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In vitro Antioxidant and Antibacterial Activity of Lamiaceae Phenolic Extracts: A Correlation Study

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Summary

Total phenols and phenolic subgroups of five Lamiaceae plant extracts (sage, thyme, lemon balm, peppermint and oregano) were determined spectrophotometrically, whereas the individual phenolics were determined by high-performance liquid chromatography. The antioxidant activity of the extracts was evaluated by means of a multiple method approach, while the antibacterial activity was tested against major foodborne pathogens such as *Campylobacter coli, Escherichia coli, Salmonella* Infantis, *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*. The highest content of total phenolics and non-flavonoids was detected in the sage extract, which also showed the best antibacterial activity, especially against Gram-positive bacteria and *C. coli*. The best reducing power and free radical scavenging activity were obtained in lemon balm extract, with the highest content of rosmarinic acid. Additionally, the effect of the phenolics, especially rosmarinic acid, on biological properties of Lamiaceae plant extracts was investigated using principal component analysis. Rosmarinic acid showed good correlation with all antioxidant parameters, confirming its significant contribution to antioxidant activity of investigated plant extracts.

Key words: Lamiaceae, phenolics, rosmarinic acid, antioxidant properties, antimicrobial activity, principal component analysis

Introduction

In the food industry there is a great demand for ingredients that effectively inhibit major causes of food deterioration, such as oxidation of food components and microbiological spoilage. Oxidation of food components is a chemical degradation which results in rancidity, loss of the nutritional quality, degradation of sensory properties of food, while the microbiological deterioration primarily affects safety of foods (*1*). These processes may be prevented, avoided or delayed by preservatives. Consumers are concerned with the possible toxicity of the conventional synthetic preservatives, so finding new, nontoxic, and what is most important, effective natural compounds/extracts with good biological properties has become a major area of scientific research. There is an increasing interest in spices because of their strong antioxidant and antimicrobial activity, which exceeds the effect of many of the currently used synthetic preservatives (*1–4*).

The use of spices has been a common practice since ancient times; they impart aroma, mask undesirable odours and can make food more pleasant and tastier. Spices are usually used as flavouring and colouring agents, but they have also been used in food preservation for centuries

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because they are known to exert antioxidant and antimicrobial activity (*5–9*). Numerous studies have been published on the antimicrobial activity of spices against a wide range of microorganisms, including foodborne pathogens (*9–12*). The results of these studies have led to suggestion that they could be used as natural preservatives. In recent years major research has been focused on the nonnutritive constituents of plants because many studies carried out showed that biological activity of plants was strongly connected with the presence of substances which usually occur in very small quantities (*3,10,11,13*). Among diverse groups of phytochemicals present in plants, their biological activity is usually attributed to the presence of phenolics and terpenoids (*9,10,14,15*). Plants from the Lamiaceae family are the most used and commercialized species with good antioxidant and antimicrobial activity. Studies on the chemical constituents of Lamiaceae plants have been mainly focused on terpenoids (essential oils), but in recent years much attention has been directed to the hydrophilic components, like phenolic compounds. The majority of the phenolics in the mint family plants are phenolic acids, exclusively caffeic acid derivatives (*3,16,17*). Although Lamiaceae plants have been widely investigated, the results of different studies are difficult to compare, usually because of different sample preparation procedures, the used methods and different strains of microorganisms. Also, in a lot of cases, contradictory data have been reported by different authors for the same compound/plant extract.

The aim of this study is to investigate and compare the phenolic composition, antioxidant and antimicrobial properties of five plants from the mint family (sage, thyme, lemon balm, peppermint and oregano) in order to find the connection between the chemical composition of the extracts and their antioxidant/antimicrobial activity. Antioxidant activity of complex mixtures, like plant extracts, is usually attributed to a group of phytochemicals present in them and rarely to the presence of a single compound. In this study, we attempt to prove the effect of rosmarinic acid, the dominant phenolic compound in the extracts, on the biological activity of plant extracts, by submitting the results to multivariate principal component analysis (PCA).

Materials and Methods

Chemicals and apparatus

All reagents and solvents used in the experiments were of adequate analytical grade and were obtained from Kemika (Zagreb, Croatia), Merck Co. (Darmstadt, Germany), Oxoid (Hampshire, UK) and Sigma-Aldrich GmbH (Steinheim, Germany). Reference substances for spectrophotometric measurements were obtained from Sigma-Aldrich GmbH (gallic acid monohydrate ≥98 %, HPLC; (+)-catechin hydrate ≥98 %, HPLC), BioChemika, Fluka AG, Sigma-Aldrich, Buchs, Switzerland ((–)-epicatechin ≥90%, sum of enantiomers, HPLC), and Riedel-de Haen AG, Seelze, Germany (L(+)-ascorbic acid ≥99.7 %, oxidimetric). Spectrophotometric measurements were performed on a UV-VIS double beam Specord 200 spectrometer (Analytik Jena GmbH, Jena, Germany) and Sunrise basic microplate reader (Tecan, Mannedorf, Switzerland). For bioluminiscence measurements, Microplate Reader Safire II (Tecan) was used. The HPLC system used consisted of a Varian 330 UV/VIS photodiode array detector, a ternary gradient liquid Pro Star 230 pump, a model 500 heater and Star chromatography workstation v. 6.0 (Varian Inc., Walnut Creek, CA, USA).

