Research Article/Araştırma Makalesi

www.dergipark.gov.tr/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES (2018) 31(2): 93-99
DOI: 10.29136/mediterranean.362163

Determination of the chemical components, antioxidant and antifungal activities of essential oil and plant extract of *Salvia candidissima* Vahl.

Salvia candidissima Vahl.'ın uçucu yağ ve ekstraktının kimyasal bileşenlerinin, antioksidan ve antifungal aktivitesinin belirlenmesi

Yusuf BAYAR[1], **Nusret GENÇ**[2]

[1]Ahi EvranUniversity, Faculty of Agriculture, Department of Plant Protection, 40200 Kirsehir, Turkey
[2]University Gaziosmanpasa, Department of Chemistry, Faculty of Science and Art, 60240 Tokat, Turkey

Corresponding author (*Sorumlu yazar*): Y. Bayar, e-mail (*e-posta*): yusufbayan@gmail.com
Author(s) e-mail (*Yazar(lar) e-posta*): nusretgenc@gmail.com

ARTICLE INFO

Received 05 December 2017
Received in revised form 02 April 2018
Accepted 12 April 2018

***Keywords*:**

Antioxidant
Antifungal
Essential oil
Chemical component
Salvia candidissima Vahl.

ABSTRACT

The aim of this study is to investigate the chemical components, antioxidant and antifungal activities of the essential oil and plant methanolic extract of *Salvia candidissima* Vahl. plant, which is one of the sage species. By using Gas Chromatography (GC) and Gas Chromatography Mass Spectrometry (GC-MS**)** method, it was determined that there are 54 components in the essential oil of *S. candidissima*. These identified components were found to constitute 99.98% of the essential oil. The main components of the essential oil were determined as Spathulenol (12.75%), Caryophyllene oxide (8.67%), Ledene oxide (6.98%) and o-Cymene (6.03%). A antifungal activity of the essential oil against *Rhizoctonia solani* and *Aternaria solani* was found. As a result of the study, it was determined that the mycelial growth of *A. solani* was inhibited by 57.92% as a result of application of 10 µL petri^{-1} essential oil, while that of *R. solani* was inhibited by 51.87%. As a result of the antioxidant study conducted with plant extract, the values of Free Radical Scavenging DPPH (lC_{50} 22.96 ± 0.45), Iron Reduction Power (FRAP 1.20 ± 0.16 mmol TE g^{-1} extract), Copper Reduction Power (CUPRAC 3.30 ± 0.12 mmol TE g^{-1} extract) and Free Cation Radical Scavenging TEAC (lC_{50} 9.25 ± 0.40 (µg ml^{-1})) were determined. The Total Phenolic (TP) and Total Flavonoid (TF) contents were found as 83.53 ± 5.92 mg GAE g^{-1} extract and 59.02 ± 3.59 mg QE g^{-1} extract, respectively. These results showed that the essential oil and plant methanolic extract of *S.candidissima* has a significant antifungal activity against plant pathogenic fungi and strong antioxidant activity.

MAKALE BİLGİSİ

Alınış tarihi 05 Aralık 2017
Düzeltilme tarihi 02 Nisan 2018
Kabul tarihi 12 Nisan 2018

***Anahtar Kelimeler*:**

Antioksidant
Antifungal
Uçucu yağ
Kimyasal bileşen
Salvia candidissima Vahl.

ÖZ

Bu çalışmanın amacı, ada çayı (*Salvia candidissima* Vahl.) bitki uçucu yağının bileşenlerinin ve bitki patojeni fungal etmenlere karşı antifungal etkinlikleri, bitki metanolik ekstraktının ise antioksidan aktivitelerinin araştırılmasıdır. *S. candidissima* uçucu yağında GC/MS kullanılarak 54 bileşen belirlenmiştir. Tanımlanan bu bileşenler uçucu yağın % 99.98'ini oluşturduğu saptanmıştır. Uçucu yağın temel bileşenleri Spathulenol (% 12,75), Caryophyllene oxide (% 8.67), Ledene oxide (% 6.98) ve o-Cymene (% 6.03) olarak belirlenmiştir. Uçucu yağın, antifungal etkinliği fungal hastalık etmenlerinden *Rhizoctonia solani* ve *Aternaria solani*'ye karşı araştırılmıştır. Çalışma sonucunda 10 µl petri^{-1} uçucu yağ uygulaması sonucunda *A. solani*'nin miselyum gelişimini % 57.92 oranında *R. solani*'nin miselyum gelişimini ise % 51.87 oranında engellediği belirlenmiştir. Bitki ekstraktları ile yapılan antioksidan çalışması sonucunda, Serbest Radikal Giderme DPPH (lC_{50} 22.96 ± 0.45), Demir İndirgeme Gücü (FRAP 1.20 ± 0.16 mmol TE g^{-1} eksrakt), Bakır İndirgeme Gücü (CUPRAC 3.30 ± 0.12mmol TE g^{-1} ekstrakt) ve Serbest Katyon Radikali Giderme TEAC ise (lC_{50} 9.25 ± 0.40 (µg ml^{-1})) olarak belirlenmiştir. Toplam Fenolik (TP) ve Toplam Flavonoid (TF) içeriği sırasıyla 83.53 ± 5.92 mg GAE g^{-1} ekstrakt ve 59.02 ± 3.59 mg QE g^{-1} ekstrakt olarak tespit edilmiştir. Bu sonuçlar; *S. candidissima* uçucu yağın bitki patojeni funguslara karşı dikkate değer bir antifungal aktiviteye sahip olmasının yanısıra bitki metanolik ekstraktları güçlü bir antioksidant kapasitesine sahip olduğunuda göstermiştir.

