

Scientific paper

Chemical and Antioxidant Profile of Hydroalcoholic Extracts of *Stachys Officinalis* L., *Stachys Palustris* L., *Stachys Sylvatica* L. from Romania

George Florian Apostolescu¹, Diana Ionela (Stegarus) Popescu², Oana Botoran², Daniela Sandru³, Nicoleta Anca Șuțan^{4,*} and Johny Neamtu⁵

¹ Doctoral school, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, 2 Petru Rareș Street, 200349 Craiova, Romania

² National Research and Development Institute for Cryogenics and Isotopic Technologies–ICSI Ramnicu Valcea, 4th Uzinei Street, 240050 Ramnicu Valcea, Romania

³ Department of Agricultural Sciences and Food Engineering, Lucian Blaga University of Sibiu, Doctor Ion Rațiu 7, 550012 Sibiu, Romania

⁴ Department of Natural Sciences, University of Pitesti, Targul din Vale 1, 110040 Pitesti, Romania

⁵ Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, 2-4 Petru Rares Str., 200349 Craiova, Romania

* Corresponding author: E-mail: anca.sutan@upit.ro
Tel.: +40 348 453 260

Received: 02-04-2023

Abstract

Stachys officinalis L., *Stachys palustris* L., *Stachys sylvatica* L. (Lamiaceae) are widely used as herbal remedies. In this study, comparative assessment of the phenolic acids, flavonoids, anthocyanin, and tannins content, together with antioxidant activity of the extracts obtained from flowers, leaves and stems was performed. Phenolic acids determined by the HPLC method reached highest values in flower extract of *S. palustris*, stem extract of *S. officinalis*, and leaf extracts of *S. sylvatica*. Flavonoids were found at values exceeding 100 mg quercetin equivalents (QE)/g dry weights in all three species, based on the spectrophotometric method. Anthocyanins were detectable only in extracts from flowers. *S. officinalis* stood out for the highest content of anthocyanins and tannins. Antioxidant activity was present in all three species studied, with *S. palustris* standing out for the most intense ferric reducing antioxidant power. The results obtained lead to the validation of applicability of these plants for curative and food purposes, given their variety and richness in bioactive compounds and antioxidants.

Keywords: *Stachys*; Phenolic Compounds; Flavonoids; Anthocyanin; Tannins; Antioxidant

1. Introduction

Many plants are known for their therapeutical effects in the treatment of certain diseases, but more and more are being discovered, and nowadays there is an ever-increasing return to nature and what it has to offer. Advanced or classical extraction technologies of valuable components lead to the completion of information in this field, the results being visible both in the scientific and commercial areas.

One of the often refferenced families in folk medicine is *Lamiaceae*, with genera and species identified world-

wide, most of them presenting exceptional curative properties. The genus *Stachys* is represented by 300–400 species, native or acclimatized, natural or ornamental, their importance and complex chemical composition being validated by the increasingly varied research that is being carried out and the possibility of superior exploitation of their bioactive potential.^{1,2}

Recent studies revealed antioxidant, enzyme inhibition, antidiabetic, anti-cholinesterase and anti-tyrosinase properties of *Stachys cretica* subsp. *mersinaea* (Boiss.) Rech.f., cytotoxic and antifungal activities of *Stachys parv-*

iflora L., *Stachys cretica* subsp. *bulgarica* Rech.f. (SC), *Stachys byzantina* K. Koch (SB), *Stachys thirkei* K. Koch, antibacterial activity against Gram-positive microorganisms of *Stachys byzantina* K.Koch, *S. officinalis* and *S. sylvatica*, nephroprotective, anti-inflammatory, hepatoprotective and anticancer properties of *Stachys pilifera* Benth, antiphlogistic effects of *S. alpina*, *S. germanica*, *S. officinalis* and *S. recta* antidepressant activity and apoptotic effect of *Stachys pilifera* Benth.^{3–11} Antioxidant activities were mentioned for all the above species. Nutritional value was also showed by a number of studies for species such as *Stachys affinis* Bunge, *Stachys lavandulifolia* Vahl. var. *lavandulifolia*, *Stachys sieboldii* Miq.^{12–14}

The chemical composition of the extracts differs depending on the species,^{6,9} on the solvent, on the different parts of plants used for extraction¹⁵ and the geographical area that the plants grow,^{16,17} and so are the antioxidant and antimicrobial properties.^{16,18}

In the central area of Romania (Sibiu County), seven species of *Stachys* genus have been identified so far: *Stachys alpina* L., present on the valleys and slopes of the Cibin and Făgăraș mountains; *Stachys annua* L. found on the montan hills at altitudes between 300 m and 700 m; *Stachys germanica* L. found on hills and montan hills at altitudes of 320–550 m; *Stachys officinalis* L. identified in hilly-mountain areas at altitudes between 330–1250 m; *Stachys palustris* L. growing sporadically at high altitudes between 300 m and 900 m; *Stachys recta* L. present in the hilly-mountainous area at altitudes between 260–800 m; *Stachys sylvatica* L. present frequent on mountain hills at high altitudes comprised 340–1470 m.¹⁹

Considering the therapeutic and nutritional potential of the species of the genus *Stachys*, this study provides a comprehensive and comparative evaluation of polyphenols and antioxidant profile of extracts obtained from flowers, leaves and stems of the three species grown in the central area of Romania (Sibiu County): *S. officinalis*, *S. palustris* and *S. sylvatica*. Although other reports include chemical profile of *Stachys* sp., this is the first study that shows the chemical and antioxidant profile differentiated according to the aerial part of the plant and provide important clues regarding the optimal exploitation of plants, through the use of plant organs with abundant bioactive compounds.

2. Experimental

2. 1. Plant Samples and Description of the Area of Interest

Plant samples: *Stachys officinalis* L. (hemicryptophyte, Eurasia), *Stachys palustris* L. (hemicryptophyte, circumpolar), and *Stachys sylvatica* L. (hemicryptophyte, Eurasia) were collected in July 2022, in the maximum flowering period from depression Mărginimii groups. The

area that was studied is located between coordinates: 45°45'23"N 23°55'28"E and 45°45'58"N 23°54'29"E, at an altitude between 560 m and 610 m that covers the media between villages Fântânele (Cacova) and Sibiel from Mărginimea Sibiului.

