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Report

Antifungal Activity of Endemic *Salvia tigrina* in Turkey

B Dulger* **and N Hacioglu**
Canakkale Onsekiz Mart University, Faculty of Science & Arts, Department of Biology, 17020 Canakkale, Turkey

Abstract

Purpose: *The ethanol extracts obtained from the leaves, rootstock and the combined formulation of endemic Salvia tigrina Hedge & Hub.-Mor. (Labiatea) have been investigated for their antifungal activities.*
Method: *The antifungal activity of the extract was tested against Candida species (C. albicans ATCC 10231, C. tropicalis ATCC 13803 and C. guilliermondii ATCC 6260 and Cryptococcus species (C. neoformans ATCC 90112 and C. laurentii 34142), Aspergillus flavus, Penicillium frequentans, Botrytis cinerea, Geotrichum candidum, Fusarium oxysporum and Alternaria alternara by the visual broth macrodilution method. Ketoconazole was used as a positive reference standard to determine the sensitivity of the strains.*
Results: *The minimum inhibitory concentration (MIC) ranged from 3.12 to 25 mg/mL. All the extracts exhibited a strong antifungal effect against the fungal cultures. The extracts exhibited greater antifungal effect against C. albicans, C. neoformans and B. cinarea.*
Conclusion: *The findings provide support for the use of this plant in traditional medicine for fungal infections especially against candidiasis.*

Key words*: Antifungal activity, Salvia tigrina, Plant extracts, Minimum inhibitory concentration*

****Corresponding author:*** *E-mail: basarandulger@yahoo.com*

INTRODUCTION

Turkey is regarded as an important gene-centre for the Labiatae family which is represented in Turkey by 45 genera, 546 species and a total of 731 taxa. The rate of endemism in the family is 44.2%. *Salvia* (Labiatae) is repesented in Turkey by 94 taxa belonging to 89 species with 50% endemism. *Salvia tigrina* Hedge & Hub.-Mor. is endemic to Turkey[1].

Several species of *Salvia* are used in folk medicine as antiseptics, astringents and spasmolytics[2]. Many studies have shown the antioxidant, antimicrobial and antiviral activities of some *Salvia* species[3-6]. *S. officinalis* and *S. fruticosa* are used as flavouring agents in perfumary[7]. The essential oil of *S. sclarea* shows significant antiinflammatory and moderate analgesic actions[8].

Although there have been many investigations on *Salvia* species, *Salvia tigrina* has not been previously studied, to the best of our knowledge. During our routine field exursion in Turkey, it was found that this plant is used to treat cold, bronchitis, urinary tract infections and, externally, boils, abscesses. Thus, the purpose of this study is to investigate the antifungal activity of selected organic solvents extracts of this plant against some pathogenic fungi.

MATERIALS AND METHODS

Plant material

The plant material was collected from Icel, Turkey in September, 2007. Voucher specimens of the plant were deposited in the Biology Department at Canakkale Onsekiz Mart University and identified by Ersin Karabacak from the same Department.

Preparation of extracts

The plant parts (leaf and rootstock) were air-dried. The dry powdered plant material (20 g), either of the leaf or the roortstock, was soaked in the %50 ethanol until complete saturation of the plant material. The extract was filtered using Whatman filter paper no. 1, and the filtrate solvent was evaporated under vacuum using a rotary evaporator at 55°C. The resulting dried extract was stored in labeled sterile screw-capped bottles at -20°C. The extract (in the form of sticky black substances) amounting to around 2 g was dissolved in 0.1 mL of DMSO (5 mg/g) (dimethyl sulfoxide) before testing. The combination of plant extracts, leaf and rootstock, (1:1 ratio) was also used in this test[9]. *Candida* species (*C. albicans* ATCC 10231*, C. tropicalis* ATCC 13803 and *C. guilliermondii* ATCC 6260) and *Cryptococcus* species (*C. neoformans* ATCC 90112 and *C. laurentii* 34142), *Aspergillus flavus, Penicillium frequentans, Botrytis cinerea, Geotrichum candidum, Fusarium oxysporum* and *Alternaria alternara* which were used as the test fungi were obtained from the Microbiology Research Laboratory in Canakkale Onsekiz Mart 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

