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Comparative analysis of bioactive phenolic compounds composition from 26 medicinal plants

Oksana Sytar [a,b,*], **Irene Hemmerich** [d], **Marek Zivcak** [c], **Cornelia Rauh** [d], **Marian Brestic** [c]

[a] *Research Center AgroBioTech, Slovak University of Agriculture, Nitra, A. Hlinku 2, 94976 Nitra, Slovak Republic*
[b] *Plant Physiology and Ecology Department, Taras Shevchenko National University of Kyiv, Institute of Biology, Volodymyrskya Str., 64, Kyiv 01033, Ukraine*
[c] *Department of Plant Physiology, Slovak University of Agriculture, Nitra, A. Hlinku 2, 94976 Nitra, Slovak Republic*
[d] *Department of Food Biotechnology and Food Process Engineering, Institute of Food Technology and Food Chemistry TU Berlin, Königin-Luise-Str. 22, 14195 Berlin, Germany*

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Abstract Bioactive phenolic compounds are powerful antioxidants in traditionally used medicinal and industrial crop plants and have attracted increased interest in the last years in their application and role in non-destructive methodology for pre-screening analysis of some stress factors. In this study the qualitative target was linked with future possible applications of received data for improving non-destructive methodology as well as for improving existing knowledge regarding antioxidant content in some plant species. Comparative analysis of total phenolics, flavonoid contents, phenolic acid composition, and antioxidant activity in known east central Europe medicinal and industrial crop plants of 26 species of families *Asteraceae*, *Rosaceae* and *Lamiaceae* was done. Among the investigated leaf extracts the highest total phenolic, total flavonoid contents and antioxidant activity have been seen for *Stachys byzantine* L. (*Lamiaceae*), *Calendula officinalis* L. (*Asteraceae*) and for *Potentilla recta* L. (*Rosaceae*). The highest syringic acid content has been found in the leaf extracts of plant family *Asteraceae* – in the range from 0.782 to 5.078 mg g^{-1} DW. The representative's family *Rosaceae* has a higher content of p-anisic acid in the range 0.334–3.442 mg g^{-1}DW compared to the leaf extracts of families *Lamiaceae* and *Asteraceae*. The comparative study showed significant differences of content of phenolic acids in the leaf extracts of different representative's families *Rosaceae*, *Asteraceae* and *Lamiaceae*. We suggest that the presence of some phenolic acids can

* Corresponding author at: Plant Physiology and Ecology Department, Taras Shevchenko National University of Kyiv, Institute of Biology, Volodymyrskya Str., 64, Kyiv 01033, Ukraine.

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be used as a possible marker for family botanical specifications of representative families *Asteraceae* and *Rosaceae*. It was supposed that some pharmacological effects can be connected with the analyzed data.

1. Introduction

Polyphenols and flavonoids are the common antioxidant natural products found in medicinal plants. Literature review shows that herbal medicines (especially from large families, *Asteraceae*, *Rosaceae* and *Lamiaceae*) have been used from ancient times as remedies for the treatment of diseases because they contain pharmacological and biological active ingredients (Saeidnia et al., 2005; Hajimehdipoor et al., 2014). Herbs have been used in many domains including medicine, nutrition, flavoring, beverages, dyeing, repellents, fragrances, cosmetics, smoking, and other industrial purposes. Furthermore, the usage of herbal extracts or active compounds (such as chlorogenic acid, ferulic acid, cinnamic, rosmarinic acids) in food, cosmetic and pharmaceutical industries have being increased in the last years, so that the biological and phytochemical study of medicinal plants is essential and an interesting area of research (Gohari et al., 2011; Bonarska-Kujawa et al., 2011; Sytar et al., 2012; Maria John et al., 2015).

Literature data show a correlation between radical scavenging capacities of plant extract families *Asteraceae* and *Lamiaceae* with total phenolic compound content (Miliauskas et al., 2004). Much research work has been done with the screening of different plant extracts for antioxidant capacity and total phenol content (Katalinic et al., 2006; Xu et al., 2014; Abbas et al., 2015). There are a few publications on phenolic content and phenolic acid composition of medicinal plants. The existing data refer usually to one or a few plant species. In addition, screened antioxidant compounds which are responsible for antioxidant activity could be isolated and then used as antioxidants for the prophylaxis and treatment of free radical-related disorders (Middleton et al., 2000; Packer et al., 1999). Therefore, research to identify antioxidative compounds is an important issue. Although it remains unclear which of the compounds of medical plants are the active ones, polyphenols recently have received increasing attention because of some interesting new findings regarding their biological activities. From pharmacological and therapeutic points of view, the antioxidant properties of polyphenols, such as free radical scavenging and inhibition of lipid peroxidation, are the most crucial. Even though a variety of herbs are known to be sources of phenolic compounds, studies on polyphenol composition and evaluating their antioxidative effects have rarely been carried out.

Lavender (*Lavandula angustifolia L.*) is an important source of a thoroughly studied essential oil, while antioxidant properties of this plant are much less documented. Data about antioxidant properties of *Salvia* plants are very scanty. The essential oils of pot marigold (*Calendula officinalis L.*) are used as medicines soothing the central nervous system and exhibiting other useful healing properties. The oil is also rich in carotenoids and used as a dye, as a lubricant and for other purposes (Marvin et al., 2000). Sweet clover (*Melissa officinalis L.*) is applied in the production of some beverages and foods (Ehlers et al., 1997). Honey of *M. officinalis* obtained during the plant flowering period was found to possess quite high antioxidant activity as it distinctly reduced polyphenol oxidase (Lei et al., 2000). Members of the *Rosaceae* family have long been used for food and medicinal purposes. The physiological functions of *Rosaceae* fruits may be partly attributed to their abundance of phenolics. Nowadays there is no available data about the phenolic composition in the leaf extracts of some representative's family *Rosacaeae*. The information on antioxidant compounds content of these plants was not presented well.

Literature data show data of antioxidant capacity and total phenolic content in selected herbs but usually no system on which part of plant was taken for analysis and in this case it is not easy to compare such results (Wojdyło et al., 2007). At the same time much research was done with antioxidant content measurement in whole plants which were usually used in the pharmaceutical industry (Nadeem et al., 2011). Nowadays with developing non-invasive techniques, which may be used in early steps of metabolomics research a special interest to have data regarding antioxidant composition in the leaves as proof or development of non-invasive approaches (Sytar et al., 2015) has increased. Such non-destructive techniques are based on simultaneous measurements of multispectrally-induced chlorophyll fluorescence (hereinafter denoted as multiplex measurements). This technique, though not yet widely used, has become more popular due to the introduction of commercially available devices in the last decade. In our previous experimental paper were published data where multiplex measurements were used for pre-screening flavonoid content in the leaves of plant species belonging to the family *Asteraceae*, *Lamiaceae* and *Rosaceae* (Sytar et al., 2015). Results of this study indicated that leaves of herbal plants belonging to families *Asteraceae*, *Lamiaceae* and *Rosaceae* can be sources of flavonoids, but more detailed biochemical analysis of their flavonoid composition is needed.

