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Research Article

Phenolic Acid Patterns in Different Plant Species of Families Asteraceae and Lamiaceae: Possible Phylogenetic Relationships and Potential Molecular Markers

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Nowadays, investigations of some specific secondary metabolites estimated near 10,000 various compounds of phenolic nature in different plant species. The interest in natural compounds is not only due to their antioxidant potential, but also to their economic impact, as most of them may be extracted from underexploited plant species. The presented research work presents an extended analysis of the most important phenolic acids of the selected known and underexploited plant species from the families *Asteraceae* and *Rosaceae* with the development of phylogenic tree analysis according to the nonparametric rate smoothing (NPRS) methods. HPLC-UV analysis revealed the original spectrum of phenolic acids in selected known and underexploited plant species of the families *Rosaceae* and *Asteraceae*. The analysis of phenolic acid's contribution from their total amount in the methanolic extract in *Asteraceae* found the high percentage of syringic acid in leaves varied between 64.13% and 95.13%. The detected high contribution of syringic acid among estimated phenolic acids in *Asteraceae* leaves suggests its possible prevalence in the representatives of the family *Asteraceae*. The content of draconic acid in the leaves of most representatives of the family *Rosaceae* which represented more than 30% of total phenolic acid content. The high presence of such phenolic acids may relate to the antioxidant activity of the studied herbal extracts.

1. Introduction

Nowadays, studies analyzing the role of some specific secondary metabolites of the phenolic group identified near 10,000 various compounds of phenolic nature in different plant species. It is known that common transitional secondary metabolites for phenolic compounds is phenylalanine or a precursor of phenylalanine-shikimic acid. Both compounds are presented mostly in conjugated forms and have one or more sugar residues connected to the hydroxyl groups. It is also known their possible connection with other compounds, like amines, lipids, carboxylic and organic acids, and other phenolic plant hormones, which develop huge phenolics biodiversity in the various plant varieties [1–3].

Several phenolic classes can be esteemed regarding to the quantity of phenol rings and specification of structural elements which also unite these rings [4]. The structural characteristics of the compounds of the most important groups of phenolic nature such as coumarins, tannins, flavonoids, and lignans are established on the existence in a core monocyclic carbon skeleton of phenolic and benzoic functional groups. The phenolic compounds have been characterized by high diversity and various ways of their

biosynthesis. Many secondary metbaolites of phenolic nature have a different structure but may have similar biological activities [5, 6].

Phenolic acids are a subgroup of phenolic compounds broadly presented in hydroxybenzoic and hydroxycinnamic acids with antioxidant capacity [7]. It is known about positive correlation between the content of phenolic compounds in plant extracts of various plants and their antioxidant potential [8–10]. Singh et al. has been discovered that gentisic acid among phenolic acids got the most higher antioxidant activity. The gentisic acid showed highest antioxidant activity. Gallic and caffeic acids got second and third places, respectively, regarding the level of antioxidant activity [11]. The salicylic acid is an important phenolic acid which participate in the responses to different abiotic stresses [12].

Plant phenolic compounds biodiversity is visible at variation of genetic lines within and between species [13, 14]. Different phenolics are intensively studied in various chemo-systematic learning with botanical, plant physiology orientation [15]. For example, phenolic compounds can be biochemical markers for identifying base species of plants in Ethiopia [16]. A taxonomic marker of *Ericaceous* species/genera (family: *Ericaceae*) is flavonol gossypetin [17]. The absence of some phenolic acids and specific phenolic compounds also can be a marker of some genus of the plants. It was typical absence of ellagic acid for the genus *Pittosporum* (*Pittosporaceae*) [18]. The connection between diversity of phenolics of bearberry (*Arctostaphylos uva-ursi, Ericaceae*) species and the lines with cytogenetic and genetic background was shown [19]. It was studied that phenolic acid derivatives may act as chemotaxonomic markers in the *Cardiocrinum* species leaves (*Liliaceae* family) [20]. Exploring the antioxidant activity of phenolic acids in a wide range of herbs can be used for further elucidation of their prospective healthful capacities in the biomembranes against oxidative stress and in chemotaxonomy studies of the genus and families [21–23].

Most of the phenolics compounds analysis, which were previously reported, are HPLC-UV qualitative analysis and less mass-spectrometry analysis. It also used the Folin–Ciocalteu method to estimate the total phenolics content of the plant extract together with the estimation of antioxidant activity [24]. It is known using common herbs and crops in human pharmaceutical and food, especially *Calendula officinalis*, *Rudbeckia* sp., *Echinacea purpurea* (*Asteraceae*), *Rosa canina*, *Rosa rubiginosa*, *Alchemilla mollis*, and *Eriobotrya japonica* (*Lamiaceae*) which have shown antioxidant properties [24–31]. The interest in antioxidant natural components is not only due to their biological capacity, but also to their economic impact, as most of them may be extracted from underexploited plant species. At the same time, analysis of the relationships among species in the plant genus or family based on the phenolic acid composition may bring new knowledge. Previously such analysis was done on complete chloroplast genomes, rbcL, and matK chloroplast genes, electrochemical fingerprints showed a series of oxidation peaks of flavonols, phenolic acids, procyanidins, alkaloids, and pigments in the plant tissue [32–34]. The proposed study presents an original phylogenetic tree analysis according to the NPRS methods based on the identified phenolic acids by high-performance liquid chromatography (HPLC) analysis of the selected known and underexploited plant genotypes from the families *Asteraceae* and *Rosaceae*.