Depression Mărginimii groups is located at the foothills of Mountains Cindrel and is formed by two depressions, one of Sibiu and the other of Săliște, separated by Măgura Beleuța with an altitude of 630 m. Depression is characterized by gradually hill Miocene aged at the foothills of mountains, meadows, and terraces, attributes that frame it in the contact area. The climate is distinguished according to the landscape, with the depression area showing warm sides, rich in precipitation, and more significant in winter. The solar radiation exceeds 115 kcal/cm²/year overall. Air temperature oscillates depending on the landscape, depression area presenting an annual average temperature of 9 °C and northwest winds. Rainfall totals over 600 mm, with summer showers. Woody and herbaceous species are specific to the foothill area. The xerophiles meadows from the Depression Mărginimii (of Săliște) stand out through boreal plant diversity, dominated by plants original from Eurasia, followed by those Europeans and Central-European. Floristic species from this area were botanically researched with results that led to a very thorough and complete inventor.¹⁹

Plant samples of *S. officinalis*, *S. palustris*, and *S. sylvatica* were recorded within the CCBIA from L. Blaga University, Sibiu, Romania under no. 314/1, 314/2, and 314/3 respectively.

2. 2. Chemicals and Reagents

The chemicals and reagents used in the process were sodium nitrite (NaNO₂) 5%, aluminum chloride hexahydrate (AlCl₃ · 6H₂O) 10%, sodium hydroxide (NaOH) 1M, quercetin, potassium chloride (KCl) 0.025M, sodium acetate (CH₃COONa) 0.4M, hydrochloric acid (HCl), cyanidin-3-glucoside, reagent Folin-Ciocalteu, sodium carbonate (Na₂CO₃) 20%, tannic acid, casein, 0.5% formic acid in H₂O, methanol (CH₃OH), ferric-tripyridyltriazine (Fe³⁺-TPTZ), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) from Fluka (Germany) and Sigma-Aldrich (Germany). The HPLC standards were caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid, m-coumaric acid, sinapic acid, trans-cinnamic acid, benzoic acid, ellagic acid, gallic acid, p- hydroxybenzoic acid, rosmarinic acid, syringic acid, and vanillic acid from Sigma-Aldrich (Germany).

2. 3. Preparation of *Stachys* sp. Extracts

Flowers, stems, and leaves of *S. officinalis*, *S. palustris*, and *S. sylvatica* were dried separately at a temperature of 40 °C until the constant mass. Each 50 g of shredded dried material was soaked in a 500 mL solution of aqueous

80% methanol for 3 days at a temperature of 18 °C in a covered container. The samples were decanted, filtered with a Buchner vacuum pump (Whatman filter paper No. 1001 090), and concentrated in a rotary evaporator. The dry extracts were resuspended in distilled water to a concentration of 1:1 mg/ml.

2. 4. Determination of Phenolic Acids (PAs)

PAs were quantified through the HPLC method proposed by Baczek et al.²⁰ slightly modified, and by consulting other methods that were already applied on plant extracts.^{21,22} Phenolic acids were identified following the HPLC system Smartline, KNAUER GmbH (Berlin, Germany), equipped with a quaternary pump, automatic injection and DAD detector, set to the following λ wavelengths: 280 nm, 320 nm, 360 nm. Briefly, C18 columns (Zorbax SB – Aq: 250 mm $\times$ 4.6 mm i.d., 5.0 μ m p.s) were used. For the mobile phase, a solution of deionized H₂O and phosphoric acid (pH 3.5) was used as eluent A, and acetonitrile (pH 3.5) as eluent B, with the follows ratio: 0.00 min – 20% B; 0.45 min – 20% B; 5.50 min – 30% B; 5.55 min – 90% B; 6.50 min – 95% B; 6.51 min – 20% B; 15.00 min – STOP. A volume of 2 μ L extract was injected into the column for chromatographic analysis, and the flow rate was 1 mL/min, at the temperature of 35 °C and 15 min total time of analysis. The identification and quantification of phenolic acids was achieved by comparison with selected standards, using calibration curves for each individual compound. The experiments were performed in triplicate and the results were expressed in μ g/g extract.²⁴

2. 5. Determination of Total Flavonoid Content (TFC)

Flavonoids were determined based on the spectrophotometric method described by Popescu et al.²³ The aqueous extracts (5 mL) were homogenized with 5% NaNO₂ solution (0.3 mL) and incubated for 5 minutes. Later a solution of AlCl₃ · 6H₂O 10% (0.5 mL) was added and the mixture was left to react in darkness. After 15 minutes of reaction 2 mL of 1M NaOH solution was added and made up to 10 mL with distilled water. The samples were read with UV-1900 SHIMADZU spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at a wavelength of 510 nm. The TFC was expressed in mg quercetin equivalents /gram of dry weight (mg QE/g DW).

2. 6. Determination of Total Monomeric Anthocyanin Pigment Content

The colorimetric method based on the difference of absorbance of anthocyanins at a change in pH (pH 1 and pH 4.5) was applied for determination of total monomeric anthocyanin pigment (MAPC) content.²⁴ Depending on their concentration, the difference in the absorbance of

MAPC was read at a wavelength of 520 nm, respectively 700 nm, using UV-1900 SHIMADZU spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Results obtained in mg/L cyanidin-3-glucoside were converted into mg/g.²⁴

2. 7. Determination of Total Tannin Content (TTC)

For the assessment of TTC, comparative quantification of total polyphenols determined through Folin-Ciocalteu method and express the results in μ g tannic acid equivalents/ml (μ g TAE/ml) and polyphenols residuals in casein was applied. The difference between the total level of polyphenols and polyphenols residuals represents the TTC expressed in mg tannic acid equivalents /g dry weight (mg TAE/g DW).^{25,26}

2. 8. Determination of Ferric Reducing Antioxidant Power (FRAP)

Antioxidant properties of the extracts were evaluated based on the reduction of Fe³⁺ -TPTZ in Fe²⁺-TPTZ by antioxidants ingredients from the samples. FRAP was monitored using the spectrophotometric method described by Lachowicz-Wisniewska et al.²⁷ Briefly, 1 mL of each aqueous extracts was homogenized with 3 mL Fe³⁺ -TPTZ, absorbance being read at a wavelength of 593 nm with UV-1900 SHIMADZU spectrophotometer (Shimadzu Corporation, Kyoto, Japan), after 10 minutes of incubation. The results are expressed in mg Trolox equivalents/g of dry weight (mg TE/g DW).