Therefore testing of bioactive components composition and antioxidant activity in the leaves of plant species belonging to family *Asteraceae*, *Lamiaceae* and *Rosaceae* is of interest, primarily in order to find new promising sources for natural antioxidants, nutraceuticals and second to use these results in future for developing a non-destructive methodology.

2. Methodology

The plants were located in the Botanical Garden Slovak agricultural university in Nitra. Leaves of medicinal herbs *Rosaceae*, *Asteraceae* and *Lamiaceae* were collected during the flowering period. Each leaf was marked as external, middle or internal considering its position within the plant, according to its length, the degree of development and level of association (Yommi et al., 2013). The longer, greener, and alternated

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leaves were considered as external. The internal leaves are connected with each other, standardly more yellowish and are not fully expanded. The rest of the leaves, with less defined features, were classified as middle. The number of leaves was taken by zone to calculate average for biological replication. For quality evaluation, a petiole section of 15 cm (measured from the node toward the bottom) of each leaf was taken. The antioxidant activity and content of total phenols and phenolic acids have been evaluated in the leaf material. The leaves were harvested and frozen in liquid nitrogen for preventing phenolic compound volatilization and were lyophilized.

2.1. Determination of DPPHÆ radical scavenging capacity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Molyneux, 2004) was utilized with some modifications. The stock reagent solution ($1 * 10^{-3}$ M) was prepared by dissolving 22 mg of DPPH in 50 mL methanol and stored at 20 °C until use.

The weight of samples was 0.02 g. All samples were assayed six times. The extraction was carried out in two steps; firstly, 0.02 g of dry material was placed in the eppendorf tubes and 1 mL of distilled water was added. The samples were heated for 15 min at 95 °C. Then the material was centrifuged for 5 min (12,000 rpm, 25 °C). The extract was replaced in a new tube. The supernatant was filled up again with 1 mL of distilled water and reheated for 10 min at 95 °C, then spun again (12,000 rpm, 25 °C, 5 min). The extract was filled into the new tube. The working solution ($6 * 10^{-5}$ M) was obtained by mixing 6 mL of the stock solution with 100 mL methanol to obtain an absorbance value of 0.8 ± 0.02 at 515 nm, using a spectrophotometer (Jenway 6505 UV/Vis). The different extracts (0.1 mL of each) were allowed to react with 3.9 mL of the DPPH solution and vortexed during 30 s and then the absorbance was measured at 515 nm, at a reaction time of 30 min. A control sample with no added extract was also analyzed and the scavenging percentage was calculated according to the following equation:

$$\text{DPPH scavenging capacity } (\%) = [(\text{A control} - \text{A sample})/\text{A control}] * 100$$

$$\text{A} = \text{absorbance at } 515 \text{ nm}$$

2.2. Determination of total phenolics

Total phenolics were determined by using Folin–Ciocalteu reagent (Singleton and Rossi, 1965). Twenty milligrams of powdered samples (freeze-dried) was extracted for 10 min with 500 μL of 70% methanol (HPLC-Gradient grade, VWR chemicals) at 70 °C. The mixtures were centrifuged at 3500*g* for 10 min and the supernatants were collected in separate tubes. The pellets were re-extracted under identical conditions. Supernatants were combined and used for total phenolics assay and for HPLC analysis. For total phenolics assay 20 μL of extract was dissolved into 2 mL of distilled water. 200 μL of dissolved extract was mixed with 1 mL of Folin–Ciocalteu reagent (previously diluted tenfold with distilled water) and kept at 25 °C for 3–8 min; 0.8 mL of sodium bicarbonate (75 g L^{-1}) solution was added to the mixture. After 60 min at 25 °C, absorbance was measured at 765 nm. The results were expressed as gallic acid equivalents.

2.3. Total flavonoids estimation

0.5 mL of each extract stock solution of 70% methanol, 1.5 mL methanol, 0.1 mL aluminum chloride, 0.1 mL potassium acetate solution and 2.8 mL distilled water were added and mixed well. Sample blank was prepared in similar manner by replacing aluminum chloride with distilled water. Sample and sample blank of all experimental extracts were prepared and their absorbance was measured at 415 nm. All prepared solutions were filtered through a filter paper before measurements. Various concentrations of standard quercetin solution were used to make a standard calibration curve. 10 mg of quercetin was dissolved in methanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100 mg mL^{-1}. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm (λ_{max} of quercetin) with a Shimadzu UV-1800 spectrophotometer.

2.4. Analysis of hydroxycinnamic acid derivatives

Analysis of hydroxycinnamic acid derivatives has been previously developed (Mewis et al., 2010). Samples were taken after finishing the freeze-drying process where the material was ground by a flint mill (20,000 g, 2 min). A total of 20 mg ground samples from leaf suspension were extracted for 15 min using 0.75 mL 70% methanol (v/v, pH 4.0, phosphoric acid) in an ultrasonic water bath on ice. Then samples were centrifuged for 5 min at 6000*g*. The supernatants were collected and the pellets were re-extracted twice more with 0.5 mL of 70% methanol (HPLC-Gradient grade, VWR chemicals). Coumaric acid or cinnamic acid (Sigma–Aldrich Chemie GmbH) (40 μL of 3 mM solution) was added as internal standard to the first extraction. The combined supernatants from each sample were reduced to near dryness in a centrifugation evaporator (Speed Vac., SC 110) at 25 °C.

Samples were added up to 1 mL with 40% acetonitrile (HPLC Ultra Gradient Grade, Roth). The samples were filtrated using 0.22-mm filters, and then analyzed with HPLC. The chromatography was performed using a Dionex UltiMate 3000 HPLC System with a diode array detector (DAD-3000) with a WPS-3000 SL auto sampler, LPG-3400SD pump and a TCC-3000RS Column Compartment (Dionex Corp., Sunnyvale, CA, USA).

Extracts (1 mL) were analyzed at a flow rate of 0.4 mL/min and a column temperature of 35 °C. The column used is Narrow-Bore Acclaim PA C16-column (3 mm, 120A, 2.1 × 150 mm, Dionex). A 49-min gradient program was used with 0.1% v/v phosphoric acid in ultrapure water (eluent A) and 40% v/v acetonitrile in ultra-pure water (eluent B) as follows: 0–5 min: 0.5% B, 5–9 min: 0–40% B, 9–12 min: 40% B, 12–17 min: 40–80% B, 17–20 min: 80% B, 20–24 min: 80–99% B, 24–32 min: 99–100% B, 32–36 min: 100–40% B, 36–49 min: 40–1% B. The gradient program was followed by a 4-min period to return to 0.5% B and a 5-min equilibration period resulting in a total duration of 39 min. Peaks were monitored at 290, 330 and 254 nm respectively. The phenolic acid quantity was calculated from HPLC peak areas at 290 nm. The retention times in the HPLC for the experiments were 12.13 min for vanillic acid, 12.72 min for chlorogenic acid, 13.29 min for caffeic acid, 15.98 min for the internal standard

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p-coumaric acid and 21.59 min for cinnamic acid. For the identification of unknown phenolic compounds, a semi-quantitative analysis was performed using HPLC coupled with mass spectrometric detection (LC/MS) and NMR (Mewis et al., 2010).