© Akdeniz Üniversitesi Ziraat Fakültesi

1. Introduction

The *Salvia* species is one of the largest species in the Labiatae family. *Salvia* L. (Lamiaceae) species consists of about 900 species which spread around the world and Turkey is an important country in terms of *Salvia* species. There are 97 species and 113 taxons in the flora of Turkey, and 51 of these species are endemic to Turkey and the endemism rate of 52.5% is quite high (Çadırcı et al. 2012; Şenkal et al. 2012; Karık 2013). Because of the chemical components that they possess, *Salvia* species are of great importance both in medicine and are economically important in terms of species and distribution. Many types of *salvia* are used in pharmaceutical, cosmetic, perfumery and food industries as well as in parks and gardens for decorative purposes. It is also known that it is commonly used in the world in alternative medicine (Chalchat et al. 1998; Demirci et al. 2003). The antibacterial, antifungal, and antioxidant activities of many *Salvia* species have been reported by various researchers (Delamare et al. 2007; Kelen and Tepe 2008; Yılar and Kadıoğlu 2016).

In nature, many environmental factors, stress and external factors influence free radicals to occur during normal metabolic activities in humans and animals. Free radicals, known as reactive oxygen species, react easily with biological molecules, such as, nucleic acids, lipids, carbohydrates and proteins in organisms due to being reactive short-lived and unstable. As a result of these radicals, aging in the human body, as well as numerous cancers, immune system diseases, liver diseases are shown as the cause (Halliwell and Gutteridge 1990; Young and Woodside 2001; Serteser and Gök 2003).

Being one of the most important in *Salvia* species, *Salvia candidissima* Vahl., a perennial herbaceous plant, grows up to 700-2000 m from sea level and can be 30-60 cm long and it grows in Central and Eastern Anatolia in our country, and can also be found on roadsides, rock, limestone and bush fields (Karabacak 2009).

This study was carried out to investigate the chemical components, antioxidant and *in-vitro* antifungal activity of the essential oil and methanolic extract of *S. candidissima* plant which is naturally grown in Kahramanmaraş province.

2. Materials and Methods

2.1. Collection of plant material

In this study, the plant material used *S. candidissima* was collected from the natural environment of Türkoğlu district of Kahramanmaraş in June of 2016. The plant diagnosis was conducted by Asst. Assoc. Dr. Melih YILAR. The collected plant material was taken in the flowering period and dried in a shady environment until chemical analysis.

2.2. Obtaining Essential Oil

Essential oil was obtained by 100 g of the dried plant material by using the water distillation method with a Clevenger apparatus. Essential oil is fused at +40 °C until use (Telci et al. 2006).

2.3. Analyzes of Gas Chromatography (GC) and Gas Chromatography Mass Spectrometry (GC-MS)

GC analysis: Component analyzes were performed with the 7890 A model GC system, 5975C inert MSD with the Triple-Axis Detector with an automatic Autosampler system. Samples were injected in split (10:1) mode as 1 µl of HP-5 (5% Phenyl Methyl Siloxan) for partitioning the components by diluting with hexane at the ratio of 1:10. The internal pressure of helium used as carrier gas is set at 5 psi. The injector temperature was planned to be 250 ºC and the detector temperature was planned to be 250 ºC. FID detector was used for quantitative values. The starting temperature of the clone was 60 ºC, the final temperature was 240 ºC, programmed to increase by 4 ºC per minute.

GC-MS analysis was conducted with the 7890 A model GC system, 5975C inert MSD with Triple-Axis Detector. For GC/MS separation, an electron ionization system having 70 eV ionization energy was used. The helium flow rate used as carrier gas is 1.0 ml min^{-1}. The clone used is HP-5Ms (30 m x 0.25 mm x 0.25 µm film), with the starting and final temperatures and the operation program is the same as GC. The injector and MS transfer temperatures are set to 230 ºC and 250 ºC, respectively. As in gas chromatography, 1.0 µl split/splitles (10:1) was given to the clone from the sample diluted with hexane.