Plant material and extraction procedure

Plant material: sage (*Salvia officinalis* L.), thyme (*Thymus serpyllum* L.), lemon balm (*Melissa officinalis* L.), peppermint (*Mentha piperita* L.) and oregano (*Origanum vulgare* L.), produced and distributed by Suban (Samobor, Croatia), were purchased from a local herbal pharmacy.

Homogenized plant material (5 g) was extracted with 250 mL of extraction solvent (ethanol/water=80:20, by volume) at 60 °C for 60 min. After cooling, the samples were filtered to remove residual particles, and the residual tissue was washed with solvent (3×10 mL). The extractions were performed in triplicate for each sample, and after the filtration, the three sample extracts were combined and evaporated to the volume of 150 mL, centrifuged and used for further analysis.

Phenolic composition analysis

Total phenols and phenolic subgroups

The total phenolic content in plant extracts was estimated using the Folin-Ciocalteu method (*18*), while the measurement of non-flavonoids was done using the method described by Kramling and Singleton (*19*). The content of flavonoids was calculated as the difference between the total phenolic and non-flavonoid content. All measurements were carried out in triplicate and the results were expressed as mg of gallic acid equivalents (GAE) per gram of dry plant material (*9,15*).

The concentration of the mixture of flavanol monomers and proanthocyanidins was determined using vanillin assay (*18*), while the content of flavanol monomers only was estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method (*20*). The results for the mixture of flavanol monomers and proanthocyanidins were expressed as mg of catechin equivalents (CE) per gram of dry plant material and for flavanol monomers as mg of epicatechin equivalents (EE) per gram of dry plant material. Each determination was performed in triplicate. Results are expressed as mean values±standard deviations (S.D.).

HPLC analysis of individual phenolic compounds

For separation, quantification and identification of individual phenolics, HPLC method was used. The standards of vanillin, *m*-hydroxybenzoic, *p*-hydroxybenzoic, protocatechuic, gallic, vanillic, syringic and cinnamic acids were obtained from Merck Co., while *o*-coumaric, *p*-coumaric, caffeic, *trans*-ferulic and rosmarinic acids, (–)-epicatechin, quercetin, luteolin and apigenin were purchased from Sigma-Aldrich GmbH. *Trans*-resveratrol was obtained from Sigma-Aldrich, Milwaukee, WI, USA. Astringin and quercetin-4'-glucoside were purchased from Polyphenols Laboratories AS (Sandnes, Norway), while (+)-catechin, kaempferol and myricetin were obtained from Fluka AG.

Polyphenolic compounds (rosmarinic acid, stilbenes, catechins, flavonols and flavones) were separated on an

UltraAqueous C_{18} column (250×4.6 mm, 5 mm, maintained at 30 °C; Restek, Bellefonte, PA, USA) using the following conditions: a gradient consisting of solvent A (water/acetic acid=98:2, by volume) and solvent B (acetonitrile/acetic acid=98:2, by volume) was applied as follows: from 92 % A at 0 min to 80 % A at 18 min, to 60 % A at 25 min, to 55 % A at 30 min, to 35 % A at 40 min, and to 20 % A at 50 min, maintaining at 20 % A for 4 min (54 min), then from 20 to 90 % A at 57 min, and maintaining at 90 % for 3 min (60 min).

Vanillin and monomeric phenolic acids were separated on a Zorbax Eclipse XDB-C18 column (250×4.6 mm, 5 mm, maintained at 25 °C, Agilent Technologies, Santa Clara, CA, USA) using the following conditions: a gradient consisting of solvent A (acetonitrile), solvent B (water/acetic acid=99:1, by volume) and solvent C (methanol) applied as follows: from 1 % A, 95 % B and 4 % C at 0 min to 5 % A, 85 % B and 10 % C at 15 min, to 15 % A, 35 % B and 50 % C at 45 min, to 20 % A, 5 % B and 75 % C at 60 min, to 1 % A, 95 % B and 4 % C at 72 min, maintaining at 1 % A, 95 % B and 4 % C for 3 min (75 min).