2.5. Statistical analysis

Means and standard deviations were calculated by the Microsoft Office Excel 2003. Significant differences of these data were calculated using analysis of variance (ANOVA-Duncan's multiple test (STATISTICA 10, StatSoft, Tulsa, USA). All results were expressed as mean ± standard deviations from replications $n = 6$.

3. Results

In our experimental work among investigated methanolic extracts of leaves of representative family *Lamiaceae Stachys byzantina* K. Koch leaves have been shown to have the highest total phenolic, total flavonoid contents and antioxidant activity (Table 1).

Among experimental extracts of a different representative family *Lamiaceae* the leaf extract of *Coleus blumei* Benth.got the second highest total flavonoids (7.8 mg of QE/mg of extract) and total phenolic content (1.174 mg g^{-1} DW) (Table 2). The methanolic extracts of leaves *Salvia officinalis* L. and *Salvia officinalis cv. purpurea* L. which were collected at beginning of the flowering period have similar content of total flavonoids and total phenolics. There is evidence that no difference exists in contents of investigated antioxidants in the genus *Salvia*. *Mentha suaveolens* Ehrh. leaf extracts have 3 times lower total flavonoid content compared to the leaf extracts of *Stachys byzantine* K.Koch. The content of total phenolics was less 8 times in the leaf extracts of *M. suaveolens* compared to the methanolic extracts of *S. byzantine* K.Koch. The leaf methanolic extracts of *Lavandula officinalis* L., *M. spicata* L., *Rosmarinus officinalis* L. and *M. officinalis* L. have lowest total phenolic and total flavonoid contents and antioxidant activity of these extracts were on the level between 75.12% and 78.95%.

The leaf extracts of *C. officinalis* L. among investigated extracts of the representative family *Asteraceae* have been shown to have the highest total phenolic, total flavonoid contents and antioxidant activity (Table 2). The methanolic extracts of leaves *Rudbeckia fulgida* Aiton and *Achillea filipendulina* Lam. have been characterized by the highest content among the experimental species of the family *Asteraceae* after *C. officinalis* L. leaf extracts. The content of total flavonoid and total phenolics in the leaves were on the same level as in the methanolic extract of *S. officinalis* L. *cv. Purpu* (*Lamiaceae*).

Among representatives of genus *Helianthus,* leaves of *Helianthus annuus* L. got the highest content of flavonoids (2.46 mg QE mg^{-1} DW) and total phenolics (0.928 mg g^{-1} DW). At the same time leaves of methanolic extract of *H. annuus* L. without ap.dom. have a very low total flavonoid content compared to the leaves of *H. annuus* L. The antioxidant activities of *H. annuus* L., *Helianthus tuberosus* L. and *H. annuus* L. without ap.dom were 68.12%, 67.16% and 64.21%, respectively. *Echinacea purpurea* (L.) Moench. is one of the most important medical herbs and in our experiment it was estimated that *Echinacea* leaf extract has the lowest total

Table 1 Total phenolic, total flavonoids contents and antioxidant activity of methanolic extracts of leaves representatives' family *Lamiaceae*.

Plant species	Total flavonoids (mg QE mg^{-1} DW)	Total phenolics (mg g^{-1} DW)	Antioxidant activity (%)
Stachys byzantina K. Koch	11.1 ± 0.003	18.64 ± 0.699	94.56 ± 0.35
Coleus blumei Benth.	7.8 ± 0.004	1.174 ± 0.074	84.03 ± 0.26
Salvia officinalis (L.) *cv.* purpur.	5.12 ± 0.001	1.958 ± 0.153	80.12 ± 0.31
Salvia officinalis L.	5 ± 0.004	2.23 ± 0.270	81.56 ± 0.29
Mentha suaveolens Ehrh.	3.9 ± 0.001	2.25 ± 0.297	78.35 ± 0.15
Lavandula officinalis Mill.	2.2 ± 0.005	0.977 ± 0.153	75.21 ± 0.23
Mentha spicata L.	1.22 ± 0.002	1.786 ± 0.153	77.34 ± 0.25
Rosmarinus officinalis L.	1 ± 0.001	1.713 ± 0.236	78.95 ± 0.34
Melissa officinalis L.	0.2 ± 0.0002	1.688 ± 0.127	75.12 ± 0.19

Table 2 Total phenolic, total flavonoid contents and antioxidant activity of methanolic extracts of leaves of representative family *Asteraceae*.

Plant species	Total flavonoids (mg QE mg^{-1} DW)	Total phenolics (mg g^{-1} DW)	Antioxidant activity (%)
Calendula officinalis L.	6.5 ± 0.004	1.125 ± 0.153	92.56 ± 0.35
Rudbeckia fulgida Aiton	4.42 ± 0.001	1.198 ± 0.112	86.03 ± 0.26
Achillea filipendulina Lam.	4.12 ± 0.003	1.098 ± 0.113	87.02 ± 0.25
Helianthus annuus L.	2.46 ± 0.003	0.928 ± 0.085	78.12 ± 0.31
Helianthus tuberosus L.	1.20 ± 0.004	0.953 ± 0.127	77.16 ± 0.29
Echinops ritro L.	1.24 ± 0.002	0.806 ± 0.001	78.35 ± 0.17
*Helianthus annuus*** L.	0.14 ± 0.001	0.855 ± 0.042	74.21 ± 0.24
Echinacea purpurea (L.) Moench	0.13 ± 0.001	0.928 ± 0.085	73.34 ± 0.26

** Plants lacking apical dominance.

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Table 3 Total phenolic, total flavonoid contents and antioxidant activity of methanolic extracts of leaves representative family *Rosaceae*.

Plant species	Total flavonoids (mg QE mg^{-1} DW)	Total phenolics (mg g^{-1} DW)	Antioxidant activity (%)
Potentilla recta L.	7.24 ± 0.003	1.933 ± 0.625	88.35 ± 0.29
Cerasus mahaleb (L.) Mill.	2.80 ± 0.002	0.928 ± 0.042	79.23 ± 0.34
Rosa canina L.	2.52 ± 0.005	4.09 ± 0.634	78.59 ± 0.38
Rosa rubiginosa L.	2.46 ± 0.002	4.93 ± 0.960	78.12 ± 0.24
Agrimonia eupatoria L.	2.16 ± 0.004	3.13 ± 0.297	76.25 ± 0.26
Alchemilla mollis (Buser) Rothm.	2.12 ± 0.003	3.53 ± 0.584	76.35 ± 0.34
Laurocerasus officinalis L.	1.60 ± 0.004	1.30 ± 0.340	71.56 ± 0.12
Cotoneaster horizontalis Decne.	1.30 ± 0.004	2.35 ± 0.641	72.35 ± 0.24
Potentilla recta L.	0.30 ± 0.002	1.713 ± 0.405	69.23 ± 0.24

flavonoid content and antioxidant activity among investigated plants species of the family *Asteraceae* (Table 2).

