



Original Scientific Paper

## Chemical composition of the essential oil of *Salvia bracteata* Banks and the biological activity of its extracts: antioxidant, total phenolic, total flavonoid, antifungal and allelopathic effects

Melih YILAR<sup>1</sup>, Yusuf BAYAR<sup>1</sup>, Ahi Alev ABACI BAYAR<sup>2</sup> and Nusret GENÇ<sup>3</sup>

<sup>1</sup> Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Plant Protection, Kırşehir, Turkey

<sup>2</sup> Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Landscape Architecture, Kırşehir, Turkey

<sup>3</sup> University Gaziosmanpaşa, Faculty of Science and Art, Department of Chemistry, 60240 Tokat, Turkey

\* correspondence: [melih.yilar@ahievran.edu.tr](mailto:melih.yilar@ahievran.edu.tr)

### ABSTRACT:

The present study was conducted with the aim of determining biological activities of the *Salvia bracteata* plant, activities such as its antioxidant, total phenolic, total flavonoid, allelopathic and antifungal effects. To this end, aboveground parts (flowers + shoots + leaves) of *S. bracteata* plants were collected in the province of Kırşehir, Turkey, during the flowering stage in 2018. As a result of GC-MS analysis, 23 constituents were identified, representing 96.21% of the essential oil. The major compounds of essential oil were identified as ledol (24.12%), camphor (15.54%) and valencene (5.64%). In ethyl acetate, methanol and hexane extracts of *S. bracteata*, total phenolic content was found to be 104.63, 121.66 and 20.97 mg of GAE/g of extract, respectively, while flavonoid content was 12.89, 10.85 and 1.13 mg of QE/g of extract, respectively. In addition to this, DPPH radical removal activity was identified and found to be at its highest in the methanol and ethyl acetate extracts. The TEAC (cation radical removal activity), FRAP (iron reduction power) and CUPRAC (copper reduction power) reduction activities of these plant extracts were also determined. The methanol extract of *S. bracteata* was found to have an allelopathic effect on *Rumex crispus* and *Taraxacum officinale*. This extract had a weak effect on development of the mycelium of *Alternaria solani* Sorauer, one of the most significant of plant-pathogenic fungi, but it was found to be ineffective on the *Sclerotinia sclerotiorum* pathogen.

### Keywords:

*Salvia bracteata*, antioxidant activity, total phenolic, flavonoid, antifungal and allelopathic effects

UDC: 665.525:582.929.4(560)

Received: 17 September 2019

Revision accepted: 15 December 2019

### INTRODUCTION

Diseases, pests, weeds and climatic factors may negatively affect the yield and quality of cultivated plants during their developmental phase. Weeds are an important factor that affects crop quality and causes economic loss in agricultural production. About half of all crop loss is caused by weeds, but the reported size of this loss varies between 10 to 90% (UYGUR 2002; ÖZEN *et al.* 2017). Chemical means are considered the most effective method of weed control and synthetic drugs are used extensively

around the world. Heavy use of synthetic pesticides has given rise to problems such as loss of plant strength, environmental damage and toxic residues (ISMAN 2000). Intensive research is therefore being conducted to find as quickly as possible alternative methods that would be less harmful to human health and the environment.

One of these alternative methods is to seek natural pesticides by determining the activity of compounds naturally present in plants. Of these natural compounds, essential oils of herbal origin are synthesised by aromatic plants as specialised metabolites (AYEB-ZAKHAMA *et al.* 2017).

Essential oils are reported to act as natural fungicides (KUMAR *et al.* 2014) and insecticides (ISMAN *et al.* 2011; ABBAD *et al.* 2014), in addition to having a phytotoxic effect on cultivated plants and weeds (SYNOWIEC *et al.* 2017). Essential oils act just like herbicides in adversely affecting both the germination process and growth of plant seedlings (AMRI *et al.* 2013).

Turkey is rich in its *Salvia* diversity and is home to 96 species and four subspecies. *Salvia* species are of economic importance, in addition to their medicinal qualities. Moreover, these plants are ornamental, with beautiful flowers commonly grown in gardens and parks (DEMİRÇI *et al.* 2003). *Salvia bracteata* Banks is a perennial herbaceous plant that spreads to almost every region in Turkey with altitudes ranging from 50 to 2000 m (ANONYMOUS 2017). The roots of *S. bracteata* contain diterpenoids (ULUBELEN *et al.* 1999) and were reported to act as an antimicrobial (CARDİLE *et al.* 2009).

In this study, total phenolic compounds and the presence of antioxidant, antifungal and allelopathic effects were determined in the plant *S. bracteata*, which has a wide natural distribution in the province of Kırşehir, Turkey.

## MATERIAL AND METHODS

**Collection and drying of plant samples.** The plant material was collected in the Kırşehir province of Turkey (village of Akçakent/Hamzabey, with coordinates of 39°36'32.01" N and 34°4'37.95" E, situated at an altitude of 1263 m) in the year 2018. Soil samples taken from the location were analysed. Collected plants were dried in shade under room conditions in facilities of the Department of Plant Protection of the Faculty of Agriculture, Ahi Evran University, Kırşehir. Dried plant samples were ground in an electric grinder.

**Extraction of essential oils.** Essential oils were obtained from the plants by hydro-distillation using a Schilcher apparatus. After the plant sample (100 g) was weighed, pure water (1:10 w/v) was added and it was boiled for 2 hours. This process was repeated several times. The obtained essential oils were preserved until tests were conducted (TELÇI *et al.* 2006).

**GC and GC-MS analysis.** The GC analyses of *S. bracteata* essential oils were performed using an Agilent 7890A instrument (model GC). Oil was diluted in acetone (1:10) and injected into a separate BPX90 column (100m × 0.25mm × 0.25µm). The carrier gas was helium at 5 psi inlet pressure. Injector and detector (FID) temperatures were 120 and 254°C, respectively. The column temperature was programmed from 60 to 120°C at 5°C/min with the initial and final temperatures held for 3 and 16 min. Diluted samples of 1.0 µL were injected in the split (1:5) mode. Total analysis time was 43 min. Quantitative data were obtained electronically from FID area percent data

without the use of correction factors.