2. 9. Multivariate Analysis

In order to explain the significant correlations between quality parameters (phenolic acids data), principal component analysis (PCA) was the main approach of multivariate statistical analysis. In order to display data as single point for each variable and to reveal the correspondence between the principal component and the direction of maximum variance, the data were mean-centered. Pearson correlations ($p < 0.05$ and $p < 0.01$) were used to identify correlations between all variables included in the dataset. All statistical analyzes were performed using Addinsoft XLSTAT software, version 2014.5.03 (Addinsoft Inc., New York, NY, USA).

3. Results and Discussions

3. 1. Phenolic Acids in *Stachys* Extracts

Through their anti-cancer, anti-inflammatory and antimicrobial action^{5,28–31} or through their positive effects on curing neurodegenerative diseases such as Alzheimer's,³ phenolic acids represent bioactive plants secondary metabolites with important preventive and curative acti-

ons. Seven hydroxybenzoic acids and eight hydroxycinnamic acids were identified in *Stachys* flower extracts, with very low (0.01 µg/g trans-cinnamic acid) or generous values (27970.53 µg/g benzoic acid).

The results presented in Table 1 indicate that benzoic acid accumulates significantly especially in flower of *S. sylvatica* (19071.32 µg/g) and *S. palustris* (27970.53 µg/g) and leaves of *S. officinalis* (4564.43 µg/g). The lowest values of benzoic acid were observed in extracts of stems, varying between a minimum of 282.28 µg/g in *S. palustris* and a maximum of 1270.16 µg/g in *S. officinalis*. At a significantly lower detected concentration (3080 µg/g extract), benzoic acid was indicated as one of the most abundant phenolic compound of *S. cretica* subsp. *Mersinaea*.³

The ellagic acid has been identified in the flowers extracts in quantities between 18.02 µg/g for *S. palustris* and 32.01 µg/g for *S. sylvatica*, the obtained values for the stems extracts being below 7 µg/g, and those for the leaves extracts reaching a maximum of 21.12 µg/g in *S. sylvatica*. Uneven amounts of gallic acid were found in the studied extracts. Gallic acid was found in values below 10 µg/g in flower extracts and it was undetected in stems. In comparison, higher content of 16.59 mg gallic acid equiv./g dry matter in *Stachys lavandulifolia* Vahl.³² or 900.61±0.06 mg gallic acid equivalent /100 g in dried herb in *Stachys aleurites* Boiss. & Heldr. was reported.³³ The p-hydroxybenzoic acid was identified at significant values in the flower extracts of *S. sylvatica* (83.15 µg/g) and in the leaves extracts of *S. officinalis* (73.43 µg/g). Salicylic acid was found in trace, with amounts between 0.22 µg/g – 9.42 µg/g in flowers extracts, and with subunit values in extracts of stems and leaves (0.27 µg/g – 0.96 µg/g), irrespectively of the species. Significantly lower amount of p-hydroxybenzoic acid

(0.006 mg g⁻¹ DW) and significantly higher amount of salicylic acid (0.168 mg g⁻¹ DW) were found in methanol extracts of leaves of *S. byzantina*, in comparison with leaves and flower extracts in our study.³⁴ These results suggest a species-specific phenolic acid pattern.

Syringic acid was fluctuated in flower extracts between 389.41 µg/g in *S. officinalis* and 569.78 µg/g in *S. palustris*, in stems extracts between 11.24 µg/g in *S. palustris* and 126.32 µg/g in *S. sylvatica*, and in leaf extracts between 9.29 µg/g in *S. palustris* and 111.11 µg/g in *S. sylvatica*. A syringic acid derivative was found in ethanol extract of dried roots of *Stachys geobombicis* C.Y.Wu.³⁵ Vanillic acid was not detected in the stems and leaves of studied extracts, and was identified only in the flower extracts at values between 266.78 µg/g (*S. officinalis*) and 343.21 µg/g (*S. palustris*).

Among the hydroxycinnamic acids identified, the most significant amounts were found in the case of chlorogenic acid with values varying in the flower extracts between 1011.78 µg/g (*S. officinalis*) and 7132.29 µg/g (*S. palustris*). Chlorogenic acid was also identified in stems (125.37 µg/g – 333.25 µg/g) and in leaves (113.48 µg/g – 452.65 µg/g) in all three species. Chlorogenic acid and vanillic acid were predominant in aerial parts extracts of *Stachys cretica* L. subsp. *vacillans* Rech. Fil.³⁰ Syringic acid and vanillic acid were identified in *Stachys* sp. aff. *Schimperii* whole plant extract.³⁶

Caffeic acid was identified in the flower extracts at values over 100 µg/g, but in leaf and stems extracts the values were only subunit, or undetectable in the stems extracts of *S. officinalis*. Caffeic acid was found as major phenolic compound for *Stachys tmolea* Boiss.³⁷ The p-coumaric acid was detected in all *Stachys* extracts, values being significantly identified in the flower extracts (35.66 µg/g –

Table 1. Phenolic acids identified and quantified in extracts obtained from flowers, stems and leaves of *S. officinalis*, *S. palustris*, *S. sylvatica*

