Research article
Curr. Pers. MAPs, (2022); 5(2): 127-135

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

The Antioxidant Capacities of Leaf Extracts from *Salvia viridis* L.

Kemal KARAMAYA[1], **Belgin COŞGE ŞENKAL**[2*]

[1] Department of Field Crops, Faculty of Agriculture, University of Yozgat Bozok, 66900, Yozgat, Turkey, E-mail: kemalkaramaya@yahoo.com , ORDID ID: 0000-0002-8598-5739
[2] Department of Field Crops, Faculty of Agriculture, University of Yozgat Bozok, 66900, Yozgat, Turkey, E-mail: belgin.senkal@yobu.edu.tr , ORDID ID: 0000-0001-7330-8098

*Corresponding author : e-belgin.senkal@yobu.edu.tr

http://doi.org/1038093/cupmap.1188295

Received :13/10/2022
Accepted : 13/12/2022

Abstract

This research was conducted at Yozgat Bozok University to define the antioxidant activity of *Salvia viridis* L. grown in the field and *in vitro* conditions. The leaves of the plants grown under field conditions were collected in the pre-flowering period. The leaf extracts prepared with methanol were used in the analysis. DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical screening activity was used to identify antioxidant activity. The total phenolic, flavonoid, and DPPH IC50 values of *S. viridis* L. grown in the field and under *in vitro* conditions were found as 184.15 ± 36.70 mg GAE g^{-1} and 66.46 ± 0.19 mg GAE g^{-1}, 212.92 ± 11.18 mg QE g^{-1} and 212.92 ± 11.18 mg QE g^{-1}, 117.51 mg $ml^{-1}$and 185.40 mg ml^{-1}, respectively. According to the findings of this study, it was determined that leaves of *S. viridis* L. grown in field conditions exhibited more antioxidant activity than *in vitro* conditions.

Key Words: *Salvia viridis* L., Sage, DPPH, Phenolic, Tissue Culture

1. Introduction

Most taxa in the family Labiatae/Lamiaceae are important sources of secondary metabolites (essential oils, alkaloids, saponins, etc.). For this reason, they have a high potential to be used in different sectors such as food, medicine, and cosmetics (Carović-Stanko et al., 2016). Sage, the largest member of the Lamiaceae family, is the general name of the species in the genus *Salvia*. It is reported that there are over 1000 species of the genus *Salvia* worldwide. In the Flora of Turkey, ninety-nine *Salvia* species are naturally distributed and fifty-one of these species are endemic *Salvia viridis* L., one of these species, is an annual plant that grows erect (11-34 cm in height), has a hairy structure, and has lilac-purple flowers. This species shows spread throughout Turkey except for Eastern Anatolia Region (Celep and Kahraman, 2012). While the seeds and leaves of this plant are used in fermentation barrels to increase the quality of the liquor, the powdered leaf of the plant is also used in the remedy of gum and throat infections (Cosge Senkal, 2019).

In the food industry, mainly synthetic antioxidants (butyl hydroxytoluene (BHT), butyl hydroxy anisole (BHA), tertiary butyl hydroxyquinone (TBHQ), and propyl gallates (PG), etc.) are used to protect foods from oxidative degradation and to extend their

storage periods. Although these synthetic antioxidants are highly effective, stable, and cheap, they have potential side effects such as mutagenic, carcinogenic, and teratogenic (Mammadov, 2014). During the functioning of normal metabolic activities in both humans and animals, free radicals occur under the influence of various external factors or environmental factors. Reactive oxygen species are short-lived and unstable. Therefore, they can easily react with various biological molecules (nucleic acids, lipids, carbohydrates, proteins, etc.) in organisms. As a result, they cause the development of many diseases (cancer, liver diseases, immune system diseases, etc.), especially aging in humans (Halliwell and Gutteridge, 1990).

It is known that antioxidant substances destroy these free radicals. For this reason, consumer preferences have led the industry to seek natural sources of antioxidants, and medicinal and aromatic plants have become increasingly important. Also, flavonoids, antioxidant substances and phenolic compounds obtained from natural plants are effective in protecting from these free radicals formed in the human body (Kahkönen et al.,1999). Plants are a major source of natural antioxidant compounds. Therefore, plants are considered important antioxidants. Among natural antioxidants, phenolic substances (flavonoids, coumarins, tocopherols, phenolic acids, cinnamic acid, etc.) are the most common. Under normal conditions, the damage caused by oxygen radicals is kept under control by the organism's effective antioxidant systems. Studies have shown that certain phenolic antioxidants prevent cell death caused by oxidative stress. Studies have shown that phenolic and flavonoid substances are abundant in *Salvia* taxa. It is stated that various phenolics (caffeic acid and chlorogenic acid etc.) and flavonoids (apigenin and luteolin etc.) are found in the extracts obtained from aerial parts of *S. viridis* (Rungsimakan and Rowan, 2014).