2. Materials and Methods

2.1. Plant Object. The herbs and plants (representatives *Asteraceae* and *Rosaceae* families) at the flowering stage were taken in the area of the Botanical Park, Nitra, Slovak republic. Plant species of the family *Asteraceae*: *Calendula officinalis* L., *Achillea filipendulina* Lam., *Helianthus annuus* L., *Helianthus tuberosus* L., *Echinops ritro* L., *Echinacea purpurea* L., and *Rudbeckia fulgida* L. Plant species of the family *Rosaceae*: *Potentilla recta* L., *Rosa canina* L., *Rosa rubiginosa* L., *Cotoneaster horizontali* Decne., *Agrimonia eupatoria* L., *Alchemilla mollis (Buser)* Rothm., and *Eriobotrya japonica* L. A 15 cm of a petiole section of each leaf was taken for quality estimation. It was determined from the node toward the bottom. After collection, leaves were stored in liquid nitrogen to avoid the volatilization of biological compounds and lyophilized.

2.2. Estimation of the Antioxidant Activity. Estimation of the antioxidant activity (DPPH analysis) was done regarding Singleton and Rossi, 1965 [35]. 1 mL of distilled water was added to 0.02 g of the plant sample material in the Eppendorf tube. Then, the mixed samples (leaf lyophilized powder) during 15 min were heated at 95°C and centrifuged for 5 min at 12,000 rpm. The extract was transferred to a new tube and the same procedure was repeated. The supernatant from the two step procedure was collected to a new tube. 3.9 mL of the DPPH working solution was mixed with 0.1 mL of the experimental supernatant, shaking for 30 s and placed for further reaction time during 30 min. The absorbance was estimated with a Jenway UV/Vis 6405 spectrophotometer (Jenway, Chelmsford, UK) at 515 nm. The next formula was used for the antioxidant activity calculation: %Inhibition = $[(A_0—A_1)/(A_0)]^*100$. A_0 was control reaction absorbance and A_1 was the sample's presence absorbance.

2.2.1. Phenolic Acid Assay. Phenolic acid assays has been advanced by Cai et al. with some adaptations [36]. 0.75 mL of 70% methanol was mixed with 0.02 g of plant material samples. The mixture was placed for 15 min with additional centrifugation during 5 min at 6000 g. The extraction was done one more time with 70% methanol (0.5 mL) with further collection of supernatants. The final supernatant was diminished to nearby dryness (at 25°C) in a Speed Vac evaporator. As an internal standard were used 40 μL of 3 mM solution of coumaric acid or cinnamic acid (Sigma–Aldrich Chemie GmbH). The samples for HPLC analysis with added 1 mL of 40% acetonitrile were filtrated using Millex-GP filter (0.22 μm). The Dionex UltiMate 3000 HPLC system with a diode array detector (DAD-3000) was used for HPLC analysis (Dionex Corp., Sunnyvale, CA, USA).

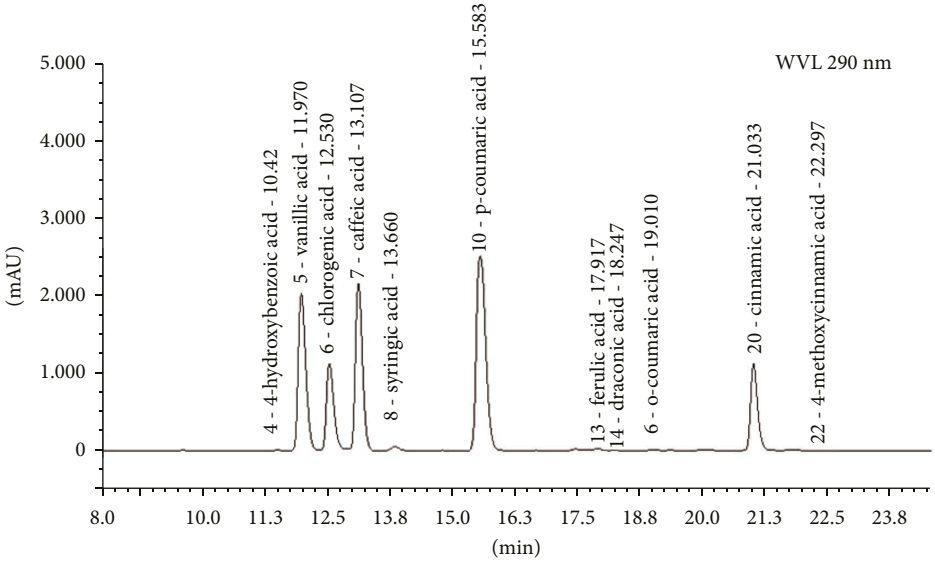

Figure 1: HPLC-UV standards chromatogram at 290 nm.