2.4. Preparation of Plant Extract

The collected *Salvia candidissima* Vahl plant was dried under suitable conditions, then grounded and pulverized. 200 mg was taken from the obtained plant sample and a 10 ml of methanol/chloroform (4/1) mixture was added. Afterwards, the mixture was kept in an ultrasonic bath at 30 °C for 30 minutes and extracted. The obtained extraction solution was removed by rotary evaporator and stock solutions including 1 mg ml^{-1} methanol were prepared. This stock solution was stored at +4 °C for use in the analyzes of total phenolic and flavonoid through antioxidant activity tests.

2.5. Free Radical Scavenging Activity (2,2-Diphenyl-1-picrylhydrazyl, DPPH)

The free radical scavenging activity was determined by making several changes in the Liyana-Pothirano method (Liyana-Pathirana and Shahidi 2005). The stock solutions of the plant extract in different quantities were placed in the test tubes and their volumes were completed to 3 ml with ethyl alcohol. 1 ml of DPPH solution (0.26 mM) was added to them and mixed by means of a vortex. After having waited for 30 minutes in the dark, their absorbances were read on a spectrophotometer at 517 nm. The obtained data were calculated in the form of IC_{50}.

2.6. Cation Radical Scavenging Activity (TEAC)

This analysis was performed according to the method implemented by Re et al. (Re et al. 1999). 2 mM ABTS prepared with phosphate buffer having 0.1 M pH 7.4 (2,2'-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) and 2.45 mM sodium persulfate ($Na_2S_2O_8$) solutions were mixed at a ratio of 1: 2 and waited for 6 hours in the dark. The stock solutions of the plant extract in different quantities were put into the test tubes and their volumes were completed to 3 ml with 0.1 M phosphate buffer (pH 7.4). By adding 1 ml of ABTS solution, it was mixed with the help of a vortex. After having waited for 30 minutes at room conditions, its absorbance value was read on a spectrophotometer at 734 nm. The obtained data were calculated in the form of IC_{50}.

© Akdeniz Üniversitesi Ziraat Fakültesi

2.7. Iron Reduction Power Activity (FRAP)

FRAP analysis was carried out by making changes in the method applied by Oyaizu (Oyaizu 1986). 0.25 ml plant extract was completed to 1.25 ml with 0.2 M phosphate buffer (pH 6.6). 1.25 ml of potassium ferricyanide [$K_3Fe(CN)_6$] solution (1%) was added on to it. This mixture was kept at 50 ˚C for 20 minutes. TCA (1.25 ml, 10%) and $FeCl_3$ (0.25 ml, 0.1%) solutions were added to the mixture cooled to room temperature. After mixing with the vortex, the spectrophotometer absorbance was measured at 70 nm. The results obtained were calculated as Troloxy equivalent (TE).

2.8. Copper Reduction Power Activity (CUPRAC)

0.1 ml was taken from the sample solution and the volume was completed with 1 ml of methanol. $CuCl_2$ (0.01 M), neocuprin ($7.5x10^{-3}$ M) and ammonium acetate solutions of 1 mL each was added on to it and mixed with the help of a vortex. After having waited at room temperature for 30 minutes, the absorbance value was read at spectrophotometer as 450 nm. The results obtained were evaluated by calculating as Troloxic equivalent (TE) (Chang et al. 2002).

2.9. Determination of Total Phenolic Compound Quantity

The total phenolic compound assay was performed with Folin-Ciocalteus reagent (Singleton et al. 1999). By taking 0.1 mL of the stock solution prepared from the plant extract, it was completed to 4.6 ml with distilled water. 0.3 ml Na_2CO_3 solution (2%) and 0.1 ml Folin-Ciocalteus reagent was added on to it and mixed with the help of a vortex. The spectrophotometer absorbance was measured by a spectrophotometer at 760 nm after having waited for 2 hours at room conditions. The results were calculated as equivalent to gallic acid (GAE).

2.10. Determination of Total Flavonoid Content

By taking 0.1 ml of the stock solution of the plant extract, it was completed to 4.8 ml with methanol. 0.1 ml of $Al(NO_3)$ (10%) and 0.1 ml of NH_4CH_3COO solutions (1 M) were added on it. After vortexing, it was stored for 40 minutes at room conditions and the absorbance was measured by spectrophotometer at 415 nm and recorded. The results obtained were calculated as quercetin equivalent (QE).

2.11. Obtaining Fungus Cultures

The plant pathogens used in the study were obtained from the stock cultures found in phytopathology laboratories of Ahi Evran University, Faculty of Agriculture, Plant Protection Department. Fungus cultures were grown in 90 mm petri dishes containing 20 ml potato dextrose agar (PDA) at 25 ± 2 °C for 7 days and then used in the study.

