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Antioxidant activity and total phenolic content of five *Salvia* species from Bulgaria

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A lot of species of the genus *Salvia* L. are used as herbal tea. They are also applied by the food flavouring, as well as in the cosmetic, perfumery and pharmaceutical industries. The most used as a medicinal plant and the best studied species of the genus is *Salvia officinalis* L. (garden sage). The aim of the present study was to determine the antioxidant activity and total phenol content of *Salvia amplexicaulis* Lam.; *Salvia pratensis* L.; *Salvia sclarea* L.; *Salvia verticillata* L. and *Salvia aethiopis* L., collected from their natural populations in the Thracian Lowland, Bulgaria. The methanolic extracts from the dried leaves and flowers of each species were tested for their radical scavenging capacity by DPPH method and the total phenol content by using of Folin-Ciocalteu reagent and gallic acid as a standard. The observed Pearson correlation between the measured quantities demonstrated a coefficient of 0.9565 at significance level $p \leq 0.01$. The tested species showed large total phenolic contents: from 906 ± 90 to 1795 ± 153 mmol GAE/kg DM and from 1746 ± 151 to 4555 ± 410 mmol GAE/kg DM of the methanolic extracts of leaves and flowers, respectively. The tested antioxidant capacities were found to be in the range from 21.8 ± 1.8 to 59.9 ± 5.0 mmol TE/kg DM and from 49.3 ± 4.5 to 89.0 ± 7.8 mmol TE/kg DM of the methanolic extracts of leaves and flowers, respectively. The results of the investigations showed that *Salvia verticillata* is the favorite *Salvia* species with the highest total phenol content and antioxidant activity

Key words: *Salvia amplexicaulis*; *Salvia pratensis*; *Salvia sclarea*; *Salvia verticilliata*; *Salvia aethiopis*; Antioxidant activity; Total phenol content

INTRODUCTION

In the recent years, the trend is synthetic antioxidants observed with negative impact on the human health to be replaced by bio-antioxidants. Of particular interest are antioxidants extracted from plants. A lot of species of the genus *Salvia* L. are used as herbal tea and also applied by the food flavouring, as well as in the cosmetic, perfumery and pharmaceutical industries [1]. *Salvia* (from the Latin *salvare* – to heal) is a genus of flowering plants belonging to the *Lamiaceae* family. It includes more than 900 species, distributed worldwide on all continents except Antarctica and Australia [2]. The most used as a medicinal plant and the best studied species of the genus is *Salvia officinalis* L. (garden sage). Some samples of sage showed a very high antioxidant activity, "with induction times more than 10-fold than that of lard used as the reference sample" [3]. *Salvia* is a rich source of phenols, 160 phenols were identified, some of which are unique to the genus [4]. A new study proved garden sage extract has a potential as a natural preservative in the meat industry [5]. The data about other *Salvia* species in the scientific literature are limited.

In Bulgaria, 19 S*alvia* species are naturally distributed [6], three of them are protected by the Biodiversity Act [7]. The aim of the present study was to determine the antioxidant activity and the total phenol content of five *Salvia* species, distributed in Bulgaria: *Salvia amplexicaulis* Lam.; *Salvia pratensis* L.; *Salvia sclarea* L.; *Salvia verticillata* L. and *Salvia aethiopis* L.

MATERIALS AND METHODS

Plant material and extract preparation

Aerial parts of five *Salvia* species: *S. amplexicaulis* Lam.; *S. pratensis* L.; *S. sclarea* L.; *S. verticillata* L. and *S. aethiopis* L. were collected in the full flowering period (from the end of June – to the begin of July 2017) from their natural populations in the Thracian Plane, Bulgaria (42°24′ N, 25°34′ E). The climate in the study area is continental Mediterranean. The soil type is Luvisols. The voucher specimens have been deposited at the Herbarium of the Agricultural University in Plovdiv and received registration numbers were as followed: *S. amplexicaulis* Lam. - SOA 062427; *S. pratensis* L. - SOA 062426; *S. sclarea* L. - SOA 062425; *S. verticillata* L. - SOA 062428 and *S. aethiopis* L. - SOA 062424

Plant material, separated in leaves and flowers, was air dried in shade at room temperature and grounded in a mechanical grinder (final powder size less than 400 μm). The samples were stored in a dark and cool room at 16 – 18 ° C prior to the analysis.