Among the investigated leaf extracts of a different representative family *Rosaceae* the methanolic leaf extracts of *Potentilla recta* L. got highest total flavonoid content (Table 3).

Leaf extracts of *Rosa canina* L. and *Rosa rubiginosa* L. got the highest total phenolic contents – 4.09 and 4.93 mg g^{-1} DW, respectively. Leaf extracts of *Agrimonia eupatoria* L. and *Alchemilla mollis* (Buser) Rothm. have the highest total phenolic and total contents compared to the leaf extracts of other representatives of families *Lamiaceae* and *Asteraceae*. Leaf extracts of *A. eupatoria* L. and *A. mollis* Buser) Rothm. have 3.13 and 3.53 mg g^{-1} DW of total phenolic content, respectively (Table 3).

In the leaf extracts of representative's families *Asteraceae*, *Rosaceae* and *Lamiaceae* have been detected next to phenolic acids: 4-Hydroxybenzoic acid, vanillic acid, chlorogenic acid, syringic acid, *o*-coumaric acid, *p*-coumaric acid, ferulic acid, hesperetic acid, *p*-anisic acid, salicylic acid, cinnamic acid, and methoxycinnamic acid (Tables 4a and 4b).

The highest content of 4-Hydroxybenzoic acid has been observed in the *R. canina* L., *R. rubiginosa* L. *(Rosaceae)*, *L. officinalis* L. *(Lamiaceae)* leaf extracts. The high content of vanillic acid has been evaluated in the leaf extracts of *L. officinalis* L., *R. canina* L., *Cotoneaster horizontalis* Decne., *L. officinalis* L., *Echinops ritro* L. – in the range from 0.258 to 0.341 mg g^{-1} DW.

The content of chlorogenic acid in the range 0.187–0.229 mg g^{-1} DW has been identified for extracts of *Cerasus mahaleb (Rosaceae)* and *S. officinalis cv. purpurea (Asteraceae)*. The smallest content of chlorogenic acid was identified in the leaf extracts of three species family *Asteraceae* (Tables 4a and 4b).

4. Discussion

In many scientific papers it has been discussed that antioxidant capacity can be influenced by total phenolic and anthocyanin content, maturity, and a variety of plant species (Prior and Cao, 2000; Kim et al., 2003). The phenolic compounds are the dominant antioxidant components which support strong antioxidant activity and stress response in the many tested plants (Cai et al., 2004; Zheng and Wang, 2001). To utilize these significant sources of natural antioxidants, further characterization of the phenolic composition is needed too (Kähkönen et al., 1999).

4.1. Family Lamiaceae

Numerous members of the *Lamiaceae* family have traditional and medicinal uses and have been used in folk medicine for many years. Most of genera of the *Lamiaceae* are rich sources of terpenoids and they also contain a considerable amount of various iridoid glycosides, flavonoids, and phenolic acids such as rosmarinic acid and other phenolic compounds (Naghibi et al., 2005). The contents of total phenolics, flavonoids and antioxidative capacities in the dried plant materials of these medicinal herbs using wet chemical analyses have been studied (Atanassova et al., 2011). But information on the contents of flavonoids, total phenolics, and phenolic acids in the leaf methanolic extract is not available as also information about the content of some antioxidants in the different plant parts (stems, inflorescences etc.). *M. officinalis* L. leaf extracts got the lowest total flavonoid and total phenolic contents among experimental plants of the family *Lamiaceae*. It was previously reported that aqueous methanolic extract of *M. officinalis* L. caused a considerable concentration-dependent inhibition of lipid peroxidation, and phenolic components present in this plant extract demonstrated antioxidant activity (Hohmann et al., 1999). Ivanova et al., 2005 have found that a*mong* extracts of 21 plants used in phytotherapy *the highest total phenolic content was that of M. officinalis* L. (*Lamiaceae*) (Ivanova et al., 2005). We can suggest that Ivanova et al. (2005) in their research work used all plants for preparing extracts therefore the total phenolic content is higher compared to the total phenolic content of leaves of the methanolic extract.

S. officinalis was used as a reference plant with well documented antioxidant activity for screening radical scavenging activity using DPPH and ABTS assays in the representative families *Lamiaceae* and *Asteraceae*. The content of total phenolic compounds, flavonoids and flavonols was measured in extracts from upper parts of the plant. The content of total phenolics that was next in order was: *S. officinalis L. (*22.6 mg g^{-1} plant extract*), Salvia pratensis L. (9.7 mg g^{-1} plant extract), L. angustifolia* Mill. *(5.4 mg g^{-1} plant extract), C. officinalis L. (6.6 mg g^{-1} plant extract), E. purpurea (L.) Moench (4.1 mg g^{-1} plant extract)* (Miliauskas et al., 2004).

4.2. Family Asteraceae

In our experimental work among investigated methanolic extracts of leaves of the representative family *Asteraceae C. officinalis* L. leaf extracts have been shown to have the highest

Table 4a Phenolic acids and their amounts in methanolic extracts (mg g^{-1} DW).

