

Original Research Article

Antifungal Activity of *Hypericum havvae* Against Some Medical *Candida* Yeast and *Cryptococcus* Species

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Abstract

Purpose: To investigate the antifungal activities of the individual as well as the combined hydroalcohol leaf and root extracts of endemic *Hypericum havvae* A. Guner (Hypericaceae).

Methods: Each dry powdered plant material (20 g) was soaked in 150 ml of aqueous ethanol (50: 50 %v/v) until complete saturation of the plant material. After the extracts were filtered and evaporated, the antifungal activity of the extracts was tested against medical yeast, *Candida* (*C. albicans* ATCC 10231, *C. tropicalis* ATCC 13808, *C. guilliermondii* ATCC 6260) and *Cryptococcus* (*C. neoformans* ATCC 90112 and *C. laurentii* 34142) species by visual broth microdilution method. Minimum inhibitory concentration (MIC) of the extract was derived from the results. Ketoconazole was used as the reference standard.

Results: MIC values ranged from 3.12 to 25.00 mg/ml. The extracts exhibited strong antifungal effect against the yeast cultures but the combination of the plant extracts (leaf + root) possessed stronger antifungal potency against *Candida albicans* and *Cryptococcus laurentii*, with the same MIC value of 1.56 mg/ml.

Conclusion: Our findings support the use of *Hypericum havvae* in traditional medicine for the treatment of fungal infections, especially Candidiasis.

Keywords: Antifungal activity, *Candida*, *Hypericum havvae*, Candidiasis.

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INTRODUCTION

Hypericum species belonging to Clusiaceae (Hypericaceae) family are widely found in Europe, Asia, Northern Africa and America [1]. This genus encompasses various species used in traditional medicine around the world. Several antifungal, antibiotic, antiviral and anticancer compounds have been isolated from *Hypericum* species [2-4]. The majority of the active compounds are phenolic in nature. These plants have a strong tendency to accumulate phenolic compounds with the phloroglucinol substitution pattern.

Hypericum havvae A. Guner is endemic to Turkey [5]. Information gathered from native healers indicate that the powdered form or water extract are used as antidepressant drug. The plant is also used for other therapeutic purposes by native Turkish people. During our routine field excursions, it was observed that this plant is used to treat colds and bronchitis, externally for boils and abscesses, and for urinary tract infections. Therefore, this study was undertaken to determine the antifungal effects of the hydroethanol extracts obtained from this endemic plant against some *Candida* and *Cryptococcus* species.

EXPERIMENTAL

Plant materials

The plant material was collected from Icel-Cehennemdere, Turkey, 37°07'36.13" N, 34°31'03.31" E, alt. 725 m in September, 2012. The plant was collected and identified by Dr. Gorkem Dulger. Voucher specimens of the plant (no. GD-056-1 for the leaves and no. GD-056-2 for the root) were deposited in herbarium of the Biology Department, Duzce University.

Preparation of extracts

The plant parts were washed and air-dried. Each dry powdered plant material (20 g) was soaked in 150 mL of aqueous ethanol (50: 50 %v/v) until complete saturation of the plant material. The extract was filtered using Whatman no. 1, the filtrate then evaporated under reduced pressure and dried using a rotary evaporator at 55 °C. The extract (in the form of sticky black substances) amounting to 2 mg was dissolved in 0.4 mL of dimethyl sulfoxide (DMSO, 5 mg/mL). The combination of plant extracts (1:1 ratio) was used in this test [6]. *Candida* species (*C. albicans* ATCC 10231, *C. tropicalis* ATCC 13808, *C. guilliermondii* ATCC 6260) and *Cryptococcus* species (*C. neoformans* ATCC 90112 and *C. laurentii* 34142) the test fungi, were obtained from Research Laboratory in Duzce University, Department of Biology, Turkey and pure cultures were maintained on Sabouraud Dextrose Agar (SDA) plates and Sabouraud Dextrose Broth (SDB) in tubes.