The extractions were prepared by maceration of 1 g powdered plant material in 10 ml methanol at room temperature for 7 days. After filtration, the residue was washed up in triplicate. Methanol was selected as a solvent because this method offers high yield of extraction of polar bioactive phenolic compounds from sage [8, 9]. Extraction technique by

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maceration is convenient and straightforward and was selected because of the high rate of extraction of polar bioactive compounds of phenolic compounds from Sage [9]. Finally, the extracts from the dried leaves and flowers of each species were adjusted to a concentration of 1 mg/ml calculated on dry matter (DM).

Determination of total phenol content

The determination of total phenol content (TPC) followed the experimental procedure described by Anesini *et al.* [10]. Briefly, 1 ml of the methanolic extract was mixed in separate tubes with 5.0 ml of Folin-Ciocalteu's reagent (1/10 dilution with water of the commercial reagent). Then, 4 ml of 7.5 % Na_2CO_3aq (w/v) was added and the tubes were left at room temperature for one hour. The absorbance at 765 nm was measured against water. Each sample was analyzed in triplicate. Gallic acid (Sigma-Aldrich, St. Louis, MO) solutions in methanol ranging from 2 to 60 μg/ml were used for calibration curve (R^2 = 0.9987). TPC of each sample was expressed as mmol GAE in 1 kg DM of starting plant material.

Determination of antioxidant activity by DPPH method

DPPH (1,1'-diphenyl-2-picrylhydrazil-radical) has a single electron on the nitrogen atom. Its solution in methanol has absorption maximum at λ = 517 nm. The mechanism of DPPH-method is based on the reaction between the test compound and DPPH-radical, wherein the potential free radical scavengers reduce DPPH-radical to 1,1'-diphenyl-2-(2,4,6-trinitrorhenyl)hydrazine by donating hydrogen atom. Resulted, the solution changed its color- from violet to yellow. DPPH was purchased from Sigma-Aldrich (St. Louis, MO).

The method described by Serpen *et al.* [11] was applied to measure radical-scavenging potential of the prepared methanolic extracts. Briefly, to 2 ml of 100 μM solution of DPPH in methanol was added 20 μl of methanolic extract. Absorbance at 517 nm was measured 30 minutes later. Since the composition of the extracts is complex, the results for their radical-binding capacity were compared with that of Trolox (water-soluble analogue of vitamin E) and calculated by regression analysis from the linear dependence between concentration of Trolox and absorption at 517 nm. Trolox standard was purchased from Sigma-Aldrich (St. Louis, MO). Standard solutions in methanol ranging from 1 to 50 μmol/l were used for calibration curve (R^2 = 0.9989). The results were expressed as mmol Trolox equivalent in 1 kg DM of plant material.

Statistical analysis

The statistical analysis was performed using Statistica 6 for Windows. All analytical determinations were performed in triplicate and the mean values ± standard deviation (SD) was reported. One–way ANOVA and Tukey post-hoc multi comparison tests were used for data analysis.

RESULTS AND DISCUSSION

The amount of total phenolic compounds varied widely: from 906 ± 90 to 1795 ± 153 mmol of gallic acid equivalents (GAE) / kg dry matter (DM) of methanolic extract prepared from the leaves and 3201 ± 296 to 4555 ± 410 mmol GAE / kg DM of methanolic extract prepared from the flowers (Table 1). The differences in TPC results between the species and the organs were not statistically significant at $P \leq 0.05$. With regards to the tested *Salvia* species, the highest level of phenol compounds was found at *Salvia verticillata*.

Table 1. Total phenolic content of different *Salvia* species (n = 3)

	GAE, mmol/kg DM (mean ± SD)	
Species	**Leaves**	**Flowers**
Salvia amplexicaulis	906 ± 90[c]	3201 ± 296[a]
Salvia pratensis	1574 ± 135[a]	3897 ± 337[b]
Salvia sclarea	996 ± 95[b]	2799 ± 255[a]
Salvia verticilliata	1795 ± 153[b]	4555 ± 410[c]
Salvia aethiopis	1735 ± 145[b]	1746 ± 151[c]
Mean	1401	3240
STD	420	1071
STD, %	30	33
P-value	0.0017	0.0025
t-value	7.5	6.8

*Dry matter; **SD – Standard Deviation; ***Different letters in the table denote significant differences between species and organs ($P \leq 0.05$)

Compared to the leaves, the flowers showed on average 2.3-fold higher phenol contents, except for *Salvia aethiopis*, were the TPC were determined to be similar: 1735 ± 145 and 1746 ± 151 mmol GAE/kg DM in the leaves and in the flowers, respectively.

Many researchers published data on TPC expressed in mg GAE/ g DM. The results to be compared, for the convenience they have to be expressed in equal units of measurement. Unification is easy using the molecular mass of gallic acid - 170.12 g/mol. So, the recalculated results of TPC ranged from 154 ± 15 to 775 ± 70 mg GAE /g DM of methanolic extracts.