The MS results were compared with the Wiley and NIST computer mass libraries. The relative peak area percentages of compounds were calculated on the basis of FID data.

**Preparation of plant extract.** For antifungal and allelopathic studies, 100 g of ground plant material was placed in a 1-liter Erlenmeyer flask and 600 ml of methanol was added. This solution was extracted at room temperature in a shaker for 24 hours. After extraction, the solution was filtered through filter paper. The methanol in the solution was removed by evaporation at 32°C until solid material was obtained. The remaining solid material was treated with acetone-water to prepare a stock solution (KADIOĞLU & YANAR 2004). The solution was stored at 4°C until the test.

For antioxidant tests, 200 mg were taken from the ground plant sample for hexane, ethyl acetate, and methanol extracts. For the hexane extract, 10 ml of hexane/chloroform (5/1) was added to the sample; for the ethyl acetate extract, 10 ml of ethyl acetate/chloroform (5/1) was added to it; and for the methanol extract, 10 ml methanol/chloroform (5/1) was added. Following the vortex, the samples were kept in an ultrasonic bath at 30°C for 30 minutes. The resulting extraction solutions were removed by a rotary evaporator and stock solutions were prepared at 1 mg/ml. These stock solutions were stored at 4°C for use in antioxidant activity tests and analyses of total phenolic and flavonoid content.

**Production of fungus cultures.** Plant pathogenic fungi were obtained from stock cultures maintained by the Phytoclinical Laboratory of the Department of Plant Protection of Ahi Evran University's Faculty of Agriculture. Used in these tests were young fungus cultures that developed for 7 days at 25±2°C in 90-mm Petri dishes containing 20 ml of potato dextrose agar (PDA).

**In vitro antifungal activity of the plant extract.** Methanol extract was dissolved in an acetone-water mixture to provide a stock solution. The final concentration of the original solutions was added to PDA media cooled to 45-50°C in batches of 50, 100, 200 and 400 ppm (ONARAN & YILAR 2012). As a control, fungi were seeded in Petri dishes containing only PDA. Moreover, a fungicide with the active ingredient thiram was used as a positive control. These different doses of PDA media were poured into 10-ml Petri dishes with a diameter of 60 mm. Mycelium discs of 5 mm diameter from plant pathogen cultures developed 7-10 days prior to the tests were seeded in Petri dishes containing extract-added PDA medium. Fungus cultures were incubated after inoculation for 7 days at 25±1°C. This study was repeated two times with four replications. The diameters of mycelia developed in the Petri dishes were measured with a digital caliper. The rate of inhibition of

mycelium growth by the extracts was calculated according to the following formula:

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

I – rate of inhibition of mycelium growth (%)

dc - mycelium growth in the control

dt - mycelium growth in the test (PANDEY *et al.* 1982)

**Allelopathic effect study.** Seeds of *Rumex crispus* L. and *Taraxacum officinale* F.H. Wigg (25 pieces each) were distributed homogeneously in 90-mm Petri dishes lined with two layers of blotting paper. Different concentrations (50, 100, 200 and 400 ppm) of plant extract and pure water for control purposes were moistened by adding 6 ml to contents of the Petri dishes. The Petri dishes were incubated at  $25 \pm 1^\circ\text{C}$  under conditions of 12 hours of light and 12 hours of darkness for 4 weeks. At the end of this period, germination rates and root and shoot lengths of the test plants were measured. The experiment was carried out in three replications and repeated two times (YILAR *et al.* 2014).

**Free radical scavenging activity DPPH (1,1-diphenyl-2-picrylhydrazyl) test.** Free radical scavenging activity was determined using several modifications of the Liyana-Pathirano method (LIYANA-PATHIRANA & SHAHIDI 2005). Stock solutions of different amounts of plant extract were put in test tubes and topped up to their final volume by adding 3 ml of ethyl alcohol. A measured volume (1 ml) of DPPH solution (0.26 mM) was then added and mixed by vortex. After it was kept in the dark for 30 minutes, absorbance was read at 517 nm. The DPPH radical scavenging activity was calculated as  $IC_{50}$ .

**Cation radical scavenging activity (TEAC).** This analysis was performed according to the method used by RE *et al.* (1999). Solutions of 2 mM ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid) and 2.45 mM sodium persulphate ( $\text{Na}_2\text{S}_2\text{O}_8$ ) prepared using 0.1 M phosphate buffer with a pH value of 7.4 were mixed at a ratio of 1:2 and kept in the dark for 6 hours. Stock solutions of different amounts of plant extracts were poured into test tubes and their volumes were topped up to 3 ml with a 0.1 M phosphate buffer (pH 7.4). Then 1 ml of ABTS solution was added, mixed by vortex and kept in room conditions for 30 minutes, after which absorbance was read at 734 nm. The ABTS cation radical scavenging activity was calculated as the  $IC_{50}$  value.

**Iron reducing power activity (FRAP).** The FRAP analysis was performed by a modified version of the Oyaizu method (OYAIU 1986). A 0.25-ml volume of plant extract was topped up to 1.25 ml with a 0.2 M phosphate buffer (pH 6.6). A measured volume (1.25 ml) of potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] solution (1%) was then added. This mixture was kept at  $50^\circ\text{C}$  for 20 minutes. Once the mixture cooled down to room temperature, TCA (1.25 ml,

10%) and  $\text{FeCl}_3$  (0.25 ml, 0.1%) were added and a vortex was applied to the mix, after which absorbance was read at 700 nm. The obtained results were calculated as the amount of trolox-equivalent matter (TE).