An initial injection volume was 40 μl at a flow rate of 0.4 mL/min. A temperature of the column (Narrow-Bore Acclaim PA C16-column (3 mm, 120A, 2.1 × 150 mm, Dionex) was 35°C. The eluent A (0.1% v/v phosphoric acid in ultrapure water) and eluent B (40% v/v acetonitrile in ultrapure water) were parts of 49-min gradient program with next steps: 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, and 36–49 min: 40-1% B. Screening of peaks was done at 290 nm. HPLC-UV standards chromatogram and UV spectrum of phenolic acids at 290 nm was used for calculation of phenolic acids quantity (mg·g^{-1} DW) (Figures 1 and 2).

2.3. Statistical Analysis. Microsoft Office Excel 2003 program was applied for the estimation of average and standard deviations. The hierarchical cluster analysis of the phenolic acid contents was done using Euclidean coefficient and WARD methods. Statistical replication of the experimental samples was 6 times.

3. Results

The HPLC-UV analysis of experimental extracts of species of the family *Asteraceae* identified the phenolic acids such as syringic acid, draconic (*p*-anisic) acid, *p*-hydroxybenzoic acid, chlorogenic acid, vanillic acid, o-coumaric acid, *p*-coumaric acid, ferulic acid, salicylic acid, cinnamic acid, and *p*-methoxycinnamic acid (Figure 3). The content of phenolic acids depends on the variety and/or the place and time of cultivation [37]. The phenolic acids as cinnamic acid, o-coumaric acid, *p*-coumaric acid, caffeic acid, sinapic acid, and ferulic acid have a phenylalanine as metabolic precursor. The phenolic acids derivatives of benzoic acid are syringic acid, *p*-hydroxybenzoic, and vanillic acids. In general, benzoic acid derivatives are reached from analogous cinnamic acid derivatives by the way of the β-oxidation enzymatic reactions. That metabolic pathway can vary between representatives of plant families and inside the group. The syringic acid among identified phenolic acids in plant species of the family *Asteraceae* had a greatest level. The syringic acid antioxidant potential is well known [38]. A significant increase of superoxide dismutase (SOD), catalase (CAT) enzymes, and glutathione (GSH) levels was observed under additional treatment with syringic acid [39].

The percentage calculation of phenolic acid amounts in the extracts of representatives of the family *Asteraceae* found high presence of syringic acid in the experimental extracts of *Asteraceae* species varied between 64.13% and 95.13% (Figure 3). Such a significant presence in the extracts of the species of the *Asteraceae* family compared to the total content of identified phenolic acids can be a possible biochemical marker of the representatives of this plant family.

The analysis of results of phenolic acid composition in the representatives of the family *Rosaceae* found a high presence of draconic (*p*-anisic) acid in the assessed leaf extracts (Figure 4).

Moreover, the HPLC-UV analysis identified in this botanical family the following phenolic acids: draconic, chlorogenic, o-coumaric, *p*-coumaric, *p*-hydroxybenzoic, vanillic, syringic, ferulic, salicylic, *p*-methoxycinnamic, and cinnamic acids. From this group, o-coumaric acid was not estimated in the leaf methanolic extracts of *Potentilla recta* L., *Rosa canina*, *Alchemilla mollis*, and *Eriobotrya japonica*. It was found that high presence of draconic acid can be a possible biochemical marker of plant species of the family *Rosaceae* (Figure 4).

The great content of the syringic acid in the representatives of the family *Asteraceae* and draconic acid in the representatives of the family *Rosaceae* can be partly connected with the studied antioxidant activity of plant extracts (Figures 5(a) and 5(b)). The highest antioxidant capacity of

