

Antifungal activity of *Salvia verticillata* subsp. *verticillata* against fungal pathogens

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ABSTRACT

Aim: It was aimed to investigate the antifungal activity of the individual as well as the combine extract (leaf+root) of *Salvia verticillata* subsp. *verticillata* L. from Lamiaceae family which has ethnobotanical use.

Material and Methods: The plant samples were collected during to flowering stage in June, Samandere village around, Duzce, Turkey. The collected plants were dried according to herbarium techniques. The powdered plant material was extraction using ethyl alcohol (96%). Antifungal effects of the extracts was evaluated for yeasts (*Candida krusei* ATCC 20298, *C. parapsilosis* ATCC 90112, *C. guilliermondii* ATCC 6260, *C. albicans* ATCC 10231, *C. tropicalis* ATCC 13808, *C. glabrata* ATCC 2001, *Cryptococcus laurentii* ATCC 34142, *C. neoformans* ATCC 90112, *Geotrichum capitatum* ATCC 28576, *Debaryomyces hansenii* DSM 70238, *Rhodotorula rubra* DSM 70403, and *Kluyveromyces fragilis* ATCC 8608) by visual broth microdilution method. Antifungal antibiotic ketoconazole was used in the study to compare the results.

Results: MIC values ranged from 1.56 to 25.00 mg/mL. The extracts have shown potential antifungal effects against the yeast cultures. Notably, the combine plant extracts (both leaves and rootstock) possessed higher antifungal potency against *C. neoformans*, *C. laurentii* and *G. capitatum*.

Conclusion: Our results demonstrated that the extracts of *Salvia verticillata* subsp. *verticillata* are used for the treatment of fungal infections as traditional medicine.

Keywords: *Salvia verticillata*; plant extracts; antifungal activity.

Salvia verticillata subsp. *verticillata* Bitkisinin Fungal Patojenlerine Karşı Antifungal Aktivitesi

ÖZ

Amaç: Bu çalışmada, Lamiaceae familyasından *Salvia verticillata* subsp. *verticillata* L. (Lamiaceae) bitkisinden elde edilen yaprak, kök ve kombine (yaprak+kök) ekstrelerinin antifungal aktivitesinin araştırılması amaçlandı.

Gereç ve Yöntemler: Bitki örnekleri, Düzce ili, Samandere köyü civarında haziran ayında çiçeklenme döneminde toplanmıştır. Toplanan bitkiler herbaryum tekniklerine göre kurutuldu. Toz haline getirilmiş bitki materyalinin, etil alkol (96%) kullanılarak ekstraksiyonu yapıldı. Tıbbi maya kültürleri (*Candida krusei* ATCC 20298, *C. parapsilosis* ATCC 90112, *C. guilliermondii* ATCC 6260, *C. albicans* ATCC 10231, *C. tropicalis* ATCC 13808, *C. glabrata* ATCC 2001, *Cryptococcus laurentii* ATCC 34142, *C. neoformans* ATCC 90112, *Geotrichum capitatum* ATCC 28576, *Debaryomyces hansenii* DSM 70238, *Rhodotorula rubra* DSM 70403 ve *Kluyveromyces fragilis* ATCC 8608) kullanılarak, görsel sıvı mikrodilüsyon yöntemi ile değerlendirildi.

Bulgular: Çalışmada sonuçları karşılaştırmak için antifungal antibiyotik ketokonazol kullanıldı. MİK değerlerinin 1.56 ile 25.00 mg / mL arasında değiştiği saptandı. Ekstreler, maya kültürlerine karşı güçlü antifungal etki sergilemiştir. Ancak bitki (yaprak+kök) ekstre kombinasyonu, *Cryptococcus neoformans*, *C. laurentii* ve *Geotrichum capitatum* maya kültürlerine karşı daha güçlü antifungal etkiye sahip olduğu belirlenmiştir.

Sonuç: Sonuçlarımız, *Salvia verticillata* subsp. *verticillata* ekstrelerinin mantar enfeksiyonlarının tedavisi için geleneksel tıpta kullanımını destekler niteliktedir.