**Copper reducing power activity (CUPRAC).** An amount of 0.1 ml was taken from the resulting sample solutions and the volume was topped up to 1 ml with methanol. Following the addition of  $\text{CuCl}_2$  (0.01 M), neocuprin ( $7.5 \times 10^{-3}$  M) and ammonium acetate solutions with a volume of 1 ml each, it was mixed by vortex. After resting for 30 minutes at room temperature, absorbance was read at 450 nm. The obtained results were calculated as the amount of trolox-equivalent matter (TE) (APAK *et al.* 2004; ELMASTAS *et al.* 2018).

**Total phenolic determination.** The total phenolic compound content was determined using the Folin-Ciocalteu reagent (SINGLETON *et al.* 1999). An amount of 0.2 ml was taken from stock solutions prepared with plant extracts and topped up to 4.6 ml with purified water. With the addition of 0.3 ml of  $\text{Na}_2\text{CO}_3$  solution (2%) and 0.1 ml of Folin-Ciocalteu reagent, it was placed in a vortex. Thereafter, it rested in room conditions for 2 hours and absorbance was measured by a spectrometer at 760 nm. Results were calculated as the amount of gallic acid-equivalent matter (GAE).

**Total flavonoid determination.** An amount of 0.2 ml was taken from the resulting sample solutions and the volume was topped up to 4.8 ml with methanol. Then 0.1 ml of  $\text{Al}(\text{NO}_3)_3$  (10%) and 0.1 ml of  $\text{NH}_4\text{CH}_3\text{COO}$  solutions (1 M) were added. After vortexing, it rested at room conditions for 40 minutes and absorbance was read at 415 nm. The obtained results were calculated as the amount of quercetin-equivalent matter (QE) (CHANG *et al.* 2002).

**Data analysis.** The significance of differences between treatments was determined through variance analysis (ANOVA), and mean values were compared using the Duncan test. All statistical analyses were performed with SPSS-15 software.

## RESULTS

**Total phenolic and total flavonoid content.** Extracts of *Salvia bracteata* obtained using different solvents were investigated. Table 1 shows the total phenolic content of methanol, ethyl acetate and hexane extracts obtained from *S. bracteata*. Significant differences were found between different extracts with respect to total content of phenolic substances. The highest phenolic content was in the methanol extract (121.66 mg of GAE/g of extract), followed by the ethyl acetate extract (104.63 mg of GAE/g of extracts) and the hexane extract (20.97 mg of GAE/g of extract).

**Table 1.** Total phenolic and total flavonoid content in *Salvia bracteata*.

Solvent of extracts	Total phenolic content (mg GAE/g extracts)	Total flavonoid content (mg QE/g extracts)
Ethyl acetate	104.63±1.02	12.89±0.90
Methanol	121.66±2.14	10.85±0.59
Hexane	20.97±0.49	1.13±0.20

**Table 2.** GC/MS analyses of the essential oil of *Salvia bracteata*.

No	RRI <sup>*</sup>	Compound name	%
1	1394	Valencene	5.64
2	1402	Isocaryophyllene	2.78
3	1417	γ-Murolene	12.4
4	1429	camphor	15.54
5	1484	10-epi-cubebol	1.28
6	1485	Cyclohexanol	0.76
7	1521	Cuneneol	0.81
8	1526	nd	1.68
9	1534	Viridiflorol	5.23
10	1475	epi-alpha-cadinol	2.38
11	1478	Caryophylleneoxide	4.79
12	1482	Ledol	24.12
13	1484	α-Cadinol	4.7
14	1506	β-Eudesmol	3.16
15	1540	Epoxy-allo-alloaromadendrene	1.2
16	1570	Tricyclo[4.4.0.0(2,7)]DEC-8-EN-4-OL	1.46
17	1602	7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene	3.33
18	1614	Isoaromadendreneepoxide	1.12
19	1622	Cydononasiloxane, octadecamethyl	0.48
20	1624	β-Oplophenone	1.8
21	1656	Benzene, 1-ethyl-3,5-dimethyl	0.52
22	1678	α-Guaiene	0.45
23	1689	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.58
<b>Total</b>			<b>96.21</b>

\* RRI: relative retention indices.

Significant differences were found between different extracts with respect to their total flavonoid content. The ethyl acetate extract was identified as the one with the highest flavonoid content (12.89 mg of QE/g of extract), followed by the methanol extract (10.85 mg of GAE/g of extract) and the hexane extract (1.13 mg of GAE/g of extracts).

**DPPH free radical activity.** As seen in Fig. 1, the DPPH radical scavenging activities of *Salvia bracteata* extracts were as follows: IC<sub>50</sub>, 24.71±0.13 µg/ml (methanol extract), IC<sub>50</sub>, 37.16±0.14 µg/ml (ethyl acetate extract) and IC<sub>50</sub>, 103.13±1.26 µg/ml (hexane extract). Their comparison with the standard antioxidants BHT (butylated hydroxytoluene) (IC<sub>50</sub>, 10.85±0.33 µg/ml), BHA (butylated hydroxyanisole) (IC<sub>50</sub>, 4.73±0.13 µg/ml) and trolox (IC<sub>50</sub>, 4.48±0.08 µg/ml) revealed high levels of DPPH radical scavenging activity for the methanol and ethyl acetate extracts (Fig. 1), whereas the hexane extract proved to have a weak DPPH radical scavenging activity.

**Reducing power.** The reducing power of compounds is indicative of their antioxidant capacity. Figure 2 shows the iron reducing power of different extracts, with the highest activity, compared to the standards BHT (4.73±0.16 µmol of TE/mg of extracts) and BHA (5.63±0.23 µmol of TE/mg of extract), found in the methanol extract (1.94±0.02 µmol of TE/mg of extracts), followed by the ethyl acetate extract (1.68±0.002 µmol of TE/mg of extract) and the hexane extract (0.018±0.005 µmol of TE/mg of extract). It was found that the methanol and ethyl acetate extracts of *S. bracteata* had a notable iron reducing power.

