Evaluation of content of phenolics in *Salvia* species cultivated in South Moravian Region

Hodnotenie obsahu fenolov vo vybraných druhoch rodu *Salvia* L. pestovaných v Juhomoravskom kraji

Original research article

Muráriková A.^{1✉}, Kaffková K.¹, Raab S.², Neugebauerová J.¹

¹Mendel University in Brno,
Faculty of Horticulture, Department of Vegetable
Growing and Floriculture, Czech Republic
²Agricultural Research, Ltd. Troubsko, Czech Republic

¹Mendelova univerzita v Brně, Zahradnická fakulta,
Ústav zelinářství a květinářství, Česká republika

²Zemědělský výzkum, spol. s r.o. Troubsko, Česká republika

Received November 30, 2014, accepted January 30, 2015

Abstract In this study, total phenolic content (TPC) and rosmarinic acid (RA) of 37 samples sage (*Salvia* L.) of extracts were determined using spectrophotometric methods. The amount of total phenols was analysed with Folin-Ciocalteu reagents. Gallic acid was used as a standard compound and the total phenols were expressed as mg.g⁻¹ gallic acid equivalents of dried plant material. The values of the extracts displayed substantial differences. All of the investigated species except *Salvia jurisicii* (990.79 mg GAE. g⁻¹ d.w.) exhibited higher content of phenolics. Among the studies, species demonstrated the highest content of phenol, followed in sequence by *Salvia tomentosa*, *Salvia fruticosa*, *Salvia triloba*, *Salvia officinalis* 'Extrakta', *Salvia officinalis*. TPC varied from 990.79 to 4459.88 mg GAE. g⁻¹ d.w. in the extracts. The total amount of RA was between 0.88 and 8.04% among species. *Salvia tomentosa*, *Salvia verticillata* and *Salvia officinalis* 'Extrakta' had the highest content of RA. The high content of phenolic compounds indicated that these compounds contribute to the antioxidant activity.

Slovak abstract V štúdiu na celkový obsah fenolových látok a kyseliny rozmarínovej bolo hodnotených 37 vzoriek šalvie (*Salvia* L.) z metanolových extraktov stanovených spektrofotometricky. Množstvo celkových fenolov bolo analyzované s pomocou Folin Ciocalteuovéhо skúmadla. Ako štandard bola použitá kyselina galová a celkové fenoly boli vyjadrené ako mg.100 g⁻¹ kyseliny galovej v sušine. Boli zobrazené podstatné rozdiely hodnotených extraktov. Všetky skúmané druhy okrem *Salvia jurisicii* (990,79 mg GAE.100 g⁻¹) vykazovali vyšší obsah fenolových látok. Najvyšší obsah fenolov vykazovali v poradí *Salvia tomentosa*, *Salvia fruticosa*, *Salvia triloba*, *Salvia officinalis* 'Extrakta' a *Salvia officinalis*. Celkový obsah fenolových látok sa pohyboval v rozmedzí od 990,79 do 4459,88 mg GAE.100 g⁻¹. Celkové množstvo kyseliny rozmarínovej bolo v rozpätí 0,88 až 8,04 %. Druhy *Salvia tomentosa*, *Salvia verticillata* a *Salvia officinalis* 'Extrakta' mali najvyšší obsah kyseliny rozmarínovej. Vysoký obsah fenolových zlúčenín indikuje, že tieto zlúčeniny prispievajú k antioxidačnej kapacite.

Keywords sage – *Salvia* L. – phenolic compounds – rosmarinic acid – south Moravian region

Kľúčové slová: šalvia – *Salvia* L. – fenolové látky – kyselina rozmarínová – juhomoravský kraj

1. INTRODUCTION

The genus *Salvia* includes about 900 species that grow in several regions all over the world (Kintzios, 2000). Sage is one of the oldest medicinal plants used by human (Muntean *et al.*, 2007). Sage has been traditionally used in medicines, flavours and fragrances (Demirci *et al.*, 2003). Curative properties of sage are particularly recognised since earliest times at is use a tonic, stimulant, carminative, antiseptic and antihydrotic is reported (Kintzios, 2000).

Plants belonging to the *Lamiaceae* family are very rich in polyphenolic compounds. Sage has been observed to have excellent properties that inhibit lipid peroxidation and this activity is attributed principally to the presence of phenolic compounds (Cuvelier *et al.*, 1996; Lu & Foo, 2001). The major phenolic compounds identified in the extracts of sage are rosmarinic acid (RA), carnosic acid, salvianolic acid and its derivatives carnosol, rosmanol, epirosmanol, rosmadial and

* E-mail: xmurarik@node.mendelu.cz

© Acta Facultatis Pharmaceuticae Universitatis Comenianae

methyl carbonate (Cuvelier *et al.*, 1996; Lu & Foo, 2001; Madsen *et al.*, 1995). Due to the variability of environmental factors, the phenolic containing extracts obtained from different sage samples show great differences in composition and consequently, differences in antioxidative power (Cuvelier *et al.*, 1996; Areias *et al.*, 2000).