MICs were performed by the visual broth macrodilution method[10]. Fungal suspensions were diluted added to RPMI-1640 medium without bicarbonate (buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid) broth supplemented with glutamine, to a concentration of approximately 0.5 x 10^5 CFU/mL (verified by colony counts in SDA). A twofold 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, 0.390–200 mg/mL. Controls used were medium without antifungal agents were used in the test. The results for the extracts were compared with a standard, ketoconazole. The tubes were then incubated at 35°C for 24 - 48h. MIC was defined as the lowest concentration that did not yield visual growth. All experiments were performed in triplicate.

RESULT

The MICs values of the extracts are presented in Table 1. The MIC results for the ethanol extract of the leaf, rootstock and the

Table 1: Minimum inhibitory concentration of the ethanol leaf and rootstock

Microorganisms	Minimum inhibitory concentration (MIC)			
	Leaf (mg/mL)	Rootstock (mg/mL)	Leaf and rootstock (1:1 ratio) (mg/mL)	Ketoconazole (50 µg/mL)
Candida albicans	6.25	12.5	3.12	0.25
Candida tropicalis	25	12.5	3.12	4
Candida guilliermondii	25	25	12.5	5
Cryptococcus neoformans	12.5	25	3.12	0.25
Cryptococcus laurentii	25	25	12.5	4
Aspergillus flavus	25	25	12.5	0.25
Geotrichum candidum	25	12.5	6.25	0.25
Fusarium oxysporum	25	25	12.5	0.12
Penicillium frequentans	25	25	12.5	2
Botrytis cinerea	12.5	6.25	3.12	0.25
Alternaria alternata	25	25	12.5	0.5

combination of both, ranged from 6.25 - 25, 12.5 - 25 and 3.12-12.5 mg/mL, respectively, and showed that activity of the extracts varied from one fungal strain to another. The extract combination (both leaf and rootstock) exhibited stronger antifungal activity than the individual extract. *Candida albicans, Cryptococcus neoformans* and *Botrytis cinarea* with MIC of 3.12 mg/mL were more susceptible to the extract combination than other fungi, followed by *Candida guilliermondii* and *Geotrichum candidum* with MIC of 6.25 mg/mL. The MIC for the other fungi was 12.5 mg/mL. However, the extracts and extract combinations were less active than the standard antifungal agent, ketoconazole.

DISCUSSION

Fungi used in this study were chosen primarily on the basis of their importance as pathogens of humans and plants. *Botrytis cinerea* is a fungus that affects many plant species, although its most notable hosts may be wine grapes. In viticulture, it is commonly known as botrytis bunch rot; in horticulture, it is usually called grey mould or gray mold. According to findings from the 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.[11]. *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.

Ethanol was found to the best solvent for extracting antimicrobial substances in a previous study [12]. The results in this study with ethanol are similar to those reported in the mentioned study. It is important to note that the concentration of extract used in the test may be correlated with a high activity of its chemical components.

The major components of *Salvia* species growing in Turkey are α-pinene, β-pinene, β-thujone, camphor, carvacrol, lynalyl acetate, sabinyl acetate and 1,8-cineole[13]. However, the composition of the essential oil of *S. tigrina* has not been reported. The mentioned substances may be responsible for the antifungal activity in *S. tigrina*. The antifungal activities of the essential oils of *Salvia*

lavandulifolia, S. officinalis and *S. sclarea* against various *Candida* species were reported to be high[14]. In another study, the essential oil of *S. multicaulis, S. kronenburgii* and *S. verticillata* were tested against *Candida albicans* and some bacteria and were foud to be very effective especially against *Candida albicans*[15]. The essential oil of *S. lachnocalyx* were investigated for antimicrobial activity against fungal cultures. The bioassays showed significant inhibition against fungi with minimum inhibitory concentration in range of 5-10 mg/mL[16]. The result of antifungal effects obtained in this study are similar to those reported in the above studies.

CONCLUSION

The results obtained in this work are in agreement with recent studies regarding antimicrobial activities of members of the Labiatae family. *Salvia tigrina* could be of importance in the search of new natural sources of bioactive compounds.

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