Due to limited quantities of production, difficulties in obtaining standard quality products, being far away from industrial areas of production areas, and high extraction and purification costs, some medicinal and aromatic plants with economic and industrial value in certain countries of the world are produced with tissue or cell techniques under controlled conditions as an alternative method. Micropropagation is the obtaining of new plants from plant parts (seed, leaf, root, stem, shoot, embryo, callus, single-cell or pollen grain, etc.) taken from a plant and have the potential to form a complete plant, in artificial nutrient environments and under aseptic conditions (Baydar, 2013).

The aim of this study was to compare the antioxidant capacities of the leaves obtained in the vegetative period of the plants *in vitro* culture and in field-grown of *S. viridis* L.

2. Material and Methods

2.1. Plant Material

In this research, *S. viridis* L. seeds collected from the collection plots in Yozgat Bozok University Gedikhasanlı Application and Research Station (35 ° 09 '34 "E-39 ° 35 '13" N, Altitude: 1135 m) in 2017 were used as material. The collected seeds were placed in a paper bag and stored in the shade in room condition.

2.2. Viability and Germination Tests

The viability of the seeds was determined with the Tetrazolium (2, 3, 5- Triphenyl Tetrazolium Chloride-TTC) test and 50 seeds were used (AOSA, 2000). Standard germination tests were set up in Petri dishes with 4 replicates using 20 seeds. The average germination rate was determined on the 21st day (Subasi and Guvensen, 2010).

2.3. Growing of Plants in Field Conditions

The research was carried out in the Yozgat Bozok University Research and Application Area/Turkey. Seeds were sown in vials

containing peat. The seedlings that grow well under greenhouse conditions and reach sufficient size were planted in the collection plot at 70x70 cm intervals (30.05.2018). The parts of *S. viridis* used in folk medicine are the leaves, flowering stems, and seeds. In this study, we used leaves for comparison with in vitro culture. Leaf samples before flowering were taken from the plants to do the necessary maintenance (hoeing, irrigation, etc.) on 15.08.2018. According to the soil analysis results, the clay, silt, and sand content of the experimental area soil is 476 g kg^{-1}, 138 g kg^{-1}, and 386 g kg^{-1}, respectively, pH is neutral (7.09), slightly salty (0.178%), lime content and ($CaCO_3$, 7.15%), organic matter content is moderate (2.49%), total nitrogen is sufficient (N, 0.15%), phosphorus (P, 78 µg g^{-1}) and potassium (K, 728 µg g^{-1}) amounts are high has been determined (Yakupoglu, 2018). During the vegetation period (June, July, and August) in the experimental area, the total precipitation was 41.8 mm, the average temperature was 22.23 ^{o}C, and the average relative humidity was 53.6% (MGM, 2019).

2.4. Production under *in vitro* Conditions

S. viridis L. seeds, which will be produced under aseptic conditions, were sown in Caisson brand MS medium on 27.08.2018. All operations during the study were carried out in an ESCO brand laminar flow sterile cabin. First of all, MS (Morishige and Skoog) medium was prepared where seeds will be sown and the pH was fixed to 5.7-8. For the surface sterilization of the seed, the first 20% sodium hypochlorite solution was prepared and 2 drops of Tween 20 for every 100 ml were dropped into it. Seeds were soaked in this solution for 10 minutes and then washed 3 times with sterilized distilled water. The seeds planted in glass plates were placed in a climate chamber operating under a 16/8-hour photoperiod at 4.000 lux light intensity during the day and 20 ° C at night. Plants germinated in Petri and then were taken into glass test tubes and their development was regularly monitored. Plants that reached the required maturity for the determination of antioxidants were taken from the tubes and taken to a shaded place to dry. Later, the samples, dried as desired, were ground and made suitable for analysis.

2.5. Preparation of Extracts

The leaves of *S. viridis* L., both grown in field (a) and in vitro conditions (b), were dried in the shade after harvest (Figure 1). The leaves of the plants grown under field conditions were collected in the pre-flowering period. Dry leaf samples were ground using a blender. This powdered sample was weighed at 0.5 g and 5 mL of methanol (1/10 w v^{-1}) was added to each sample. The incubation time and temperature of the samples in the oven (Elekto-mag M 5040 P) were set as 24 h and 40 ^{o}C, respectively. The samples removed from the oven were filtered through filter paper (Whatman No1). Methanol was removed from the samples with a rotary evaporator (Heating Bath B-491, BUCHI). 2 ml methanol was added into the balloon flasks that were dried in the oven for 24 h and vortexed. The extracts were preserved at 4 °C until the analysis began (Zakaria et al., 2019).

