Turkish Journal of Agricultural and Natural Sciences
Special Issue: 2, 2014

TÜRK
TARIM ve DOĞA BİLİMLERİ
DERGİSİ

TURKISH
JOURNAL of AGRICULTURAL
and NATURAL SCIENCES

www.turkjans.com

The Determination of Antioxidant Activity of Some Sage Populations of In The Marmara Region

[a]Sevinç Seçil ERDOĞAN, [b]Ünal KARIK, [c]Kemal Hüsnü Can BAŞER
[a]Department of Food Technology, Atatürk Central Horticultural Research Institute, Yalova, Turkey
[b]Department of Industrial Plants, Aegean Agricultural Research Institute, Menemen, İzmir, Turkey
[c]Department of Pharmacognosy, Faculty of Pharmacy Anadolu University, Eskişehir, Turkey
Corresponding author: ssecil@yalovabahce.gov.tr

Abstract

Methanolic extracts of 39 different population of three species of Salvia (*Salvia fruticosa* Mill. 20 sample, *Salvia pomifera* Mill. 4 sample and *Salvia tomentosa* Mill. 15 sample) were analyzed for their antioxidant properties. Samples were collected from different natural ecological areas in Marmara Region in Turkey. The antioxidant activity (AA) was investigated with the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and expressed as trolox equivalents (TE). The amount of total phenolics was determined by using Folin–Ciocalteu method and flavonoid contents in the extracts were determined by a colorimetric method. The AA values of the spices ranged from 287. 57 to 450.51 µmol trolox equivalent (TE) / 100 g dry weight (DW). The total phenolic and flavonoid content ranged from 488.07 to 1654.89 mg of gallic acid equivalents (GAE) / 100 g DW and 664.03 to 3693.20 mg of catechin equivalents (CE) / 100 g DW respectively.

Key words: Sage, *Salvia fruticosa* Mill., *Salvia pomifera* Mill., *Salvia tomentosa* Mill., antioxidant activity

Introduction

Salvia, the largest genus of Lamiaceae, includes about 900 species, widespread throughout the world. This genus is represented, in the Flora of Turkey, by 88 species and 93 taxa, 45 of which are endemic (Ozhatay et al., 2011). In the past few decades however, sage has been the subject of an intensive study for its phenolic antioxidant components. Several studies have shown sage to contain a range of potent antioxidants Due to the essential oil and antioxidant components of sage it is an economically interesting plant to study. The essential oil and flavourants of sage are used as basic material for various food, cosmetic and pharmaceutical preparations. Sage antioxidants can be used as an alternative to the well-known rosemary antioxidants for the protection and preservation of certain food and nutraceutical products to extend their shelf life (Durling et al., 2007). Pizzale et al., (2002) were determined that the antioxidant activities of sage samples were, on average, higher than those of oregano samples. Some samples of sage had a very high antioxidant activity, with induction times more than 10-fold higher than that of lard used as the reference sample. The superoxide scavenging activities of the rosmarinic acid derivatives were 15–20 times stronger than trolox. Being the major polyphenols in sage (or sage residue), the rosmarinic acid derivatives were more likely to be responsible for most of the observed antioxidant activity of the nonditerpenoid component in sage (Lu and Foo, 2001).

This study was to compare the antioxidant activity, total phenolics and flavonoid contents of dried herbs of 39 different population of three species of *Salvia* (*Salvia fruticosa* Mill. 20 samples, *Salvia pomifera* Mill. 4 sample and *Salvia tomentosa* Mill. 15 samples) species from the Lamiaceae family. Salvia is an important genus widely cultivated and used in flavouring and folk medicines. The genus has attracted great interest so much so that it has been the subject of numerous chemical studies. It is a rich source of polyphenols, with an excess of 160 polyphenols having been identified, some of which are unique to the genus (Lu and Foo, 2002).

Material and Methods

Samples were collected from different natural ecological areas in Marmara Region in Turkey. *S. tomentosa* were collected from towns and villages, Bursa, Balıkesir, Canakkale; *S. fruticosa*

from towns and villages, Tekirdağ, Balıkesir; *S. Pomifera* from towns and villages, Çanakkale, Turkey, (on May, June 2010). Collected plant materials were dried in oven (35 °C, 48 hour, 10-12 % moisture) then packed in paper bags and stored for 7 months.