Minimum inhibitory concentration (MIC) determination

MIC's were performed by the visual broth macrodilution method [7]. Fungal suspensions were diluted into RPMI 1640 medium (with L-glutamine, without bicarbonate) (Sigma Aldrich Chemie GmbH, Steinheim, Germany) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) to a concentration of approximately 0.5×10^5 CFU/mL, verified by colony count 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 in the range 0.39 – 200.0 mg/mL. Controls with medium without antifungal samples were included in the test. For comparison, ketoconazole was used as standard. The tubes were 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

The MICs of the extracts are presented in Table 1. The MIC results for the ethanol extracts of leaf, root and their combination ranged from 3.12 - 25, 6.25 - 25 and 1.56 - 12.5 mg/mL, respectively; the results show that the susceptibility of the extracts varied from one fungal strain to another. The combined plant extracts (leaf plus root) exhibited stronger antifungal activity than the others. *Candida albicans* and *Cryptococcus laurentii* were more susceptible than other fungi, followed by *Candida tropicalis*, *Candida guilliermondii* and *Cryptococcus neoformans*, with the same MIC of 3.12 mg/mL. The combined extracts with the same MIC of 12.5 mg/mL have shown weak activity against *Candida glabrata* and *Candida parapsilosis*. Notably, the combined extracts displayed stronger antifungal activity than the standard antifungal antibiotic, ketoconazole, against *Candida albicans*, *Candida tropicalis* and *Cryptococcus laurentii*.

DISCUSSION

Fungi used in this study were chosen primarily on the basis of their importance as opportunistic pathogens of humans. According to the findings of National Nosocomial Infection Surveillance System (NNIS), 61 % of reported nosocomial fungal infections were due to *Candida albicans*, followed by other *Candida* spp. and *Cryptococcus* spp. [8]. In a previous study, ethanol (95 %) was observed as the best solvent for extracting antimicrobial substances [9]. The results in this study, obtained with 50 % aqueous ethanol extract, are similar to those reported in that study. It is important to bear in mind that the concentration of extract used in the test may correlate with the activity of its chemical components.

In another study, n-hexane, ethyl acetate, ethanol and aqueous extracts of *Hypericum havvae* were tested for their antimicrobial activity against *Escherichia coli*, *Enterobacter aerogenes*, *Alcaligenes faecalis*, *Salmonella typhimurium*, *Citrobacter freundii*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus brevis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Micrococcus luteus*, *Micrococcus flavus*, *Candida albicans*, *Rhodotorula rubra* and *Kluyveromyces fragilis* [10]. While extracts of the plant have shown

Table 1: Minimum inhibitory concentration of hydroethanol leaf and root

Microorganism	Minimum inhibitory concentration (MIC)			
	Leaf (mg/mL)	Root (mg/mL)	Leaf and root (1:1 ratio) (mg/mL)	Ketoconazole (µg/mL)
<i>Candida albicans</i>	3.12	6.25	1.56	0.25
<i>Candida tropicalis</i>	6.25	12.5	3.12	4
<i>Candida guilliermondii</i>	6.25	12.5	3.12	5
<i>Candida krusei</i>	12.5	25	6.25	4
<i>Candida glabrata</i>	25	25	12.5	2
<i>Candida parapsilosis</i>	25	25	12.5	2
<i>Cryptococcus neoformans</i>	6.25	12.5	3.12	0.25
<i>Cryptococcus laurentii</i>	3.12	6.25	1.56	4

strong antimicrobial activity against the test bacteria, they exhibited weak activity against the test yeast cultures.

Aqueous and ethanolic extracts obtained from *H. havvae* have been investigated previously for its ability to inhibit 35 hospital isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). Both aqueous and ethanol extracts of the plant were effective against MRSA. MIC and MBC values of the ethanol extract were 0.8 - 3.2 mg/mL and 1.6 - 6.3 mg/mL, respectively [11]. Based on our findings, the extracts of *H. havvae* showed strong antifungal activity against both *Candida* and *Cryptococcus* species. The difference between our results and those of other investigations may be due to several factors, such as intra-specific variability in the production of secondary metabolites. In addition, there may be differences in the extraction protocols used to recover the active metabolites as well as differences in the assay methods.

Hypericum species contain many phenolic compounds (hypericin, hyperforin and their derivatives, hyperoside, quercetin, chlorogenic acid, flavonols and flavones), suggesting that they could have important antioxidant properties [12]. Hypericin has shown antibacterial and anti-inflammatory activity and hyperforin is the main compound involved in antidepressant activity [13].

The results indicate that *H. havvae* exerted significant activity against both *Candida* and *Cryptococcus* species, especially *Candida albicans* and *Cryptococcus laurentii*. This activity may be indicative of the presence of metabolic toxins or the above-mentioned plant compounds. Therefore, this plant extract should be analyzed further, as it might provide new compounds that are effective against pathogens. According to the latest report from the National Nosocomial Infection Surveillance System (NNIS), 61 % of reported nosocomial fungal infections are due to *Candida albicans*, followed by other *Candida* spp. and *Cryptococcus* spp. [14].

CONCLUSION

This study provides data on the antifungal properties of the hydroethanol extracts of *H. havvae* against some clinically relevant fungi. These extracts may be further developed for treatment fungal infections, especially candidiasis.

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