Before the injection into a C_{18} guard column, extracts were filtered (0.45 μm) and adequately diluted with methanol. Each sample was injected twice in the chromatographic system. The applied flow rate in both separation procedures was 1.0 mL/min. Chromatographic peaks of the detected phenolic compounds were identified by comparing their retention times and absorption spectra with those acquired for the corresponding standards analyzed under the same chromatographic conditions. Selected samples were spiked with the standard compounds to confirm the peak identity. The compounds were quantified from the areas of their peaks at 280 nm using external standard calibration curves.

Antioxidant activity

The reducing power of spice extracts was measured as ferric reducing ability of plasma (FRAP) as described by Benzie and Strain (*21*). A standard curve was prepared using different concentrations of vitamin C and the results are expressed in millimoles of vitamin C per litre of extract.

Free radical scavenging ability was determined using stabile synthetic 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) according to the procedure reported by Katalinić *et al*. (*11*,*15*), and using 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) radical cation (ABTS) according to the original procedure reported by Re *et al.* (*22*). The final results were expressed as inhibitory concentrations (IC_{50}), mg of GAE per L of extract needed to reduce the radical concentration by 50 %.

The chelating of ferrous ions by the sample was estimated using the colorimetric ferrozine-based assay described by Yen *et al.* (*23*). In this method different dilutions of extracts (concentration of phenolics) were added to the reaction mixture. The concentration of the extracts (in mg of GAE per L) that is effective in the chelation of metal ions by 50 % (IC_{50}) was calculated from the dose-response curve plotting between the percentage of chelating activity and the concentration.

The ability of plant extracts to stop the oscillations in Briggs-Rauscher system was estimated in our recently published papers (*9*,*13*). The analyzed plant extracts were diluted to a total phenol concentration of 100 mg of GAE per L, and the results are expressed as the inhibition time (in min) of the oscillations.

The antioxidant activity of Lamiaceae plant extracts in an aqueous emulsion system of linoleic acid and β-carotene was determined according to a method of Moure *et al*. (*24*). The tested plant extracts were diluted to total phenol concentration of 1000 mg of GAE per L. The oxidation of the emulsion of linoleic acid and β-carotene was monitored spectrophotometrically by measuring the absorbance of the sample and control at 470 nm at regular time intervals (40 min).

Each determination of antioxidant activity was performed in triplicate, and the results are expressed as mean values±S.D.

Antimicrobial activity

Bacterial strains and growth conditions

Antimicrobial activity was screened against six bacterial strains, namely *Campylobacter coli* ATCC 33559 (pig faeces isolate), *Escherichia coli* O157:H7 ŽM370 (clinical isolate), *Salmonella* Infantis ŽM9 (poultry meat isolate), *Listeria monocytogenes* ŽM58 (IHM, Würzburg, Germany), *Bacillus cereus* WSBC 10530 (clinical isolate) and *Staphylococcus aureus* ATCC 25923 (clinical isolate). *C. coli* was incubated microaerobically at 42 °C in Müller Hinton broth, with 5 % of defibrinated horse blood (Oxoid, Hampshire, UK), while the other bacterial cultures were incubated aerobically at 37 °C in Müller Hinton broth or agar (MHB or MHA; Oxoid). For inoculum preparation all bacteria were incubated for 20 h in MHB and for antibacterial assays, 1 mL of each inoculum was appropriately diluted with MHB to approx. 10^5–10^6 CFU/mL.

Determination of the minimum inhibitory concentration by broth microdilution method

For the broth microdilution test, bacterial culture (50 μL) in the early stationary phase was added to the wells of a sterile 96-well microtiter plate containing 50 μL of a twofold serially diluted phenolic plant extracts in MHB. The plant extracts were previously diluted to 40 % (by volume) stock solutions in MHB. To indicate respiratory activity, the presence of colour was determined after adding 10 μL of INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) (2 mg/mL) and incubating for 30 min in the dark (*25*). The absence of a bioluminescence signal was measured by a Microplate Reader Safire II after adding 100 μL of BacTiter-Glo™ reagent per well and a 5-minute incubation in the dark (*26*). The minimum inhibitory concentration (MIC) value, in mg of dry plant material per mL of growth medium, was the lowest concentration where no metabolic activity was observed. The presented data (MIC value) are the result of three measurements.

Statistical analysis

Statistical analysis was performed using STATISTICA (Data Analysis Software System, v. 8, StatSoft, Inc, Tulsa, OK, USA) and GraphPad InStat3 (GraphPad Software,

San Diego, CA, USA) software packages. Statistical differences between the different sets of data were determined by one-way ANOVA and followed by a least significant difference test at 95 % confidence level. Pearson's correlation coefficient was used to determine the relation between the variables. For more detailed insight in the relations between the variables, results were submitted to multivariate principal component analysis (PCA). All data were expressed as mean values±S.D.