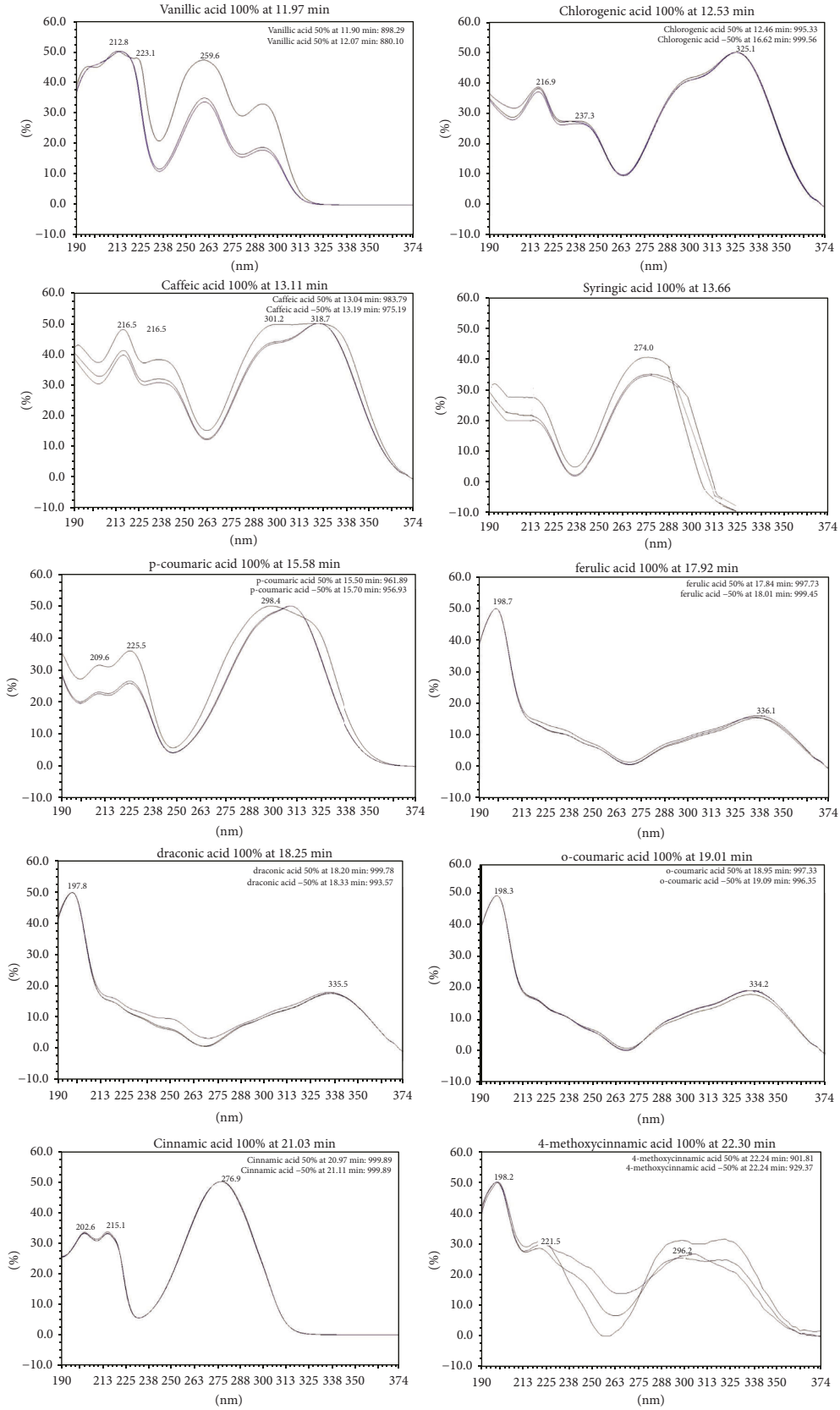

Figure 2: Phenolic acids UV spectrum at 290 nm.