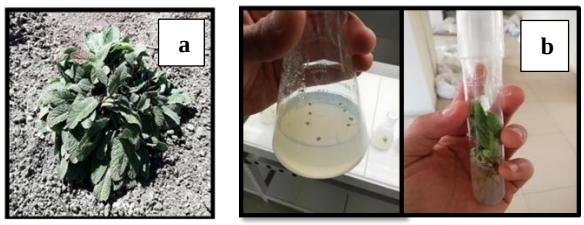

Figure 1. *Salvia viridis* in field (a) and *in vitro* (b) conditions

2.6. Determination of DPPH Radical Scavenging Activity

The antioxidant capacity of the extracts was detected using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical (Frezzini et al., 2019). In order to be able to compare the samples, first, the amount to neutralize a

certain amount of DPPH radical was determined. 16 mg of DPPH was dissolved in 100 ml of CH_3OH (methanol) and adjusted to 0.1 µM. DPPH readings were made in the spectrophotometer at 517nm. Dilution with methanol was done until the absorbance value was 1000. Samples prepared at six different concentrations (10, 20, 40, 80, 160, 320) were incubated for 30 minutes. The prepared samples were incubated for 30 minutes in the dark. In the study, in which BHT (butylhydroxytoluene) and BHA (butyl hydroxyanisole) were used as controls, analyzes were carried out with four replications. The equation used to calculate the %DPPH radical scavenging activity of the samples: "% DPPH scavenging efficiency = [(A control - Extract) / A control] x100". PerkinElmer Lambda 25 UV / VIS spectrophotometer device was used for spectrophotometric measurements.

2.7. Determination of Total Phenolic Content

The total phenolic content of the extracts was determined using the Folin-Ciocalteu Reagent (FCR) method (Gezer et al., 2006). 100 ml (Na_2CO_3) solution was prepared for the study. 40 µL of the extract was added to glass tubes containing 2.4 mL of distilled water. In the control application, the same amount of methanol was added instead of the extract. The samples were added 200 µL of folin and 600 µL of saturated Na_2CO_3, and then 760 µL of distilled water was added and vortexed. The absorbance measurements of the samples incubated for 2 hours at room temperature were made at 765 nm. Gallic acid was used for standard phenolic control. The values obtained are expressed as Gallic acid equivalents. To prepare the Gallic acid solution, 3 mg of Gallic acid was dissolved well in 15 mL of methanol. Then, by dilution, 100, 125, 150, 175, and 200 µg mL^{-1} control groups were prepared and the Gallic acid curve was drawn. The procedures were carried out in four replications. The total phenolic contents of the samples were determined as a result of the measurements in the PerkinElmer Lambda 25 UV/VIS spectrophotometer device.

2.8. Determination of Total Flavonoid Content

The total flavonoid compound amounts of the extracts were determined by optimizing the aluminum chloride colorimetric method of Biju et al. (2014). 50 µL of the previously prepared 1mg mL-1 extract was put into glass tubes. 950 µL methanol was added to it. Then 4 mL of distilled water was added and mixed for thorough dissolution. Subsequently, 0.3 mL sodium nitrate $NaNO_2$ of 5% was added and incubated for 5 minutes. Then 0.3 mL of 10% aluminum chloride (AlCl**3**) was added and incubated for 6 minutes. After incubation, 2mL of 1mol L^{-1} sodium hydroxide (NaOH) was added. 2.4 mL of distilled water was added to the obtained solution and it was completed to 10mL. The solution was incubated for 15 minutes and then absorbance was measured at 510 nm. For the Quercetin standard, 1 mg mL-1 was prepared as the main stock, and 6 different concentrations (10, 20, 40, 60, 80,100 µg mL^{-1}) were obtained by dilution. The analysis was carried out in four replications. The total flavonoid content of the samples was determined according to the measurements made in the PerkinElmer Lambda 25 UV/VIS spectrophotometer device.

2.9. Statistical Analysis

All studies were done in 4 repetitions. DPPH results of the samples were evaluated by analysis of variance and the LSD (Least Significant Difference) test was applied for the differences between the averages, and the extraction yields, phenolic, and flavonoid content were compared with the t-test. For analysis used the TARİST statistics program, and the results were presented as mean ± standard deviation

(SD). Also, the bilateral relationships were detected using the MINITAB 19 package program (Acikgoz et al.,2004).

