. MFC was the well showed no inhibition after culturing on Sabouraud dextrose agar medium (CLSI 2008).

Anti-Elastase activity

The anti-elastase activity of *S. khuzistanica* oil or extracts reaction was contained: *S. khuzistanica* oil or extracts concentrations in 0.2 M Tris-HCl buffer (pH 8.0) (80 µl), 0.1 unit Porcine Pancreatic Elastase type IV (10 µl), were incubated for 10 min at 25 ºC. Then, 0.8 mM Suc-Ala-Ala-Ala-pNA (10 µl) was inserted to the above reaction. The reaction was incubated for 20 min at 25 ºC. The control reaction was the concentration of oil in 0.2 M Tris-HCl buffers (pH 8.0) without elastase. The Optical Density (OD) was measured at 405 nm in a plate reader. The Inhibition (%) was calculated as $(1-B/A)$ ×100; Where A is the enzyme activity without oil, and B is the enzyme activity in the presence of the inhibitor (Mori et al. 2002).

Statistical analysis

The means of inhibition zone, MIC and MFC values were analyzed by GraphPad prism and were presented as means ± Standard Deviation (SD).

Results

The GC analysis of oil showed the presence of carvacrol (94.5%) as the main component of *S. khuzistanica* essential oil.

The antifungal activity evaluation of *S. khuzistanica* extracts by disc diffusion assay showed that the aqueous extract of *S. khuzistanica* had no activity against dermatophytes while its ethanol and essential oil had inhibition zone diameter around the discs. The antifungal activity of *S. khuzistanica* essential oil was higher than its ethanol extract. The inhibition zone diameters were observed for *S. khuzistanica* oil at 0.5 mg of essential oil in the ranges of 11.7±1.1 to 25.1±0.6 mm while at this concentration; *S. khuzistanica* ethanol extract had the inhibition zone diameter for *T. schoenleinii* (9.3±0.21 mm) and had no any inhibition zone diameter for other studied fungi. *S. khuzistanica* ethanol extract had the highest inhibition zone against *T. mentagrophytes*, followed by *M. canis*, *T. verrucosum, M. gypseum*, respectively. *T. schoenleinii* and *T. rubrum* had the less sensitivity to *S. khuzistanica* ethanol extract. *S. khuzistanica* essential oil showed the higher inhibition zone diameter against *T. mentagrophytes*, *T. verrucosum* and *M. gypseum*, respectively (Table 1).

In micro broth dilution assay, the MIC and MFC evaluations of different extracts from *S. khuzistanica* showed that *S. khuzistanica* aqueous extract had no activity against dermatophytes (MIC>3100 µg/ml) according to its disc diffusion results. Among to others (essential oil or ethanol extract), the MIC and MFC for *S. khuzistanica* essential oil was lower than the MIC and MFC of *S. khuzistanica* ethanol extract. The MIC values for *S. khuzistanica* ethanol extract were in the ranges of 40-770 µg/ml, while MIC values were in the ranges of 40-190 µg/ml for *S. khuzistanica* essential oil. The MFC values were in the ranges of 90-770 and 90-380 µg/ml for ethanol extract and essential oil, respectively. *M. canis* had same sensitivity to *S. khuzistanica* essential oil and ethanol extract (MIC, MFC= 40 and 90 µg/ml). *S. khuzistanica* essential oil had the low MIC values for *M. canis* (40 µg/ml), *T. schoenleinii* (40 µg/ml), followed by *T. mentagrophytes*, *M. gypseum*, *T. verrucosum* (90 µg/ml). *T. rubrum* had less sensitivity to *S. khuzistanica* essential oil (190 µg/ml). *S. khuzistanica* ethanol extract had the lower MIC values for *M. canis* (40 µg/ml), *T. verrucosum* (90 µg/ml), followed by *T. mentagrophytes*, *T. rubrum*, *T. schoenleinii* (190 µg/ml). *M. gypseum* had less sensitivity to *S. khuzistanica* ethanol extract (770 µg/ml) (Table 2).

The anti-Elastase activity evaluation of *S. khuzistanica* extracts showed that *S. khuzistanica* essential oil had the less anti-elastase activity. Although, *S. khuzistanica* aqueous extract had weak anti-dermatophyte activity, but it had the best anti-elastase activity. The anti-elastase activity of *S. khuzistanica* aqueous extract was lower than *S. khuzistanica* ethanol extract.

Table 1. The antifungal activity of *S. khuzistanica* extracts by disc diffusion assay.