2.12. Antifungal Effect of the Essential Oils in In Vitro

PDAs prepared in 500 ml erlenmeyers were autoclaved and cooled to 40 °C and transferred to 60 mm diameter petri dishes (10 mm each). A 5-mm diameter well was opened in the petri dishes to which the PDA was transferred. The mycelium discs of disease (5 mm) were transferred at equal distances directly across the wells. Plant essential oils were dropped into the wells opened with a micropipette at concentrations of 0, 1, 5 and 10 µl/petri. The lids of the petri dishes were closed thoroughly with a parafilm and left to incubate for 7 days at 23 °C. At the end of the period, the mycelial growth values of diseases in petri dishes were measured with a compass. The experiments were carried out 4 times with 2 repetitions. According to Pandey et al. (1982), the mycelium development percentage was calculated by comparing the inhibition with the development in the control (Pandey et al. 1982).

$$I=100\times(dc-dt)/dc$$

I: percentage of inhibition of mycelium growth dc: mycelium development of the control dt: mycelial growth of the fungus treated with essential oil.

2.13. Statistical analysis

SPSS 15 statistics program was performed for all calculations. Data from the in vitro culture test experiments were processed with the analysis of variance (ANOVA). When the ANOVA was significant ($P\leq 0.05$), means were separated with Duncan's Multiple Range Test.

3. Results and Discussion

3.1. GC and GC-MS Analysis results

The chemical components of *Salvia candidissima* Vahl. essential oil naturally grown in Kahramanmaraş were obtained by GC and GC-MS analysis, and the results are given in Table 1. As a result of the analysis carried out, 54 components were determined in the essential oil and 99.98% of the total fat component was determined. The main components of essential oil were determined as Spathulenol (12.75%), Caryophyllene oxide (8.67%), Ledene oxide (6.98%) and o-Cymene (6.03%). Table 1.

In the studies conducted earlier, it was reported that the total amount of components in the essential oil was 70 in *Salvia candidissima* Vahl. and the main components were reported as camphor (28.94%), bornyl acetate (12.80%), borneol (9.44%), ß-cadinene (5.88%), α-caryophyllene (5.40%), 1,8-cineole (5.15%), ß-pinene (4.93%) and α-pinene (4.89%) (Özler et al. 2009). In addition, the essential oil components of the *S. candidissima subsp. candidissima* were 39, the amount of these compounds in the essential oil was reported to be 89.2%. The main components of the essential oil were determined as α-pinene (11.2%), 1,8-cineole (9.9%), *p*-cymene (7.4%), myrtenal (6.5%), pinocarvone (6.2%) and camphene (5.7%) (Bayrak and Akgün 1987). In the study conducted by Yılar 2014 in Tokat province, 32 components were defined in the *S. candidissima* subsp. *candidsisima* essential oil and Estrogole was determined as the highest content matter with a 64.55% value, which is followed by Caryophyllene-oxide (13.97%), Isocaryophyllene (7.42%), Spathulenol (6%) and α-Amorphene (2.77%). The studies conducted showed that the main components of *S. candidissima* Valh. are Estragole, Camphor, Caryophyllene-oxide, Spathulenol, Borneol, and these compounds vary by region. In the current study, the main components of *S. candidissima* Valh. were determined as Spathulenol and Caryophyllene oxide. In this respect, the studies conducted are similar. As in these studies, the amounts of plant essential oil and its main components vary according to the regions. The main reasons for changes in the amounts of essential oils and the main components are affected by environmental conditions and other variables (height, precipitation, stress conditions, etc.).

© Akdeniz Üniversitesi Ziraat Fakültesi

Table 1. The chemical components of the essential oil of *Salvia candidissima* Vahl.