**CUBRAC.** Figure 3 shows results of determining the copper reducing power of extracts. The tested extracts had fairly high copper reducing activity compared to the standard antioxidant substances BHT (5.68±0.15 µmol of TE/mg of extract) and BHA (11.18±0.35 µmol of TE/mg of extract), with the highest activity found in the methanol extract (4.00±0.05 µmol of TE/mg of extract), followed by the ethyl acetate extract (2.99±0.01 µmol of TE/mg of extract) and the hexane extract (1.34±0.02 µmol of TE/mg of extract).

**ABTS cation radical scavenging activity.** Figure 4 shows the results of determining the levels of ABTS cation radical scavenging activity. Cation radical scavenging activities of the tested extracts in descending order were as follows: IC<sub>50</sub>, 11.20±0.20 µg/ml (methanol extract); IC<sub>50</sub>, 14.54±0.10 µg/ml (ethyl acetate extract); and IC<sub>50</sub>, 112.57±0.12 µg/ml (hexane extract). Comparisons with BHT (IC<sub>50</sub>, 4.71±0.03 µg/ml), BHA (IC<sub>50</sub>, 3.86±0.03 µg/ml) and trolox (IC<sub>50</sub>, 6.92±0.07 µg/ml) reveal a notable ABTS cation radical scavenging activity for the methanol and ethyl acetate extracts.

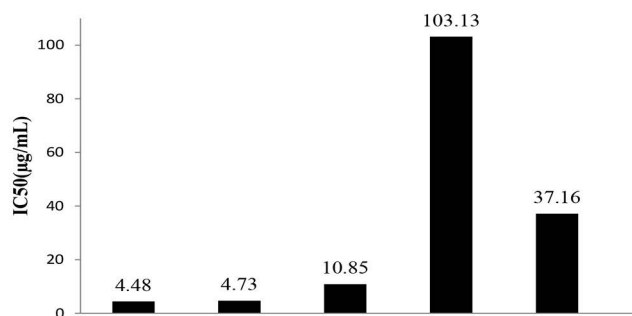


Fig. 1. *Salvia bracteata* extracts - DPPH free radical activity.

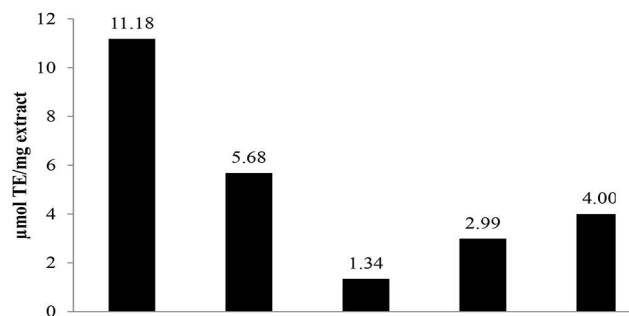


Fig. 3. *Salvia bracteata* extracts – C copper reducing power activity.

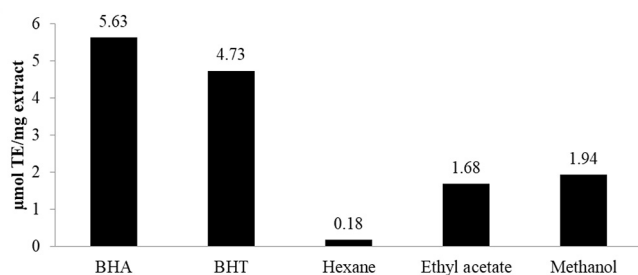


Fig. 2. *Salvia bracteata* extracts- reducing power activity.

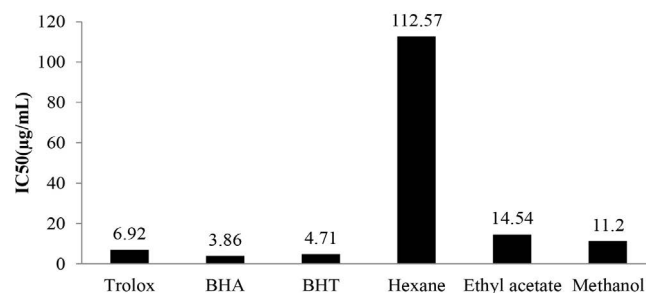


Fig. 4. ABTS cation radical scavenging activity.

Table 3. Effects (%) of *Salvia bracteata* methanol extract on seed germination and seedling growth.

Doses	<i>Rumex crispus</i>			<i>Taraxacum officinale</i>		
	% Germination	Root Length	Shoot Length	% Germination	Root Length	Shoot Length
Control	100.0±0.0 <sup>a</sup>	1.67±0.07 <sup>a</sup>	2.07±0.10 <sup>a</sup>	94.66±3.52 <sup>a</sup>	1.08±0.10 <sup>a</sup>	1.03±0.11 <sup>a</sup>
50 ppm	100.0±0.0 <sup>a</sup>	1.56±0.05 <sup>a</sup>	1.46±0.02 <sup>b</sup>	74.66±2.66 <sup>b</sup>	0.57±0.04 <sup>b</sup>	0.83±0.12 <sup>ab</sup>
100 ppm	100.0±0.0 <sup>a</sup>	1.20±0.05 <sup>b</sup>	1.07±0.02 <sup>c</sup>	69.33±2.58 <sup>b</sup>	0.42±0.14 <sup>bc</sup>	0.72±0.04 <sup>ab</sup>
200 ppm	98.66±1.33 <sup>a</sup>	0.63±0.05 <sup>c</sup>	0.99±0.09 <sup>c</sup>	28.00±5.04 <sup>c</sup>	0.32±0.06 <sup>bc</sup>	0.62±0.14 <sup>b</sup>
400 ppm	86.66±2.33 <sup>a</sup>	0.32±0.02 <sup>d</sup>	0.63±0.03 <sup>d</sup>	25.33±3.52 <sup>c</sup>	0.15±0.04 <sup>c</sup>	0.56±0.06 <sup>b</sup>

\*Means in the same column with the same letter were not significantly different as indicated by ANOVA (a = 0.05).