3. Results and Discussion

3.1. Viability and Germination in Seeds

Some physical properties were determined as well as the vitality and germination values of the seeds. The width, length, and 1000 seed weight of seeds were determined as 1.05 mm, 1.96 mm, and 2.56 g, respectively. Yilar and Altuntaş (2017) reported that they recorded the length, width, and thickness values of *S. viridis* seeds between 2.57-2.88 mm, 1.52-1.64 mm, and 1.04-1.24 mm, respectively. It was observed that there was no germination problem in the seeds of the plant and the germination value was 93% on average. According to the results of the Tetrazolium test; the viability rate of seeds belonging to the species was determined as 76%. Seeds belonging to the genus Salvia have a mucilaginous seed coat. For this reason, seeds either do not germinate at all or germinate very little (Hayta and Arabaci, 2011, Ozcan et al., 2014). However, a high germination value was recorded in the seeds of *S. viridis* in our study.

3.2. Extraction Yield of Samples

The extract yields of the samples are presented in Table 1. When these data are examined, it is seen that the extract yield of the plants grown in the field is higher than those grown under in vitro conditions.

Table 1. Extract yield of *S. viridis* leaves

	Field-Grown	***in vitro***
Dry Matter Amount (g)	0.5	0.5
Extract Amount (g)	0.0685±0.216	0.0509±0.0056
Extract Ratio (%)	13.03±4.0284	9.45±1.0401
t-value	1.229^{ns}	

^{ns}: not significant

3.3. Antioxidant Capacities of the Samples

This test is based on the spectrophotometric determination of the characteristic purple discoloration by scavenging the stable free radical 2,2-diphenylpicrylhydrazyl (DPPH) by these chemicals in the presence of electron or hydrogen atoms-donating antioxidant chemicals (Cuendet et al., 1997, Esmaeili et al., 2015). The % scavenging activity obtained as a result of the determination of DPPH free radical scavenging activities of extracts obtained from the samples were subjected to linear regression analysis and the IC50 value was calculated. Accordingly, the IC50 values obtained in the analysis were determined in terms of mg extract mL^{-1}. The concentration density required to scavenge half of the DPPH radical from the medium is expressed as the IC50 value. A low IC50 value indicates that the antioxidant activity of the sample is high, while a high value indicates that the activity of the sample is low (Aqil et al., 2006). In terms of DPPH values in our study, the difference between samples was statistically significant at the 1% level. Considering that the antioxidant activity increases as the DPPH radical scavenging activity value decrease, it was observed that the antioxidant activity of the plants grown in the field is higher than the plants grown under in vitro conditions. On the other hand, it was determined that the DPPH radical scavenging activity of both samples included in the study was much lower than the synthetic antioxidants used as control (Table 2).

While the phenolic substance contents of the samples were found statistically significant at a 1% level, the flavonoid substance contents were not significant. However, the leaves of plants grown in the field had 51.163 mg QE g-1 higher flavonoid content than in vitro conditions. While the phenolic substance contents of the samples were found statistically significant at a 1%

level, the flavonoid substance contents were not significant.

Table 2. Antioxidant capacities of the samples

	Total Phenolic (mg GAE g^{-1})	Total Flavonoid (mg QE g^{-1})	DPPH IC_{50} (mg ml^{-1})
Field-Grown	184.15±36.70	212.92±11.18	117.51±6.60 [b]
in vitro	66.46±0.19	161.76±56.13	185.40±4.78 [c]
BHA (uq ml^{-1})			19.662 [a]
BHT (uq ml^{-1})			13.818 [a]
F-value			1275.76**

** Statistically significant at the 0.01% level

However, it was recorded that the leaves of plants grown in the field had 51.163 mg QE g-1 higher flavonoid content than in vitro conditions (Figure 2, Figure 3).