Preparation of the methanol extracts

The leaves of plants were separated from the stem, and ground in a grinder. Extraction The dried sample was chopped into a pot and 5 g exactly was weighed into a 250 ml Erlenmeyer flask; 100 ml of methanol was added and the sample was left to infuse overnight. The sample was then refluxed for 1 h. After cooling to room temperature, the mixture was filtered through Whatman No 4 filter paper in a Buchner funnel. The filtered solution was evaporated under reduced pressure (Rotavapor, T <40°C) and the extract was further dried in a desiccator, under vacuum, to constant weight. The extract was then weighed, dissolved in 50ml of methanol and transferred to a 50ml volumetric flask. The solution was stored -18°C (Pizzale et al., 2002).

Determination of antioxidant activity using DPPH assay

The antioxidant activity was investigated with the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and expressed as TE after Burits ve Bucar, 2000; Wojdyło et al., 2007. The DPPH test was carried out as described before. 50 mL of extract were mixed with 5 mL of a 0.004% methanol solution of DPPH. After an incubation period of 30 min, the absorbance of the samples was read at 517 nm using a Hitachi spectrophotometer. The results were expressed in µmol TE / 100 g dry matter. All determinations were performed in triplicate.

Determination of total phenolic compounds

The amount of total phenolics was determined by using Folin–Ciocalteu method. Each sample (0.5 ml) was mixed with 2.5 ml FCR (diluted 1:10 v/v) followed by 2 ml of Na_2CO_3 (7.5 % v/v) solution. After incubation at 30 ºC for 90 min, the absorbance was measured at 765 nm. Results were expressed as gallic acid equivalents (mg gallic acid/g dried extract) (Asadi et al., 2010).

Determination of total flavonoids

Flavonoid contents in the extracts were determined by a colorimetric method. 0.5 ml extract was mixed with 2 ml distilled water and 0.15 ml of a 15 % $NaNO_2$ solution. After 6 min, 0.15 ml of a 10 % $AlCl_3$ solution was added. After 6 min, 2 ml of a 4 % NaOH solution was added to the mixture, which was adjusted to 5 ml with distilled water. The solution was mixed well and the intensity of pink color was measured at 510 nm 15 min later. (+)-Catechin was used to calculate the standard curve and the results were expressed as mg of (+)-Catechin equivalents per gram of extract (Asadi et al., 2010).

Results and Discussion

The amount of total phenolics varied widely in sages and ranged from 488.07 ± 14.92 to 1654.89 ± 36.88 mg of gallic acid equivalents (GAE) / 100 g DW (Table 1). The highest level of phenolics were found at *Salvia pomifera* sample collected from Dereağzı, Uludağ, Bursa, while the lowest was at *Salvia fruticosa* sample collected from Gündoğdu, Marmara, Balıkesir. The highest level of flavonoids were found in *Salvia pomifera* (3693.20 ±77.11 mg CE / 100 g DW) from Kestanealan village, İnegöl, Bursa, while the lowest was in *Salvia fruticosa* (664.03 ± 44.20 mg CE / 100 g DW) collected from Gündoğdu, Marmara, Balıkesir (Table 1). The antioxidant activity, TEAA values of the samples ranged from 288. 57± to 450.51±1.19 µmol (TE) / 100 g DW (Table 1). The highest AA value was determined at *Salvia pomifera* sample collected from Topağaç, Marmara, Balıkesir, while the lowest was at *Salvia fruticosa* sample from Kumbağ forest camp, Tekirdağ. Total phenolics and antioxidant activity showed a linear correlation while flavonoid contents and antioxidant activity showed a poor linear correlation with the correlation coefficients of 0.79 and 0.51, respectively. Some authors (Cai et al., 2004; Djeridane et al., 2006; Katalinic et al., 2006; Katsube et al., 2004) have demonstrated a linear correlation between the content of total phenolic compounds and their antioxidant capacity, while others (Czapecka et al., 2005; Wong et al., 2006) showed poor linear correlation of total antioxidant activity and phenolic content.

According to a study carried out by Tepe et al., (2005), total phenolic of *Salvia tomentosa* was 200 GAE µg ml^{-1} in an aqueous methanolic extract (7.83 %, w/w). *S. tomentosa* was collected from Söğütlügöl plateau (1000 m), Düziçi-Osmaniye, Turkey.

Total phenolics of *Salvia officinalis* and *Salvia elegans* were determined as 1.34 ± 0.09, 1.31 ± 0.08 mg GAE/g of fresh weight (FW);

Table 1 Antioxidant activity, total phenolic and flavonoid content of Some sage populations of *Salvia fruticosa* Mill., *Salvia pomifera* Mill. and *Salvia tomentosa* Mill. in the Marmara Region.

