

# Anticandidal activity of endemic *Salvia potentillifolia* Boiss. and Heldr. ex Bentham and *Origanum hypericifolium* Schwartz and P.H. Davis in Turkey

A. Celik,  
C. Ergin<sup>1</sup>,  
I. Arslan,  
T. Kartal

Departments of Biology and <sup>1</sup>Microbiology, Pamukkale University, Denizli, Turkey.

**Address for correspondence:**

Dr. A. Celik, Department of Biology, Pamukkale University, Denizli, Turkey.  
E-mail: acelik@pau.edu.tr

DOI: 10.4103/0976-9668.71668

## Abstract

This study established baseline data on lytic anticandidal activities of endemic species *Origanum hypericifolium* and *Salvia potentillifolia* naturally distributed in Denizli and its environment. Stream distillation was used to isolate the unfatty polar part and clinical isolated *Candida* spp. strains were subcultured to sabouraud dextrose agar. Lytic anticandidal activities of unfatty polar parts were evaluated by enzyme-linked calorimetric method against 93 clinical isolates belonging to *Candida albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. Kefyr*, and *C. parapsilosis*. As a result, two (2.15%) strains of *C. glabrata* among tested pathogenic 93 clinical isolates of *Candida* strains were found to be sensitive to *S. potentillifolia*. However, each strain of *C. albicans* and *C. tropicalis* was found to be sensitive to *O. hypericifolium*. Results indicated that *O. hypericifolium* and *S. potentillifolia* had a potential of being used in food and medicine because of their anticandidal activity.

**Key words:** Anticandidal activity, lytic, *Origanum hypericifolium*, *Salvia potentillifolia*, yeast

## INTRODUCTION

The genus *Origanum* L. (Lamiaceae) is represented by 26 taxa in Turkey, including 15 endemic species.<sup>[1-3]</sup> *Origanum hypericifolium* Schwartz and P.H. Davis are endemic species with limited distribution and included in the lower risk and conservation-dependent category in red data book of Turkey.<sup>[4]</sup> [Figure 1]

The genus *Salvia* (sage) is an important genus of the Lamiaceae family and comprises about 900 species, widespread throughout the world. Some members of this genus are also cultivated to use as flavoring agents in perfumery, cosmetics as well as in food. There are about 90 species of *Salvia* in the Turkish flora, of which 45 are endemic.<sup>[1]</sup>

*Candida* spp. are important healthcare-associated pathogens especially immunocompromised host. Antifungal resistance, increasing costs, hospitalization time, and treatment difficulties of candidal infections are worldwide problems. Antifungal strategies are mainly based on targeting molecules on yeast cell wall, inhibition of cytochrome



Figure 1: *Origanum hypericifolium*

oxidases, antimetabolite effect to nucleus activities, and gene-regulated efflux system blocking. Novel antifungal molecules may help in the treatment of yeast infections.<sup>[5-7]</sup>

## MATERIALS AND METHODS

### Plant materials

The aerial parts of *O. hypericifolium* were collected during the flowering stage from July to August, 2007, on Mount Sandras (elevation 1860 m), Beyağaç-Denizli, where it is endemic. Dr. Ali Celik further identified all the collected plants. The voucher specimens are deposited at the herbarium of Pamukkale University, Faculty of Science and Art, Biology Department (herbarium no. AÇE 2545). The samples were air-dried and stored in a polyethylene bag until use.

### Extraction

The air-dried and finely ground sample was extracted by using the method described previously (Sokmen, Jones, and Erturk, 1999). The resulting extract (13.11%, w/w) was suspended in water and partitioned with chloroform (CHCl<sub>3</sub>) to obtain water-soluble (polar) (10.86%, w/w) and water-insoluble subfractions (2.25%, w/w), which were then lyophilized and kept in the dark at +4°C until tested.

### Microbial strains

For screening anticandidal activity; *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida kefir*, *Candida krusei*, and *Candida glabrata* strains have been isolated from clinical samples and identified by API 20C AUX (Bio-Mérieux, France). Each yeast strain was subcultured onto Sabouraud dextrose agar for two days at room temperature.

Strain susceptibility of polar parts of plant extract have been tested by rapid yeast lysis assay microtiter method described by Jewell *et al.*<sup>[8]</sup> Briefly, each yeast strain was grown in YEPM (1% yeast extract, 2% peptone, and 2% maltose) broth medium at 28°C for 48 h with shaking. Yeasts were centrifuged at 5000 × *g* for 20 min at 4°C. The supernatant broth was discarded and yeast pellet was suspended in buffered saline gelatin (0.1% gelatin, 0.9 NaCl, 0.03% KH<sub>2</sub>PO<sub>4</sub>, 0.06% Na<sub>2</sub>HPO<sub>4</sub>). Turbidity of each yeast suspension was adjusted to Mc Farland No.1 standard. Lysis-sensitive yeast cells were freshly tested without stock procedure.