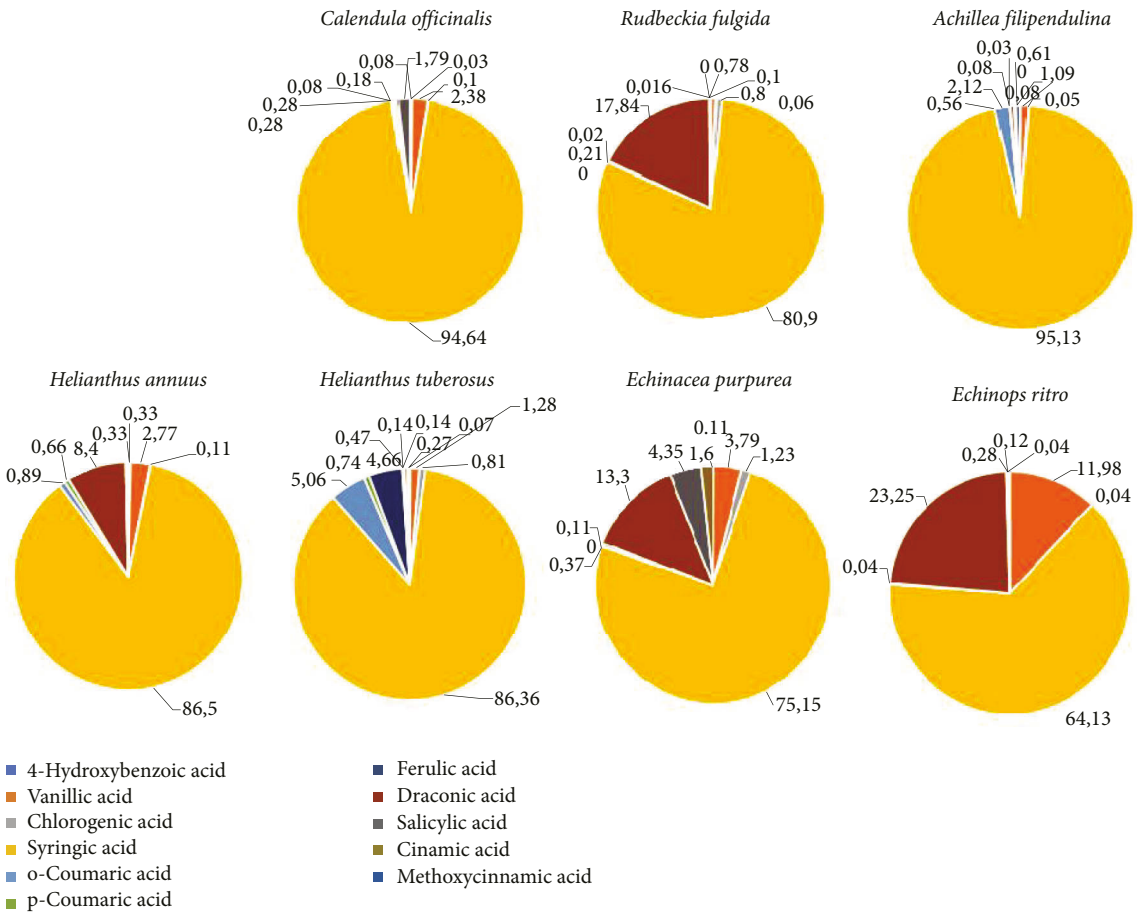

Figure 3: Percentage of phenolic acids amounts in the methanolic extract of representatives of the family *Asteraceae* (%).

experimental extracts was noticed in the extracts of *Potentilla recta* L., *Rosa canina*, and *Rosa rubiginosa*, where the percentage of draconic acid was 33.99%, 53.39%, and 57.76%, respectively. The low antioxidant activity among representatives of the family *Rosaceae* was identified in *Eribotrya japonica* L. Among representatives of the family *Asteraceae*, the significant high antioxidant potential was noticed in the extract of *Calendula officinalis* L, *Rudbeckia fulgida Aiton*, and *Achillea filipendulina* L.

At the same time, to detail the presence of phenolic acids in connection with analyzed plant species, the cluster analysis dendrogram (Figure 6) was created based on the analysis of clusters using the Euclidean coefficient and WARD method. The analogical analysis formed by the obtained data of phenolics composition and content in plant cultivars with a comparable phenolics concentration in another plant cultivars was previously used by Krochmal–Marczak et al. [37]. The cluster analysis group objects based on the data describing the objects and their relationships. The objects within a group are similar (or related) to one another but different or unrelated from the objects in other groups.

Cluster 1 in Figure 6(a) (family *Asteraceae*) contains data describing the similar phenolic acids composition in *Calendula officinalis* and *Achillea filipendulina* leaves; cluster 2 contains the similar phenolic acids composition in *Helianthus annuus* and *Helianthus tuberosus* species. Cluster 3 includes the similar phenolic acid composition in *Echinops ritro* and *Echinacea purpurea* leaves, whereas cluster 4 describes the phenolic acids composition in *Rudbeckia fulgida* L., which is significantly different from other representatives of the family *Asteraceae*.

Based on the cluster analysis, it is evident that the concentration of phenolic acids inside one family was differentiated by the plant species' genetic properties. The significant presence of syringic acid was a prevailing trait for all experimental representatives of the family *Asteraceae*.

The apparent data structure matches with the cluster analysis of the concentration of phenolic acids in the representatives of the family *Rosaceae*. As shown in Figure 6(b), intergroup relations reveal four main clusters characterized by the similar composition of phenolic acids inside each cluster. Cluster one in Figure 6(b) (family *Rosaceae*) contains data describing the similar phenolic acid composition in *Potentilla recta* L., *Alchemilla mollis*, and *Eriobotrya japonica*. Cluster 2 contains a similar phenolic acid composition in *Rosa rubiginosa* and *Cotoneaster horisontalis*, which are closer to representatives of cluster 1.

Interestingly, the *Rosa canina* is in cluster 4, and it is characterized by the different phenolic acid composition compared to the other studied representatives of the family