Compound number	RT	RI	%	Compound name	Identification technique
1	14.761	1001	6.03	o-Cymene	MS, RI
2	15.046	1010	0.44	Eucalyptol	MS, RI
3	17.162	1073	0.31	β-Linalool	MS, RI
4	19.741	1147	1.71	Borneol	MS, RI
5	20.084	1157	1.14	4-Terpineol	MS, RI
6	20.261	1162	0.25	p-Cymen-8-ol	MS, RI
7	20.501	1169	0.33	α-Terpineol	MS, RI
8	21.499	1196	1.86	β-Citronellol	MS, RI
9	26.803	1355	3.04	alfa-Copaene	MS, RI
10	27.204	1367	3.34	β-copaene	MS, RI
11	27.512	1377	0.64	Benzenebutanal, γ,4-dimethyl	MS, RI
12	28.293	1400	3.29	Caryophyllene	MS, RI
13	28.541	1408	0.51	β-copaene	MS, RI
14	28.899	1420	1.59	Aromandendrene	MS, RI
15	29.345	1435	0.41	4,5-di-epi-aristolochene	MS, RI
16	29.92	1453	0.41	γ-Muurolene	MS, RI
17	30.16	1461	0.64	Germacrene D	MS, RI
18	30.384	1468	0.42	β-Selinene	MS, RI
19	30.599	1475	0.34	γ-Gurjunene	MS, RI
20	31.095	1490	0.37	γ-Cadinene	MS, RI
21	31.277	1496	0.99	δ-Cadinene	MS, RI
22	31.981	1520	0.54	9-Methoxycalamenene	MS, RI
23	32.258	1530	1.74	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	MS, RI
24	32.566	1540	0.14	α-Calacorene	MS, RI
25	32.8	1548	2.53	Palustrol	MS, RI
26	33.095	1558	12.75	Spathulenol	MS, RI
27	33.317	1566	8.67	Caryophyllene oxide	MS, RI
28	33.58	1575	1.38	Viridiflorol	MS, RI
29	33.986	1588	2.88	Caryophyllene oxide	MS, RI
30	34.361	1600	0.25	4-epi-cubedol	MS, RI
31	34.452	1604	0.38	Ledene oxide	MS, RI
32	34.808	1617	3.06	tau-Cadinol	MS, RI
33	34.899	1620	1.31	Isoaromadendrene epoxide	MS, RI
34	35.181	1631	6.98	Ledene oxide	MS, RI
35	35.632	1647	1.10	Aromadendrene oxide	MS, RI
36	35.842	1654	2.12	nd	MS, RI
37	36.107	1664	1.65	Ledene oxide	MS, RI
38	36.328	1671	0.72	Spiro-6-(bicyclo[3.2.1]octane)-2'-(oxirane), 7,8-di(hydroxymethyl)-5-methyl-2-isopropyl-	MS, RI
39	36.669	1683	0.51	Aromadendrene oxide	MS, RI
40	37.052	1697	0.69	Ledene oxide	MS, RI
41	37.579	1717	0.75	Murolan-3,9(11)-diene-10-peroxy	MS, RI
42	37.728	1722	0.82	Isoaromadendrene epoxide	MS, RI
43	37.905	1729	0.75	Murolan-3,9(11)-diene-10-peroxy	MS, RI
44	38.273	1743	1.39	9-Isopropyl-1-methyl-2-methylene-5-oxatricyclo[5.4.0.0(3,8)]undecane	MS, RI
45	39.796	1799	2.57	Isoaromadendrene epoxide	MS, RI
46	40.149	1814	1.85	2(1H)-Naphthalenone, 4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-4,8a-dimethyl	MS, RI
47	40.444	1826	1.33	1-Hexadecanol, 2-methyl-	MS, RI
48	40.727	1837	2.11	Platambin	MS, RI
49	41.446	1866	1.93	14-Oxatricyclo[9..2.1.0(1,10)]tetradecane, 2,6,6,10,11-pentamethyl-	MS, RI
50	41.872	1883	0.82	geranyl-α-terpinene	MS, RI
51	42.079	1891	0.66	Androstan-17-one, 3-ethyl-3-hydroxy	MS, RI
52	42.515	1908	2.76	Retinol, acetate	MS, RI
53	42.681	1915	2.72	geranyl-p-cymene	MS, RI
54	44.094	1973	2.06	5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-enal	MS, RI
Total			99.98		

© Akdeniz Üniversitesi Ziraat Fakültesi

3.2. Antioxidant Activity Results

Total phenolic substance analysis was performed using Folin-Ciocalteu reagent (FCR) of *Salvia candidissima* Vahl. plant essential oil. A graphic was created by using the Gallic acid as a standard in the analysis. By using this graph, the amount of phenolic substance in the extract was calculated as mg Gallic acid (mg GAE g^{-1} extract). As a result of the phenolic substance analysis, it was determined that there is 83.53 ± 5.90 mg GAE g^{-1} extract. In the study conducted by Diri (2006), the total phenolic substance amounts of extracts of *S. candidissima* were reported as 59.19 ± 0.13 for hexane extract, 49.15 ± 1.17 for ethyl acetate and 63.27 ± 0.01 µg (Pirokatechol mg^{-1} extract) for ethanol extract. In addition, in the study conducted by Tosun et al. (2009), the total phonochemical content of the methanol extract of *S. candidissima* was determined as 87.1 mg GAE g^{-1} DW.

A was graphic was created using quercetin for the total flavonoid content *Salvia candidissima* Vahl. as a standard and the content of flavonoid substance in the extract was calculated as mg quercitin using this graph. As a result of the analysis, it was determined that the total flavonoid content of the methanol extract of *S. candidissima* Vahl. was 59.02 ± 3.59 mg QE g^{-1} extract. The DPPH radical is used in the evaluation of free radical scavenging activity tests of natural antioxidants. The antioxidant activities of methanol extracts of *S. candidissima* Vahl. prepared in different concentrations and DPPH radical scavenging standards lC_{50} (µg ml^{-1}) are given in Figure 1.

As a result of the DPPH analysis, higher antioxidant activity than BHT, BHA and Trolox, which are used as standard, shows a weaker radical scavenging power in terms of antioxidant capacity. In a previous study, the DPPH radical scavenging activity of *S. candidissima* was reported to be IC_{50}= 33.4 (Tosun et al. 2009). In another study, the radical scavenging activity of *S. candidissima* subsp. *candidissima* was determined as IC_{50}= 49.7 (Tepe et al. 2004).