RA is a water-soluble ester of 3,4-dihydroxycinnamic acid and 3,4 dihydroxyphenyllactate. It is found mainly in the family of *Lamiaceae*, although it has also been described in other plant families such as *Apiaceae*. RA is the major phenol compounds of sage, also found in many other plants used as seasoning (Petersen & Simmonds 2003). RA has been reported to have antiviral, antibacterial, antihepatitis, antimutagenic, anti-allergic, anticarcinogenic and anti-inflammatory properties (Ito *et al.* 1998; Cao *et al.* 2005).

In this study, the content of phenolic and RA from *Salvia* L. samples from Czech Republic were investigated.

MATERIAL AND METHODS

Plant material

The plant material (aerial parts) was collected on two locations in the Czech Republic in South Moravian Region (Lednice and Troubsko) from May to July of 2012. The plant material was dried in bundles, in the shade in an airy place and then stored in paper bags and kept at room temperature.

Chemicals

Folin–Ciocalteu's reagent, sodium carbonate, distilled water, ethanol, hydrochloric acid, sodium nitrate, sodium molybdate, sodium hydroxide

Extract preparation

Dry and pulverised plant material (5 g) was extracted using 75% methanol. The samples were filtered after 24 hours of extraction at room temperature with filter paper (Shan *et al.*, 2005). The obtained extracts were kept in sample tubes and stored in a refrigerator.

Analysis of total phenolics

Total phenolic content (TPC) was determined using Folin–Ciocalteu's method. The appropriate amount of filtered methanol extracts were oxidised with Folin–Ciocalteu's reagents and after 5 minutes, the reaction was neutralised with saturated sodium carbonate. The resulting blue complex was then measured at 756 nm after 90 minutes of incubation at room temperature. Gallic acid was used as the standard and the contents of phenolics were expressed as g gallic acid equivalents (GAE.100 g⁻¹ d.w.).

Analysis of rosmarinic acid

Stock solution: To 0.200 g of the powdered drug, add 80 ml of ethanol (50% V/V) R. Boil in a water-bath under a reflux condenser for 30 minutes. Allow to cool and filter. Rinse the filter with 10 ml of ethanol (50% V/V) R. Combine the filtrate and the rinsings in a volumetric flask and dilute to 100.0 ml with ethanol (50 % V/V) R.

Test solution: To 1.0 ml of the stock solution add 2 ml of 0.5 M hydrochloric acid, 2 ml of a solution prepared by dissolving 10 g of sodium nitrite R and 10 g of sodium molybdate R in 100 ml of water R and then add 2 ml of dilute sodium hydroxide solution R and dilute to 10.0 ml with water R; mix.

Compensation solution: Dilute 1.0 ml of the stock solution to 10.0 ml with water R. Measure immediately the absorbance of the test solution at 505 nm.

RESULTS AND DISCUSSION

The results of TPC of *Salvia* species are shown in Table 1 and Figure 1. Content of RA are shown in Table 1 and Figure 2.

The TPC of the samples were determined at 1441.26–5136.70 mg GAE.100 g⁻¹ d.w. Extract of *Salvia jurisicii* exhibits the lowest phenolic content (1441.26 mg GAE.100 g⁻¹ d.w.) and the concentration of RA (0.88%). Plant extract of *Salvia tomentosa* had higher phenolic content (5136.70 mg GAE.100 g⁻¹ d.w.) and the concentration of RA (8.04%).

Authors Tosun *et al.* (2009) investigated the content of total phenols in eight species of sage. The amount of the total phenolics was highest in *Salvia verticillata* (167.1 mg GAE.100 g d.w.), followed by *Salvia virgata* (101.2 mg GAE.100 g d.w.) and *Salvia candidissima* (100.3 mg GAE.100 g d.w.). *Salvia microstegia* had the lowest TPC (50.3 mg GAE.100 g d.w.). Several studies have been carried out with the *Salvia* species and TPC has been found to be between 41–134 mg GAE.100 g d.w. basis (Kosar *et al.*, 2008; Ogutcu *et al.*, 2008). The results are comparable to those obtained by Miliauskas *et al.* (2004), who studied some culinary plants and obtained ranges of 4.30–37.90 mg. GAE.100 g. Total content of phenolic compounds in *Salvia sclarea* extracts was 24.0 ± 1.1 (mg GAE.100 g d.w.). Wagensteen *et al.* (2004) obtained 19 mg GAE.100 g total phenolic compounds in some coriander plants. Ismail *et al.* (2004) detected ranges of 11.07–71.67 mg in GAE.100 g selected vegetables. Variation of phenolic compounds content arises due to several factors, which include the area of cultivation and other environmental stresses (Makkar, 1999).