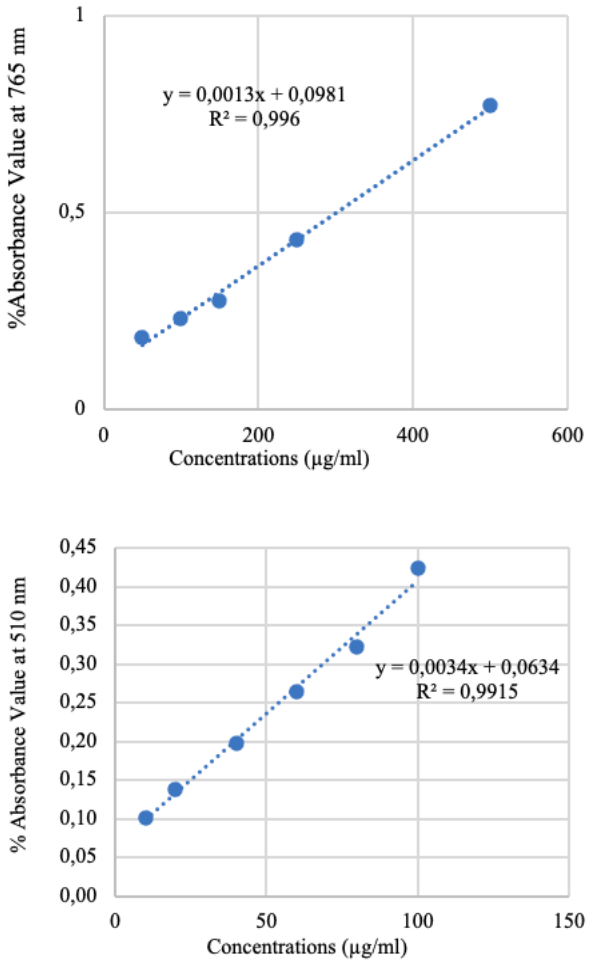

Figure 2. Gallic acid (on top) and Quercetin (bottom) standard curves

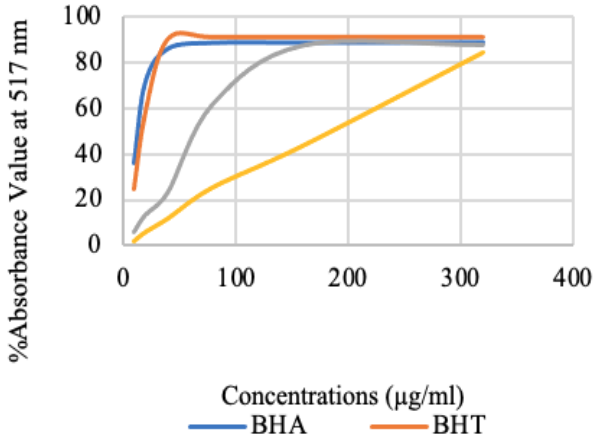

Figure 3. DPPH radical scavenging activity capacities of samples

Similarly, in this study that antioxidant activity was investigated in both wild plants and callus cultures of three *Ephedro* spp. was observed that both wild plants and callus of all three *Ephedro* species had antioxidant activity, but callus exhibited lower activity than wild species. In addition, the phenol content of wild plants was found to be higher than callus (Parsaeimehr et al., 2010).

Due to its reproducibility and low cost, the DPPH test is the most commonly used method to evaluate the antioxidant potential of plant materials. It has been used to test the activity of extracts obtained from aerial parts of a large number of Salvia species (*S.officinalis, S.aethiopis, S.candidissima, S. verticillate, S.virgata, S. hypargeia, S.sclarea, S. limbate, S. microstegia,* and *S.glutinosa*, etc.) (Tosun et al.,2009; Tepe et al., 2006; Veličković et al. 2011). Bayan and Genc, (2016), in their research to define the antioxidant capacity of methanol extract on *S. verticillata* species, determined the IC50 value of DPPH activity as 11.47±0.30 mg ml^{-1}. They also recorded total phenolic and total flavonoid contents as 140.18±8.73 mg GAE g^{-1} extract and 51.56±1.18 mg QE g^{-1} extract, respectively. The antioxidant capacities, total phenolic and flavonoid contents of *Salvia* species exhibit broad variation. For example, in the study carried out with different *Salvia*

species, the lowest and highest antioxidant capacity values were found in *S. dichroantha* (73.855 mg GAE $g^{-1)}$ and in *S. heldreichiana* (80.207 mg AAE g^{-1}), respectively. The highest total phenolic content was found in *S. tomentosa* (13.316 mg GAE 100ml^{-1}), while the lowest total phenolic content was recorded in *S. halophila* (6.168 mg GAE 100 ml^{-1}) (Er, 2012)

Phenols are very important plant components that have free radical scavenging ability due to their hydroxyl groups (Hatano et al., 1989). Therefore, the phenolic content of plants directly contributes to their antioxidant effect and flavonoid biosynthesis (Bendini et al.,2006; Dlugosz et al.,2006; Wojdylo et al.,2007; Parsaeimehr et al.,2010). In our study, it was recorded that there was a positive correlation between the DPPH activities of the extracts and the total phenolic and flavonoid amounts (Figure 4). Similar findings were also expressed by Tosun et al. (2009).