**Properties of soil at the collection site.** Soil samples obtained from the location where *S. bracteata* plants were collected had the following values: water saturation 58.3%, pH 8.09, total salinity 0.008, lime ratio 14.074%, organic matter 3.501%, K<sub>2</sub>O (kg/da<sup>-1</sup>) 98.766 and P<sub>2</sub>O<sub>5</sub> (kg/da<sup>-1</sup>) 3.914.

**GC and GC-MS analysis.** According to GC/MS analyses, 96.26% of the essential oil of *S. bracteata* was obtained and 23 components were identified. Ledol (24.12%), camphor (15.54%) and valencene (5.64%) were identified as the main components (Table 2).

Table 4. Antifungal effects (%) of *Salvia bracteata* methanol extract.

Doses (ppm)	<i>A. solani</i>	<i>S. sclerotiorum</i>
Control <sup>+</sup>	100±0.00 <sup>a</sup>	100±0.00 <sup>a</sup>
Control <sup>-</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>
50	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>
100	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>
200	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>
400	26.11±3.01 <sup>b</sup>	0.00±0.00 <sup>b</sup>

\*Means in the same column with the same letter were not significantly different as indicated by ANOVA (a = 0.05).

**Allelopathic effect.** The methanol extract showed an allelopathic effect on test plants, although at different levels. With an increase of dose, there was an increase of negative effects. Table 3 shows the allelopathic effect of the plant extract on test plants.

The methanol extract of *S. bracteata* did not have a statistically significant effect ( $p < 0.005$ ) on germination of *R. crispus* seeds compared to the control group. It reduced the rate of germination by 13.34%. However, *R. crispus* prevented root and shoot growth of the seedlings at a statistically significant level ( $p < 0.005$ ) compared to the control group.

*Taraxacum officinale* was found to be more sensitive to the methanol extract of *S. bracteata*. As compared to the control group, seed germination in *T. officinale* was affected to an extent of 69.33% by *S. bracteata* extract, root growth to an extent of 86.11% and shoot growth to an extent of 45.63% (Table 3).

**Antifungal activity.** The study did not show 100% inhibition of mycelium development by the plant pathogens used. The methanol extract of the plant showed the highest effect on *A. solani* with a value of 26.11% as compared to the negative control. On the other hand, it was determined that the doses used had no effect on the other pathogen, *Sclerotinia sclerotiorum* (Lib.) de Bary (Table 4).

## DISCUSSION

Phenols are very important plant components with their activities of free radical scavenging thanks to their hydroxyl groups (HATANO *et al.* 1989). The phenolic content of plants can therefore directly contribute to their antioxidant activities. The main phenolic substances identified in sage extracts are rosmarinic acid, carnosic acid, salvianolic acid and derivatives, carnosol, rosmanol, epirosmanol, rosmadial and methyl carnosate (WU *et al.* 1982; MADSEN & BERTELSEN 1995; LU & FOO 2001). The DPPH scavenging capacity of plant extracts is mostly associated with phenolic hydroxyl groups. However, the properties of these assumed antioxidants are linked to a variety of mechanisms, including inhibition of initiation of the radical chain, binding of catalysts for transition of metal ions, separation of peroxides, inhibition of continuous hydrogen abstraction and radical cleaning (DIPLOCK 1997).

The DPPH radical scavenging activities of the ethyl acetate and dichloromethane extracts of *S. bracteata* were demonstrated in previous studies. The ethyl acetate extract was reported to display a DPPH radical activity of  $13.95 \pm 0.32$  at  $250 \mu\text{g ml}^{-1}$ ,  $16.29 \pm 0.22$  at  $500 \mu\text{g ml}^{-1}$  and  $21.04 \pm 0.32$  at  $1000 \mu\text{g ml}^{-1}$ , whereas the same values for the dichloromethane extract were reported as  $3.99 \pm 0.32$  at  $250 \mu\text{g ml}^{-1}$ ,  $3.92 \pm 0.47$  at  $500 \mu\text{g ml}^{-1}$  and  $4.79 \pm 0.01$  at  $1000 \mu\text{g ml}^{-1}$  (ORHAN *et al.* 2013). In the same study, the

authors determined total phenolic and flavonoid content of the acetate, methanol and dichloromethane extracts of *S. bracteata*. They did not determine total phenolic content of the ethyl acetate and dichloromethane extracts of *S. bracteata*, but the total phenolic content of its ethanol extract was found to be  $63.25 \pm 9.85$ . In the same study, ORHAN *et al.* (2013) reported that total flavonoid content for the dichloromethane, ethyl acetate and ethanol extracts was  $81.4 \pm 7.99$ ,  $134.99 \pm 3.01$  and  $101.57 \pm 6.02$ , respectively. Thus, both the current study and previously published data confirm that *S. bracteata* has notable antioxidant activity thanks to the phenols it contains.

Similar studies have reported chemical composition of the essential oil of *S. bracteata*. In a study from Iran, 46 components were identified in the essential oil of this species, including  $\beta$ -caryophyllene (10.7-41.6%),  $\gamma$ -muurolene (27.1-36.3%), bicyclogermacrene (1.8-9.9%), caryophylleneoxide (1.5-9.6%) and  $\alpha$ -humulene (1.1-9.4%) (SEFIDKON *et al.* 2007). In a study performed with different parts of *S. bracteata*, a total of 41 components were detected in the plant's essential oils obtained before flowering. The main components of this oil were identified as  $\alpha$ -pinene (29.60%), myrcene (9.70%), limonene (7.10%),  $\beta$ -pinene (6.50%) and germacrene-D (5.96%). In the flowering phase, 50 components were identified, including  $\alpha$ -pinene (28.90%), myrcene (7.65%), limonene (7.17%) and  $\beta$ -pinene (7.90%). After flowering, 39 components were identified in the oil obtained from plants, with  $\alpha$ -pinene (19.40%), myrcene (9.45%), limonene (13.93%) and bornyl acetate (5.44%) as the main ones (AMIRI 2007).