Sample collection site	Species	Antioxidant activity (µmol TE/100g DW)	Total phenol (mg GAE/100 g DW)	Flavonoid (mg CE/100g DW)
TEKIRDAĞ-MERKEZ KUMBAĞ FOREST CAMP-1	*S.fruticosa*	287.57±2.16	1159.83±36.53	1943.89±3.47
TEKIRDAĞ-MERKEZ KUMBAĞ FOREST CAMP-2	*S.fruticosa*	330.164±1.55	920.11±35.93	1457.81±18.40
TEKIRDAĞ-ŞARKÖY UÇMAKDERE VILLAGE	*S.fruticosa*	337.98±0.35	991.96±25.30	1826.91±13.36
TEKIRDAĞ-ŞARKÖY GAZİKÖY	*S.fruticosa*	328.93±1.89	1170.18±59.23	1812.63±43.80
TEKIRDAĞ-ŞARKÖY GAZİKÖY	*S.fruticosa*	352.80±1.55	1123.99±45.33	1582.52±63.79
TEKIRDAĞ-ŞARKÖY GAZIKÖY	*S.fruticosa*	371.93±0.94	931.28±56.25	1567.85±6.38
TEKIRDAĞ-MERKEZ UÇMAKDERE	*S.fruticosa*	319.46±3.04	987.40±18.54	1506.08±44.81
BALIKESIR-MARMARA GÜNDOĞDU	*S.fruticosa*	355.27±0.94	488.70±14.92	694.53±24.36
BALIKESİR-MARMARA GÜNDOĞDU	*S.fruticosa*	358.97±0.71	769.18±51.92	928.11±15.19
BALIKESIR-MARMARA GÜNDOĞDU	*S.fruticosa*	341.07±0.36	772.77±49.44	950.11±26.08
BALIKESIR-MARMARA GÜNDOĞDU	*S.fruticosa*	358.97±2.92	666.84±23.73	723.87±31.45
BALIKESİR-MARMARA GÜNDOĞDU	*S.fruticosa*	371.93±1.28	711.44±19.36	664.03±44.20
BALIKESİR-MARMARA GÜNDOĞDU	*S.fruticosa*	373.58±2.69	772.37±13.61	810.35±27.52
BALIKESİR-MARMARA TOPAĞAÇ	*S.fruticosa*	371.52±4.11	882.28±47.12	851.66±25.56
BALIKESİR-MARMARA TOPAĞAÇ	*S.fruticosa*	450.51±1.19	986.37±14.24	1104.15±9.19
BALIKESİR-MARMARA VİRANKÖY	*S.fruticosa*	345.60±2.17	800.44±19.33	1283.69±59.21
BALIKESİR-MARMARA VİRANKÖY	*S.fruticosa*	364.73±1.55	1047.14±3.78	1282.15±39.22
BALIKESİR-MARMARA VİRANKÖY	*S.fruticosa*	348.48±1.88	1132.75±5.99	1316.12±54.95
BALIKESİR-MARMARA YANADA	*S.fruticosa*	358.56±2.34	1143.10±16.70	1231.18±27.47
BALIKESİR-MARMARA ÇINARLI	*S.fruticosa*	341.69±3.40	1049.92±48.10	1250.49±55.55

antioxidant activity (ORAC) 13.28 ± 0.40, 11.55 ± 0.42 µmol of TE/ g FW, respectively (Zheng and Wang, 2001).

Sage extracts obtained by Soxhlet extraction were screened for their possible antioxidant activity using DPPH free radical scavenging assay. According to the findings, the most active plant was found as S. *verbenaca* (14.30 ± 1.42 µg mg^{-1}), followed by *S. virgata* (65.70 ± 2.12 µg mg^{-1}). *S. staminae* exhibited the weakest antioxidant activity with IC_{50} value of 75.40 ± 0.57 µg mg^{-1}. Collection sites were as follows: *S. virgata*: Gardaslar Hill (1350 m), Sivas–Turkey; 16th July 2004. *S. staminea*: Kilickaya district, Yusufeli, Artvin–Turkey; 06th September

2004. *S. verbenaca*: Kilickaya district, Yusufeli, Artvin–Turkey; 06th September 2004 (Tepe 2008).

Tepe et al. (2007) investigated the methanol extracts of *Salvia verticillata* subsp. verticillata and *S. verticillata* subsp. *amasiaca* for their possible antioxidant activity using DPPH free radical scavenging assay. According to the findings, *S. verticillata* subsp. *verticillata* was superior to that of subsp. *amasiaca* with an IC_{50} value of 14.5 ± 1.21 µg mg^{-1}.Collection sites for the samples were as follows: *S. verticillata* subsp. *verticillata:* Seyitler Village, Artvin-Turkey; 13th August 2004. *S. verticillata* subsp. *amasiaca*: Seyitler Village, Artvin-Turkey; 13th August 2004.

