Original article

Antifungal activity of *Satureja khuzestanica* (Jamzad) leaves extracts

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Abstract

Introduction and objective: Opportunistic fungal infections have been a common cause of morbidity and mortality in the immunosupresed individuals such as AIDS and organ recipients. Treatment of these infections is a great challenge, thus antifungal therapy is playing a greater role in their health care. Traditional plants are a valuable source of novel antifungals. The aim of this study was to assess *in vitro* antifungal activity of the ethanolic extract of *Satureja khuzestanica* leaves.

Materials and methods: In the current experimental study the Minimum Inhibitory Concentration (MIC) of the ethanolic extract of *S. khuzestanica* leaves was evaluated against saprophytic fungi isolates such as *Aspergillus flavus, A. niger, Penicillium* sp., *Fusarium* sp., *Alternaria* sp., *Rhizopus* sp., and *Mucor* sp. Antifungal susceptibilities were determined using the agar well diffusion method and amphotericine B was used as positive control as gold therapeutic agent.

Results: Our findings showed that the ethanolic extract of *S. khuzestanica* leaves exhibited antifungal activity against all tested saprophytic fungi with MIC values (625-5000µg/ml).

Conclusion: The Results demonstrated that this plant has strong antifungal potential against all tested fungi.

Keywords: Antifungal agents, *Satureja khuzestanica*, Fungi, Saprophytic fungi

Introduction

Due to the increase of the number of immunocompromised individuals, fungal infections have increased in the last two decades [1]. Among them, opportunistic systemic mycoses are associated with high mortality rates [2]. This is essential for systemic mycoses that are typically in immunocompromised patients as toxicities are induced by commercial antifungal drugs. The side effects are often observed in these patients because of the dosage and prolonged therapy [3]. Herbal healers

suggest that their medicines are cheaper and more efficient than commercial ones [4].

There are many drugs for the treatment of fungal diseases; however, there are a limited number of efficacious antifungal drugs [5]. They posses a series of limitations such as undesirable side effects and low sensitivity against these fungal infections [6,7]. Hence, new antifungal agents still require improvement to be effective against opportunistic infections. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries. Traditional healers usually are cheaper and sometimes more effective than modern chemical medicine [8].

This study was designed to investigate the effect of antifungal activity of *Satureja khuzestanica* leaves by using the well diffusion method. *S. khuzestanica* is a natural plant, which is extensively grown in south of Iran. This plant is known for its medical application. The *Satureja* genus is related to the family *Lamiaceae*, subfamily *Nepetoideae* [9]. During recent years, antiviral [10], antibacterial and antifungal [11-12], antispasmodic and antidiarrhea [13] and vasodilatory [14] uses are recognized in various species of *Satureja* in many parts of the world. There are a few studies which are carried out on *S. khuzestanica* essential oil (SKEO) [15]. The antimicrobial activity of *Satureja* species was first reported during 1950s. It was established that the inhibitory effect of thymol and carvacrol, which are known as the most competent plant antibacterial drugs [16].

Material and methods

Plant material

The aerial parts of plant were collected in April 2006 during the flowering stage of plant from Dezful in Khuzestan province. The plant was obtained from Sedigheh Nanaei, a Botanist of Agricultural and Natural Resources of the Research Centre of Khuzestan, Ahvaz, Iran.

Preparation of plant extracts

The healthy leaves were dried in shade condition and to avoid decomposition of chemical constituents dried leaves were powdered and stored in clean and dry airtight containers for further studies. Leaves powder were macerated in 80% ethanol (10g /100ml 80% ethanol) for 72 hours [17] and then filtered using Buckner funnel and Whatman filter paper #1. The ethanol extract was evaporated at room temperature. Then 1gm of the dried plant extract was dissolved in 5ml 20% dimethylsulfoxide (DMSO) to obtain a final concentration of 200 mg ml^{-1}.

Preparation of inoculum

Environmental isolates of six genera of fungi including *A. niger*, *A flavus, Penicillium, Fusarium*, *Alternaria*, *Rhizopus and Mucor* were subcultured and used in this study. All isolates were subcultured and prepared for the assessment of plant extract activity. These fungi were: *A. niger*, *A flavus, Penicillium, Fusarium*, *Alternaria*, *Rhizopus and Mucor*. Stock cultures were maintained at 4°C on slopes of Sabouraud dextrose agar (Merck, Germany). Active cultures for experiments were prepared with removing a loopful of cells from the stock cultures to test tubes of sterile distilled water to prepare 10^6 colony forming units (CFU/ml).

Antifungal activity and minimal inhibitory concentration evaluation

Agar diffusion method was carried out for the assessment of the ethanolic extract of *S. khuzestanica* according Perez *et al*. [18] study. One hundred microlitres of inoculums (10^6 CFU/ml; 0.5 McFarland) of each test saprophytic fungi [19] evenly was spread using a sterile glass spreader onto Sabouraud dextrose agar plates. The plates

have been kept to dry and a sterile borer (7 mm in diameter) was then used to punch wells in the agar medium. Subsequently, wells were filled with 100µl of the plant extract [20] at concentration of 3.12-100 mg/ml and allowed to diffuse at room temperature for 2h.