Results and Discussion

Lamiaceae plants were a subject of numerous research because of their good antioxidant properties and high content of phenolics (*6*,*7*,*9*,*10*,*14*,*27*). The results of this study also confirmed relatively high content of these compounds in all investigated plant extracts. As shown in Fig. 1, the highest mass fraction of total phenols was detected in sage extract ((267.7±2.0) mg of GAE per g), which also contained the highest content of non-flavonoids ((236.4±0.5) mg of GAE per g). Non-flavonoid mass fraction was the dominant one in all investigated extracts, especially in sage. This is in accordance with our previous study reporting that the mass fraction of flavonoid compounds in sage extracts ranges from 4 to 17 %, depending on the phenophase in which the plant material was collected (*9*,*13*). The content of total phenols extracted from the sage used in this study was higher than in that reported by Generalić *et al.* (*9*) probably due to the origin of the plant material. On the other hand, the oregano extract was the richest in flavonoids (92.8 mg of GAE per g or 12 % of the total phenols), while the lowest mass fraction of this group of phytochemicals was detected in peppermint and lemon balm extracts, *i.e.* 3 and 9 %, respectively. Thyme extract had the highest fraction of flavanol monomers. All extracts contained small fraction of flavanol monomers and proanthocyanidins (ranging from 0.01 to 0.13 mg of EE per g); some of them were even negligible, like in sage and lemon balm (Fig. 1).

The HPLC analysis, a common method for identification of individual plant phenolics, was used to detect major phenolic compounds in the investigated plant extracts. Same as for the total phenols and phenolic subgroups, considerable variations were observed between spices in terms of individual phenolics. A total of twenty-four phenolic compounds was identified and quantified, including simple phenolics, phenolic acids, stilbenes, catechins, flavanols and flavones (Table 1).

Vanillin, a single compound from the group of simple phenolics, was investigated, and it was detected in the extracts of sage ((0.54±0.01) mg/g), thyme ((1.30±0.03) mg/g) and peppermint ((0.14±0.03) mg/g), while its presence in the remaining two extracts was not confirmed. All plant extracts contained extremely high mass fractions of phenolic acids, both hydroxybenzoic and hydroxycinnamic derivatives, but the content of the monomer forms was significantly lower compared to that of rosmarinic acid. Gallic acid was the only acid from the group of hydroxybenzoic acids which was detected in all spice extracts (ranging from 0.05 to 0.13 mg/g). Among all the investigated phenolic acids, only protocatechuic acid was not detected in sage extract, which is in accordance with

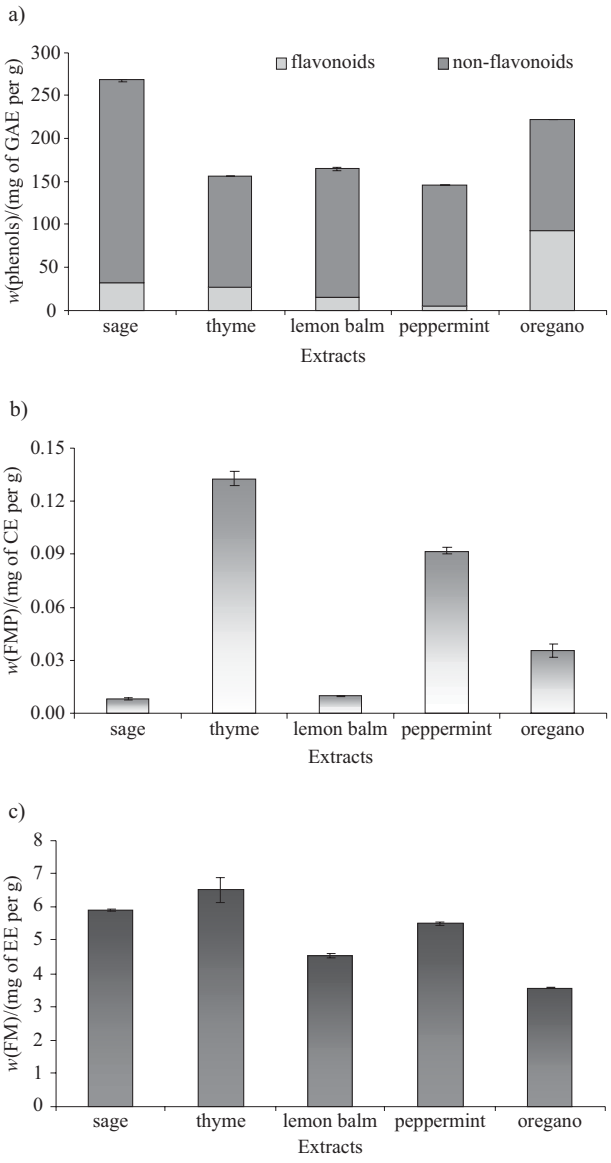

Fig. 1. The mass fractions of the main phenolic subgroups in the plant extracts: a) flavonoids and non-flavonoids, b) mixture of flavanol monomers and proanthocyanidins (FMP) and c) flavanol monomers (FM); GAE=gallic acid equivalents, EE=epicatechin equivalents, CE=catechin equivalents