Phenolic acid	<i>S. officinalis</i> (µg/g)			<i>S. palustris</i> (µg/g)			<i>S. sylvatica</i> (µg/g)		
	Flowers	Stems	Leaves	Flowers	Stems	Leaves	Flowers	Stems	Leaves
Hydroxybenzoic acid									
Benzoic acid	12464.34	1270.16	4564.43	27970.53	282.28	2225.44	19071.32	347.79	3447.22
Ellagic acid	24.25	4.56	12.34	18.02	1.27	2.97	32.01	6.96	21.12
Gallic acid	7.33	n.d	0.27	2.48	n.d	0.22	9.34	n.d	n.d
P-hydroxybenzoic acid	25.39	2.11	73.43	49.27	8.54	57.14	83.15	n.d	4.04
Salicylic acid	5.23	0.96	0.35	9.42	0.27	0.22	1.22	0.29	0.77
Syringic acid	389.41	34.12	22.44	569.78	11.24	9.29	487.76	126.32	111.11
Vanillic acid	266.78	n.d	n.d	343.21	n.d	n.d	312.22	n.d	n.d
Hydroxycinnamic acid									
Caffeic acid	102.56	n.d	0.01	176.53	0.02	0.15	149.28	0.04	0.11
Chlorogenic acid	1011.78	125.37	452.65	7132.29	234.23	217.02	2119.69	333.25	113.38
P- coumaric acid	35.66	10.21	12.92	46.22	9.22	16.78	55.19	22.33	24.21
Ferulic acid	821.32	247.77	293.99	916.16	196.78	241.39	441.02	133.44	188.07
M-coumaric acid	5.66	1.21	2.92	4.22	n.d	n.d	5.19	2.33	4.21
Rosmarinic acid	9.56	n.d	n.d	4.93	n.d	n.d	4.55	n.d	n.d
Sinapic acid	7.99	n.d	n.d	3.23	n.d	0.03	7.13	n.d	0.05
Trans-cinnamic acid	0.01	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Total	15177.27	1696.47	5435.75	37246.29	743.85	2770.65	22779.07	972.75	3914.29

Values are expressed as mean (n = 3), n.d = not detected

55.19 µg/g), and lower in stems and leaves (9.22 µg/g – 24.21 µg/g). In all assessed extracts a significant amount of ferulic acid were quantified. The values determined in the flower extracts varied between 441.02 µg/g and 916.16 µg/g, and in the stem extracts up to a maximum of 247.77 µg/g. Ferulic acid were identified in other species, such as *Stachys germanica* L.,³⁸ *Stachys pumila* Banks & Sol.,³⁹ *S. byzantine*³⁴ and in *Stachys thirkei* K. Koch was found as major phenolic compounds along with chlorogenic acid, caffeic acid and rosmarinic acid.³⁷

In the extracts obtained from flowers have been detected m-coumaric acids (4.22 µg/g – 5.66 µg/g), rosmarinic acid (4.55 µg/g – 9.56 µg/g), sinapic acid (3.23 µg/g – 7.29 µg/g), trans-cinnamic acid (0.01 µg/g for *Stachys Officinalis* L). Other authors analyzed phenolics compounds, respectively PAs from various *Stachys* extracts, results being noted in the case of species *S. officinalis*,^{20,40,41,42} *S. palustris*,^{12,41} *S. sylvatica*,^{12,38} *Stachys cretica* ssp. *anatolica* Rech. Fil.,³¹ *Stachys lavandulifolia* Vahl.,⁴³ *Stachys tmolea* Boiss.⁴⁴

3. 2. Total Flavonoid Content in Flower, Stem and Leaf Extracts

Flavonoids are important bioactive compounds identified in all extracts, irrespective of plant species or organ used. As noted in table 2, the highest value of 51.66 mg QE/g DW were identified in flower extract of *S. palustris*, followed by flower extracts of *S. officinalis* (45.36 mg QE/g DW) and *S. sylvatica* (39.48 mg QE/g DW). TFC was lower in the extracts obtained from the stems and leaves regardless of the species. Similar or lower TFC was identified by other authors in *Stachys* species. Sarikurkcu et al. reported a TFC between 39.24 mg Re/g extract (routine equivalents) and 47.70 mg Re/g for *S. byzantina* extract,⁴⁵ and Ahmadvand et al. referenced a TFC of 17.09 mg QE/g extract and 31.18 mg QE/g for *Stachys inflata* Benth extract.⁴⁶

3. 3. Anthocyanins Content in Flower, Stem and Leaf Extracts

Anthocyanins are water-soluble, colored and bioactive compounds, associated with the red color of the flower petals of the three studied species. In this study, anthocyanins were identified at an average value of 32.61 mg/g extract in *Stachys officinalis* flowers, 19.88 mg/g extract in *Stachys palustris* flowers and 27.72 mg/g extract in *Stachys sylvatica* flowers. Table 2 shows the lack of anthocyanins in the extracts from stems and leaves. Anthocyanins were also detected by Lachowicz-Wisniewska et al.²⁷ in the flowers of the species *Stachys palustris* at an average amount of 20 mg/100 g d.m. or by Bursal et al.⁴⁷ in the extracts of *Stachys annua* at an average value of 34.3 µg/g, but also by other authors who highlighted their antioxidant and anti-inflammatory qualities.⁴⁸

Table 2. Flavonoids, anthocyanins, tannins and antioxidant activity of *S. officinalis*, *S. palustris*, *S. sylvatica*

Species	Aerial part	Total flavonoids (mgQE/g DW)	Anthocyanins (mg/g DW)	Total tannins (mg TAE/g DW)	FRAP (mg TE/g DW)
<i>S. officinalis</i>	Flowers	45.36	32.61	87.55	71.34
	Stems	39.45	n.d	77.39	56.38
	Leaves	31.22	n.d	84.27	83.22
	Total	38.67	10.87	83.07	70.31
<i>S. palustris</i>	Flowers	51.66	19.88	75.54	93.76
	Stems	19.78	n.d	44.97	66.09
	Leaves	32.67	n.d	71.76	76.21
	Total	34.70	19.88	64.09	78.68
<i>S. sylvatica</i>	Flowers	39.48	27.72	101.33	87.54
	Stems	29.07	n.d	56.39	63.27
	Leaves	31.63	n.d	51.15	75.77
	Total	34.70	27.72	69.62	75.52

3. 4. Determination of Total Tannin Content

Tannins are phenolic compounds produced as secondary metabolites by terrestrial and aquatic plants.⁴⁹ Table 2 stands out the fact that tannins vary in the flower extracts from 75.54 mg TAE/g DW to 101.33 mg TAE/g DW, the significantly higher value being attributed to *S. sylvatica*. In the extracts derived from stems, TTC was quantified to a value of 44.97 mg TAE/g DW and 77.39 mg TAE/g DW, the lowest value being defining for the species *S. palustris*. Lachowicz-Wisniewska et al. identified 36 hydrolysable tannins in *S. palustris* flower extracts, 32 in stem extracts and 31 in leaf extracts.²⁷ TTC varied between the level of 1.72% and 2.91% pyrogallol equivalent in *S. officinalis*, depending of the vegetative stage of plant development.²⁰