Plant species	4-Hydroxybenzoic acid	Vanillic acid	Chlorogenic acid	Syringic acid	o-Coumaric acid	p-Coumaric acid
Family Lamiaceae						
Stachys byzantina K. Koch	0.006 ± 0.002	0.113 ± 0.037	0.002 ± 0.0002	2.368 ± 0.311	0.013 ± 0.004	0.006 ± 0.001
Coleus blumei Benth.	0.002 ± 0.0004	0.061 ± 0.025	0.001 ± 0.0002	4.086 ± 0.485	0.097 ± 0.018	0.003 ± 0.001
Salvia officinalis (L.) *cv.* purpur.	0.002 ± 0.000	0.119 ± 0.027	0.229 ± 0.011	0.017 ± 0.002	2.08 ± 0.86	0.007 ± 0.000
Salvia officinalis L.	0.010 ± 0.001	0.163 ± 0.021	0.038 ± 0.007	0.013 ± 0.004	3.243 ± 0.907	0.015 ± 0.005
Mentha suaveolens Ehrh.	0.001 ± 0.000	0.099 ± 0.057	0.034 ± 0.005	0.082 ± 0.017	0.927 ± 0.122	0.007 ± 0.001
Lavandula officinalis Mill.	0.181 ± 0.021	0.319 ± 0.064	0.003 ± 0.001	1.365 ± 0.256	0.081 ± 0.019	3.543 ± 0.067
Mentha spicata L.	0.001 ± 0.000	0.020 ± 0.011	0.034 ± 0.005	0.004 ± 0.001	0.627 ± 0.089	0.023 ± 0.001
Rosmarinus officinalis L.	0.005 ± 0.001	0.025 ± 0.003	0.024 ± 0.002	0.014 ± 0.006	0.523 ± 0.085	0.029 ± 0.000
Melissa officinalis L.	*	0.073 ± 0.036	0.006 ± 0.001	0.008 ± 0.003	0.563 ± 0.074	0.0206 ± 0.005
Stachys byzantina K. Koch	0.001 ± 0.000	0.020 ± 0.011	0.034 ± 0.005	0.004 ± 0.001	0.627 ± 0.089	0.023 ± 0.001
Coleus blumei Benth.	0.005 ± 0.001	0.025 ± 0.003	0.024 ± 0.002	0.014 ± 0.006	0.523 ± 0.085	0.029 ± 0.000
Salvia officinalis (L.) *cv.* purpur.	*	0.073 ± 0.036	0.006 ± 0.001	0.008 ± 0.003	0.563 ± 0.074	0.0206 ± 0.005
Family Asteraceae						
Calendula officinalis L.	0.006 ± 0.001	0.092 ± 0.001	0.004 ± 0.001	3.653 ± 0.712	0.007 ± 0.001	0.011 ± 0.002
Rudbeckia fulgida Aiton	0.001 ± 0.000	0.049 ± 0.014	0.005 ± 0.0003	5.078 ± 0.804	0.013 ± 0.005	0.001 ± 0.000
Achillea filipendulina Lam.	0.003 ± 0.0002	0.041 ± 0.019	0.002 ± 0.0006	3.593 ± 0.780	0.080 ± 0.015	0.001 ± 0.000
Helianthus annuus L.	0.003 ± 0.000	0.025 ± 0.012	0.001 ± 0.0001	*	0.008 ± 0.003	0.006
Helianthus tuberosus L.	0.001 ± 0.000	0.019 ± 0.012	0.012 ± 0.001	1.279 ± 0.345	0.075 ± 0.019	0.011 ± 0.001
Echinops ritro L.	*	0.302 ± 0.074	0.001 ± 0.0005	1.616 ± 0.242	*	0.003 ± 0.001
*Helianthus annuus*** L.	0.011 ± 0.002	0.021 ± 0.008	0.002 ± 0.0007	0.782 ± 0.070	0.001 ± 0.0004	0.001 ± 0.000
Echinacea purpurea (L.) Moench	0.003 ± 0.0004	0.102 ± 0.065	0.033 ± 0.006	2.023 ± 0.075	*	0.003 ± 0.001
Family Rosaceae						
Potentilla recta L.	0.005 ± 0.001	0.027 ± 0.01	0.030 ± 0.004	0.015 ± 0.001	*	0.015 ± 0.005
Cerasus mahaleb (L.) Mill.	0.093 ± 0.015	0.043 ± 0.011	0.187 ± 0.041	0.176 ± 0.027	0.026 ± 0.001	0.526 ± 0.108
Rosa canina L.	0.279 ± 0.017	0.258 ± 0.039	0.032 ± 0.003	0.132 ± 0.000	*	0.078 ± 0.022
Rosa rubiginosa L.	0.262 ± 0.021	0.088 ± 0.011	0.027 ± 0.004	0.033 ± 0.005	0.043 ± 0.004	0.053 ± 0.009
Agrimonia eupatoria L.	0.014 ± 0.002	0.08 ± 0.008	0.001 ± 0.0003	0.012 ± 0.003	0.060 ± 0.015	0.067 ± 0.019
Alchemilla mollis (Buser) Rothm.	0.046 ± 0.009	0.046 ± 0.015	0.006 ± 0.001	0.011 ± 0.001	*	0.050 ± 0.019
Laurocerasus officinalis L.	0.032 ± 0.009	0.275 ± 0.086	0.014 ± 0.000	0.009 ± 0.000	0.001 ± 0.000	0.012 ± 0.004
Cotoneaster horizontalis Decne.	0.023 ± 0.005	0.335 ± 0.025	0.009 ± 0.003	0.003 ± 0.0003	0.003 ± 0.001	0.008 ± 0.002
Potentilla recta L.	0.009 ± 0.001	0.341 ± 0.078	0.008 ± 0.001	0.004 ± 0.001	*	0.024 ± 0.011

* Not determined.
** Plants lacking apical dominance.

total phenolic, total flavonoid contents and antioxidant activity. Butnariu and Coradini, 2012 identified and characterized the full range of phenolic and flavonoid compounds in *C. officinalis* flowers (Butnariu and Coradini, 2012). Total flavonoids ranged between 44.91 and 76.44 mg QE/g DW in leaf and flower extracts of *C. officinalis* (Marigold) growth in Tunisia, respectively (Rigane et al., 2013). Unfortunately as authors did not present information on which vegetation period they collected samples we suggest that results of total flavonoid and total phenolic contents can be different at the beginning and end of the flowering period. The total flavonoid content can depend on cultivars too. Content of total flavonoids in nine varieties of *C. officinalis* leaves was in the range 6.11–15.74 mg g^{-1} dry weight in their leaf ethanolic extracts (Olennikov and Kashchenko, 2014).

Third place regarding flavonoids, total phenolics contents among the investigated representative family *Asteraceae* was given to the leaf extract of *A. filipendulina* Lam. It is known that extracts prepared from *Achillea millefolium* L. flowers, leaves and seeds had effective H_2O_2 radical scavenging activity, total antioxidant activity, and total phenolic content (Keser et al., 2013). It was suggested that quantitative and qualitative differences in total polyphenolic and flavonoid contents between the subspecies of *Achillea distans* Waldst. & Kit. Ex Willd. can be used as a potential taxonomic marker in order to distinguish the species. Luteolin, apigenin, quercetin, caffeic and chlorogenic acids were present in the two extracts of various subspecies of *A. distans* Waldst. & Kit. Ex Willd., but in different amounts (Benedec et al., 2013).

The leaves of *H. annuus* L. among representatives of genus *Helianthus* (*Asteraceae*) have the highest content of total flavonoids (2.46 mg QE mg^{-1} DW) and total phenolics (0.928 mg g^{-1} DW). Literature data about content of total phenolic and total flavonoids for sunflower plants give

Table 4b Phenolic acids and their amounts in methanolic extracts (mg g^{-1} DW).