RA content in sage plants grown in other countries was reported in literature. In research Bandoniene *et al.*, (2005), the content of RA in dried leaves of the sage species were 13.3–47.3 mg.100 g. *Salvia glutinosa* and *Salvia sclarea* had the highest concentration of RA. The presence of RA was also reported in field - grown as well as various in-vitro cultures

Table 1. Total phenolic content and rosmarinic acid in 37 samples of sage

No.	Scientific name	Growing area	TPC [mg GAE.100 g ⁻¹] d.w.	RA [%]
1.	<i>Salvia officinalis</i> spp. <i>lavandulifolia</i>	Troubsko	3017.08	3.1942
2.	<i>Salvia coccinea</i>	Troubsko	3407.53	3.6872
3.	<i>Salvia virgata</i>	Lednice	2181.63	4.4509
4.	<i>Salvia fruticosa</i>	Troubsko	2758.92	3.5657
5.	<i>Salvia tomentosa</i>	Troubsko	5136.70	8.0377
6.	<i>Salvia pratensis</i>	Troubsko	2573.34	2.8315
7.	<i>Salvia pratensis</i>	Troubsko	1853.09	3.0208
8.	<i>Salvia transsylvanica</i>	Lednice	1723.99	1.7669
9.	<i>Salvia amplexicaulis</i>	Troubsko	2959.13	3.7507
10.	<i>Salvia verticillata</i>	Troubsko	2351.14	4.7440
11.	<i>Salvia nemorosa</i> ssp. <i>tesquicola</i>	Lednice	3055.43	2.6245
12.	<i>Salvia argentea</i>	Troubsko	1232.71	1.1440
13.	<i>Salvia verticillata</i>	Troubsko	2814.64	5.5786
14.	<i>Salvia tilifolia</i>	Troubsko	1675.88	2.0976
15.	<i>Salvia aethiopsis</i>	Troubsko	2095.31	1.7017
16.	<i>Salvia reflexa</i>	Troubsko	2442.06	2.7001
17.	<i>Salvia officinalis</i>	Lednice	3699.76	4.7746
18.	<i>Salvia aethiopsis</i>	Troubsko	1934.45	2.2794
19.	<i>Salvia nemorosa</i> 'Rosenwein'	Troubsko	2242.00	3.5474
20.	<i>Salvia fruticosa</i>	Lednice	4208.93	3.5252
21.	<i>Salvia nemorosa</i> 'Blaukönigin'	Troubsko	2723.48	4.4589
22.	<i>Salvia pratensis</i>	Troubsko	2019.42	2.6790
23.	<i>Salvia azurea</i>	Troubsko	3280.25	3.6280
24.	<i>Salvia kopetdaghensis</i>	Troubsko	2258.25	3.4739
25.	<i>Salvia officinalis</i> 'Krajová'	Troubsko	3226.07	3.7312
26.	<i>Salvia officinalis</i>	Troubsko	3247.35	3.3207
27.	<i>Salvia jurisicii</i>	Troubsko	1441.26	1.0443
28.	<i>Salvia jurisicii</i>	Troubsko	990.79	0.8809
29.	<i>Salvia transcaucasica</i>	Troubsko	1589.25	3.4137
30.	<i>Salvia nemorosa</i>	Troubsko	2386.01	2.7090
31.	<i>Salvia nemorosa</i> 'Violetkönigin'	Lednice	2298.70	3.1573
32.	<i>Salvia cadmica</i>	Troubsko	2579.21	3.4794
33.	<i>Salvia officinalis</i> 'Extrakta'	Lednice	3905.49	5.0139
34.	<i>Salvia pratensis</i>	Lednice	2411.13	2.9563
35.	<i>Salvia triloba</i>	Lednice	3922.15	4.3200
36.	<i>Salvia officinalis</i> 'Extrakta'	Lednice	3178.62	3.4880
37.	<i>Salvia sclarea</i> 'Vatican White'	Lednice	2980.74	3.7251

TPC: total phenolic content, RA: rosmarinic acid

Figure 1. Total phenolic content (%) in tested samples
TPC: total phenolic content

Figure 2. The content of rosmarinic acid (%) in tested samples

of *Salvia officinalis* plants originating in Poland. The content of RA in that study was about 1.2–1.9% (Grzegorzczak *et al.*, 2007).