3. 5. Principal Component Analysis of Flowers, Stems and Leaves Sample of *S. Officinalis*, *S. Palustris* and *S. Sylvatica*

The results obtained through HPLC method were analyzed and interpreted to explain and to identify the relationships and the patterns of chemical compounds characteristic of *S. officinalis*, *S. palustris* and *S. sylvatica* flowers, leaves and stems. The first principal component (PC1) corresponds to 68% of the total variation, while the second principal component (PC2) explains only approximately 11% (Figure 1). The analysis of the PCA from flowers, leaves and stems showed a separation of the samples depending on their chemical composition, leaves and stems of *S. sylvatica*, stems of *S. officinalis* being located on the negative semiaxes. The location of the *S. officinalis* flower sample in quadrant II, away from the other samples, suggests the highest content of anthocyanins, flavonoids, rosmarinic acid, M-coumaric acid, etc. A similar content of

PAs are indicated by the close location of samples from flowers of *S. palustris* and *S. sylvatica* in the first quadrant, on the one hand, and samples from leaves of *S. palustris* and *S. officinalis* in the IV quadrant, on the other side (Figure 1a). The main components of positive side of PC1 were p-coumaric acid, flavonoids, ellagic acid, rosmarinic acid, anthocyanin, salicylic acid, syringic acid, sinapic acid and trans-cinnamic acid, while the positive side of the PC2 is identified with vanillic acid, tannins, caffeic acid, ferulic acid, p-hydroxybenzoic acid (Figure 1b).

The results are confirmed by the Pearson correlation coefficient. In the heatmap presented in Figure 2, a significant positive correlation can be observed between the chemical variables identified in the analyzed samples, very rarely being identified a weak negative correlation between the chemical components, such as between trans-cinnamic acid and p-hydroxybenzoic acid or FRAP.

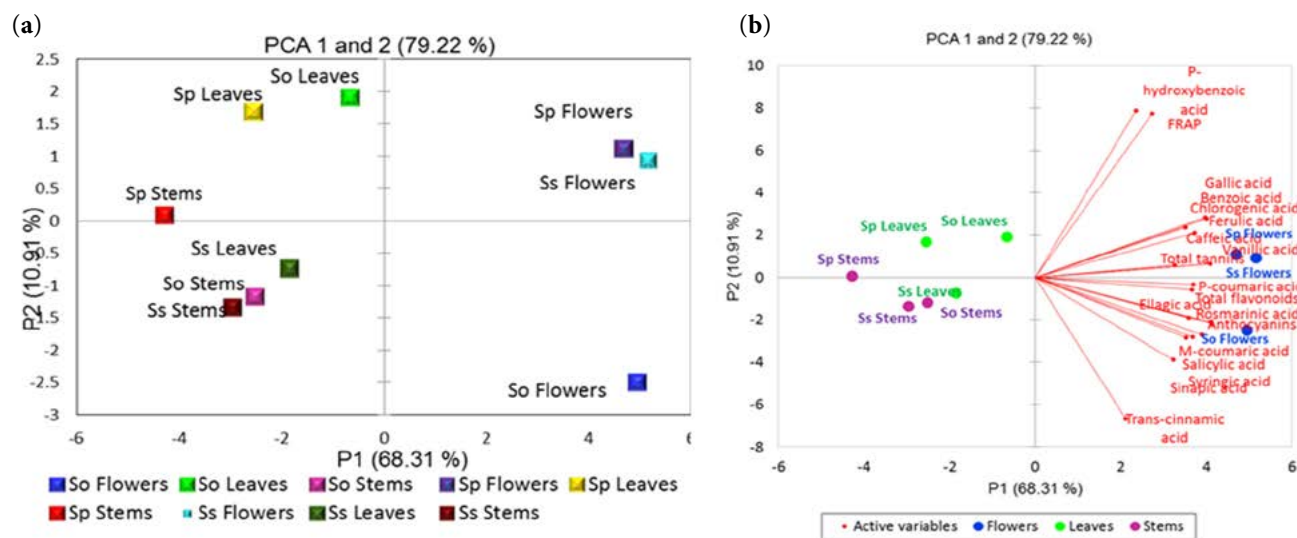


Figure 1. Differentiation of flowers, leaves and stems sources based on the compositional profile; (a) PCA score plot illustrating differentiation of flowers, leaves and stems sources based on the compositional profile. Colored symbols correspond to the flowers, leaves and stems of the three species addressed in this study (So – *S. officinalis*, Sp – *S. palustris* and Ss – *S. sylvatica*). The first two principal axes explained approximately 79% of the variance; (b) PCA loading plot showing the multivariate variation among the flowers, leaves and stems of the three species in terms of chemical compositional variables

3. 6. FRAP of Flower, Stem and Leaves Extracts

In the flower, stem and leaf extracts of *S. officinalis*, *S. palustris*, *S. sylvatica*, FRAP values varied between 56.38 mg TE/g DW and 93.76 mg TE/g DW (Table 2). It is noted that this activity is more significant for flower extracts obtained from *S. palustris*, followed by *S. sylvatica*, and then by *S. officinalis*. Regarding the leaf extracts, a more pronounced activity is on *S. officinalis* (83.22 mg TE/g extract), followed by *S. palustris* (76.21 mg TE/g extract) and *S. sylvatica* (75.77 mg TE/g extract). Significant values were also obtained on *Stachys cretica* L. extract (12.98±0.11 mg TE/g extract, 236.44±2.96 mg TE/g extract, 254.40±8.58 mg TE/g extract, 127.20 mg TE/g extract).^{3,29,30,31,47} Cüce et al. established for micropropagated plants of *Stachys an-*

nua L. that FRAP values varied between 334.5 mg TE/g extract and 1409.5 mg TE/g extract.⁵⁰ Other studies revealed that FRAP values of *Stachys thirkei* K. Koch. and *Stachys turcomanica* Trautv. extracts varied depending on the solvent type and on the concentration of the solvent used for extraction, respectively.^{5, 51}

4. Conclusions

Extracts obtained from flowers, leaves and stems of *S. officinalis*, *S. palustris*, *S. sylvatica* have a chemical composition rich in phenolic compounds. The comparative analysis has completed the literature data with new and comprehensive information about the phenolic and antioxidant profile. Compared to stems and leaves, these bioactive compounds are more abundant in flowers, but to-

gether they create a generous profile. Flavonoids, anthocyanins and tannins are found in the most significant amounts in *S. officinalis*, followed by *S. palustris* and *S. sylvatica*. The PCA analysis revealed significant differences in chemical composition of flowers, leaves and stems. Valuable elements such as hydroxybenzoic or hydroxycinnamic acids, the plenteous load of natural antioxidants in the assessed extracts, place them in the recommended list for their further use in pharmaceutical, cosmetic and food industries.