Tosun *et al.* [12] screened eight *Salvia* species from Turkey for their TPC and antioxidant properties. Under the tested sage species were *S. verticillata* and *S. aethiopis*. The authors found 82 mg GAE/kg and 50 mg GAE/kg of metanolic extracts prepared from the leaves of the two species, respectively. Compared to the results of the present study: 295 ± 25 mg GAE/g for the leaves extract of *S. aethiopis* and and 305 ± 25 mg GAE/g for the leaves extract of *S. verticallita*, the sage species from Bulgaria demonstrated higher TPC values. Firuzi *et al.* [13] reported TPC and the dependence on their antioxidant properties of eleven Salvia species from Iran, including *Salvia sclarea* and *Salvia aethiopis*, which demonstrated 15 ± 1 mg GAE/g DM and 14 ± 1 mg GAE/g DM of the methanol extracts from aerial parts, respectively. The results obtained in the present study showed definitely much larger TPC- from 170 ±16 mg GAE/g DM (leaves extract from *S. sclarea*) to 295 ± 25 mg GAE/g DM (leaves extract from *S. aethiopis*). Marurikova *et al.* [14] investigated the total phenol content of 37 Salvia species, sampled in the Czech Republic. Under the tested Salvia species were *S. pratensis* from 2 different locations with TPC values of 2573 and 1853 mg GAE/100 g DM; *S. aethiopis* from 2 different locations with TPC values of 1934 and 2095 mg GAE/100 g DM; *S. amplexicaulis* with TPC value of 2959.13 mg GAE/100 g DM and *S. verticallita* from 2 different locations with TPC values of 2351 and 2815 mg GAE/100 g DW (dry weight plant material). In the present study the found lowest TPC of the methanol extracts from the leaves and flowers of *S. pratensis*, *S. aethiopis*, *S. amplexicaulis* and *S. verticallita* was the TPC of the extract from leaves *S. amplexicaulis*- 15 400 ± 1 530 mg GAE/100 g DM (dry extract material). Derakhshini *et al.* [15] reported TPC of *S. sclarea* from Iran- 16 mg GAE/g fresh material. Jasicka-Misiak *et al.* [16] explored two varieties of *Salvia sclarea*, growth in Poland for their total phenol content. The found values were 96 ± 3 and 134 ± 10 mg GAE / g DM. Tekeli *et al.* [17] tested *S. verticallita* from Turkey for its TPC and found 348 mg GAE / g extract from aerial plant parts. Compared to the results obtained in this study, the *S. verticallita* from Bulgarian showed lower TPC of the leaves extract (305 ± 26 mg GAE / g DM), but much higher TPC of the flowers extract (775 ± 70 mg GAE / g DM). Erbil *et al.* [18] investigated also *S. verticallita* from Turkey and determined 120 mg GAE / g DM of the methanol extract from the aerial plant parts.

Many researchers were studied leaves and flowers separate for their TPC and AA. Alimpilic *at al.* [19] determined these values of methanol extracts of *S. amplexicaulis*, sampled in Macedonia. The results were 154 ± 1 mg GAE/g DM for the leaves and 140 ± 1 mg GAE/g DM for the flowers and showed the less advantage for the leaves. The results obtained in the present study were equal for the leaves: 154 ±15 mg GAE/g DM, but for the flowers were found TPC in value of 545 ±50 mg GAE/g DM- nearly 3.5-fold higher. The reason of this inconformity was probably the harvesting time point. Alimpilic *at al.* [19] sampled the plant material at the end of the flowering period and our research team – in the full flowering period. Tusevski *et al.* [20] compared the AA of leaves, stems and flowers of 20 Macedonian medicinal plants, under these was *Salvia verticillata* L. The results obtained showed the antioxidant potential, based on phenolic compounds, extracted from the flowers was significant higher than those extracted from leaves.

It is known *Salvia* species are characterized by phenolic compounds that have a high antioxidant activity. Rosmarinic acid and caffeic acid have supremacy [4, 21]. Such phenolic compounds can donate hydrogen atoms to DPPH-radical and scavenge it and the superoxide scavenging activities of the rosmarinic acid derivatives were 15-20 times stronger than trolox, the water-soluble analog of vitamin E [22].