As seen in previous investigations and the present study, there are differences between the percentages of main components in the essential oil of *S. bracteata*. These differences are a result of different ecological conditions, properties of soil (pH, organic matter, salinity, etc.) at the locations where the plants grow, characteristics of the plants (flowering time, harvesting time, post-harvest and drying conditions) and extraction conditions (SENATORE *et al.* 1997; SEFIDKON & MIRZA 1999; ALIZADEH 2013; MOADELI *et al.* 2013; RAPPOSELLI *et al.* 2015).

As the dose of *S. bracteata* methanol extract increased, so did its negative effects on test plants (AYDIN & TURSUN 2010). Very few studies have been conducted on the plant *S. bracteata*, and the existing ones are all on its antimicrobial activity. The antimicrobial activity of *S. bracteata* was reported by CARDILE *et al.* (2009). There are studies on the allelopathic effects of members of the genus *Salvia*. According to the findings of these studies, aboveground exudants of the species *Salvia namaensis* Schinz, *S. fallax* Fernald, *S. disermas* L., *S. chamaedryoides* Cav., *S. confertiflora* Pohl., *S. × jamensis* J. Compton, *S. buncananii* Hedge, *S. wargneriana* Polak, *S. scabra* Linn. fil., *S. miniata* Fernald, *S. cacaliaefolia* Benth., *S. adenophora* Fernald and *S. rutilans* Carriere have varying phytotoxic effects on *Papaver rhoeas* L. (poppy) and *Avena sativa* L. (oats) (Bisio *et al.* 2010); the essential oil of *S.*

*officinalis* inhibits germination and root development in *Lepidium sativum* L. (cress) (BOUAJAJ *et al.* 2013); *S. miniata* stops the germination of *Papaver rhoeas* L. and *Avena sativa* in Petri applications (Bisio *et al.* 2011); the essential oil of *S. leucophylla* is effective on the germination of *Brassica campestris* (NISHIDA *et al.* 2005); *Hordeum vulgare* (barley) and *Portulaca oleracea* (purslane) show reduced germination in the aqueous extract of *S. officinalis* (BAJALAN *et al.* 2013); and the aqueous extract of *S. macrosiphon* affects the germination and seedling development of *Zea mays* L. (corn) (ROWSHAN & KARIMI 2013). Furthermore, HASSANNEJAD & GHAFARBI (2013) showed that a 5% dose of the aqueous extract of *S. officinalis* reduced the germination of *Cuscuta campestris* Yunck by 25%.

*Salvia bracteata* had antimicrobial activity against the pathogens *S. aureus*, *E. coli*, *M. smegmatis* and *C. albicans*; and the MIC values against these pathogens were determined as 1.1, 0.5, 1.1 and 1.1 mg/ml, respectively (KILIÇ *et al.* 2005). CARDILE *et al.* (2009) reported that the essential oil of *S. bracteata* had an antimicrobial effect on human-pathogenic Gram-positive and Gram-negative bacteria. It was also shown by various researchers that many plant species of the genus *Salvia* exhibited biological activity (BAYAR & GENÇ 2018; YILAR *et al.* 2018).

Results of the present study show that *S. bracteata* extracts possess promising antioxidant properties *in vitro*. *Salvia bracteata* was shown to be rich in important phenolics with a potential to directly affect the presence of antioxidants. We suggest that the methanol extract of *S. bracteata* may represent an alternative to synthetic chemicals in the control of weeds and plant-pathogenic fungi. It is considered to have a significant potential, especially for cultivated plants widely grown in organic farming, which is of increasing importance in Turkey, as well as on a global scale.

**Acknowledgements** – This work was supported by the Ahi Evran University Scientific Research Projects Unit (Project No. ZRT.A3.17.005).

## REFERENCES

- ABBAD A, KASRATI A, JAMALI CA, ZEROUAL S, BA M'HAMED T, SPOONER- HART R & LEACH D. 2014. Insecticidal properties and chemical composition of essential oils of some aromatic herbs from Morocco. *Natural Product Research* **28**: 2338–2341.
- ALIZADEH A. 2013. Essential oil constituents, antioxidant and antimicrobial activities of *Salvia virgata* Jacq. from Iran. *Journal of Essential Oil Bearing Plants* **16**(2):172–182.
- AMIRI H. 2007. Quantative and qualative changes of essential oil of *Salvia bracteata* Bank et Sol. in different growth stages. *DARU Journal of Pharmaceutical Sciences* **15**(2): 79–82.
- AMRI I, HAMROUNI L, HANANA M & JAMOSSI B. 2013. Reviews on phytotoxic effects of essential oils and their individual components: news approach for weeds management. *International Journal of Applied Biology and Pharmaceutical Technology* **4**: 96–114.
- ANONYMOUS 2017. Taxon Page. *Salvia bracteata* Banks et Sol. <http://tubives.com/index.php?> [Accessed 10.08.2017].
- APAK R, GÜÇLÜ K, OZYUREK M & KARADEMIR SE. 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry* **52**: 7970–7981
- AYDIN O & TURSUN N. 2010. Determination of allelopathic effects of some plant originated essential oils on germination and emergence of some weed seeds. *Kahramanmaraş Sütçü Imam University Journal of Nature Science* **13**(1): 11–17.
- AYEB-ZAKHAMA AE, SAKKA-ROUIS L, FLAMINI G, JANNET HB & HARZALLAH-SKHIRI F. 2017. Chemical composition and allelopathic potential of essential oils from *Citharexylum spinosum* L. grown in Tunisia. *Chemistry & Biodiversity* **14**: 1–10.
- BAJALAN I, ZAND M & REZAEI S. 2013. Allelopathic effects of aqueous extract from *Salvia officinalis* L. on seed germination of barley and Purslane. *International Journal of Agriculture and Crop Sciences* **5**(7): 802–805.
- BAYAR Y & GENÇ N. 2018. Determination of the chemical components, antioxidant and antifungal activities of essential oil and plant extract of *Salvia candidissima* Vahl. *Mediterranean Agricultural Sciences* **31**(2): 93–99.
- BISIO A, DAMONTE G, FRATERNALE D, GIACOMELLI E, SALIS A, ROMUSSI G, CAFAGGI S, RICCI D & TOMMASI ND. 2011. Phytotoxic clerodane diterpenes from *Salvia miniata* Fernald (Lamiaceae). *Phytochemistry* **72**: 265–275.
- BISIO A, FRATERNALE D, GIACOMINI M, GIACOMELLI E, PIVETTI S, RUSSO E, CAVIGLIOLI G, ROMUSSI G, RICCI D & TOMMASI ND. 2010. Phytotoxicity of *Salvia* spp. exudates. *Crop Protection* **29**: 1434–1446.
- BOUAJAJ S, BENYAMMA A, BOUAMAMA H, ROMANE A, FALCONIERI D, PIRAS A & MARONGIU B. 2013. Antibacterial, allelopathic and antioxidant activities of essential oil of *Salvia officinalis* L. growing wild in the Atlas Mountains of Morocco. *Natural Product Research* **27**(18): 1673–1676.
- CARDILE V, RUSSO A, FORMISANO C, RIGANO D, SENATORE F, ARNOLD NA & PIAZOR F. 2009. Essential oils of *Salvia bracteata* and *Salvia rubifolia* from Lebanon: Chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. *Journal of Ethnopharmacology* **126**: 265–272.
- CHANG CC, YANG MH, WEN HM & CHERN JC. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis* **10**: 178–182.
- DEMIRCI B, BAŞER KHC, YILDIZ B & BAHCICIOĞLU Z. 2003. Composition of the essential oils of six endemic *Salvia* spp. from Turkey. *Flavour and Fragrance Journal* **18**: 116–121.

