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Determination of Antifungal and Antioxidant Activities of *Salvia tomentosa* Mill

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ABSTRACT: In this study, was determined antifungal and antioxidant activity of *Salvia tomentosa*. The essential oil of *Salvia tomentosa* was determined of the percentages of mycelium inhibition agains plant pathogenic fungi *Rhizoctonia solani* and *Alternaria solani* 40.63% and 60.13% respectively. To determine the antioxidant capacity, Free radical reduction (scavenging), iron reduction power (FRAP), copper reduction power (CUPRAC) and free radical cations reduction (TEAC), total phenolic (TP) and total flavonoids (TF) tests were performed. The results of the tests, scavenging activity DPPH lC_{50} 15.08±1.28 (μg mL^{-1}), FRAP 1.81±0.27 mmol TE g^{-1} extract, CUPRAC 5.57±0.20 mmol TE g^{-1} extract and TEAC ise 3.30±0.33 lC_{50} (μg mL^{-1}) was determined. Total phenolic and total flavonoid was determined to be extracted content 131.34±8.39 mg GAE g^{-1} of extract and 35.05±2.82 QA g^{-1} respectively.

Keyword; Antifungal, antioxidant, *Salvia tomentosa*, total phenolic, essential oil

Salvia tomentosa Mill'in Antifungal ve Antioksidant Aktivitesinin Belirlenmesi

ÖZET: Bu çalışma, *Salvia tomentosa*'nın antifungal ve antioksidant aktivitesi belirlenmesi amacıyla yürütülmüştür. *Salvia tomentosa*'nın uçucu yağı bitki patojeni fungus olan, *Rhizoctonia solani* ve *Alternaria solani* 'nin miselyum gelişim sırasıyla % 40.63 ve % 60.13 engellemiştir. Antioksidan kapasitesini belirlemek için, Serbest Radikal Giderme (DPPH), Demir İndirgeme Gücü (FRAP), Bakır İndirgeme Gücü (CUPRAC) ve Serbest Katyon Radikali Giderme (TEAC) antioksidan aktivite testleri ile Toplam Fenolik (TP) ve Toplam Flavonoid (TF) miktarı analizleri yapılmıştır. Yapılan antioksidan testleri sonucunda, DPPH lC_{50} 15.08±1.28 (μg mL^{-1}), FRAP 1.81±0.27 mmol TE g^{-1} eksrakt, CUPRAC 5.57±0,20 mmol TE g^{-1} ekstrakt ve TEAC ise 3.30±0.33 lC_{50} (μg mL^{-1}) olarak belirlenmiştir. Toplam fenolik ve toplam flavanoid içeriği sırasıyla 131.34±8.39 mg GAE g^{-1} ekstrakt ve 35.05±2.82 mg QE g^{-1} ekstrakt olarak tespit edilmiştir.

Anahtar Kelimeler; Antifungal, antioksidant, *Salvia tomentosa*, toplam fenolik, uçucu yağ

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INTRODUCTION

Numerous plants have been used for various purposes, like perfumery, drugs and food. Salvia species, a member of Lamiaceae family, one of the most significant species of this group. Salvia species, usually used as sage, have been used since old times for more primarily to treat colds, bronchitis, tuberculosis, cancer, diabetes, bleeding and menstruous diseases. In addition, this species are used as traditional medicines all around the world, in possession of antitumor, antidiabetic, antioxidant, and antibacterial features. (Heath, 1981;Topcu, 2006; Celep et al., 2009). Salvia species incorporate differend secondary metabolites like diterpenoids, essential oils, sterols, flavonoids, sesquiterpenoids, sesterpenoids, and flavonoids (Ulubelen et al., 1997; Esquivel et al., 2000). The some of phenolic compounds of plants member of this genus have shown perfect antimicrobial activity. Besides, Salvia species have scavenging activity of active oxygen, as in superoxide anion radicals, singlet oxygen and hydroxyl radicals (Masaki, et al., 1995; Tepe, et al., 2005)

Salvia tomentosa is a species common in Turkey. This plant is a perennial flowering plants in April-August. Body upright and 1 m long, four-cornered, and often It is branched. Flower stalks 5-10 mm, calyx is 12-16 mm (Davis, 1982). Considering have a significant medical potential of plants in Turkey, information of the this issue and works on plants are limited (Tepe, et al., 2005).

In this study, was aimed to determine of the antioxidant and antifungal activity of the constituents of *S. tomentosa* grown naturally in Kahramanmaras, Turkey.