CONCLUSION

In conclusion, methanol extract of sage grown in Lednice and Troubsko has high content of phenolic compounds. Extract of *Salvia jurisicii* exhibits the lowest phenolic content (990.79 mg GAE.100 g⁻¹ d.w.). Plant extract of *Salvia tomentosa* has higher

phenolic content (5136.70 mg GAE.100 g⁻¹ d.w.). The total amount of RA was between 0.88 and 8.04% among species. Mainly, extracts of *Salvia tomentosa*, *Salvia fruticosa*, *Salvia triloba*, *Salvia officinalis* 'Extrakta', *Salvia officinalis* can be used as natural antioxidants.

ACKNOWLEDGEMENTS

This study was supported by grant OPVKCZ.1.07/2.4.00/31.0089 of the Ministry of Education of the Czech Republic.

References

- [1] Areias F, Valentao P, Andrade PB, Ferres F, Seabra RS. Flavonoids and phenolic acids of sage: Influence of some agricultural factors. *J. Agric Food Chem.* 2000;48:6081-6084.
- [2] Bandoniene D, Murkovic M, Venskutonis PR. Determination of Rosmarinic Acid in Sage and Borage Leaves by Hight-Performance Liquid Chromatography with Different Detection Methods. *J Chromatogr Sci.* 2005;43:372-376.
- [3] Cao H, Cheng WX, Li C, Pan XL, Xie XG, Lie TH. DFT study on the antioxidant activity of rosmarinic acid. *J.Mol. Struct.* 2005;719:177-183.

- [4] Cuvelier ME, Richard H, Berset C. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. *J. Am. Oil Chem. Soc.* 1996;79:645-652.
- [5] Demirci B, Baser HC, Yildiz B, Bahcecioglu Z. Composition of the essential oils of six endemic *Salvia* spp. from Turkey. *Flavour Frag. J.* 2003;18:116-121.
- [6] Grzegorzyc I, Matkowski A, Wysokinska H. Antioxidant activity of extract from *in vitro* cultures of *Salvia officinalis* L. *Food Chem.* 2007;104:536-541.
- [7] Ismail A, Marjan ZM, Foong CW. Total antioxidant activity and phenolic contents in selected vegetables. *Food Chem.* 2004;87:581-586.
- [8] Ito H, Miyazaki T, Ono M, Sakurai H. Antiallergic activities of rabdosiin and its related compounds: Chemical and biochemical evaluations. *Bioorg. Med. Chem.* 1998;6:1051-1056.
- [9] Kintzios SE. *Sage: The Genus Salvia*. Amsterdam, Md: CRC Press; 2000.
- [10] Kosar M, Goger F, Baser KHC. In vitro antioxidant properties and phenolic composition of *Salvia virgata* Jacq. from Turkey. *J. Agric. Food Chem.* 2008;56:2369-2374.
- [11] Lu Y, Foo LY. Salvianolic acid. A potent phenolic antioxidant from *Salvia officinalis*. *Tetrahedron Letters.* 2001;42:8223-8225.
- [12] Madsen HI, Bertelsen G. Spices as antioxidant. *Trends in Food Science and Technology.* 1995;6:271-277.
- [13] Makkar HPS. Quantification of tannins in tree foliage: A laboratory manual for FAO/IAEA. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, 1999; p. 1-29.
- [14] Miliuskas S, Venskunones PR, Van Beek TA. Screening of radical scavenging activity of some medicinal plant extracts. *Food Chem.* 2004;85:231-237.
- [15] Muntean LS, Tamas M, Muntean S, Muntean L, Duda MM, Vărbăni DI, Florian S. *Tratat de plante medicinale cultivate și spontane*. Cluj-Napoca, Md: Risoprint; 2007.
- [16] Petersen M, Simmonds MSJ. Rosmarinic acid. *Phytochemistry.* 2003;62:121-125.
- [17] Ogutcu H, Sokmen A, Sokmen M, Polissiou M, Serkedjieva J, Daferera D, Sahin F, Baris O, Gulluce M. Bioactivities of the various extracts and essential oils of *Salvia limbata* C.A. Mey and *Salvia sclarea* L.. *Turkish Journal of Biology.* 2008;32:181-192.
- [18] Shan B, Cai YZ, Mei S, Corke H. Antioxidant Capacity of 26 spice extracts and characterization of their phenolic constituents. *J. Agric. Food Chem.* 2005;53:7749-7759.
- [19] Tosun M, Ercisli S, Sengul M, Ozer H, Polat T, Ozturk E. Antioxidant Properties and Total Phenolic Content of Eight *Salvia* Species from Turkey. *Biol. Res.* 2009;42:175-181.
- [20] Wagensteen H, Samuelsen BA, Malterude EK. Antioxidant activity in extracts from coriander. *Food Chem.* 2004;88:293-297.