	Ethanol extract (mg)				Aqueous extract (mg)				Essential oil (mg)		Ketoconazole (µg)
	0.5	1.0	2.5	3.0	0.5	1.0	2.5	3.0	0.5	1.0	15
T. mentagrophytes	-	10.2±0.9	40.3±0.6	56.3±0.6	-	-	-	-	25.1±0.6	41.1±1.3	33.3±0.58
T. rubrum	-	11.8±0.3	19.6±0.7	23.5±0.9	-	-	-	-	11.7±1.1	14.7±1.3	39.9±0.12
M. canis	-	13.1±0.3	25.3±0.3	37.7±1.2	-	-	-	-	12.7±1.1	16.7±1.3	39.3±0.58
M. gypseum	-	8.2±1.1	21.3±0.6	30.4±0.5	-	-	-	-	17.1±1.3	23.3±1.7	13.3±0.5
T. verrucosum	-	11.1±1.3	23.7±0.6	34.6±1.2	-	-	-	-	20.2±1.4	28.8±1.0	31.7±0.46
T. schoenleinii	9.3±0.2	10.9±0.2	23.8±0.7	28.5±0.3	-	-	-	-	12.3±0.2	14.7±0.6	40.8±0.68

(-): no effect

Table 2. The antifungal activity of *S. khuzistanica* extracts by broth dilution assay.

	Ethanol extract		Aqueous extract		Essential oil		Ketoconazole	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
T. mentagrophytes	190	770	>3100	>3100	90	380	0.5	1
T. rubrum	190	190	>3100	>3100	190	380	0.25	0.25
M. canis	40	90	1550	>3100	40	90	0.25	0.25
M. gypseum	770	150	>3100	>3100	90	380	1	1
T. verrucosum	90	770	>3100	>3100	90	380	1	2
T. schoenleinii	190	380	>3100	>3100	40	190	0.5	1

MIC= Minimal Inhibitory Concentration (µg/ml), MFC=Minimal Fungicidal Concentration (µg/ml)

As the results are shown, very high anti-elastase activities were exhibited by *S. khuzistanica* ethanol and aqueous extracts which inhibited over 75% of elastase enzyme activity. Relatively moderate anti-elastase activity was exhibited by *S. khuzistanica* essential oil (Fig. 1).

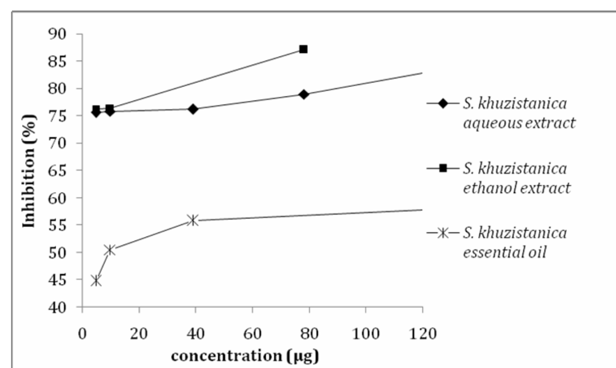

Figure 1. The anti-Elastase activity of *S. khuzistanica* extracts.

Discussion

The results of our screening showed *S. khuzistanica* ethanol extract and its essential oil showed the high antifungal activity against dermatophytes especially against *M. canis*. The antimicrobial activity of essential oils or other extracts is related to their components. As mentioned before, the main component of *S. khuzistanica* essential oil was carvacrol (95%). Hydroxyl group of carvacrol functions as trans membrane carrier across the membranes (Ben Arfa et al. 2006) and interacts with membrane proteins and peri-plasmic enzymes (Juven et al. 1994). The anti-dermatophyte activity of carvacrol has been proved against dermatophytes (Pinto et al. 2006). Furthermore, the anti-dermatophyte activity of *Satureja montana* oil with carvacrol as the main component of oil (around 60%) were confirmed against *T. violaceum*, *T. rubrum*, *T. mentagrophytes* (Haloc et al. 2014).

Production of elastase by dermatophytes is reported to have essential role in their pathogenesis (Okumura et al. 2007). Therefore, the elastase inhibitors can be a new candidate for exploring the potential antifungal agents. The high anti-elastase activity of *S. khuzistanica* ethanol extract and also its high antifungal activity make it as suitable candidate for further evaluation about its mechanism of action.

Therefore, according to the antifungal activity of *S. khuzistanica* essential oil, the oil can be used as the topical treatment for control or treatment of skin fungal infections.

Acknowledgement. Financial support for Medicinal Plant, Research Center of Barij Essence was essential for conducting this study.

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