MATERIALS AND METHODS

Plant Material

The flowered branches of *S. tomentosa* were collected from Turkoglu /Kahramanmaras, Turkey, flowering stage in Jun 2016. Plant materials were dried under shadow and room temperature. The plant specimens were identified by Dr. Melih Yılar.

Extraction of Essential Oils

The wet plant material was exposed to hydro distillation for 2 h using a Neos essential oils system. The essential oil from dried parts samples were preserved in a sealed vial at 4 °C until analysis.

Total Phenolic Contents

The total phenolic compound was determination by Folin-Ciocalteus reagent with small modification (Slinkard and Singleton, 1977)**.** For this purpose, plant extract 0.1 ml was mixed with distille water 4.6 ml. On 0.3 ml of Na2CO3 solution (2%) and 0.1 ml of Folin-Ciocalteus reagent was added to the mixed. It was incubated for 2 hours at room conditions. Absorbance was measured at 760 nm. As result of gallic acid equivalents (GAE) g^{-1} of extract was calculated.

Determinations of Total Flavonoid Assay

The plant sample total flavonoid contents were determined by (Chang, at al., 2002). Plant extract 0.1 ml was mixed with methanol 4.8 ml. 0.1 ml Al (NO_3) (10%) and 0.1 ml NH_4CH_3COO solution (1M) was added to the mixed. It was incubated for 45 munite at room conditions. After the absorbance were measured at 415 nm. As result of quarcitin equivalents (QE) g^{-1} of extract was calculated.

DPPH· Free Radical-Scavenging Activity

The free radical scavenging activity were determined by Liana-Pathirana made a few changes in the method (Liyana-Pathirana and Shahidi, 2005). Plant methanol ekstracts was differend amounts stock solutions by placing of test tube and volume was completed to 3 ml with ethyl alcohol. Over them 1 ml of DPPH· solution (0.26 mM) was added and stirred with the help of vortex. After standing for 30 minutes in a dark ambient, the absorbance was read at 517 nm. The data obtained were expressed as IC_{50}. The radical scavenging activity was calculated from the equations.

$$I_{DPPH=}100.[A_{control}-(A_{sample}-A_{blank})] / A_{control}$$

Iron Ion Reducing Antioxidant Power (FRAP)

The FRAP analysis method applied by Oyaiz (1986) was performed through amendments. 0.25 ml plant extract of 0.2 M phosphate buffer (pH 6.6) with 1.25 mL complete. On 1.25 ml of potassium ferricyanide [K_3 Fe $(CN)_6$] solution (1%) was added. The mixture was incubated at 50 ° C for 20 minutes. The mixture cooling to room temperature TCA (1.25 mL, 10%) and FeCl3 (0.25 mL, 0.1%) solution were added. After stirring vortex was measured absorbance

at 70 nm. The results obtained was Trolox equivalent (TE), calculated.

Cupric Reducing Antioxidant Capacity (CUPRAC)

The cunpric antioxsidant capacity was determined by Chang (2002), with slight modifications. Results were compared with used in standard antioxidants BHA and BHT absorbance.

Fungal Cultures

The plant pathogenic fungi used were obtained from the stock cultures of the Department of Plant Protection, Faculty of Agriculture, University of Ahi Evran, Turkey.

***In Vitro* Antifungal Effect of the Essential Oils**

The antifungal activities of essential oil were determined by the agar well diffusion method (Tepe, et al., 2005). The PDA were autoclaved and cooled to 40^0. Later were transferred to 60 mm petri dishes (10 ml petri^{-1}). 5 mm diameter wells were opened on the PDA inside the petri dishes. The plant essential oils were transferred 1, 5 and 10 µl petri^{-1} inside the wells. Mycelium disks of 5 mm were later placed at equal distances to these wells. The fungi transferred petri dishes were incubated at 22±2°C. According to mycelium inhibitions was calculated the formulated.

$$I=100\times(DC-DT)/DC$$

I: Inhibition percentage compared to the control (mycelium development)

DC: Mycelium development in the control

DT: Mycelium development in essential oils applications

RESULT AND DISCUSSION

***In Vitro* Antifungal Results**

Essential oils of the *S. tomentosa*, were found to be significantly effective mycelium grow on *R. solani* and *A. solani*. The antifungal activity properties of essential oil of the *S. tomentosa in-vitro* are shown in table 1.

Table 1. Antifungal activity values (Inhibition (%) and Inhibition zone) for *S. tomentosa* Mıll. essential oil.