- DIPLOCK AT. 1997. Will the good fairies please prove us that vitamin E lessens human degenerative disease. *Free Radical Research* **27**: 511–532.
- ELMASTAS M, CELIK SM, GENÇ N, AKSİT H, ERENLER R & GÜLCİN İ. 2018. Antioxidant activity of an Anatolian herbal tea—*Origanum minutiflorum*: isolation and characterization of its secondary metabolites. *International Journal of Food Properties* **21**: 374–384.
- HASSANNEJAD S & GHAFARBI SP. 2013. Allelopathic effects of some Lamiaceae on seed germination and seedling growth of dodder (*Cuscuta campestris* Yunck.). *International Journal of Biosciences* **3**(3): 9–14.
- HATANO T, EDAMATSU R, MORI A, FUJITA Y, YASUKARA T & YOSHIDA T. 1989. Effects of the interaction of tannins with co-existing substances. VI.: effects of tannins and related polyphenols on superoxide anion radical, and on 1, 1-Diphenyl-2-picrylhydrazyl radical. *Chemical and Pharmaceutical Bulletin* **37**: 2016–2021.
- ISMAN BM. 2000. Plant essential oils for pest and disease management. *Crop Protection* **19**(8-10): 603–608.
- ISMAN MB, MİRESMAİLİ S & MACHALİ C. 2011. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. *Phytochemistry Reviews* **10**: 197–204.
- KADIOĞLU İ & YANAR Y. 2004. Allelopathic effects of plant extracts against seed germination of some weeds. *Asian Journal of Plant Sciences* **3**(4): 472–475.
- KILIÇ T, DIRMENÇİ T, SATIL F, BİLSEL G, KOCAGÖZ T, ALTUN M & GÖREN AC. 2005. Fatty acid compositions of seed oils of three Turkish *Salvia* species and biological activities. *Chemistry of Natural Compounds* **41**(3): 276–279.
- KUMAR V, MATHELA CS, TEWARI G, SINGH D, TEWARI AK & BISHT KS. 2014. Chemical composition and antifungal activity of essential oils from three Himalayan *Erigeron* species. *LWT - Food Science and Technology* **56**: 278–283.
- LIYANA-PATHIRANA CM & SHAHİDİ F. 2005. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *Journal of Agricultural and Food Chemistry* **53**: 2433–2440.
- LU Y & FOO LY. 2001. Salvianolic acid L, a potent phenolic antioxidant from *Salvia officinalis*. *Tetrahedron Letters* **42**(46): 8223–8225.
- MADSEN HL & BERTELSEN G. 1995. Spices as antioxidant. *Trends in Food Science and Technology* **6**(8): 271–274.
- MOADELİ SN, ROWSHAN V & ABOTALEBİ A. 2013. Comparison of the essential oil components in wild and cultivate population of *Salvia virgata*. *International Research Journal of Applied and Basic Sciences* **4**(2): 337–340.
- NISHIDA N, TAMOTSU S, NAGATA N, SAITO C & SAKAI A. 2005. Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: Inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. *Journal of Chemical Ecology* **31**(5): 1187–1203.
- ONARAN A & YILAR M. 2012. Antifungal activity of *Trachystemon orientalis* L. aqueous extracts against plant pathogens. *Journal of Food Agriculture & Environment* **10**(3): 287–291.
- ORHAN İE, SENOL FS, ERCETİN T, KAHRAMAN A, CELEP F, AKAYDIN G, SENER B & DOĞAN M. 2013. Assessment of anticholinesterase and antioxidant properties of selected sage (*Salvia*) species with their total phenol and flavonoid contents. *Industrial Crops and Products* **201**(41): 21–30.
- OYAIZU M. 1986. Studies on product of browning reaction prepared from glucose amine. *Japan Journal of Nutrition* **44**: 307–315.
- ÖZEN F, YALDIZ G & ÇAMLICA M. 2017. Allelopathic Effects of Some Aromatic Plants Essential Oils in Weed Control. *Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi* **3**(1): 40–48.
- PANDEY DK, TRIPATHI NN, TRIPATHI RD & DIXIT SN. 1982. Fungitoxic and phytotoxic properties of essential oil of *Hyptis suaandolens*. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection* **89**: 344–349.
- RAPPOSELLI E., MELITO S, BARMINA GG, FODDAI M, AZARA E & SCARPA GM. 2015. Relationship between soil and essential oil profiles in *Salvia desoleana* populations: preliminary results. *Natural Product Communications* **10**(9): 1615.
- RE R, PELLEGRINI N, PROTRGGENTE A, PANNALA A, YANG M & RICE-EVANS C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* **26**: 1231–1237.
- ROWSHAN V & KARİMİ S. 2013. Essential oil composition and allelopathic affect of *Salvia macrosiphon* Boiss. on *Zea mays* L. *International Journal of Agriculture: Research and Review* **3**(4): 788–794.
- SEFİDKON F, HOOSHİDARY F & JAMZAD Z. 2007. Chemical variation in the essential oil of *Salvia bracteata* Banks & Soland from Iran. *Journal of Essential Oil Bearing Plants* **10**(4): 265–272.
- SEFİDKON F & MİRZA M. 1999. Chemical composition of the essential oils of two *Salvia* species from Iran, *Salvia virgata* Jacq. and *Salvia syriaca* L. *Flavour and Fragrance Journal* **14**: 45–46.
- SENATORE F, DE FUSCO R & DE FEO V. 1997. Essential oils from *Salvia* spp. (Lamiaceae). I. Chemical composition of the essential oils from *Salvia glutinosa* L. growing wild in Southern Italy. *Journal of Essential Oil Research* **9**: 151–157.
- SINGLETON VL, ORTHOFER R & LAMUELA-RAVENTOS RM. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology* **299**: 152–178.
- SNOWIEC A, KALEMBA D, DROZDEK E & BOCIANOWSKI J. 2017. Phytotoxic potential of essential oils from temperate climate plants against the germination of selected weeds and crops. *Journal of Pest Science* **90**: 407–419.
- TELÇİ İ, BAYRAM E, YILMAZ G & AVCI B. 2006. Variability in essential oil composition of Turkish basil (*Ocimum basilicum* L.). *Biochemical Systematics and Ecology* **34**: 489–497.
- ULUBELEN A, OKSUZ S, KOLAK U, TAN N, BOZOK-JOHANSSON C, CELİK C, KOHLBAU HJ & VOELTER W. 1999. Diterpenoids from the roots of *Salvia bracteata*. *Phytochemistry* **52**: 1455–1459.