Plant species	Ferulic acid (hesperetic acid***)	p-Anisic acid	Salicilic acid	Cinnamic acid	Methoxy-cinnamic acid
Family Lamiaceae					
Stachys byzantina K.Koch	0.001 ± 0.000 (0.030 ± 0.006)	0.053 ± 0.004	0.168 ± 0.023	0.045 ± 0.004	0.056 ± 0.025
Coleus blumei Benth.	0.003 ± 0.001 (0.019 ± 0.005)	0.096 ± 0.024	0.105 ± 0.013	0.011 ± 0.002	0.002 ± 0.0006
Salvia officinalis (L.) *cv.* purpur.	0.003 ± 0.001	0.038 ± 0.003	0.008 ± 0.000	0.018 ± 0.003	0.004 ± 0.001
Salvia officinalis L.	0.0103 ± 0.004	0.369 ± 0.056	*	0.009 ± 0.003	0.005 ± 0.001
Mentha suaveolens Ehrh.	0.010 ± 0.001	0.203 ± 0.045	0.112 ± 0.016	0.475 ± 0.055	0.018 ± 0.004
Lavandula officinalis Mill.	3.328 ± 0.769	0.033 ± 0.011	*	*	0.002 ± 0.0003
Mentha spicata L.	0.005 ± 0.001	0.329 ± 0.046	0.004 ± 0.000	0.010 ± 0.002	0.030 ± 0.002
Rosmarinus officinalis L.	0.016 ± 0.006	0.020 ± 0.003	0.122 ± 0.034	0.029 ± 0.005	0.836 ± 0.033
Melissa officinalis L.	0.037 ± 0.021	0.043 ± 0.016	0.008 ± 0.002	0.006 ± 0.002	0.143 ± 0.025
Family Asteraceae					
Calendula officinalis L.	0.003 ± 0.000 (0.076 ± 0.009)	0.011 ± 0.001	0.069 ± 0.012	0.003 ± 0.001	0.001 ± 0.0002
Rudbeckia fulgida Aiton	*	1.120 ± 0.110	0.006 ± 0.001	0.004 ± 0.001	*
Achillea filipendulina Lam.	0.003 ± 0.000	0.021 ± 0.000	0.010 ± 0.002	*	0.023 ± 0.004
Helianthus annuus L.	*	0.076 ± 0.012	*	0.003 ± 0.00	*
Helianthus tuberosus L.	0.069 ± 0.006	0.002 ± 0.000	0.002 ± 0.000	0.007 ± 0.001	0.004 ± 0.0004
Echinops ritro L.	0.001 ± 0.000	0.586 ± 0.088	0.007 ± 0.000	0.003 ± 0.0003	0.001 ± 0.0001
*Helianthus annuus*** L.	*	0.033 ± 0.005	*	0.008 ± 0.002	*
Echinacea purpurea (L.) Moench	0.010 ± 0.002	0.358 ± 0.047	0.117 ± 0.024	0.043 ± 0.003	*
Family Rosaceae					
Potentilla recta L.	0.342 ± 0.049	0.364 ± 0.135	0.047 ± 0.013	0.215 ± 0.014	0.011 ± 0.003
Cerasus mahaleb (L.) Mill.	0.005 ± 0.000	0.049 ± 0.017	0.018 ± 0.006	0.006 ± 0.000	*
Rosa canina L.	0.006 ± 0.000	3.442 ± 0.397	1.526 ± 0.164	0.148 ± 0.032	0.546 ± 0.086
Rosa rubiginosa L.	0.056 ± 0.006	1.042 ± 0.092	0.033 ± 0.008	0.148 ± 0.015	0.022 ± 0.0006
Agrimonia eupatoria L.	0.064 ± 0.018	0.279 ± 0.049	1.673 ± 0.288	0.024 ± 0.005	0.041 ± 0.008
Alchemilla mollis (Buser) Rothm.	0.046 ± 0.008	0.334 ± 0.073	0.023 ± 0.003	0.063 ± 0.013	0.028 ± 0.005
Laurocerasus officinalis L.	0.002 ± 0.001	0.013 ± 0.003	0.026 ± 0.002	0.008 ± 0.001	0.001 ± 0.0005
Cotoneaster horizontalis Decne.	0.004 ± 0.001	1.541 ± 0.329	0.209 ± 0.029	0.005 ± 0.001	0.030 ± 0.006
Eriobotrya japonica	0.007 ± 0.001	0.410 ± 0.134	0.065 ± 0.005	0.003 ± 0.001	0.006 ± 0.0006

* Not determined.
** Plants lacking apical dominance.
*** In some species shown also values of hesperedic acid (in brackets).

information about their content in the seeds or kernels (Nadeem et al., 2011; Žilić et al., 2010). It was found that sunflower sprouts are rich in phenolic compounds and with germination increased the total phenolic and flavonoid levels, as well as the antioxidant activity of the seeds (Pająk et al., 2014).

E. purpurea L. is one of the most important medical herbs and is a kind of *Asteraceae* native to and perennially grown in North America, which is used pharmacologically and for esthetic enjoyment. In our research *E. purpurea* L. leaf extract showed the lowest total flavonoid content and antioxidant activity among investigated plants species of the family *Asteraceae*. Normally for the estimation of antioxidant content in *E. purpurea* (L.) Moench used harvested whole plants and information regarding flavonoid content in the leaves which can be used for monitoring and pre-screening did not exist. The total phenolic content for whole plants was 22.3 mg of GAE/g and total flavonoid content was 86.0 mg of QE equivalent/g (Lee et al., 2010). Wojdyło et al., 2007 has estimated the total phenolic content in the leaf extract of *E. purpurea* (L.) Moench which was similar with results of our experimental analysis – 15.15 mg of GAE/100 g of DW (Wojdyło et al., 2007).

4.3. Family Rosaceae

Methanolic leaf extracts of *P. recta* L. got the highest total flavonoid content among investigated leaf extracts of different representative's family *Rosaceae*. Extracts from the aerial and/or underground parts of *P. recta* L. have been applied in traditional medicine and exhibit antioxidant, hypoglycemic, anti-inflammatory, antitumor and anti-ulcerogenic properties. To develop a new methodology for pre-screening some antioxidants with the aim to control changes of antioxidants during the vegetation period it is also important to know the content of total phenolics and flavonoids in the leaf extracts of *P. recta* L. The highest content of identified phenolic compounds (hyperoside, (+)-catechin, caffeic acid, ferulic acid, rutin and ellagic acid) was observed in the whole plant of *Potentilla parvifolia* Fisch. ex Lehm. (14.17 mg g^{-1}), followed by

P Potentilla fruticosa (L.) Rydb. (10.01 mg g^{-1}) and *Potentilla glabra* hort. Lodd. (7.01 mg g^{-1}). *The* whole plant extracts of *P.fruticosa* possessed the highest content of total phenolic and total flavonoids which were correlated with antioxidant activity parameters (Wang et al., 2013), similar to our results with leaf extracts of *P. recta* L.