Anahtar Kelimeler: *Salvia verticillata*; bitki ekstreleri; antifungal aktivite.

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INTRODUCTION

Salvia L. (Lamiaceae) is represented by ninety-eight species, four subspecies and three varieties, of which fifty-six are endemic in Turkey (1,2). *Salvia* species are widely used in Anatolia for the treatment of stomach pains, colds and sore throats (3-5). In addition, these plants are used to stop bleeding and treat inflammatory skin diseases, or for wounds as an antiseptic (6).

Salvia verticillata subsp. *verticillata* is named as "dadirak" and subsp. *amasiaca* is locally known as "hart şalbası" in Turkey (7). Both subspecies are known as "Karabaş otu" in the Eastern part of Turkey and are used to treat cold and gastrointestinal disorders (8).

During routine excursions, it was determined that *Salvia verticillata* subsp. *verticillata* is used for colds, stomach aches and as an antiseptic for wounds. The aim was to reveal the antifungal effect of the ethanol extracts obtained from different part of the plant against the fungal disease agents.

MATERIAL AND METHODS

Plant Materials

The plant samples were collected during to flowering stage in June, 2020 at an altitude of 780 m, Samandere village around, Duzce, Turkey. The plant was identified by Dr. Ersin Karabacak from Canakkale Onsekiz Mart University, Canakkale, Turkey. A voucher sample was kept in author's personal collections (voucher number; GD.109-5)

Preparation of Extract

Each dry powdered plant material (50 g) was extracted with 80% ethanol by maceration. The extract was concentrated and filtered using a rotary evaporator at 40°C (Yield: 5.6% on dried weight). The extract was dissolved in DMSO to a final concentration of 1 g/mL for preliminary screening. The combine extract (1:1 ratio) was used in the study (9). The yeast cultures (*Candida krusei* ATCC 20298, *C. tropicalis* ATCC 13808, *C. guilliermondii* ATCC 6260, *C. albicans* ATCC 10231, *C. glabrata* ATCC 2001, *C. parapsilosis* ATCC 90112, *Cryptococcus neoformans* ATCC 90112, *C. laurentii* ATCC 34142, *Geotrichum capitatum* ATCC 28576, *Rhodotorula rubra* DSM 70403, *Kluyveromyces fragilis* ATCC 8608 and *Debaryomyces hansenii* DSM 70238) were used for evaluating antifungal activity.

MIC (Minimum Inhibitory Concentration) Determination

MICs were applied according to the method described by Dulger (2009) (10) by the visual broth macrodilution method (11). The concentration was 0,390-200 mg/mL. Medium without antifungal samples were used as controls. Antifungal antibiotic ketoconazole was used in the study to compare the results. Tubes not providing visual growth were determined as the lowest concentration. All of the experiments were done in triplicate.

RESULTS

All of the extracts were found to be active against the yeasts with MIC values ranging between 0.25 mg/mL and 25 mg/mL (Table 1). The MIC findings for the leaf, rootstock and the combine extract ranged from 3.12-25, 6.25-25 and 1.56-12.5 mg/mL, respectively. As

compared the findings with those of the antibiotic Ketoconazole, the combine extract (leaf+rootstock) was exhibited more potential antifungal effect than those of the other extracts.

The combine extracts against *C. laurentii* have shown stronger antifungal effect than the standard antibiotic ketoconazole with MIC value 1.56 mg/mL. Notably, *Cryptococcus neoformans* and *Geotrichum candidum* have shown the same MIC value 1.56 mg/mL but these values were far ineffective than those of the standard antibiotic ketoconazole with MIC values 0,25 and 1.00 mg/mL, respectively.