the results reported by Kivilompolo and Hyötyläinen (*3*). The highest mass fraction of total hydroxybenzoic acids was found in the sage extract, especially syringic ((1.61±0.01) mg per g) and *p*-hydroxybenzoic ((1.04±0.02) mg per g) acids. Extracts of thyme and oregano contained all monomeric phenolic acids from the group of hydroxycinnamic acids, while the highest fractions of these compounds were detected in the extracts of lemon balm and peppermint. Among hydroxycinnamic acids, rosmarinic acid, an ester of caffeic acid, is one of the most abundant phenolic acids occurring in plants, especially in Lamiaceae species, so it is often used as chemotaxonomic marker of that plant family (*3*,*7*,*9*,*16*). The mass fraction of this compound was extremely high in all investigated extracts, ranging from 17.46 (oregano) to 72.4 mg/g (lemon balm), as expected. Lemon balm ex-

Table 1. Quantitative HPLC analysis of individual phenolics per dry mass of plant material

Phenolic compound	*w* (extract)/(mg/g)				
	Sage	Thyme	Lemon balm	Peppermint	Oregano
Simple phenols					
vanillin	(0.54±0.01)^a	(1.30±0.03)^b	n.d.	(0.14±0.03)^c	n.d.
Hydroxybenzoic acids					
m-hydroxybenzoic	(0.23±0.03)^a	n.d.	(0.41±0.02)^b	n.d.	n.d.
p-hydroxybenzoic	(1.04±0.02)^a	(0.24±0.02)^b	(0.12±0.01)^c	(0.56±0.02)^d	n.d.
protocatechuic	n.d.	(0.13±0.01)^a	(0.34±0.02)^b	n.d.	n.d.
gallic	(0.09±0.01)^a	(0.13±0.01)^b	(0.07±0.00)^c	(0.06±0.01)^c	(0.05±0.00)^d
vanillic	(0.58±0.02)^a	n.d.	(0.16±0.01)^b	n.d.	(0.42±0.02)^c
syringic	(1.61±0.01)^a	n.d.	n.d.	(0.19±0.01)^b	(0.72±0.01)^c
Hydroxycinnamic acids					
cinnamic	(0.04±0.00)^a	(0.10±0.00)^b	(0.96±0.03)^c	(0.05±0.00)^a	(0.06±0.01)^a
o-coumaric	(0.21±0.01)^a	(0.24±0.02)^b	(0.77±0.01)^c	(1.67±0.01)^d	(0.40±0.01)^e
p-coumaric	(0.07±0.00)^a	(0.21±0.03)^b	n.d.	n.d.	(0.20±0.01)^b
caffeic	(0.70±0.01)^a	(0.09±0.00)^b	(0.59±0.00)^c	(0.90±0.02)^d	(0.30±0.04)^e
trans-ferulic	(0.20±0.01)^a	(0.29±0.01)^b	(0.61±0.02)^c	n.d.	(0.14±0.04)^d
rosmarinic	(25.2±0.5)^a	(45.8±0.7)^b	(72.4±0.2)^c	(51.8±0.8)^d	(17.46±0.09)^e
Stilbenes					
astringin	(0.01±0.00)^a	(0.47±0.01)^b	(0.11±0.01)^c	n.d.	n.d.
trans-resveratrol	(0.14±0.00)^a	n.d.	n.d.	(0.12±0.00)^a	n.d.
Catechins					
(+)-catechin	(0.90±0.07)^a	(1.3±0.1)^b	(0.63±0.02)^c	(2.7±0.2)^d	n.d.
(–)-epicatechin	(1.32±0.09)^a	n.d.	n.d.	(0.98±0.00)^b	(0.41±0.02)^c
Flavonols					
quercetin	(0.58±0.02)^a	n.d.	n.d.	n.d.	n.d.
quercetin-4′-glucoside	(4.89±0.00)^a	(3.72±0.07)^b	n.d.	(1.67±0.07)^c	(15.0±0.1)^d
kaempferol	(0.15±0.01)^a	(0.37±0.02)^b	n.d.	n.d.	(0.11±0.01)^c
myricetin	n.d.	n.d.	n.d.	n.d.	n.d.
Flavons					
luteolin	(0.7±0.2)^a	n.d.	(1.31±0.04)^b	n.d.	(0.24±0.02)^c
apigenin	n.d.	(0.42±0.02)	n.d.	n.d.	n.d.

Results are expressed as mean values±standard deviations (S.D.). Different letters (a, b, c, d) in superscript in the same row denote significant difference ($p<0.05$); n.d.=not detected

tract was also rich in cinnamic, caffeic and *trans*-ferulic acids. High amounts of ferulic acid in lemon balm extracts were reported by Wojdylo *et al*. (*7*). Zgórka and Glowniak (*16*) found high amounts of *p*-hydroxybenzoic acid in peppermint extract, and did not detect the presence of *p*-coumaric and *trans*-ferulic acids, which is in accordance with our results.