Leaf extracts of *R. canina* L. and *R. rubiginosa* L. got the highest total phenolic content – 4.09 and 4.93 mg g^{-1} DW, respectively (Table 3). Literature data present biochemical characteristics of fruit genus *Rosa*. Eight Rose hip fruit species were compared taking into consideration the ascorbic acid, total polyphenols, total flavonoid contents and their antioxidant activity. The total polyphenol content varied from 575 mg/100 g frozen pulp (var. *transitoria* f. *ramosissima*) to 326 mg/100 g frozen pulp (var. *lutetiana* f. *fallens*). The total flavonoid content showed the highest value for var. *assiensis* variant 163.3 mg/100 g frozen pulp and the lowest value was attributed to var. *transitoria* f. *montivaga* 101.3 mg/100 g frozen pulp. The most important substances are acids, phenolic compounds such as tannin. Fruits are famous for high vitamin C and antioxidant properties (Roman et al., 2013; Aptin et al., 2013). At the same time based on the studies conducted by Nowak and Gawlik-Dzikib (2007) may assume that the extracts of rose leaves are a rich source of natural antioxidants and could be used to prevent free-radical-induced deleterious effects. Remarkably high antioxidant activity of *R. canina* L. and *R. rubiginosa* L. leaf extracts has been found to be 95.7% and 92%, respectively. The total phenolic content in the % of dry weight was for *R. canina* L. leaf extract 13.9% and for *R. rubiginosa* L. leaf extract 10.8%, respectively. The quercetin content in extracts of rose leaves ranging from 3.68 to 15.81 mg g^{-1} DW and kaempferol content from 1.25 to 9.41 mg g^{-1} DW was found in rose leaves (Nowak and Gawlik-Dzikib, 2007).

Leaf extracts of *A. eupatoria* L. and *A. mollis* (Buser) Rothm. have the high total phenolic and total contents compared to the leaf extracts of other representatives of family *Lamiaceae* and *Asteraceae*. *C. horizontalis* Decne. and *Eriobotrya japonica* (Thunb.) Lindl. got higher total phenolic content (2.35 and 1.71 mg g^{-1} DW, respectively) compared to the total flavonoid content on the level of experimental leaf extract families *Lamiaceae* and *Asteraceae* (Tables 1–3). The average contents of flavonoids and total phenolics of loquat flower ethanol extracts of five cultivars *E. japonica* (Thunb.) Lindl. were 1.59 ± 0.24 and 7.86 ± 0.87 mg g^{-1} DW, respectively (Zhou et al., 2011). Quantitative determination of the total polyphenols and flavonoids of aerial parts of *C. horizontalis* Decne family *Rosaceae* was performed colorimetrically using Folin–Ciocalteu and aluminum tri-chloride methods respectively and the concentration of total polyphenols 14 mg g^{-1} for plant extract GAE was determined, while the concentrations of flavonoid and flavonol contents expressed as rutin equivalents were 6.8 and 2.2 mg g^{-1} for plant extracts respectively (Shaza et al., 2012).

It has been shown that the antioxidant activity of *A. eupatoria* L. and *A. mollis* (Buser) Rothm. is associated with high polyphenolic content (Ivanova et al., 2005; Trendafilova et al., 2011). The different ways of leaf extraction have been shown different contents of total phenolics in the leaf extract of *A. eupatoria* L. For example leaf infusion extract and leaf boiling extracts have values of 0.117 and 0.242 g L^{-1} gallic acid equivalents of total phenolic which were some of the highest among experimental extracts of 48 different medical plants (Gião et al., 2007).

4.4. Phenolic acids composition

Chlorogenic acid is a hydroxycinnamic acid, a member of a family of naturally occurring esters of polyphenolic caffeic acid and cyclitol (−)-quinic acid. It is an important biosynthetic intermediate (Wout et al., 2003) and phenolic acid which was used in medicine and the food industry. The chlorogenic acid content strongly correlated with total phenols in sunflower extracts (*Asteraceae*). Other marked phenolics of all sunflower hybrids were caffeic acid, ferulic acid, rosmarinic acid, myricetin and rutin. All these nutrients with antioxidant properties influenced the capacity of $DPPH^{\cdot}$ scavenging. Accordingly, sunflower kernels had a higher $DPPH^{\cdot}$ scavenging activity, and a higher nutritive value than sunflower seeds (Žilić et al., 2010).

The highest syringic acid content has been found in the leaf extracts of plant family *Asteraceae* – in the range from 0.782 to 5.078 mg g^{-1} DW compared to the species of families *Lamiaceae* and *Rosaceae*. Leaf extract of *R fulgida* Aiton (*Asteraceae*) has the highest content (5.078 mg g^{-1} DW) among all experimental extracts of families *Asteraceae*, *Lamiaceae* and *Rosaceae*. The leaf extract of *C. blumei* Benth. (*Lamiaceae*) has the second highest content (4.086 mg g^{-1} DW) among experimental leaf extracts. *S. byzantine* K. Koch (*Lamiaceae*) leaf extracts also have been characterized by the high content of syringic acid (2.368 mg g^{-1} DW). In the leaf extracts of the representative family *Rosaceae* has been identified as syringic acid but in a small quantity compared to the leaf extracts of the family *Asteraceae*. In the methanolic extracts of dried aerial parts of 16 species of *Helichrysum* (*Asteraceae*) have been identified syringic acid. To compare with our results the *H. plicatum Mill.* subsp. content of syringic acid was 4.31 mg g^{-1} DW and *Helichrysum graveolens* −*2.87* mg g^{-1} DW. At the same time among 16 *Helichrysum* (*Asteraceae*) species the content of syringic acid has been varied (Albayrak et al., 2010). Syringic acid, vanillic acid, caffeic acid, chlorogenic acid, protocatechuic acid and *p*-coumaric acid were identified in the three extracts (heptane, ethyl acetate and methanol) of *C. officinalis L.* (*Asteraceae*) (Matysik et al., 2005).

o-Coumaric acid is a hydroxycinnamic acid, an organic compound that is a hydroxy derivative of cinnamic acid. There are three isomers of coumaric acids *o*-coumaric acid, *m*-coumaric acid, and *p*-coumaric acid- that differ by the position of the hydroxy substitution of the phenyl group. In the experimental leaf extract of plant species families *Asteraceae*, *Rosaceae*, *Lamiaceae* were identified *o*-coumaric and *p*-coumaric acids (Table 4a). The highest content of *o*-coumaric acid has been identified in the leaf extracts of *S. officinalis* L. (3.243 mg g^{-1} DW) and *S. officinalis purpurascens* L. (2.080 mg g^{-1} DW). In the methanolic extracts of the aerial parts of *Salvia halophila o*-coumaric acid has been identified in a concentration of 63.7 mg kg^{-1} and 107.8 mg kg^{-1} for the extract of *Salvia virgate (Akkol et al., 2008)*. The highest content of *p*-coumaric acid among investigated plant species families *Asteraceae*, *Lamiaceae*, *Rosaceae* has been found in the leaf extract of *L. officinalis* L. (3.543 mg g^{-1} DW). At the same time Zgórka and Głowniak, 2001 have observed the content of *p*-coumaric acid in the flowers of *L. officinalis* near

200 μg g^{-1} DW. For four plant organs (*L. officinalis* L. flowers, the herbs of *T. vulgaris* L. and *Hyssopus officinalis* L. and *R. officinalis* L. leaves), the concentration levels of this compound ranged from 100 to 200 mg g^{-1} DW, whereas for *Satureja hortensis* L. herb the content was above 2000 μg/g (0.2%) dry weight (Zgórka and Głowniak, 2001).