When the ABTS radical scavenging activity of the methanol extract of *S. candidissima* Vahl. was 9.25 ± 0.40 lC_{50} (µg ml^{-1}) was compared with BHT 9.40 ± 0.55 lC_{50} (µg ml^{-1}), BHA 3.30 ± 0.05 lC_{50} µg ml^{-1}) and Trolox 4.80 ± 0.06 lC_{50} (µg ml^{-1}), which are used as standard, it was determined that the BHA standard has the highest radical scavenging activity. The ABTS radical scavenging activity of the methanol extract of *S. candidissima* Vahl. was found to be low for BHA and Trolox standards and high for the BHT standard (Figure 2).

The reduction of Fe^{+3} ion is indicative of electron-emitting ability, which is important for the antioxidant activity of a compound, and is closely related to other antioxidant mechanisms. The prepared methanol extract of *S. candidissima* Vahl. was compared with BHA and BHT antioxidants, which are used as standard (Figure 3).

The reduction power of *S. candidissima* Vahl. is 1.20 ± 0.16 mmol TE g^{-1} extract, while it was calculated for BHA and BHT, which were used as a control, 6.60 ± 0.16 mmol TE/g extract and 2.74 ± 0.35 mmol TE g^{-1} extract, respectively. Considering this result, it was determined that *S. candidissima* Vahl. is a good reducing antioxidant (Figure 3).

The CUPRAC reduction capacity of *S. candidissima* Vahl. was 3.30 ± 0.12 mmol TE g^{-1} extract compared to the standard used BHT 15.25 ± 0.65 and BHA 15.99 ± 1.07 mmol TE g^{-1} extract. As a result of the analysis performed, it was determined that *S. candidissima* Vahl. had a good CUPRAC reductive antioxidant capacity compared to the controls used (Figure 4).

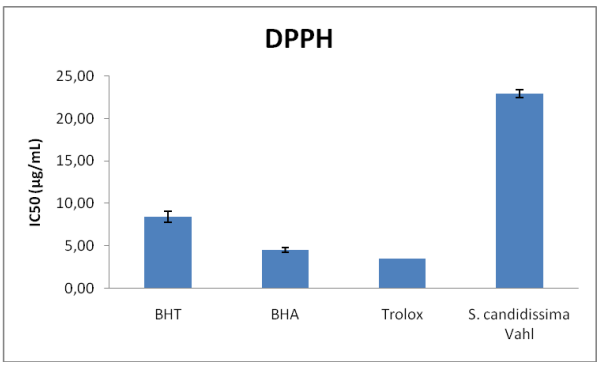

Figure 1. The radical scavenging activity of *S. candidissima* extract as a result of DPPH.

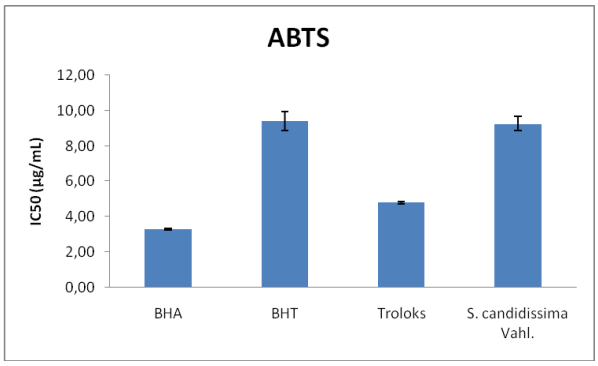

Figure 2. The radical scavenging activity of *S. candidissima* extract as a result of ABTS analysis.

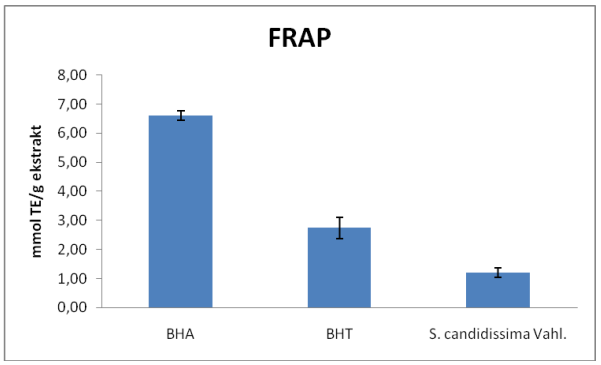

Figure 3. The reduction power of *S. candidissima* extract.