The combine extracts against *C. glabrata* have susceptible to the extract with MIC values 3.12 mg/mL, followed by *C. albicans* and *C. guilliermondii* with MIC values 6.25 mg/mL. *Candida tropicalis*, *C. parapsilosis*, *D. hansenii* and *K. fragilis* have shown a moderate activity with MIC value 12.5 mg/mL. Besides, the combine extract has shown potent effect against *C. krusei* and *R. rubra* (MIC value; 6.25 mg/mL). It can be said clearly, the combine extracts have strong antifungal effects against *Cryptococcus laurentii*, *C. neoformans* and *Geotrichum candidum*.

Table 1. Minimum inhibitory concentration of the ethanol leaf and root stock extracts

Microorganisms	Minimum Inhibitory Concentration (MIC)			
	Leaf (mg/mL)	Root stock(mg/mL)	Leaf and Root stock (mg/mL)	The Standard Antibiotic (mg/mL)
<i>Candida albicans</i>	6.25	12.5	6.25	0.25
<i>Candida tropicalis</i>	12.5	12.5	12.5	4.00
<i>Candida guilliermondii</i>	12.5	25	6.25	5.00
<i>Candida krusei</i>	25	25	25	4.00
<i>Candida glabrata</i>	6.25	12.5	3.12	2.00
<i>Candida parapsilosis</i>	25	25	12.5	2.00
<i>Cryptococcus neoformans</i>	3.12	6.25	1.56	0.25
<i>Cryptococcus laurentii</i>	3.12	6.25	1.56	4.00
<i>Geotrichum capitatum</i>	3.12	6.25	1.56	1.00
<i>Rhodotorula rubra</i>	25	25	25	2.00
<i>Debaryomyces hansenii</i>	25	25	12.5	4.00
<i>Kluyveromyces fragilis</i>	6.25	12.5	12.5	4.00

DISCUSSION

In a previous study, it is reported that the essential-oil of *S. verticillata* consisted mainly of germacrene D, the sesquiterpenes, α -humulene and B-caryophyllene (2). Besides, germacrene D (10-16%) was determined as the main compound for *S. verticillata* subsp. *verticillata* from Turkey (12). In another study, the major components were hexahydrofarnesyl acetone (7.8%), limonene (4.1%), α -pinene (8.2%) and spathulenol (31.0%) were reported for the same plant (2). On the other hand, 1,8-cineole, limonene, β -phellandrene and β -pinene were the main components for essential oil of *S. verticillata* subsp. *amasiaca* (13).

There is limited the antimicrobial activity studies on *S. verticillata*. The methanol extracts obtained from flowers and leaves of *S. verticillata* L. subsp. *amasiaca* (Frey&

Bronm). Bornm. have been investigated for their antibacterial activity against nine test bacteria by the macro broth dilution. The extracts have strong antimicrobial effects against *P. aeruginosa*, *S. enteridis*, *E. coli*, *B. cereus*, *B. subtilis* and *S. aureus* (14). In another study, essential oil of *S. verticillata* subsp. *amasiaca* has shown potential activity with MIC values 196 mg/mL against *Mycobacterium tuberculosis* H37Ra (5). In addition, it is reported that 27 aqueous extracts of twenty-one plants (including *S. verticillata*) used to treat respiratory tract infections as folk remedy were evaluated for their antioxidant properties, total phenolic contents and antimicrobial activity (15). These extracts (including *S. verticillata*) have stronger antifungal effect against *Candida krusei* than ketoconazole used as reference antibiotics and 6 extracts obtained from the plants exhibited antifungal effect against *Epidermophyton floccosum* and three extracts were potential effective against *Trichophyton rubrum*. Based on our findings, the extract of *S. verticillata* subsp. *verticillata* shown strong antimicrobial activity against the tested fungal species. The findings of the present research have also shown similar antifungal activity with better efficiency. The similarities show that the investigated plant possess broad spectrum antifungal effects. However, due to the environmental conditions in which the plant grows and the test fungi used, there is a difference between our findings and the reported data.

CONCLUSION

S. verticillata subsp. *verticillata* used in this study could be prospective source of novel antifungal antibiotics. Besides, this study validates the use of *S. verticillata* subsp. *verticillata* as antifungal agent in traditional medicine in Turkey.

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