- UYGUR FN. 2002. Weeds and biological control. *Proceedings of the V Turkish National Congress of Biological Control*. Atatürk University, Faculty of Agriculture. 4-7<sup>th</sup> September, Erzurum.
- WU JW, LEE MH, HO CT & CHANG SS. 1982. Elucidation of the chemical structures of natural antioxidants isolated from rosemary. *Journal of the American Oil Chemists' Society* 59(8): 339–345.
- YILAR M, KADIOGLU I & TELCI I. 2018. Chemical composition and antifungal activity of *Salvia officinalis* (L.), *S. cryptantha* (Montbret et Aucher ex Benth.), *S. tomentosa* (Mill.) plant essential oils and extracts. *Fresenius Environmental Bulletin* 27(3): 1695–1706.
- YILAR M, ONARAN A, YANAR Y, BELGUZAR S & KADIOGLU I. 2014. Herbicidal and antifungal potential of *Trachystemon orientalis* (L.) G. Don (Kaldırık). *Iğdır University Journal of the Institute of Science and Technology* 4(4): 19–28.

## REZIME



Botanica  
SERBICA

## Hemijski sastav esencijalnog ulja *Salvia bracteata* i biološka aktivnost njenih ekstrakata: antioksidansni, ukupni fenolni, ukupni flavonoidni, antifungalni i alelopatski efekti

Melih YILAR, Yusuf BAYAR, Ahu Alev ABACI BAYAR i Nusret GENÇ

Ovo istraživanje je sprovedeno s ciljem utvrđivanja bioloških aktivnosti vrste *Salvia bracteata*, kao što su njeni antioksidansni, ukupni fenolni, ukupni flavonoidni, alelopatski i antigljivični efekti. U tu svrhu, nadzemni delovi (cvetovi + izdanci + listovi) biljaka *S. bracteata* sakupljeni su u provinciji Kirsehir, Turska, u fazi cvetanja, tokom vegetacijske sezone 2018. Prema rezultatima analize GC-MS identifikovano je 23 sastojka, koji predstavljaju 96.21% esencijalnog ulja. Otkrivena su glavna jedinjenja esencijalnog ulja, kao što su ledol (24.12%), kamfor (15.54%) i valenin (5.64%). Ekstrakti etil acetata, metanola i heksana dobijeni su iz biljaka i određeni su njihov ukupni fenolni (104.63, 121.66 i 20.97 mg GAE/g ekstrakta), kao i sadržaj flavonoida (12.89, 10.85 i 1.13 mg QE/g ekstrakta). Zatim je identifikovana aktivnost uklanjanja radikala DPPH, koja je najveća u ekstraktima metanola i etil acetata. Takođe, kod ovih biljnih ekstrakata su utvrđene aktivnosti TEAC (radikalno uklanjanje katjona), aktivnosti FRAP (redukcija gvožđa) i aktivnosti smanjenja bakra. Utvrđeno je da metanolni ekstrakt *S. bracteata* ima alelopatski učinak na biljke *Rumex crispus* i *Taraxacum officinale*. Iako je metanolni ekstrakt biljaka imao malo uticaja na razvoj micelija *Alternaria solani*, jedne od značajnih patogenih gljivica kod biljaka, pokazalo se da nije delotvoran na patogen *Sclerotinia sclerotiorum*.

**Ključne reči:** *Salvia bracteata*, antioksidativno delovanje, ukupno fenolno-flavonoidno delovanje, antifungalno i alelopatsko delovanje