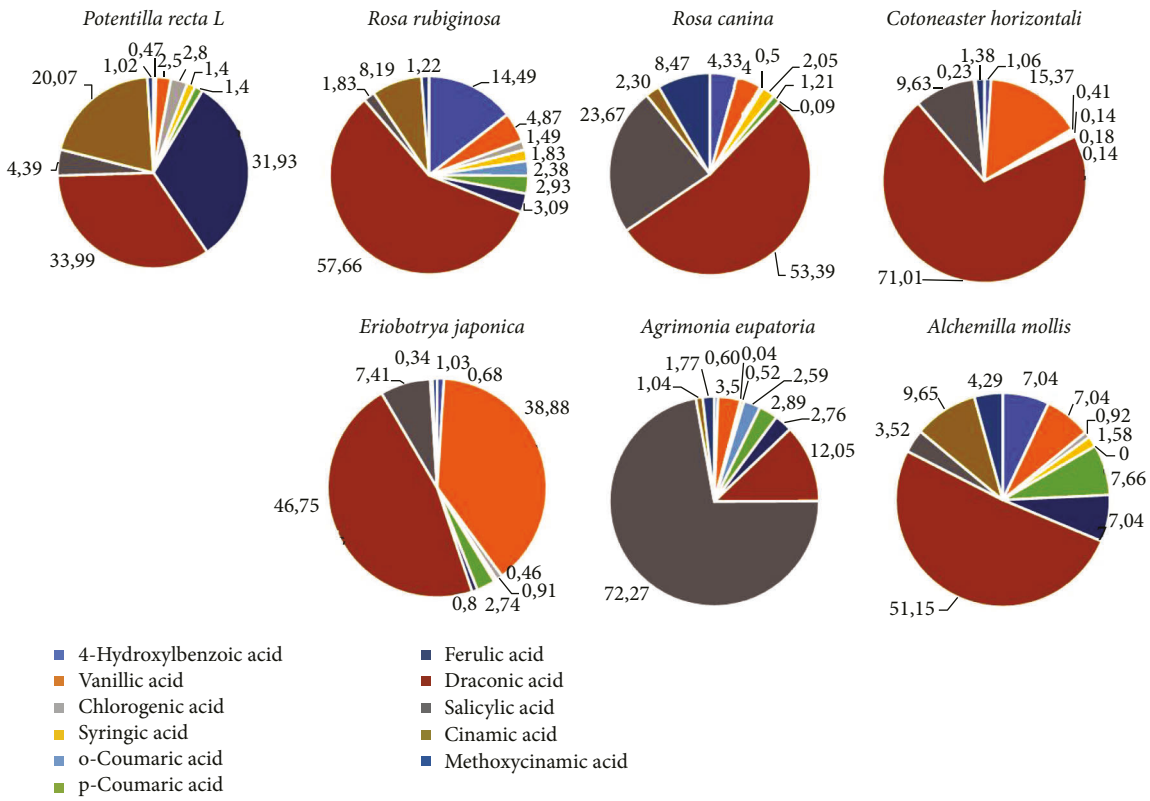

Figure 4: Percentage of phenolic acids amounts in the methanolic extract of representatives of the family *Rosaceae* (%).

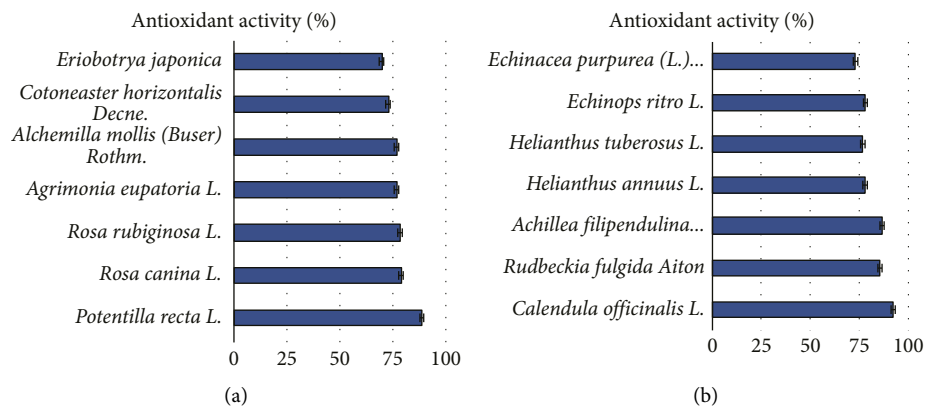

Figure 5: antioxidant activity of the experimental extracts of plant species *Rosaceae* (a) and *Asteraceae* (b) families.

Rosaceae. Cluster 3 includes the phenolic acid composition presented by *Agrimonia eupatoria*. The analysis of phenolic acids composition showed the substantial accumulation of specific phenolic acids in the plant leaf may indicate about their participation in plant defense.

4. Discussion

The percentage of phenolic acids can be calculated regarding phenolic acid amounts in the methanolic extract of the representatives *Asteraceae* family. It was found high syringic acid level in the experimental extracts of *Asteraceae* species.

Between 64.13% and 95.13%, such significant presence in the leaves of representatives of *Asteraceae* family can be a possible biochemical marker of plant species of this family. The formation of specific secondary metabolites in phylogenetic close families and genera of plants is possible due to the similarity of metabolic processes [39, 40]. The presence of syringic acid may be scientifically applied both as a specific marker for experimental plant species and as a source of this substance in the studied plant species in biofortification processes.