### Evaluation of antifungal activity

Undiluted *O. hypericifolium* and *S. potentillifolia* polar parts of plant extract have been tested in separate microtiter plate. Each microtiter plate has been prepared as a total of 100 µl test extract. Following extract preparation in well, 100 µl of the maltase-induced *Candida* sp. suspensions was added to wells except for controls. The microtiter plates were sealed with polyethylene tape and incubated at 28°C with orbital shaking at 100 r/min. After 30 min of incubation, the film was removed and 40 µl of filter-sterilized, 4 mg *p*-nitrophenyl- $\alpha$ -D-glucopyranoside/ml in water was added

**Table 1: Anticandidal activity of *O. hypericifolium* and *S.potentillifolia***

Microorganisms	<i>Origanum hypericifolium</i>		<i>Salvia potentillifolia</i>	
	Activity (±)	(%)	Activity (±)	(%)
<i>Candida albicans</i>	+	2.15 (n = 1)	-	-
<i>Candida tropicalis</i>	+	2.15 (n = 1)	-	-
<i>Candida parapsilosis</i>	-	-	-	-
<i>Candida kefir</i>	-	-	-	-
<i>Candida glabrata</i>	-	-	+	2.15 (n = 2)
<i>Candida krusei</i>	-	-	-	-

---: no activity

to each well. The plate was resealed and incubated at 37°C without agitation. After 10 min of incubation, 60 µl, 1 M Na<sub>2</sub>CO<sub>3</sub> was added to stop the reaction. Yellow color has been accepted as positive result by unaided eye. Only test extract and only *p*-nitrophenyl  $\alpha$ -D-glucopyranoside (PNPG) substrate with test extract (for spontaneous degradation) wells have been accepted as test controls.

## RESULTS AND DISCUSSION

Anticandidal activity of *O. hypericifolium* have been found to two (2.15%) yeast strain (one *C. albicans* and *C. tropicalis*). It can be seen from Table 1 that only two (2.15%) *C. glabrata* strains have been killed by *S. potentillifolia* extract.

In this study, lytic antifungal activity from crude extracts of *O. hypericifolium* and *S. potentillifolia* have been described against some *Candida* species. Only 2.5% of yeast population are not having satisfactory results because of not reflecting wide populations. Antimetabolite activity, gene-regulated efflux systems, and enzymatic inhibition of yeasts by plant extracts may play a crucial role in antifungal effect. However, tested method of antifungal activity is focused on yeast cell lysis. Chemical-genetic relationships of compounds should be investigated with further research. Our results indicate that *O. hypericifolium* and *S. potentillifolia* extract's antifungal activity should be analyzed for activity constituents by chemical analysis for newer molecules to improve the therapy strategies in medicine.

## REFERENCES

1. Davis PH. Flora of Turkey and East Aegean Island. Vol. 7. Edinburgh: Edinburgh University Press. 1982 p. 297.
2. Davis PH, Tan K, Mill R. Flora of Turkey and the East Aegean Islands. Vol. 10. Edinburgh: Edinburgh University Press; 1988 p. 206.
3. Guner A, Özhatay N, Ekim T, Başer HC. Flora of Turkey and the East Aegean Islands. Vol. 11. Edinburgh: Edinburgh University Press;

2000 p. 207

4. Ekim T, Koyuncu M, Vural M, Duman H, Aytac Z, Adiguzel N. Red Data Book of Turkish Plants. Ankara: Bariscan offset; 2000 p. 246.
5. Miranda LN, van der Heijden IM, Costa SF, Sousa AP, Sienna RA, Gobara S, *et al.* Candida colonisation as a source for candidaemia. J Hosp Infect 2009;72:9-16.
6. Sanglard D, Odds FC. Resistance of *Candida* species to antifungal agents: Molecular mechanisms and clinical consequences. Lancet Infect Dis 2002;2:73-85.
7. Espinel-Ingroff A. Novel antifungal agents, targets or therapeutic strategies for the treatment of invasive fungal diseases: A review of the literature (2005-2009). Rev Iberoam Micol 2009;26:15-22.
8. Sokmen, A., Jones, B.M., Erturk., M. 1999. The *in vitro* antibacterial activity of Turkish medicinal plants. Journal of Ethnopharmacology, 67, 79–86.
9. Jewell SN, Waldo RH, Cain CC, Falkinham JO. Rapid detection of lytic antimicrobial activity against yeast and filamentous fungi. J Microbiol Methods 2002;49:1-9.

**Source of Support:** Nil, **Conflict of Interest:** None declared.