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Antifungal Activity of Endemic *Satureja icarica*

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The methanol extract obtained from *Satureja icarica* P.H. Davis (Lamiaceae) have been investigated for their antifungal activity. Antifungal activity was determined with *Candia albicans, Candida tropicalis, Candida guilliermondii, Cryptococcus neoformans, Cryptococcus laurentii, Aspergillus flavus, Aspergillus niger, Alternaria alternate, Geotrichum candidum, Fusarium oxysporum, Penicillium frequentans, Penicillium canescens* and *Botrytis cineriae* by the well-in-agar method and the visual broth macrodilution method. The diameter of inhibition zones varied from 11.6 to 18.8 mm. The MIC values ranged from 6.25 to 25 μg/mL. The extracts exhibited a strong antifungal effect against the tested fungi. *Candida albicans* is more susceptible to the extract. The present findings provide a support to the use of this plant in traditional medicine for fungal infections especially against candidiasis.

Key Words: Antimicrobial activity, *Satureja icarica,* P.H. Davis.

INTRODUCTION

The rate of increase of opportunistic fungal infections in immunocompromized patient, including those with AIDS, recipients of solid organ transplant and cytotoxic chemotherapy, has created new challenges in the area of antifungal drug therapy. Although there are several effective preparations available, some conditions such as ringworm infections of nails or recurrent vaginal candidiasis are frequently intractable to treatment[1].

The genus *Satureja* (Lamiaceae) is represented in Turkey by 15 species of which 5 are endemic[2]. Several *Satureja* species are locally known as keklik otu, kiliç kekik, firubu, çatli or kekik in the regions where they grow and used as culinary or medicinal herbs in various regions of Turkey[3]. Leaves, flowers and stem of *Satureja* species are used as folk remedies to treat various ailments such as cramps, muscle pains, nausea, indigestion, diarrhae and infectious diseases. In addition, *Satureja* species have showed antispasmodic, antidiarrhoeal, antioxidant, sedative as well as antimicrobial properties[4].

Satureja icarica P.H. Davis is endemic to Turkey[2]. During routine excur-sions, it is determined that this plant is used to treat diarrhae, cold, bronchitis and externally for boils and abscesses. Therefore, the aim was to determine the antifungal effects of extracts obtained from this endemic plant against common pathogenic and saprophytic fungi.

EXPERIMENTAL

Aerial parts of *Satureja icarica* was collected from Gokceada, Canakkale, Turkey during the months of September-October 2007. Voucher specimen of the plant was deposited in the Biology Department at Canakkale Onsekiz Mart University, Canakkale, Turkey and identified by Dr. Emin Ugurlu from Celal Bayar University, Manisa, Turkey.

Plant extraction: The plant parts were air-dried. Each dry powdered plant material (20 g) was extracted with 150 mL of 80 % methanol (Merck, Darmstadt, Germany) for 24 h by using Soxhlet equipment[5]. The extract was filtered using Whatmann filter paper no. 1 and the filtrate were then evaporated under reduced pressure and dried using a rotary evaporator at 55 °C. Dried extract were stored in labelled sterile screw-capped bottles at -20 °C. The extract amounting to around 2 g was dissolved in DMSO before testing. *Candida albicans, Candida tropicalis, Candida guilliermondii, Cryptococcus neoformans, Cryptococcus laurentii, Aspergillus flavus, Aspergillus niger, Alternaria alternate, Geotrichum candidum, Fusarium oxysporum, Penicillium frequentans, Penicillium canescens* and *Botrytis cineriae* as the test fungi were obtained from Microbiology Researh Laboratory at Uludag University, Department of Biology, Bursa, Turkey and pure cultures were maintened on Sabouraud Dextrose Agar (SDA) plates and Sabouraud Dextrose Broth (SDB) in tubes.

Detection of antifungal activity: The screening for antifungal activity was done using a modified version of the well-in-agar method[6]. Each test fungus (from SDB medium) was aseptically swabbed on the SDA medium to get uniform of 0.5 cm diameter was made in the inoculated medium and 0.2 mL of each extract was filled into the well and the plates were kept in room temperature for an hour to allow spread of extract in the medium. Later, the agar plates were incubated for 7 d at 25 ± 2 °C. The presence of zones of inhibition around the wells was observed and interpreted were performed in triplicate. Ketocanazole diluted at 50 µg/mL was used as a standard antifungal for the positive control and sterilized distilled water used as the negative control. DMSO was also tested for solvent effect.