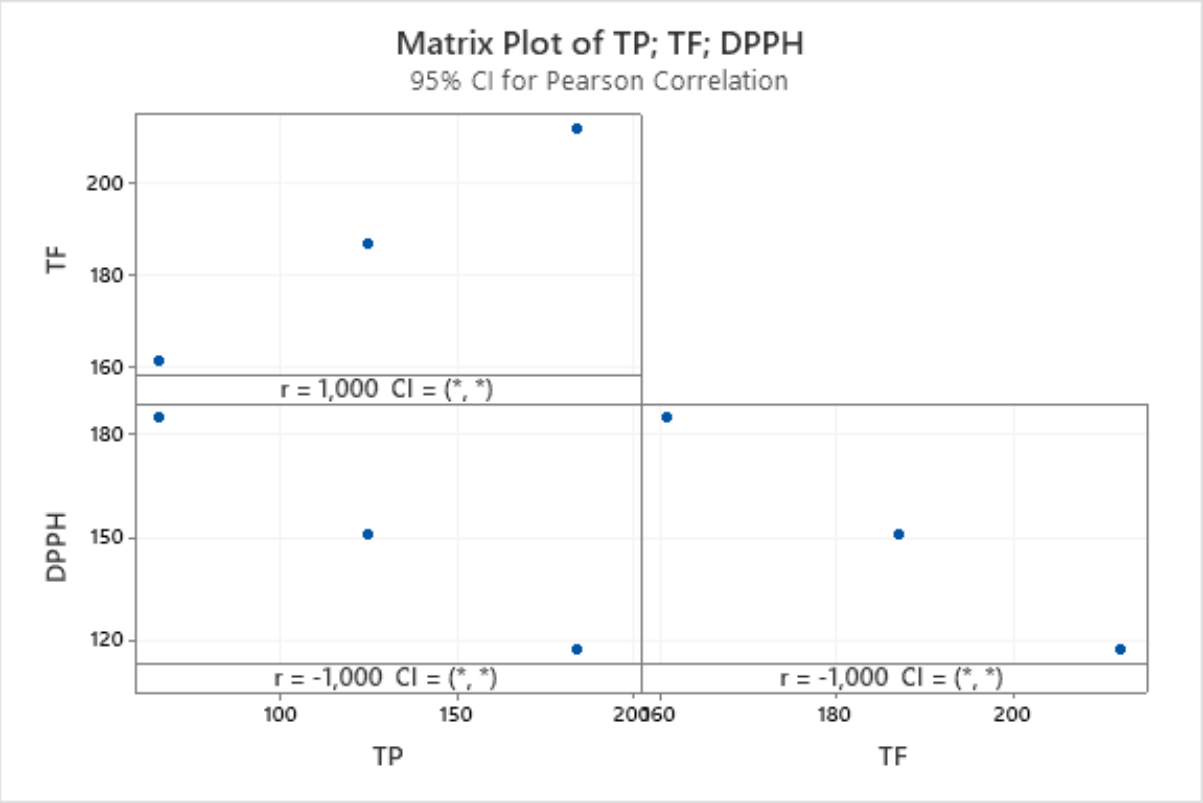

Figure 4. Bilateral Relations among TP (Total Phenolic), TF (Total Flavonoid) and DPPH

Plants that are members of the Lamiaceae family contain many polyphenolic compounds. Rosmarinic acid ($C_{18}H_{16}O_8$), carnosic acid ($C_{20}H_{28}O_4$), and salvianolic acid ($C_{26}H_{22}O_{10}$), are the main phenolic components identified in extracts from *Salvia*. Rosmarinic acid is present in the above-ground parts and roots of *S. viridis*, but the rosmarinic acid concentration in its leaves was very low (Fotovvat et al., 2019, Zengina et al.,2019) We only used leaves in our study. And it was determined that the antioxidant capacity of the leaves was low.

4. Conclusion

In this research, the antioxidant activity of leaves obtained from in vitro and field-grown plants of *S. viridis* was compared for the first time. Our findings showed that the antioxidant capacity determined in the leaves of field-grown *S. viridis* was higher than in vitro conditions. As it is known, various environmental stresses often cause an increase in plant secondary metabolites. In previous studies, it has been explained that aerial parts and roots of *S. viridis* show strong

antioxidant activity. Therefore, economically important raw materials from this species can be produced. This study showed that desirable bioactive substances for pharmaceutical purposes can be obtained from plants grown in vitro. However, in order to bring this species to the economy, its natural compounds and biological activity should be clarified with comprehensive studies.