Funding

This work was supported by the grant POCU/993/6/13 -153178, co-financed by the European Social Fund within

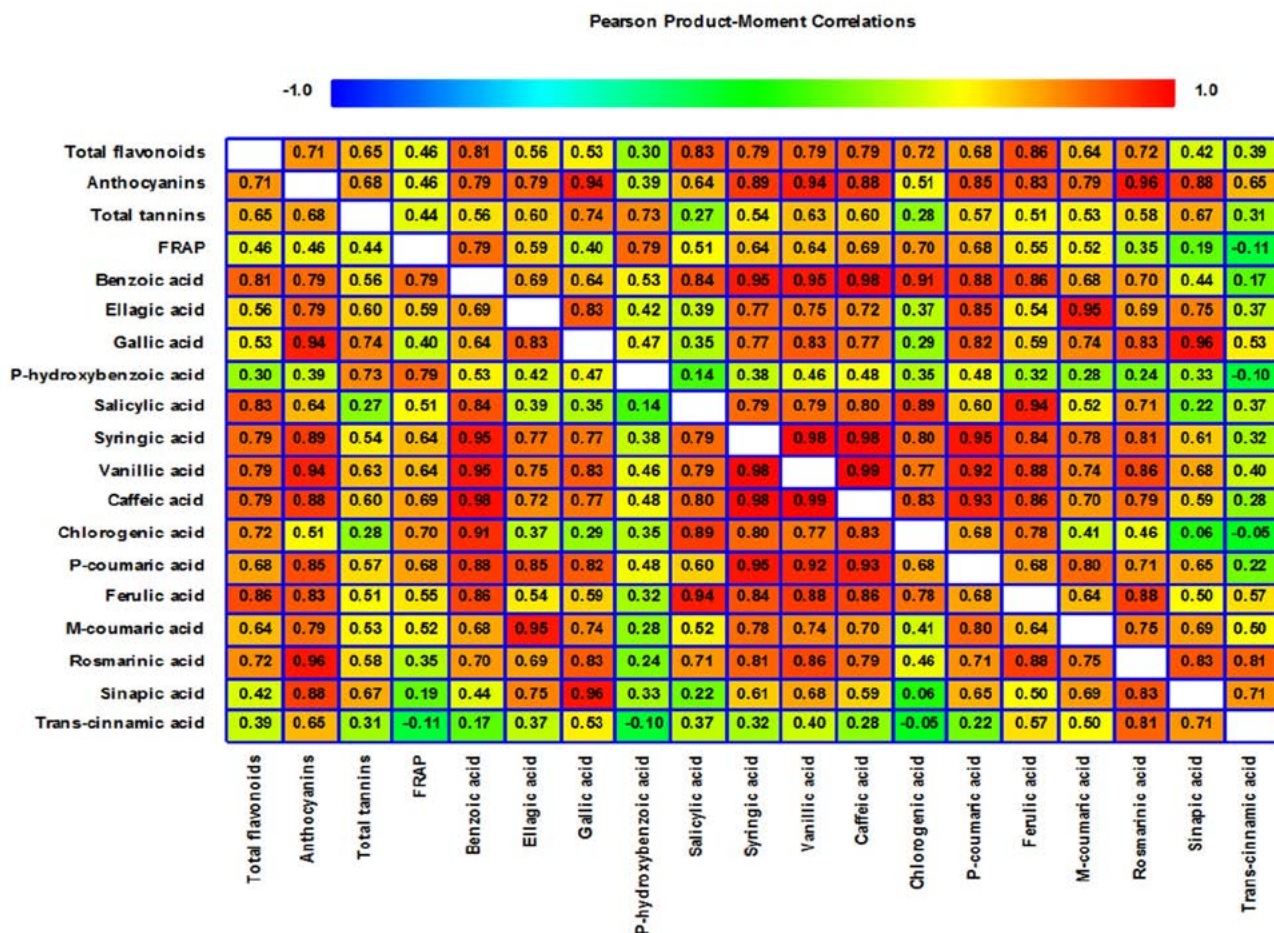


Figure 2. Heatmap of Pearson correlation coefficient obtained from chemical compositional variables analyzed from flowers, leaves and stems of *S. officinalis*, *S. palustris* and *S. sylvatica*.

the Sectorial Operational Program Human Capital 2014 – 2020, PN 23150301 (The implementation of integrated isotopic-chemical-nuclear analytical method-ologies for the authentication of traditional Romanian food product-s).N.A.Ş. gratefully acknowledges the support obtained through project number PN-III-P4-ID-PCE-2020-0620, within PNCDI III, a grant from the Romanian Ministry of of Education and Research, CNCS–UEFISCDI.