Besides phenolic acids, we also investigated the presence of the non-flavonoids from the group of stilbenes: astringin and *trans*-resveratrol. Over the past several years, stilbenes and especially resveratrol and its derivatives, have attracted immense attention of biologists and chemists due to their numerous biological activities and possible pharmacological applications. In this study, the presence of these phytochemicals was not confirmed only in the oregano extract. Kulišić-Bilušić *et al.* (*28*) also reported the presence of *trans*-resveratrol in sage and lemon balm extracts. Significant mass fraction of astringin was found in thyme extract (0.47 mg/g), while sage and peppermint contained 0.14 and 0.12 mg/g of *trans*-resveratrol, respectively. In our previous study on sage, we confirmed the presence of stilbenes in the investigated extracts, but the dominant compound was *cis*-resveratrol (*9*).

From the group of flavonoids, sage extract was rich in quercetin (0.58 mg/g), quercetin-4′-glucoside (4.89 mg/g), kaempferol (0.15 mg/g) and luteolin (0.7 mg/g). Wojdylo *et al*. (*7*) reported similar results for quercetin and luteolin in their study. Thyme extract contained high mass fraction of quercetin-4′-glucoside (3.72 mg/g), and it was the only extract in which apigenin was found (0.42 mg/g). The presence of apigenin in thyme extracts was also reported by Boros *et al*. (*29*). Peppermint extract was rich in catechines, while oregano extract was rich in quercetin-4′-glucoside (15.0 mg/g).

In order to draw conclusions about the antioxidant properties of a natural plant extract, a multiple method approach is necessary. For this reason, the ferric reduc-

ing ability of plasma (FRAP), free radical scavenging activity (using stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals), chelating ability, activity of extracts in preventing lipid oxidation determined by β-carotene method and antioxidant activity of plant extracts determined using Briggs-Rauscher oscillating reaction were tested. The results obtained using these methods are presented in Table 2.

The FRAP values of the investigated plant extracts ranged from 13.2 (oregano) to 31.8 (lemon balm) mmol of vitamin C equivalents per L of extract. Although peppermint extract had the lowest mass fraction of total phenolics, good reducing ability of that extract could be related to the presence of high levels of rosmarinic acid. Very significant linear correlation was found between the content of rosmarinic acid and FRAP values (R=0.9858). Katalinić *et al*. (*14*) investigated reducing capacity of 70 medicinal plants and also reported best results for lemon balm among all investigated plants. Free radical scavenging assays, DPPH and ABTS, showed a wide variation among the examined spices, with lemon balm having the highest antioxidant activity, probably due to its high content of phenolics, namely rosmarinic acid (DPPH, R=–0.9197; ABTS, R=–0.9102). Capecka *et al.* (*30*) also reported the best free radical scavenging activity of lemon balm among the investigated Lamiaceae plants. FRAP, DPPH and ABTS are single electron transfer-based assays. This could be the reason for good correlation between the rosmarinic acid content and the obtained antioxidant properties (*31*). The position and/or number of OH groups on the benzene ring also affect the extent of direct electron transfer reactions. Rosmarinic acid has two *ortho*-dihydroxy groups (catechol structures), and that could be the reason why lemon balm extract, with the highest mass fraction of rosmarinic acid, showed the best results. The catechol structure is the most important structural feature for strong chelating activity, so it is not surprising that the lowest chelating IC_{50} value was detected for lemon balm extract. A negative correlation was found between the content of rosmarinic acid and chelating activity (R=–0.8831). The lipid peroxidation inhibition potential of the Lamiaceae plant extracts was tested using the β-carotene bleaching method. Using this method, oregano extract was shown to be superior to the other studied extracts. A negative correlation was found between the content of rosmarinic acid and β-carotene inhibition percentage (R=–0.8801), which could mean that this compound is not so effective antioxidant in lipid media. The last method used to provide information about the antioxidant activity of the extracts was Briggs-Rauscher method. The inhibition time of the Briggs-Rauscher oscillations depends on the free radical scavenging ability of the added antioxidant compound/mixture (*9*). In our study thyme extract provided the longest inhibition (53.7 min).