Quantitative analysis of phenolic compounds of other *Asteraceae* plants such as *Achillea millefolium L.* (common

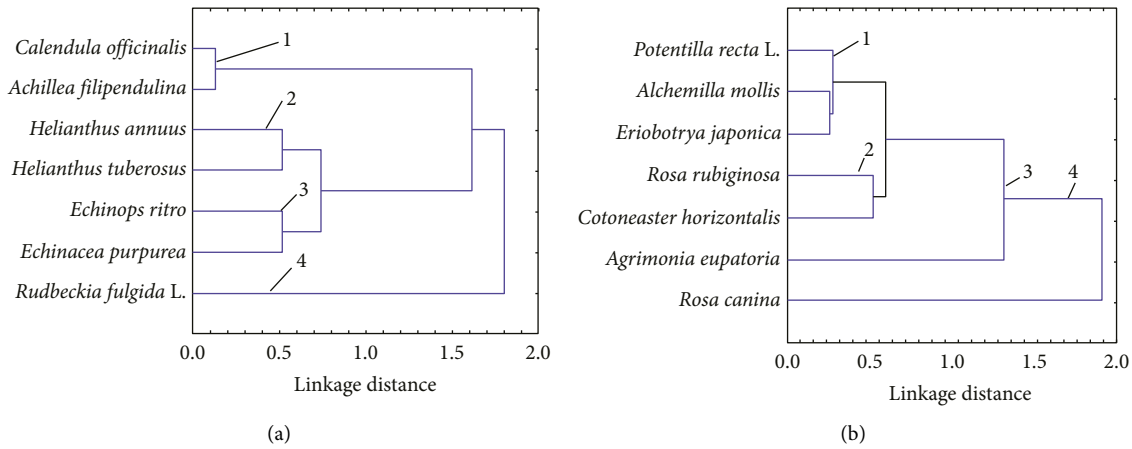

FIGURE 6: Dendrogram of the species family Asteraceae (a) and Rosaceae (b) obtained after applying hierarchical cluster analysis to the phenolic acids contents using Euclidean coefficient and WARD methods. 1, 2, 3, and 4 clusters. The similar composition of phenolic acids inside of each cluster.

yarrow), *Helichrysum arenarium L.* (immortelle) also shown high presence of syringic acid. At the same time, phenolic acids compostion was different between these studied plant species. For example, common yarrow got high level of rosmarinic acid but immortelle has no romarinic acid but high level of caffeic acid [41]. Syringic acid was discovered in some plants, such as Schumannianthus dichotomus (*Marantaceae*) and *Ardisia* elliptica (*Primulaceae*) [42, 43]. Syringic acid is one of the transitional compounds of the plant pigment malvidin. Syringic acid and plant anthocyanidin malvidin were described in vinegar and red wine [44]. The presence of methoxy groups attributes the therapeutic activity of syringic acid with the places 3 and 5 of the aromatic ring. Syringic acid is able to regulate the modifications of some biological functions such as factors of growth, transcriptional factors, and the level of signaling molecules implicated in the development of various human diseases such as cancer, liver damage, and diabetes. In the eantime, syringic acid has also huge spectrum of applications in industry counting on bioremediation and photocatalytic ozonation [38, 45].

It was confirmed anesthetic and sedative activities of syringic acid in the *Quercus infectoria* plant extract (Dar M.S., Ikram, 1979). The correlation between the percentage level of syringic acid and antioxidant activity has been observed for the studied representatives' family *Asteraceae—Calendula officinalis*, *Achillea filipendulina*, and *Rudbeckia fulgida* has shown a high percentage of syringic acid and also a high level of antioxidant potential (Figures 3 and 5). The intense antioxidant activity of syringic acid was discussed by Srinivasulu et al.[38]. The antioxidant activity potential can relate to the anti-inflammatory activity of the studied plant extracts [46]. Anti-inflammatory and antioxidant potential of plants from the *Asteraceae* family was noted [47]. The unique mechanisms of action of biological markers for the anti-inflammatory activity was described with assistance of the metabolic approach. The huge network and connections between the diversified range of tribes and genera of the family *Asteraceae* with the help of HPLC-ESI-HRMS metabolomic approach was described [48].

In our study, intergroup relations using cluster analysis reveal four main clusters characterized by shared variance in the representatives of the family *Asteraceae* (Figure 7(a)). The cluster analysis indicated that the identified phenolic acid concentrations were differentiated by the genetic properties of the plant species inside one family. The high presence of syringic acid in the experimental extracts of the family *Asteraceae* was a significant trait for all experimental plant species.

The further study with percentage calculation of phenolic acid amounts in methanolic extract of representatives of the family *Rosaceae* found that percentage of draconic acid in the studied leaf extracts of *Rosaceae* vary between 12.05% and 71.01%. Such significant presence in the leaves of mostly all representatives of the family *Rosaceae* which was higher than 30% of the total amount of identified phenolic acids can be used as a species-specific biochemical marker.

Draconic acid is another name, p-anisic acid or 4-methoxybenzoic acid. It is one of the isomers of draconic acid characterized by antioxidant and antiseptic properties [49]. It is also recommended to use as a transitional compound in the formation of more composite natural compounds. Draconic acid is found naturally in anise, fruits of figs *Ficus mucuso* (*Moraceae*) [50], leaves extract of *Rhododendron ferrugineum (Ericaceae)* [51], and in the mycelium of *Cordyceps sinensis* (*Ophiocordycipitaceae*) [52]. In our study all experimental plant extracts were characterized by the presence of draconic acid. The draconic acid content in the leaf extracts of the studied representatives was a great level in *Rosa canina* L., *Rosa rubiginosa* L.