Total phenol content of *Salvia officinalis* was determined as 8.25 ± 0.09 mg GAE/100 g of DW, 41.2 ± 1.11 µM trolox/100 g DW. According to quantitative analysis of major phenolic compounds identified in *Salvia officinalis;* phenolic acids: cafeic acid 296 ± 0.00, neochlorogenic acid 53.1 ± 0.11, p-coumaric acid; 10.3 ± 0.17, ferulic acid, 13.5 ± 0.11 178 ± 1.11; flavonoids: quercetin 178 ± 1,11, luteolin 49.6 ± 0.03, apigenin 22.1 ± 0.03 (Wojdyło et al., 2007).

Total phenolic compounds and total flavonoids were determined in *Salvia officinalis, Salvia sclarea, Salvia glutinosa, Salvia pratensis* as 22.6±0.9, 24.0±1.1, 17.1±0.6, 9.7±0.4 GAE mg/g plant extract; 3.5±1.6, 4.8±0.5, 5.7±0.3, 1.4±0.1 mg/g plant extract rutin equivalent (RE), respectively (Miliauskas et al., 2007).

Cai et. al. (2004) was determined antioxidant activity (TEAC) 761.5 µmol trolox /100g DW and total phenolic content 4.26 GAE g/100 g DW in *Salvia miltiorrhiza.*

Quality parameters such as phenolics content and composition, and antioxidant activity of wild *Salvia fruticosa* were investigated in six-month storage period for two consecutive years. The phenolic components were found to be either lower or not changed in consecutive harvesting year. They also did not significantly change during the storage period. The antioxidant activity of the samples was found to be higher for the second harvesting year. The total phenolic content of the samples were found in the range of 41.58–43.20 mg GAE g^{-1} DW. Harvesting year and storage period did not change it. The total flavonoid contents of the samples were found between 25.79 and 38.61 mg of CE g^{-1} DW depending on the harvesting year, growing condition and storage period. In overall evaluation, the flavonoid content of the samples decreased by the second harvesting year. However, it was not influenced by the storage period (Dinçer et al. 2012).

The antioxidant properties and total phenolics of different extracts of thyme (*Thymus vulgaris* L.) and sage (*Salvia officinalis* L.) randomly collected from Shambolia farm located at Fayoum area in Egypt, and sweet marjoram (*Origanum majorana* L.) were examined using 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH•) free radical scavenging method and Folin–Ciocalteu method, respectively. Methanol exhibited the highest extraction ability for such phenolic compounds, where the total phenols were 8.10, 5.95, and 5.20 (mg GAE / g DW) for thyme, sage, and marjoram, respectively and also exhibited the strongest antioxidant capacity (Roby and et. al., 201).

Antioxidant activities and phenolic compositions of the active fractions of *Salvia virgata* Jacq. from Turkey were examined. According to the data, the highest total phenols were obtained also from the aqueous methanol and water extracts 212.30 ± 0.43, 116.22 ± 0.84 GAE mg/g DW, respectively. Total flavonoid was determined 3.57 ± 0.05, 3.87 ± 0.06 RE mg/g DW respectively (Kosar and et. al., 2008).

Tawaha et al. (2007) reported that the total phenolic content of *S. fruticosa* was 24.1 mg GAE g^{-1} DW.

Papageorgiou et al. (2008) have reported that the total phenolic content of leaves of *S. fruticosa* in different year and season ranged between 63.7 mg GAE g^{-1} DW (determined in May) and 144 mg GAE g^{-1} DW (determined in August). These differences can be related with plant parts and storage conditions before analysis and they stored their samples under nitrogen. On the other hand, Tawaha et al. (2007) used all aerial parts of the plants. It has been reported that extraction methods employed, geographical coordinates, climate, UV radiation, soil characteristics, and other ecological conditions may also cause such differences in total phenolic content (Kallithraka et al., 2009; Papageorgiou et al., 2008).

References

Asadi S., Ahmadiani A., Esmaeili M.A., Sonboli A., Ansari N., Khodagholi F., 2010**.** In vitro antioxidant activities and an investigation of neuroprotection by six Salvia species from Iran: A comparative study. Food and Chemical Toxicology, 48, 341–1349

Burits, M., ve Bucar, F., 2000. Antioxidant activity of Nigella sativa essential oil. Phytotheraphy Research, 14, 323–328.