Acknowledgments

We thank Dr. Tansu Uskutoğlu for his contributions to the laboratory studies.

Author Contribution

This manuscript has been prepared from the master thesis.

KK: Supply of materials required for research, field, and laboratory. He contributed to the establishment and execution of the experiments, the acquisition and evaluation of data, and the writing of the Master's Thesis.

BCS: Planning the research, supplying the plant materials required for the research, establishing and conducting field and laboratory trials, obtaining data and making statistical analyzes. He contributed to the writing of the Master Thesis and the conversion of the Thesis into an article.

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

1. Acikgoz, N., Ilker, E., Gokcol, A. (2004). Biyolojik Araştırmaların Bilgisayarda Değerlendirilmeleri, Ege University. Seed Technology Application and Research Center Publication, İzmir, pp. 56-160.
2. AOSA, (2000). Association of Official Seed Analysts. AOSA/ SCST Tetrazolium Testing Handbook. Lincoln, NE: Association of Official Seed Analysts.
3. Aqil, F., Ahmad, I., Mehmood, Z. (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turkish Journal of Biology, 30, 77-183.
4. Bayan, Y., Genc, N. (2016). Salvia verticillata subsp. amasiaca'nın toplam fenolik madde ve antioksidan kapasitesinin belirlenmesi, Nevşehir Bilim ve Teknoloji Dergisi, 5,158-166.
5. Baydar, H. (2013). Tıbbi ve Aromatik Bitkiler Bilimi ve Teknolojisi, Süleyman Demirel University Faculty of Agriculture Publication, Isparta,pp.23-141.
6. Bendini, A., Cerretani, L., Pizzolante, L., Gallina-Toschi, T., Guzzo, F., Cedolo, F., Andereetta, F., Levi, M. (2006). Phenol content related to antioxidant and antimicrobial activity of Passiflora spp. Extracts. Eur Food Res Technol, 223, 102–109.
7. Biju, J., Sulaiman, C.T., Satheesh, G., Reddy, V.R.K. (2014). Total phenolics and flavonoids in selected medicinal plants from Kerala. International Journal of Pharmaceutical Research, 6, 406-408.
8. Carović-Stanko, K., Petek, M., Grdiša, M., Pintar, J., Bedeković, D., Ćustić, M.H., Satovic, Z. (2016). Medicinal plants of the family Lamiaceae as functional foods – a Review. Czech Journal of Food Sciences, 34,377–390.
9. Celep, F., Kahraman, A. (2012). *Salvia* L. In: Güner, A., Aslan, S., Ekim, T., Vural, M. and Babac, M.T. Türkiye Bitkileri Listesi (Damarlı Bitkiler), Nezahat Gökyiğit Botanik Bahçesi ve Flora Dizisi. (Turkish Plant List Vein Plants, İstanbul: Nezahat Gökyiğit Botanic Garden and Floristics Research Society Publication., in Turkish).
10. Cosge Senkal, B. (2019). The effect of cuttings stages on components and content of essential oils from *Salvia viridis* L. KSU Journal of Agriculture and Nature, 22, 71-77.
11. Cuendet, M., Hostettmann, K., Potterat, O.H. (1997). Iridoid glucosides with free radical scavenging properties from Fagraea blumei. Helvetica Chimica Acta, 80,1144-1152.
12. Dlugosz, A., Lembas-Bogaczyk, J., Lamer-Zarawska, E. (2006). Antoxid increases ferric reducing antioxidant power (FRAP) even stronger than vitamin C. Acta Poloniae Pharm, 63, 446–448.
13. Er, M. (2012). Biochemical and bioactive features of some Salvia L. (Adaçayı) species growing in Konya, The Graduate School of Natural and Applied Science of Selçuk University, Konya, pages 31.
14. Esmaeili, A., Taha, R.M., Mahajer, S., Banisalam, B. (2015). Antioxidant activity and total phenolic and flavonoid content of various solvent extract from In Vivo and In Vitro grown Trifolium pratense L. (Red Clover). BioMed Research International, ID 643285.
15. Fotovvat, M., Radjabian, T., Saboora, A. (2019). HPLC fingerprint of important phenolic compounds in some Salvia L. species from Iran. Records of Natural Products, 13,37-49.
16. Frezzini, M.A., Castellani, F., De Francesco, N., Ristorini, M., Canepari, S. (2019). Application of DPPH assay for assessment of particulate matter reducing properties. Atmosphere, 816,1-14.
17. Gezer, K., Duru, M.E., Kıvrak, I., Turkoglu, A., Mercan, N., Turkoglu, H., Gulcan, S. (2006). Free-radical scavenging capacity and antimicrobial