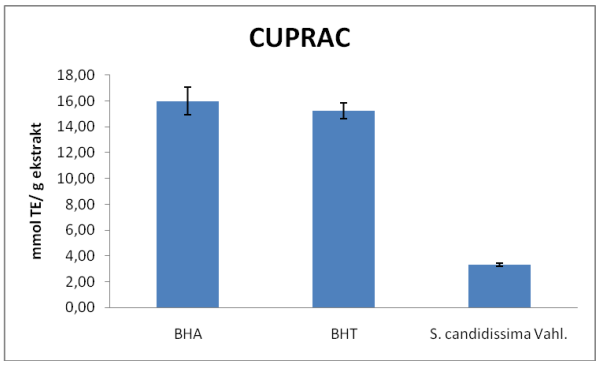

Figure 4. CUPRAC Copper (II) Reduction power of *S. candidissima* extract.

© Akdeniz Üniversitesi Ziraat Fakültesi

3.3. Antifungal Effect Results in In Vitro

As a result of the current study, it was determined that the essential oil of *S. candidissima* Vahl. had a significant antifungal activity on mycelial growth of *R. solani* and *A. solani*. The results of the obtained antifungal effects are given in Table 2.

Table 2. The antifungal activity of *Salvia candidissima* essential oil on mycelial gowth inhibition (%) of fungal disease agents *R. solani* and *A. solani.*

Doses (µl petri^{-1})	Plant pathogens			
	R. solani		*A. solani*	
	I$^{(\%)}$	Mg $^{(mm)}$	I$^{(\%)}$	Mg$^{(mm)}$
0	0.00^{c*}±0.0	60	0.00^{c}±0.0	60
1	0.00^{c}±0.0	60	0.00^{c}±0.0	60
5	18.23^{b}±0.96	49.05	23.31^{b}±1.57	46.01
10	51.87^{a}±1.23	28.87	57.92^{a}±3.38	25.25

I$^{(\%)}$; Inhibition (%) Mg $^{(mm)}$; mycelial growth

No effect was observed on mycelial growth in both pathogens at the dose of 1 (µl petri^{-1}) of *Salvia candidissima* Vahl. In the case of essential oil at a dose of 5 (µl petri^{-1}), the mycelial growths of both fungus species were decreased; however, inhibition of mycelial growth of *A. solani* more than those recorded for *R. solani*. A dose of 10 (µl petri^{-1}) of the essential oil affected mycelial growth in both fungi; however, no complete inhibition (100%) was observed. In studies conducted earlier, the seed fatty acids of *S. candidissima* subsp. *candidissima* were shown to be effective against certain microorganisms and because of their richness in the amount of linoleic acid present in fatty acids, they were found to exhibit antimicrobial activity at different levels against microorganisms (Kılıç et al. 2005). Furtehermore, *Salvia* species were reported to have a strong antifungal activity in many studies. In the study conducted by Yıldırım et al (2010), they investigated the antifungal activity of the methanol, ethanol, hexane and water extracts of the *Salvia* species and reported that the hexane extract had the most effective antifungal activity. In another study, it was found that the essential oils of *S. lavandulifolia*, *S. officinalis* and *S. sclarea* species exhibited high antifungal activity against *Candida* species (Seyoum et al. 2006). As indicated in the studies mentioned above, the current study also supports the result that the essential oil of *Salvia candidissima* has significant antifungal activity against the fungal disease agents *R. solani* and *A. solani*.

4. Conclusion

As a result of the study, the chemical composition of *Salvia candidissima* Vahl. was determined. As a result of the antioxidant analysis carried out, it was determined that the methanol extract had a significant antioxidant capacity. As a result of GC and GC-MS analysis, 54 components were found in the essential oil, and the main components were determined as Spathulenol, Caryophyllene oxide, Ledene oxide and o-Cymene. The antifungal effect of the essential oil against *R. solani* and *A. solani* was found to be quite high.

References

Bayrak A, Akgül A (1987) Composition of essential oils from Turkish *Salvia* species. Phytochemistry; 26: 846–847.

Chalchat JC, Michet A, Pasquier B (1998) Study of the clones of *Salvia officinalis* L. yields and chemical composition of essential oil. Flavour And Fragrance Journal, Vol. 13, 68-70.

Chang CC, Yang MH, Wen HM, Chern JC (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods". *J. Food Drug Analysis*, 10, 178-182.

Çadırcı E, Süleyman H, Gürbüz P, Kuruüzüm UA, Güvenalp Z, Demirezer LÖ (2012) Anti-inflammatory effects of different extracts from three *Salvia* species. Turk. J. Biol., 36: 59-64.

Delamare APL, Moschen-Pistorello IT, Artico L, Atti-Serafini L, Echeverrigaray S (2007) Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chem., 100 pp. 603–608.

Demirci B, Başer KHC, Yıldız B, Bahçecioğlu Z (2003) Composition of the essential oils of six endemic *Salvia* spp. from Turkey. Flavour Fragr. J., 18, 116–121.

Diri HA (2006) *Salvia candidissima* VAHL. Characterization Of Essential Compositions and Determınation Of Antioxidant Activity of *Salvia candidissima* Vahl. MUGLA University Institute of Science and Technology. pp. 104.