The antioxidant activity (AA) was expressed as mmol of Trolox equivalents (TE) in 1 kg DM of the methanolic extract. It ranged from 21.8 ± 1.8 to 89.0 ± 7.8 mmol TE / kg DM (Table 2). The differences in AA results between the species and the organs were not statistically significant at $P \leq 0.05$. The flowers showed on average 1.8-fold higher AA than the leaves. *Salvia aethiopis* differed again: its extracts from leaves and flowers showed similar AA, the values were 44.5 ± 3.8 and 49.3 ± 4.5 mmol TE/kg DM, respectively. Under all tested in the present study by the DPPH-method *Salvia* species, *S. verticillata* again was favorite. Its extracts showed the highest antioxidant activity. No wonder, a

Table 2. Radical scavenge activity of different *Salvia* species (n = 3)

	TE mmol/kg DM* (mean ± SD**)	
ID	**Leaves**	**Flowers**
Salvia amplexicaulis	21.7 ± 1.8[b]	75.0 ± 7.1[a]
Salvia pratensis	40.8 ± 3.5[a]	79.1 ± 6.7[a]
Salvia sclarea	30.9 ± 2.8[a]	69.0 ± 5.9[a]
Salvia verticilliata	59.9 ± 5.0[c]	89.0 ± 7.8[b]
Salvia aethiopis	44.5 ± 3.8[a]	49.3 ± 4.5[c]
Mean	39.6	72.3
STD	14.4	14.8
STD, %	36	20
P-value	0.00357	0.00039
t-value	6.1	10.9

*Dry matter; **SD – Standard Deviation; ***Different letters in the table denote significant differences between species and organs ($P \leq 0.05$)

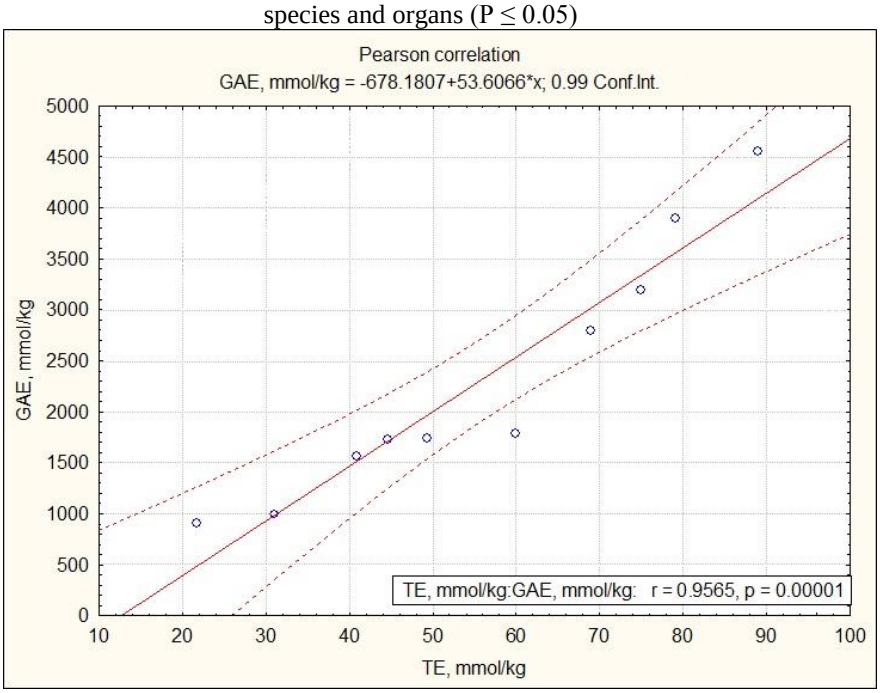

Figure 2. Pearson correlation between TPC and antioxidant activity, $P \leq 0.01$ (2-tailed)

number researchers investigated the antioxidant activity and TPC of different *Salvia* species and defined *S. verticallita* as favorite one [12, 23, 24].

CONCLUSIONS

Methanol extracts obtained from leaves and flowers of five *Salvia* species, sampled in South Bulgaria: *Salvia amplexicaulis Lam.; Salvia pratensis L.; Salvia sclarea L.; Salvia verticillata L. and Salvia aethiopis L.*, were tested for their radical scavenging capacity and the total phenol content. The observed results witnessed higher TPC and antioxidant activity of the tested species compared to *Salvia* species harvested in East European and Middle East region and studied by other researchers. *Salvia verticillata* proved to be the favorite *Salvia* species, again. Compared to the leaves, the flowers showed higher phenol concentration and antioxidant activity. Person correlation between the total phenolic content and the antioxidant activity of the methanolic extracts was observed with a high positive coefficient, so the resulting suggestion is: these compounds are responsible for the antioxidant activity. However, the obtained results evidenced that the studied *Salvia* species, notably *S. verticillata*, can be used as promising source of natural antioxidant additives in the food and pharmaceutical industries.

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