activity of wild edible mushroom of Turkey. African Journal of Biotechnology, 5,1924-1928.
18. Halliwell, B., Gutteridge, J.M.C. (1990). A Role of free radicals and catalytic metal ions in human disease: An overview. Methods in Enzymology,186, 1-85.
19. 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 2,2'-diphenylpicrylhydrazyl. Chemical and Pharmaceutical Bulletin, 37, 2016-2021.
20. Hayta, E., Arabaci, O. (2011). Kekik olarak adlandırılan bazı bitki cinslerinin tohumlarında farklı çimlendirme yöntemlerinin belirlenmesi. ADÜ Ziraat Fakültesi Dergisi, 8, 91-101.
21. Kahkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry, 47, 3954-3962.
22. Mammadov, R. (2014). Tohumlu Bitkilerde Sekonder Metabolitler. Nobel Akademik Yayıncılık, Ankara, pp.15-129.
23. Meteoroloji Genel Müdürlüğü (MGM).2019. https://www.mgm.gov.tr, Access Date: April 30, 2019.
24. Ozcan, I.I., Arabaci, O., Ogretmen, N.G. (2014). Bazı adaçayı türlerinde farklı tohum çimlendirme uygulamalarının belirlenmesi. Türk Tarım-Gıda ve Teknoloji Dergisi, 2,203-207.
25. Parsaeimehr, A., Sargsyan, E., Javidnia, K. (2010). A comparative study of the antibacterial, antifungal and antioxidant activity and total content of phenolic compounds of cell cultures and wild plants of three endemic species of Ephedra. Molecules, 15,1668–1678.
26. Rungsimakan, S., Rowan, M.G. (2014). Terpenoids, flavonoids and caffeic acid derivatives from *Salvia viridis* L. var. Blue Jeans. Phytochemistry, 108,177-188.
27. Subasi, U., Guvensen, A. (2010). Seed germination studies on rare endemic Salvia smyrnaea Boiss. (Lamiaceae). Biological Diversity and Conservation, 3,126-132.
28. Tepe, B., Sokmen, M., Akpulat, H.A., Sokmen, A. (2006). Screening of the antioxidant potentials of six Salvia species from Turkey. Food Chemistry, 95,200-204.
29. Tosun, M., Ercisli, S., Sengul, M., Ozer, H., Polat, T., Ozturk, E. (2009). Antioxidant properties and total phenolic content of eight Salvia species from Turkey. Biological Research, 42,175-181.
30. Veličković, D.T., Karabegović, I.T., Stojičević, S.S., Lazić, M.L., Marinković, V.D., Veljković. V.B. (2011). Comparison of antioxidant and antimicrobial activities of extracts obtained from Salvia glutinosa L. and Salvia officinalis L. Hemijska Industrija, 65,599–605.
31. Wojdylo, A., Oszmianski, J., Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem, 105, 940–949.
32. Yakupoglu, T. (2018). Bozok Yöresinde Araştırma Amaçlı Kullanılan Tarım Arazilerinin Bazı Toprak Özellikleri ve Bölgesel Kalkınmaya Katkı Sağlayacak Araştırmalar Açısından Çeşitli Öneriler, III. Uluslararası Bozok Sempozyumu, Yozgat, Bildiri Kitabı, pp. 1338-1343.
33. Yilar,M., Altuntas, E. (2017). Determination of some physical properties of the annual and perennial sage (*Salvia viridis L., Salvia cryptantha* Montbret et Aucher) varieties seeds. Mediterr Agric Sci, 30,137-141.
34. Zakaria, N.A., Ibrahim, D., Sulaiman, S.F., Supardy, N.A. (2011). Assessment of antioxidant activity, total phenolic content and in vitro toxicity of Malaysian red seaweed, Acanthophora spicifera. Journal of Chemical and Pharmaceutical Research, 3,182-191.
35. Zengina, G., Mahomoodally, F., Picot-Allain, C., Diuzheva, A., Jekő, J., Cziáky, Z., Cvetanović, A., Aktumsek, A., Zeković, Z., Rengasamy, K.R.R. (20199. Metabolomic profile of *Salvia viridis* L. root extracts using HPLC_MS/MS technique and their pharmacological properties: a comparative study. Industrial Crops and Products, 131,266-280.