Halliwell B, Gutteridge JMC (1990) A Role of free radicals and catalytic metal ions in human disease: An overview. In: Methods in Enzymology, 186, 1-85.

Karabacak E (2009) Türkiye'nin Avrupa-Sibirya fitocoğrafik bölgesindeki *Salvia* L. (Lamiaceae) cinsinin revizyonu. (Doktora Tezi). Çanakkale Onsekiz Mart Üniversitesi. Fen Bilimleri Enstitüsü, Çanakkale, s. 205.

Karık M (2013) Marmara Bölgesindeki Anadolu Ada Çayı (*Salvia fruticosa* Mill.) Populasyonlarının Morfolojik ve Kalite Özelliklerinin Belirlenmesi, Kültüre Alınma Olanaklarının Araştırılması. Doktora tezi T.C Namık Kemal Üniversitesi Fen Bilimleri Enstitüsü Tarla Bitkileri Ana Bilim Dalı. 2013, s. 4-5.

Kelen M, Tepe B (2008) Chemical composition, antioxidant and antimicrobial properties of the essential oils of three *Salvia* species from Turkish flora. Biores. Technol., 99, pp. 4096–4104.

Kılıç T, Dirmenci T, Satil F, Bilsel G, Kocagoz T, Altun M, Goren AC (2005) Fatty acid composition of seed oils of three Turkish Salvia species and biological activities. Chemistry of Natural Compounds, 41, 276-279.

Liyana-Pathirana CM, Shahidi F (2005). Antioxidant activity of soft commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions". J. Agric. Food Chem; 53: 2433–2440.

Oyaizu M (1986) Studies on product of browning reaction prepared from glucose amine. Japan Journal of Nutrition, 44, 307-315.

Özler MA, Duru ME, Diri HA, Harmandar M (2009) Antioxıdant Activity And Chemical Composition of The Essential oil of *Salvia Candidissima* Vahl. Grownig Wild in Turkey. I International Medicinal and Aromatic Plants Conference on Culinary Herbs.

Pandey DK, Tripathi NN, Tripathi RD, Dixit SN (1982) Fungitoxic and phytotoxic properties of essential oil of *Hyptis suaveolens*. Z Pflanzenkrankheiten Pflanzenschutz 89: 344–349.

Re R, Pellegrini N, Protrggente, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Madicine, 26: 1231-1237.

Serteser A, Gök V (2003) Doğal Antioksidanların Biyoyararlılığı. 3. Gıda Mühendisliği Kongresi. 2-4 Ekim, S. 83-98, Ankara.

Seyoum A, Asres K, El-Fiky FK (2006) Structure-radical scaveng-ing activity relationship of flavonoids. Phytochemistry. 67: 2058-2070.

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folinciocalteu reagent". *Methods in Enzymology*, 299: 152-178.

Şenkal BC, İpek A, Gürbüz B (2012) Türkiye florasında bulunan adaçayı (*Salvia* L. spp.) türlerinin uçucu yağ içeriklerinin değerlendirilmesi. *Tıbbı ve Aromatik Bitkiler Sempozyumu* 13-15 Eylül 2012, s. 166-176.

© Akdeniz Üniversitesi Ziraat Fakültesi

Telci I, Bayram E, Yilmaz G, Avci B (2006) Variability in essential oil composition of Turkish basils (*Ocimum basilicum* L.). Biochemical Systematics and Ecology, 34, 489-497.

Tepe B, Sokmen M, Akpulat HA, Sokmen A (2006) Screening of the antioxidant potentials of six Salvia species from Turkey. Food Chemistry 95 200–204.

Tosun M, S Ercisli, Sengul M, Ozer H, Polat T, Ozturk E (2009) Antioxidant Properties and Total Phenolic Content of Eight *Salvia* Species from Turkey. Biol Res 42: 175-181.

Yılar M (2014) Tokat Ve Çevresinde Yaygın Olarak Görülen *Salvıa* Türlerinin Antifungal Ve Biyoherbisidal Aktivitelerinin Belirlenmesi, Doktora Tezı T.C Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsü Bitki Koruma Anabilim Dalı. s. 58.

Yılar M, Kadıoglu I (2016) Antifungal Activities of some *Salvia* Species Extracts on *Fusarium oxysporum* f. sp. *radicis- lycopersici* (Forl) Mycelium Growth *In-vitro*. Egyptian Journal of Biological Pest Control, 26(1), 115-118.

Yıldırım N, Bekler FM, Yıldırım NC (2010) In Vıtro Antimicrobial Evaluation of Commercial Tea Extracts Against Some Pathogen Fungi and Bacteria. Dig. J. Nanomat. Biostruc. 5 (4), 821-827.

Young IS, Woodside J V (2001) Antioxidants in Health and Disease". Journal of Clinical Pathology, 54: 176-186.

© Akdeniz Üniversitesi Ziraat Fakültesi