5. References

- M.S. Kocak, M.C. Uren, M. Calapoglu, A. Sihoglu Tepe, A. Mocan, K.R.R. Rengasamy, C. Sarikurkcü, *S. Afr. J. Bot.* **2017**, *113*, 128–1321. DOI:10.1016/j.sajb.2017.08.005
- E.M. Tomou, C. Barda, H. Skaltsa, *Medicines* **2020**, *7*, 63. DOI:10.3390/medicines7100063
- M.B. Bahadori, B. Kirkan, C. Sarikurkcü, *Ind. Crop Prod.* **2019**, *127*, 82–87. DOI:10.1016/j.indcrop.2018.10.066
- A. Shakeri, G. D'Urso, S.F. Taghizadeh, S. Piacente, S. Norouzi, V. Soheili, J. Asili, D. Salarbashi, *J. Pharm. Biomed. Anal.* **2019**, *168*, 209–216. DOI:10.1016/j.jpba.2019.02.018
- G. Gülsoy Toplan, T. Taşkın, E. Mataracı Kara, G.E. Genç, *Istanbul J. Pharm.* **2021**, *51*, 341–347. DOI:10.26650/IstanbulJPharm.2021.974035
- D.I. Stegăruş, E. Lengyel, G.F. Apostolescu, O.R. Botoran, C. Tanase, *Plants* **2021**, *10*, 2710. DOI:10.3390/plants10122710
- H. Sadeghi, D. Rostamzadeh, E. Panahi Kokhdan, A. Asfaram, A.H. Doustimotlagh, N. Hamidi, S. Hossein, *Evid. Based Complement. Alternat. Med.* **2022**, 7621599. DOI:10.1155/2022/7621599
- H. Sadeghi, M. Mansourian, E.P. Kokhdan, Z. Salehpour, I. Sadati, K. Abbaszadeh-Goudarzi, A. Asfaram, A.H. Doustimotlagh, *J. Food Biochem.* **2020**, *44*, e13190. DOI:10.1111/jfbc.13190
- E. Háznagy-Radnai, Á. Balogh, S. Czige, I. Máthé, J. Hohmann, G. Blazsó, *Phytother. Res.* **2012**, *26*, 505–509. DOI:10.1002/ptr.3582
- R. Jahani, D. Khaledyan, A. Jahani, E. Jamshidi, M. Kamalnejad, M. Khoramjouy, M. Faizi, *Res. Pharm. Sci.* **2019**, *14*, 544–553. DOI:10.4103/1735-5362.272563
- E. Panahi Kokhdan, H. Sadeghi, H. Ghafoori, H. Sadeghi, N. Danaei, S. Salaminia, M.R. Aghamaali, *Armaghane Danesh* **2019**, *24*, 17–30.