The plates were incubated at 25°C for 72h. The minimum inhibitory concentration (MIC) is regarded as the lowest concentration of the plant extract that inhibits the growth of the test organisms. Sterile DMSO used as negative control. Amphotericin B was used in the assay as positive control. Drug-free solution was also used as a blank control. The antifungal activity was evaluated to determine the inhibition zone (Fig. 1). The experiments were replicated three times and the mean of the inhibition zone of each tested fungi was measured.

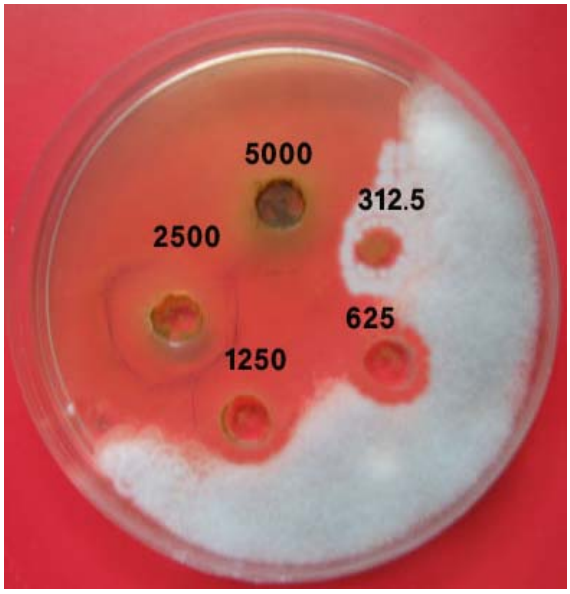

Fig. 1: *In vitro* antifungal activity of the ethanolic extract of *Satureja khuzestanica* leaf against *Mucor* (MIC=625µg/ml)

Results and discussion

Table 1 shows the MIC of ethanolic extract of *S. khuzestanica* leaves against six tested fungi. The inhibition zone of ethanolic extract of *S. khuzestanica* against tested fungi showed in table 2. The plant extract exhibited strong activity against *A. flavus* with MIC values 1250-5000µg/ml and inhibition zone of 20-40mm. Plant extract with the same concentrations revealed that *Penicillium, Fusarium*, *Alternaria*, *Rhizopus* and *Mucor* showed MIC values ranging from 625 to 5000µg/ml with inhibition zone of 5-30mm. The less antifungal activity was against *A. niger* with MIC values of 2500 to 5000µg/ml and inhibition zone of 15-24mm.

Table 1: Antifungal activity of *Satureja khuzestanica* and amphotericin B against selected fungi

Fungi	MIC (µg/ml)	
	S. khuzestanica	Amphotericin B
A. flavus	1250	2000
A. niger	2500	2000
Penicillium	625	2000
Fusarium	625	1000
Alternaria	625	2000
Rhizopus	625	2000
Mucor	625	2000

In the current study, the positive control, amphotericin B, showed antifungal activity with MIC values 1000-2000µg/ml for all fungi (Table 1). The MIC of the ethanolic extract of *S. khuzestanica* leaves against *A. niger*, *Penicillium*, *Fusarium*, *Alternaria*, *Rhizopus* and *Mucor* was more potent than amphotericin B while against *A. flavus* it was less than amphotericin B. The extracts of *S. khuzestanica* leaves were active with the concentrations less than 2000µg/ml against the most of tested fungi. It is interesting to note that the previous work also revealed that the methanolic extracts of *S. khuzestanica* displayed inhibitory activity against Gram-negative and Gram-positive bacteria and were also active against the fungi *Candida albicans* and *A. niger* [21].

Table 2: Zone of inhibition and MIC of the ethanolic extract of *Satureja khuzestanica* leaf against saprophytic fungi

Organisms	Zone of inhibition (mm) concentration (mg/ml)						MIC (µg/ml)
	100	50	25	12.5	6.25	3.12	
Aspergillus flavus	40	30	25	20	0	0	1250
Aspergillus niger	24	20	15	0	0	0	2500
Penicillium	26	22	20	18	16	0	625
Fusarium	25	20	16	15	8	0	625
Alternaria	30	25	22	17	15	0	625
Rhizopus	25	20	18	15	13	0	625
Mucor	25	20	17	15	5	0	625

The results in the present study suggest that *S. khuzestanica* leaves extract may possess some compounds such as Carvacrol and Thymol which are phenolic aromatic compounds. These compounds are well known as powerful antibacterial and antifungal properties [22]. We suggest to perform *in vivo* investigations to find out more information about the treatment of fungal infections.

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