According to the obtained results, it can be confirmed that levels of rosmarinic acid are responsible for most of the observed *in vitro* antioxidant activities of the investigated Lamiaceae plant extracts. In this study, PCA was used for better visualisation of data sets obtained from the determinations of ten studied variables: total phenols, flavonoids, non-flavonoids and rosmarinic acid content, as well as antioxidant activity (FRAP, DPPH and ABTS assay, chelating activity, β-carotene and Briggs--Rauscher method) (Figs. 2a and b). The use of this unsupervised classification method often permits a simple representation of different sample data and their correlations. The first two principal components (PCs) described 92.34 % of the initial data variability, while the remaining PCs each accounted for less than 1 % of the total variance. The correlation loadings of the first two PCs showed high correlations of all studied parameters (Fig. 2a). All the variables studied were characterised by PC1, with the exception of non-flavonoids. Parameters that showed the highest values of factor coordinates, with the highest variable contributions based on the correlation, were FRAP, DPPH IC_{50} and ABTS IC_{50} for PC1 and non--flavonoids for PC2. Rosmarinic acid showed good correlation with all antioxidant parameters confirming its significant contribution to the antioxidant activity of the investigated plant extracts. Other non-flavonoid compounds contained in the investigated plant extracts showed lower impact on the antioxidant properties. Antioxidation parameters based on the concentration of total phenols which causes 50 % of radical/metal inhibition (IC_{50} value) were grouped on the right side of the loading plot.

The score plot (Fig. 2b) showed the projection of five cases (investigated plant extracts) in the multivariate space of the first two PCs. The clear separation of the

Table 2. Antioxidant properties of the plant extracts determined as ferric reducing ability of plasma (FRAP), DPPH and ABTS radical scavenging activities, chelating ability (CHEL) and β-carotene bleaching assays, and Briggs-Rauscher (BR) oscillating reaction

Assays	Extracts				
	Sage	Thyme	Lemon balm	Peppermint	Oregano
FRAP/mmol of vitamin C per L	(15.5±0.6)^a	(25.7±0.1)^b	(31.8±0.3)^c	(25.7±0.2)^b	(13.2±0.3)^d
DPPH IC_{50}/mg of GAE per L	(413±20)^a	(251±27)^b	(233±27)^b	(254±21)^b	(474±14)^c
ABTS IC_{50}/mg of GAE per L	(388±15)^a	(249±12)^b	(232±17)^b	(237±20)^b	(453±31)^c
CHEL IC_{50}/mg of GAE per L	(658±31)^a	(315±17)^b	(305±11)^b	(406±20)^b	(940±143)^c
β-carotene inhibition/%	(77.6±0.4)^a	(77±3)^a	(72.2±0.7)^b	(72±3)^b	(78.8±0.6)^a
BR/min	(18.3±0.4)^a	(53.7±0.1)^b	(38±2)^c	(45±3)^d	(12.8±0.5)^e

Results are expressed as mean values±S.D.; different letters (a, b, c, d) in superscript in the same row denote significant difference ($p<0.05$); GAE=gallic acid equivalents