12. A. Venditti, C. Frezza, D. Celona, A. Bianco, M. Serafini, K. Cianfaglione, D. Fiorini, S. Ferraro, F. Maggi, A.R. Lizzi, G. Celenza, **2017**, *Food Chem.* **221**, 473–481. DOI:10.1016/j.foodchem.2016.10.096
13. M. Tunc Turk, R. Tunc Turk, U. Karik, T. Eryigit, *Int. J. Agric. Environ. Food Sci.* **2019**, **3**, 5–8. DOI:10.31015/jaefs.2019.1.2
14. J.K. Lee, J.-J. Lee, Y.-K. Kim, Y. Lee, J.-H. Ha, *Nutrients* **2020**, **12**, 2063. DOI:10.3390/nu12072063
15. K. Namvar, E.A. Salehi, N. Mokhtarian, *Bioscience Journal* **2018**, **34**, 1349–1356. DOI:10.14393/BJ-v34n5a2018-41517
16. C. Georgescu, A. Frum, L.I. Virchea, A. Sumacheva, M. Shamtsyan, F.G. Gligor, N.K. Olah, E. Mathe, M. Mironescu, *Molecules* **2022**, **27**, 4986. DOI:10.3390/molecules27154986
17. V.B. Vundać, A.H. Brantner, M. Plazibat, *Food Chem.* **2007**, **104**, 1277–1281. DOI:10.1016/j.foodchem.2007.01.036
18. C. Popescu, C. Popescu, B. Popescu, D. Daas, C. Morgovan, N.K. Olah, *Farmacia* **2014**, **62**, 743–752. DOI:10.1016/j.foodchem.2007.01.036
19. C. Drăgulescu (Ed.), „Lucian Blaga” University Sibiu, **2010**, pp.450–452.
20. K. Bączek, O. Kosakowska, J.L. Przybył, Z. Węglar, *Herba Pol.* **2016**, **62**, 7–16. DOI:10.1515/hepo-2016-0007
21. F.G. Gligor, A. Frum, L.G. Vicas, M. Totan, C. Roman-Filip, C.M. Dobrea, *Anal. Lett.* **2020**, **53**, 1391–1406. DOI:10.1080/00032719.2019.1708373
22. V.I. Craciun, F.G. Gligor, A.M. Juncan, A.A. Chis, L.L. Rus, *Rev. Chim.* **2019**, **70**, 3202–3205. DOI:10.37358/RC.19.9.7516
23. D.I. Popescu, E. Lengyel, F.G. Apostolescu, L.C. Sun, O.R. Botoran, N.A. Şuţan, *Horticulturae* **2022**, **8**, 952. DOI:10.3390/horticulturae8100952
24. J. Lee, R.W. Durst, R.E. Wrolstad, *J. AOAC Int.* **2005**, **88**, 1269–1278. DOI:10.1093/jaoac/88.5.1269
25. E.L.C. Amorim, J.E. Nascimento, J.M. Monteiro, T.J.S.P. Sobrinho, T.A.S. Araujo, U.P. Albuquerque, *Functional Ecosystems and Communities* **2008**, 88–94.
26. C.L. Gomes, C.C.A.R. Silva, C.G. De Melo, M.R.A. Ferreira, L.A.L. Soares, R.M.F. Da Silva, L.A. Rolim, P.J. Rolim Neto, *An. Acad. Bras. Cienc.* **2021**, **93**, e20190373. DOI:10.1590/0001-3765202120190373
27. S. Lachowicz-Wisniewska, A. Pratap-Singh, I. Kapusta, A. Kruszyńska, A. Rapak, I. Ochmian, T. Cebulak, W. Zukiewicz-Sobczak, P. Rubinski, *Pharmaceuticals* **2022**, **15**, 785. DOI:10.3390/ph15070785
28. V. Amalan, V. Natesan, I. Dhananjayan, R. Arumugam, *Bio-med. Pharmacother.* **2016**, **84**, 230–236. DOI:10.1016/j.biopha.2016.09.039
29. M.B. Bahadori, B. Kirkan, C. Sarikurkcü, O. Ceylan, *Ind. Crops Prod.* **2019**, **131**, 85–89. DOI:10.1016/j.indcrop.2019.01.038
30. B. Kirkan, C. Sarikurkcü, O. Ceylan, *Ind. Crop and Prod.* **2019**, **131**, 85–89. DOI:10.1016/j.indcrop.2019.01.038
31. I. Carev, C. Sarikurkcü, *Plants* **2021**, **10**, 1054. DOI:10.3390/plants10061054
32. S. Rahimi Khoigani, A. Rajaei, S.A.Goli, *Nat Prod. Res.* **2017**, **31**, 355–358. DOI:10.1080/14786419.2016.1233410
33. G. Ozkan, R.S. Gokturk, O. Unal, S. Celik, *Chem. Nat. Comp.* **2006**, **42**, 172–174. DOI:10.1007/s10600-006-0070-1
34. O. Sytar, I. Hemmerich, M. Zivcak, C. Rauh, M. Brestic, *Saudi J Biol.* **2018**, **25**(4), 631–641. DOI:10.1016/j.sjbs.2016.01.036
35. X. Zhou, S. Huang, P. Wang, Q. Luo, X. Huang, Q. Xu, X. Qin, J. Qin, C. Liang, X. Chen, *Nat. Prod. Res.* **2017**, 1–6. DOI:10.1080/14786419.2017.1405413
36. M. Abdel-Mogib, H.S.M. Al-Zahrani, *JKAU: Sci.* **2005**, **17**, 77–82. DOI:10.4197/Sci.17-1.8
37. T. Askun, E.M. Tekwu, F. Satil, S. Modanlioglu, H. Aydeniz, *BMC Complement. Altern. Med.* **2013**, **13**, 365. DOI:10.1186/1472-6882-13-365
38. S.S. Mitic, M. Stojkovic, J.L. Pavlović, M. Mitić, B.T. Stojanović, *Oxid. Commun.* **2012**, **35**, 1011–1021.
39. R.A. Kepekçi, S. Polat, G. Çoşkun, A. Çelik, A.S. Bozkurt, Ö. Yumrutaş, M. Pehlivan, *J. Food Biochem.* **2017**, **41**, 12286. DOI:10.1111/jfbc.12286
40. I. Šliumpaite, P. Venskutonis, M. Murkovic, O. Ragažinskienė, *Ind. Crop Prod.* **2013**, **50**, 715–722. DOI:10.1016/j.indcrop.2013.08.024
41. V. B. Vundać, **2019**, *Plants (Basel)*, **8**, 32. DOI:10.3390/plants8020032
42. J.S. Lazarević, A.S. Đorđević, D.V. Kitić, B.K. Zlatković, G.S. Stojanović, *Chem. Biodivers.* **2013**, **10**, 1335–1349. DOI:10.1002/cbdv.201200332
43. M.N. Bingol, E. Bursal, *Int. Lett. Nat. Sci.* **2018**, **72**, 28–36. DOI:10.18052/www.scipress.com/ILNS.72.28
44. W. Elfalleh, B. Kirkan, C. Sarikurkcü, *Ind. Crop Prod.* **2019**, **127**, 212–216. DOI:10.1016/j.indcrop.2018.10.078
45. C. Sarikurkcü, M.S. Kocak, M.C. Uren, M. Calapoglu, A.S. Tepe, *Eur. J. Integr. Med.* **2016**, **8**, 631–637. DOI:10.1016/j.eujim.2016.04.010
46. H. Ahmadvand, S. Farajollahi, H. Amiri, A. Amiri, *Herb. Med. J.* **2017**, **2**, 97–104. DOI: 10.22087/hmj.v0i0.623
47. S.E. Bursal, P. Taslimi, A.C. Gören, I. Gülçin, *Biocatal. Agric. Biotechnol.* **2020**, **28**, 101711. DOI:10.1016/j.bcab.2020.101711
48. G. Paun, E. Neagu, V. Moroeanu, C. Albu, T.-M. Ursu, A. Zanfrescu, S. Negres, C. Chirita, G.L. Radu, *Rev. Bras. Farmacogn.* **2018**, **28**, 57–64. DOI:10.1016/j.bjp.2017.10.008
49. M. Fraga-Corral, P. Otero, J. Echave, P. Garcia-Oliveira, M. Carpena, A. Jarbou, B. Nuñez-Estevéz, J. Simal-Gandara, M.A. Prieto, *Foods* **2021**, **10**, 137. DOI:10.3390/foods10010137
50. M. Khanavi, M. Hajimahmoodi, M. Cheraghi-Niroomand, Z. Kargar, Y. Ajani, A. Hadjiakhoondi, M. R. Oveisi, *Afr. J. Biotechnol.* **2009**, **8**, 1143–1147. DOI:10.5897/AJB2009.000-9182
51. M. Cüce, T. Bekircan, A.H. Laghari, M. Sökmen, A. Sökmen, E.Ö. Uçar, A.O. Kılıç, *Indian J. Tradit. Knowl.* **2017**, **16**, 407–416.
52. K. Namvar, A. Mohammadi, E.A. Salehi, P. Feyzi, *Pharm. Sci.* **2017**, **23**, 244–248. DOI:10.15171/PS.2017.36

Povzetek

Stachys officinalis L., *Stachys palustris* L., *Stachys sylvatica* L. (Lamiaceae) se pogosto uporabljajo kot zdravila rastlinskega izvora. V tej raziskavi je bila opravljena primerjalna ocena vsebnosti fenolnih kislin, flavonoidov, antocijaninov in taninov ter antioksidativne aktivnosti izvlečkov, pridobljenih iz cvetov, listov in stebel. Fenolne kisline, določene z metodo HPLC, so dosegle najvišje vrednosti v izvlečku cvetov *S. palustris*, izvlečku stebela *S. officinalis* in izvlečku listov *S. sylvatica*. Na podlagi spektrofotometrične metode so bile pri vseh treh vrstah ugotovljene vrednosti flavonoidov, ki so presegale 100 mg ekvivalentov kvercetina (QE)/g suhe snovi. Antocijanini so bili zaznani le v izvlečkih iz cvetov. *S. officinalis* se je odlikoval z najvišjo vsebnostjo antocijaninov in taninov. Antioksidativna aktivnost je bila prisotna pri vseh treh proučevanih vrstah, pri čemer se je vrsta *S. palustris* odlikovala z najintenzivnejšo antioksidativno sposobnostjo reduciranja železovih ionov. Dobljeni rezultati so zaradi raznolikosti in bogastva bioaktivnih spojin in antioksidantov pripeljali do potrditve uporabnosti teh rastlin v zdravilne in prehranske namene.



Except when otherwise noted, articles in this journal are published under the terms and conditions of the Creative Commons Attribution 4.0 International License