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a representative of the hydroxycinnamate group. Ferulic acid, a ubiquitous natural phenolic phytochemical is present in seeds and leaves, both in its free form and covalently conjugated to the plant cell wall polysaccharides, glycoproteins, polyamines, lignin and hydroxy fatty acids (Kumar and Pruthi, 2014). Ferulic acid exhibits a wide variety of biological activities such as antioxidant, antiinflammatory, antimicrobial, antiallergic, hepatoprotective, and anticarcinogenic activities (Kroon and Williamson, 1999). Leaf extract of *L. officinalis* L. (*Lamiaceae*) got the highest ferulic acid content (3.328 mg g^{-1} DW) among the investigated species. Major phenolic acids identified in the analyzed medicinal plants including oregano (*Origanum vulgare* L.), lavender (*L. angustifolia* L.) and lemon balm (*M. officinalis* L.) were ferulic, rosmarinic, *p*-coumaric and caffeic acid (Spiridon et al., 2011).

p-Anisic acid, also known as 4-methoxybenzoic acid or draconic acid, is one of the isomers of anisic acid. *p*-Anisic acid has antiseptic properties (Friedman et al., 2003; Bhimba et al., 2010). It is also used as an intermediate in the preparation of more complex organic compounds. In all leaf extracts of species *Asteraceae*, *Lamiaceae* and *Rosaceae* was identified *p*-anisic acid but the contents were different (Table 4b). For example the representative family *Rosaceae* has a higher content of *p*-anisic acid in the range 0.334–3.442 mg g^{-1} DW compared to the leaf extracts of families *Lamiaceae* and *Asteraceae*. The leaf extract of *R. canina* L. got 3.442 mg g^{-1} DW of *p*-anisic acid content and leaf extract of *R. rubiginosa* L. −1.042 mg g^{-1} DW respectively. The leaf extract of *C. horizontalis* Decne. has 1.541 mg g^{-1} DW of *p*-anisic acid. The *R. fulgida* Aiton leaf extract of family *Asteraceae* has been characterized by high *p*-anisic acid content (1.120 mg g^{-1} DW) and also the highest syringic acid content. β-resorcylic acid, *p*-coumaric acid, caffeic acid, 5-O-(E)-caffeoylquinic acid and 5-O-(E)-pcoumaroylquinic acid methyl ester were found in the MeOH-soluble flower extracts of *Rudbeckia hirta* (Michaela et al., 2014). Some of these metabolites were isolated for the first time from the genus *Rudbeckia*. Syringic acid which was identified in the leaf extracts of *R. fulgida* Aiton was also found in the açaí palm (*Euterpe oleracea* Mart.) and oil palm (Pacheco-Palencia et al., 2008). It was studied that accumulation of phenolic acids, especially syringic acid, may prove a useful trait in breeding resistant oil palm cultivars to the *Ganoderma boninense* Pat. (Chong et al., 2012). Syringic acid is a naturally occurring *O*-methylated trihydroxybenzoic acid which can be enzymatically polymerized and can change rhizosphere bacterial and fungal community structures (Zhou et al., 2014).

The leaf extracts of *R. canina* L. and *R. rubiginosa* L. showed high contents of salicylic and cinnamic acids (Table 4b). The leaf extract of *A. eupatoria* L. is also characterized by the highest salicylic acid content. At the same time for experimental species *Salvia*, *L. officinalis* L. *and Helianthus* sp. salicylic acid was not detected. In recent years salicylic acid has been the focus of intensive research due to its function as an endogenous signal mediating local and systemic plant defense responses against pathogens. It has also been found that salicylic acid plays a role during the plant response to abiotic stresses such as drought, chilling, heavy metal toxicity, heat, and osmotic stress (Rivas-San Vicente and Plasencia, 2011).

In the leaf extracts of *M. suaveolens* Ehrh. (*Lamiaceae*) and *P. recta* L. (*Rosaceae*) have been found the highest content of cinnamic acid and also high contents of chlorogenic acid which can depend on the content of cinnamate. Cinnamic acid has low toxicity and in the search for novel pharmacologically active compounds, cinnamic acid derivatives are important and promising compounds with high potential for development into drugs (Sova, 2012). A major use of cinnamic acid is in the manufacturing of the methyl, ethyl, and benzyl esters for the perfume industry too (Budavari, 1996). The major route of synthesis of chlorogenic acid from cinnamate is shown to be: cinnamic acid → *p*-coumaric acid → *p*-coumaroylquinic acid → chlorogenic acid, and the secondary route cinnamic acid → *p*-coumaric acid → caffeic acid → chlorogenic acid (Steck, 1968).

R. canina L. extract showed the high methoxycinnamic content (0.546 mg g^{-1} DW). But the leaf extract of *R. officinalis* L. showed 0.836 mg g^{-1}DW of methoxycinnamic content. Gallic acid, catechin, procyanidin-B2 and hydroxycinnamic acid derivatives (chlorogenic, *t*-caffeic, *p*-coumaric, ferulic and sinapic acids) were principal for all rose hip species (*R. canina L.*, *R. dumalis L.*, *R. gallica L.*, *Rosa dumalis* subsp.*boissieri* and *R. hirtissima* Lonacz.) (Demir et al., 2014) and presented information about phenolic acid composition in the leaf extract of *Rose* species.

5. Conclusions

We have determined the phenolic, flavonoid profile, phenolic acid composition and the antioxidant activities for 26 leaf extracts of plant species families *Rosaceae*, *Lamiaceae* and *Asteraceae* and we have completed the literature data with new information concerning the polyphenolic compounds and their bioactivity. The simultaneous determination of a wide range of phenolic acids was performed using a rapid, highly accurate and sensitive HPLC method for detection and identified next phenolic acids: 4-Hydroxybenzoic acid, vanillic acid, chlorogenic acid, syringic acid, *o*-coumaric acid, *p*-coumaric acid, ferulic acid, hesperetic acid, *p*-anisic acid, salicylic acid, cinnamic acid, and methoxycinamic acid. The comparative study showed differences of content of phenolic acids in the leaf extracts of different representative families *Rosaceae*, *Asteraceae* and *Lamiaceae*. This study suggests that leaf extracts of *R. fulgida*, *C. officinalis*, *E. purpurea (Asteraceae)*, *R. canina*, *R. rubiginosa (Rosaceae)*, *S. officinalis*, *S. officinalis cv. Purpurea*, *L. officinalis*, (*Lamiaceae*) can be the source of some phenolic acids. The highest syringic acid content has been found in the leaf extracts of plant family *Asteraceae* – in the range from 0.782 to 5.078 mg g^{-1} DW. The representative family *Rosaceae* has a higher content of *p*-anisic acid in the range 0.334–3.442 mg g^{-1} DW compared to the leaf extracts of families *Lamiaceae* and *Asteraceae*. We suggest that presence of some phenolic acids can be used as possible markers for the families *Asteraceae* and *Rosaceae*.

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