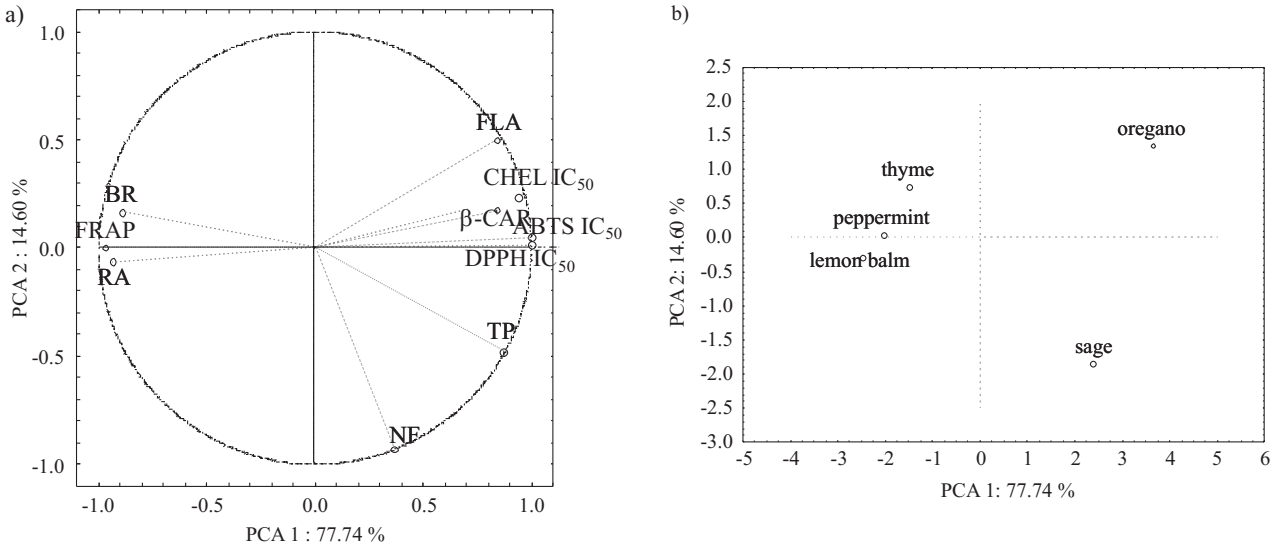

Fig. 2. Correlation loading plot (a) and score plot (b) of PCA describing data sets obtained from the spectrophotometric determinations of the antioxidant activity (ferric reducing ability of plasma (FRAP), results of β-carotene (β-CAR) and Briggs-Rauscher (BR) oscillating reaction assays, sample concentration providing 50 % inhibition of DPPH (DPPH IC_{50}) and ABTS (ABTS IC_{50}) free radicals, and chelating activity (CHEL IC_{50})) and the phenolic composition (total phenols (TP), non-flavonoids (NF), flavonoids (FLA) and rosmarinic acid (RA) content) of five Lamiaceae plant extracts

extracts pointed out the main similarities between them. The three extracts grouped on the left (lemon balm, peppermint and thyme) were characterised by high mass fraction of rosmarinic acid, and good reducing, free radical scavenging and chelating activities. Vertical separation along the PC2 (presenting non-flavonoids) indicated small differences in non-flavonoid content between the extracts. Oregano and sage extracts, positioned on the right, are characterised by low mass fraction of rosmarinic acid, low FRAP values and short inhibition time of Briggs-Rauscher oscillations. The obvious vertical separation along the PC2 was observed, caused by the domination of non-flavonoid fraction in the sage extract and high content of flavonoids in the oregano extracts. The results of the PCA analyses were in accordance with the results obtained by analysis of variance (Table 2). Also *t*-test showed statistically significant difference ($p<0.05$) in the non-flavonoid content between all extracts, which corresponded to vertical separation of the extracts in the score plot.

The antibacterial activity of plant extracts against different bacterial strains is well documented, but studies that include activity of the plant extracts from the same botanical family against major foodborne pathogens are scarce. In this study, antibacterial activity of mint extracts was evaluated by the broth microdilution method against Gram-negative (*C. coli*, *E. coli* and *Salmonella* Infantis) and Gram-positive (*B. cereus*, *L. monocytogenes* and *S. aureus*) bacterial strains, and the results are presented in Table 3.

Minimum inhibitory concentrations (MICs) of all plant extracts were detected against all tested Gram-positive bacteria and they ranged from 0.34 to over 6.85 mg per mL. The most effective was the sage extract, which inhibited *S. aureus* at the concentration of 0.34 mg per mL of growth medium. Sage extract was also effective against other Gram-positive strains, but at fivefold higher concentrations (1.68 mg per mL of growth medium). Other plant extracts provided almost twofold higher MIC values against *B. cereus* and *L. monocytogenes*. Good antibacterial properties of sage against *B. cereus* and *S. aureus* had also been reported in our previous study (*9*). Lemon balm extract also showed good antimicrobial activity against *S. aureus* (MIC value of 2.43 mg per mL of growth medium), while the MICs of other extracts ranged from 5.60 to 6.85 mg per mL of growth medium. It has already been established that Gram-positive organisms are more sensitive than Gram-negative to the antimicrobial

Table 3. Antibacterial activity of plant extracts expressed as minimum inhibitory concentration (MIC) in mg of dry plant material per mL of growth medium

	Bacterium	MIC/(mg/mL)				
		Sage	Thyme	Lemon balm	Peppermint	Oregano
Gram(–)	*Campylobacter coli*	0.82	3.40	3.40	1.71	1.70
	Escherichia coli	6.72	>6.73	6.73	6.71	>6.72
	Salmonella Infantis	6.72	>6.73	>6.73	>6.71	>6.72
Gram(+)	*Bacillus cereus*	1.68	6.73	6.73	6.71	3.36
	Listeria monocytogenes	1.68	6.73	6.73	6.71	6.72
	Staphylococcus aureus	0.34	6.48	2.43	6.85	5.60

compounds in spices, probably due to the presence of outer membrane surrounding the cell wall in Gram-negative bacteria. The most resistant organism to the tested extracts was *S.* Infantis. The MIC value against this microorganism was detected only in the sage extract (6.72 mg per mL of growth medium), while for all other extracts the tested range of concentrations was not sufficient to acquire approximated MIC values. Also, it was impossible to test more concentrated plant extracts due to the susceptibility of microorganisms to higher concentrations of ethanol.

Conclusions

In this work, all investigated Lamiaceae plants were found to have high levels of phenolics, mainly rosmarinic acid, and thus provided good antioxidant and antimicrobial properties. The principal component analysis (PCA) proved to be a very useful tool to identify the most effective variables and their relationships. The combination of phenolic and antioxidant analyses of the plant extracts and the application of PCA allowed the interpretation of the results indicating the existing relationship between the extract active component, rosmarinic acid, and the related biological activity. The application of these plant extracts as food preservatives in some real food systems, the mechanism of their activity and interaction with other food components should be the objective of future research.

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