Cai Y., Luo Q., Sun M., Corke H. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants

associated with anticancer. Life Sciences 74, 2157–2184

Czapecka, E., Mareczek, A., Leja, M. 2005. Antioxiadant activity of fresh and dry herbs of some Lamiaceae species. Food Chemistry, 93, 223–226.

Dincer C., Topuz A., Sahin-Nadeem H., Sultan Ozdemir K., Cam I.B., I.Tontul, Gokturk R.S., Tugrul Ay S. 2012. A comparative study on phenolic composition, antioxidant activity and essential oil content of wild and cultivated sage (Salvia fruticosa Miller) as influenced by storage. Industrial Crops and Products 39, 170 – 176

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal, N. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry, 97, 654–660

Durling, N. E., Catchpole, O. J., Grey, J. B., Webby, R. F., Mitchell, K. A., Foo, L. Y., Perry, N. B., 2007. Extraction of phenolics and essential oil from dried sage(Salvia officinalis) using ethanol-water mixtures" , Food Chemistry, 101: 1417-1424.

Kallithraka S. Salacha M.I, Tzourou I. 2009. Changes in phenolic composition and antioxidant activity of white wine during bottle storage: Accelerated browning test versus bottle storage. Food Chemistry 113, 500–505

Katalinic, V., Milos, M., Jukic, M. 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chemistry, 94, 550–557.

Katsube, T., Tabata, H., Ohta, Y., Yamasaki, Y., Anuurad, E., Shiwaku, K., et al. 2004. Screening for antioxidant activity in edible plant products: Comparison of low-density lipoprotein oxidation assay, DPPH radical scavenging assay, and Folin–Ciocalteu assay. Journal of the Agricultural and Food Chemistry, 52, 2391–2396.

Kosar M., Göger F., Baser K.H.C. 2008. In Vitro Antioxidant Properties and Phenolic Composition of *Salvia virgata* Jacq. from Turkey. Journal of the Agricultural and Food Chemistry, 56, 2369–2374

Lu Y, Foo LY. 2001. Antioxidant activities of polyphenols from sage (Salvia officinalis) Food Chemistry, 75: 197-202.

Lu, Y., Foo, L.Y., 2002. Polyphenolics of Salvia—a review. Phytochemistry. 75, 197–202.

Miliauskas G., Venskutonis P.R., Van Beek T.A. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts Food Chemistry. 85, 231–237

Özhatay F. N., Kültür Ş., Gürdal M. B., 2011. Check-list of additional taxa to the supplement Flora of Turkey V. Turk J Bot 35, 1-36

Papageorgiou V., Gardeli C., Mallouchos A., Papaıoannou M., Komaitis M.2008. Variation of the Chemical Profile and Antioxidant Behavior of *Rosmarinus officinalis* L. and *Salvia fruticosa* Miller Grown in Greece. J. Agric. Food Chemistry, 56, 7254–7264

Pizzale, L., Bortolomeazzi, R., Vichi, S., Überegger, E., Conte, L.S, 2002. Antioxidant activity of sage (*Salvia officinalis and S fruticosa*) oregano (*Origanum onites and O indercedens*) extracts related to their phenolic compund content. Journal Science Food Agricultural. 82, 1645-1651.

Roby M.H.H., Sarhana M.A., Selima K.A.H., Khalela K.I. 2013. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (Thymus vulgaris L.), sage (Salvia officinalis L.), and marjoram (Origanum majorana L.) extracts. Industrial Crops and Products 43, 827– 831

Tawaha K., Alali F. Q., Gharaibeh M., Mohammad M., El-Elimat T. 2007. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chemistry 104, 1372–1378

Tepe B, Daferera D, Sökmen A, Sökmen M, Polissiou M., 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae) Food Chemistry, 90: 333-340.

Tepe B, Eminagaoglu O, Akpulat H. A, Ay E. 2007. Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of *Salvia verticillata* (L.) subsp. *verticillata* and S. *verticillata* (L.) subsp. *amasiaca* (Freyn & Bornm.) Bornm. Food Chemistry 100, 985–989

Tepe B. 2008. Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of *Salvia virgata* (Jacq), *Salvia staminea* (Montbret & Aucher ex Bentham) and *Salvia verbenaca* (L.) from Turkey Bioresource Technology 99, 1584–1588

Wojdyło A, Oszmian´ski J., Czemerys R., 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry 105, 940–949

Wong, C.-C., Li, H.-B., Cheng, K.-W., Chen, F. 2006. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric

reducing antioxidant power assay. Food Chemistry, 97, 705–711.

Zheng W., Wang S.Y. 2001. Antioxidant activity and phenolic compounds in selected herbs. Journal Agricicultural Food Chemistry, 49 (11), 5165-51