Doses (µl petri^{-1})	**Plant Pathogens**			
	****R.s**		**A.s**	
	I(%)	**Iz (mm)**	**I(%)**	**Iz(mm)**
NC	0.00^{c*}±0.0	60	0.00^{d}±0.0	60
1	0.00^{c}±0.0	60	16.46^{c}±1.16	50.12
5	20.06^{b}±0.17	47.96	29.50^{b}±1.21	42.3
10	40.63^{a}±2.71	35.61	60.12^{a}±1.43	23.92

* According to DUNCAN, the averages with different letters in the same column are different at the significance level of p<0.05
***A. solani* (A.s), *R. solani* (R.s) Negative control (NC)

The essential oil had antifungal activity agains plant pathogenic fungi *R.solani* and *A. solani* 40.63% and 60.13% respectively. The essential oil a great had antifungal activity of agains *A. solani* and the least agains *R. solani*. Provious differet studies of the *S. tomentosa*, was reported antifungal activity. Yılar and Kadioglu (2016) *S. tomentosa* essantial oil, methanol and aquatic extract, have reported antifungal activity of *F. oxysporum* f. sp. *radicis lycopersici*. Haznedaroglu et al (2001) essential oil of the *S. tomentosa* have reported antibactericidal effects. This study and similar previous studies showed that essential oil of the *S. tomentosa* are effective mycelium development on *R. solani* and *A. solani*.

Antioxidant Activity Results

Table 2. Antioxidant activity of *S. tomentosa* of the methanol extract by TP, TF, DPPH, TEAC, Reducing power and CUPRAC[a].

Sample	TP mg GAE g^{-1} extract	TF mg QE g^{-1} Extract	DPPH· IC_{50} (µg mL^{-1})	TEAC IC_{50} (µg mL^{-1})	Reducing power mmol TE g^{-1} extract	CUPRAC mmol TE g^{-1} extract
Methanol extract	131.34±8.39	35.05±2.82	15.08±1.28	3.30±0.33	1.81±0.27	5.57±0.20
BHA[b]			4.52±0.67	3.30±0.05	6.60±0.16	15.99±1.07
BHT[b]			8.45±0.67	9.40±0.55	2.74±0.35	15.25±0.61
Trolox[b]			3.50±0.03	4.80±0.06		

[a] IC50 values represent the means the standard deviation of three parallel measurements ($p < 0.05$).
[b] Standart compounds

The total flavonoid and total phenolic, antioxidant activity of *S. tomentosa* methanol extract are shown in Table 2. The results of total phenolics content of the sample analysed was 131.34±8.39 gallic acid equivalents mg (GAE) g^{-1} of extract. Total phenolic content of *S. tomentosa* extracted with various solvents ranged widely, among 10 and 275 µg GAE mg^{-1} (Tepe et al., 2005; Erdogan-Orhan, et al., 2010). In our studies result, was 131.34 mg (GAE) g^{-1}, which is support to with our results.

The total flavonoid content of *S. tomentosa* methanolic extract was 35.05±2.82 quarcitin equivalents mg (QE) g^{-1} of extract table 3. Previous studies, Dincer et al (2013) reported that the total flavonoid content of *S. tomentosa* 40.83 mg of CE g^{-1} dw, which is support to with our results. Also total pheneolic and flavonoid content, results maybe effected of the geographical positions of the plants, ecological conditions, and climate (Papageorgiou et al., 2008).

The plant seconder metabolites, sach as phenolics and flavonoids, have antioxidant activity because thire redox feature (Baba et al., 2015). The methanol extract IC_{50} values of *S. tomentosa* 15.08 mg/ml DPPH· table. Dincer et al (2013) have reported that IC_{50} values of *S. tomentosa* methanol extract 2.29 mg dw mg^{-1}. ABTS scavining ativity of *S. tomentosa* methanol extract are presented table 3. The ABTS activity of methanol extract were found to be beter than standart BHT and Trolox, but with same BHA. The table.. showed the reducin power of the methanol extract and standart, such as BHA and BHT. The excellent reducing power methanol crude extract 1.81±0.27mmol TE g^{-1} extract, compare with standart BHA and BHT. CUPRAC reducin power of methanol extract sample 5.57±0.20 mmol TE g^{-1} extract was determined. The CUPRAC reducin power activity of methanol extract were found to be better than standart BHA and BHT table 2. *S. tomentosa* methanol extract was exhibited strong of the antioxidant actvity, fonolics compoud or flavonoids may be also play important roles in the activity.

CONCLUSION

In this study, was determined antifungal and antioxidant activities *S. tomentosa* The results of this work have shown essentail oil significant antifungal activity against *R. solani* and *A. solani*. As a result of, methanol extract from *S. tomentosa* was showed considerable antioxidant activity. Also, methanol extract was determined content total phenolic and flavonoid. These studies, can be used in management of plant pathogenic fungi diseases control.

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