Minimum inhibitory concentration (MIC) determination: MICs were performed by the visual broth macrodilution method[7]. Fungal suspensions were diluted into RPMI-1640 medium without bicarbonate (pH 7.0 with 0.165 morpholinepropanesulfonic acid) broth supplemeted with

glutamine, to a concentration of approximately 0.5×10^5 CFU/mL, verified by colony counts in SDA. A two-fold serial dilution of 0.2 mL each of extract was added to 1.8 mL of the RPMI-1640 medium. The concentrations were 0.390-200 mg/mL. Controls with medium without antifungal samples were used in the test. To compare the results with standard, ketaconazole was used. Tubes were then incubated at 35 °C for 24-48 h. MIC was defined as the lowest concentration which did not yield visual growth. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

The results obtained in this study are given in Table-1. The extracts of the *S. icarica* are shown strong antifungal activity against the tested fungal strains. The diameter of inhibition zone by the well-in-agar varied from 11.6 to 18.8 mm. The MIC values ranged from 6.25 to 25 μg/mL. *Candida albicans* is more susceptible to the extract as compared with standard antibiotic. As a general result, all the fungi assayed showed inhibition when tested against the extract of *S. icarica*. The essential oil of *S. icarica* was reported to contain carvacrol (52.0-56.0 %), borneol (5.0-5.8 %), γ-terpinene (5.8-6.9 %), *p*-cymene (13.1-17.0 %) as main constituents[8]. In another study[9], antibacterial activity of the essential oils of *S. wiedemanniana* obtained from various samples was shown. Carvacrol and thymol were shown to inhibit pathogenic microorganisms. Furthermore, antimicrobial activities

TABLE-1
ANTIFUNGAL ACTIVITY OF *Satureja icarica*

Microorganisms	Diamater of Inhibition zones (mm)		Minimum Inhibitory concentration (MIC)	
	Methanol extract (mg/mL)	Ketaconazole (50 μg/mL)	Methanol extract (mg/mL)	Ketaconazole (50 μg/mL)
Candida albicans	18.8	17.4	6.25	0.25
Candida tropicalis	17.6	19.2	12.50	4.00
Candida guilliermondii	14.7	19.0	25.00	5.00
Cryptococcus neoformans	16.8	18.2	12.50	0.25
Cryptococcus laurentii	15.4	18.0	25.00	4.00
Aspergillus flavus	12.8	19.2	25.00	0.25
Aspergillus niger	15.2	21.4	12.50	0.50
Alternaria alternate	11.6	18.8	25.00	0.50
Geotrichum candidum	15.4	17.6	12.50	0.25
Fusarium oxysporum	14.2	20.2	12.50	0.12
Penicillium frequentans	17.6	19.6	12.50	2.00
Penicillium canescens	16.2	18.2	12.50	2.00
Botrytis cineriae	13.4	19.8	25.00	0.25

of different *Satureja* species were reported in previous studies[10-12]. The results obtained from this study are parallel to the mentioned studies. In an earlier work[3], the essential oil of *S. icarica* showed antifungal effects against the tested fungi. While, it showed a minimal inhibitory concentration value of 62.5 µg/mL against *Candida albicans*, it determined that MICs value is 6.25 µg/mL in this study. This difference may be attributing to fact that microorganisms variable sensitivity to chemical substances relates to different resistance level between the strains[13].

Fungi used in this study were chosen primarily on the basis of their importance as opportunistic pathogens of humans. According to findings from the National Nosocomial Infection Surveillance System (NNIS)[14], 61 % of reported nosocomial fungal infections were due to *Candida albicans*, followed by other *Candida* spp. and *Cryptococcus* spp. *Candida albicans*, while naturally occurring in the intestinal flora, can cause oral thrush and systemic infections. *Cryptococcus neoformans* causes cryptococcosis, an opportunistic infection of the lungs especially in AIDS patients.

The results obtained confirm the therapeutic potency of *Satureja icarica* used in traditional medicine. In addition, the result of the present study supports the folkloric usage of the studied plant and suggests that the plant extract possess certain constituents with antifungal properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases especially against *Candida* infections.

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