Literature analysis has been revealed the presence of studies on the topic phenolic content and antioxidant activity mostly in the fruit of the representatives' family *Rosaceae*, but not many in the leaf extracts [53, 54]. Just one comparable study of phenolics extraction of black cherry leaves and flowers with simulating different traditional extraction procedures was done. Black cherry leaves were extracted with methanol, which is a standard extraction of phenolics. It was identified in the leaf extracts next phenolic

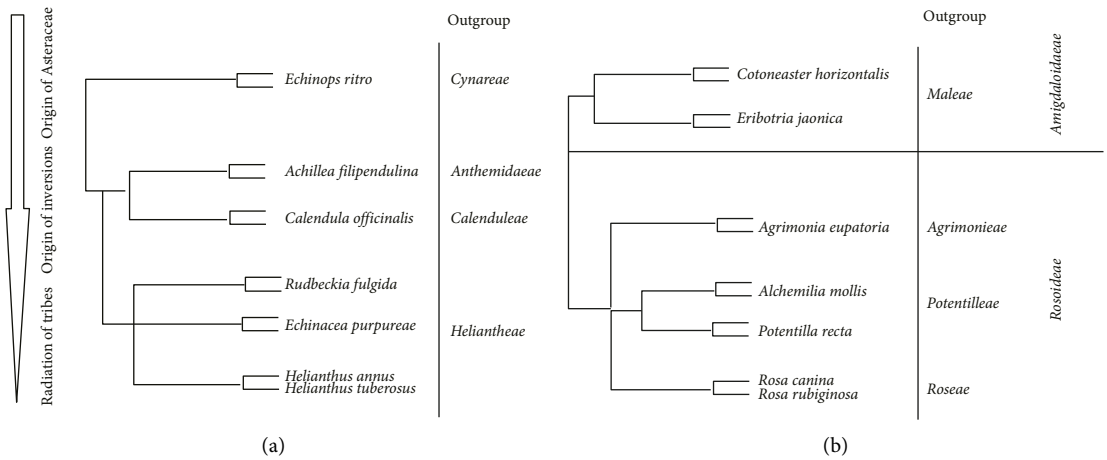

Figure 7: Presentations of phylogeny trees studied representatives' families *Asteraceae* (a) and *Rosaceae* (b).

acids: caffeic acid hexoside1,2, caffeic acid, p-coumaric hexoside 1,2,3, 5-Caffeoylquinic acid, 5-p-coumaroylquinic acid, 5-feruloylquinic acid, and dicaffeoylquinic acid [55].

The high level of syringic acid in members of the family *Asteraceae* and draconic acid in *Rosaceae* can be partly connected with the studied antioxidant capacity of plant extracts. The use of extracts in the study of antioxidant properties, antimicrobial effects may get different results compared to the study of the same effects from isolated biologically active compounds of the same plant extract. It is known that the impacts of activities of the compounds solute in extracts can have synergistic or antagonistic response [56, 57]. So, it is not correct to state that just major phenolic acid present in the leaf sample can influence the antioxidant potential of the plant extract.

The obtained data structure correlates with the cluster analysis of the concentration of phenolic acids in the family *Rosaceae* representatives. It is evident from the intergroup relations (Figure 7(b)) that the four main clusters appeared, characterized by similar phenolic acids inside each cluster. The *Rosa canina* is in cluster 4 and characterized by other phenolic acid composition than *Rosa rubiginosa,* representing the same genus. Medicinal herbs associated with the same genus are continually simply distracted because to their quite identical metabolites and structure. To comprehend them, naturally specific biological markers with assistance developed method which use HPLC analysis were analyzed profiles of metabolites [58]. Cluster analysis based on the composition and level of the identified flavonoids, ascorbic and citric acids suggested the effectiveness of the division of sect. Caninae into three subsections. Regarding this analysis, *Rosa rubiginosa* and *Rosa canina* were chosen in the different subsections [59]. The described data confirm the data of cluster analysis based on phenolic acid composition for *Rosa canina* and *Rosa rubiginosa*.

5. Conclusions

The plant extracts of the studied seven species family *Asteraceae* and seven species family *Rosaceaes* contained 11 phenolic acids. It was noticed species-dependent variations of phenolic acids composition. Based on the cluster analysis, it is evident that the level of the determined phenolic acids was differentiated by the genetic properties of the plant species inside one family. The experimental plant species can be the sources of specific phenolic acids with compatible antioxidant activity. The presented data regarding phenolic acids composition reveal that their profiles are significant biochemical markers of authenticity indication of the studied plant species. It was found significant appearance of syringic acid in the extracts of representatives of the family *Asteraceae*. At the same time, draconic acid have shown major presence in the extracts of *Rosaceae* family species. The explanation of the results is creative with applied scientific background for further studies of plants and herbs botanical, physiological features related to the phenolic acids composition and presence of other specific secondary metabolites.

Data Availability

The data analyzed in this study were a reanalysis of the existing data, which are openly available at locations cited in the reference section.

Ethical Approval

This paper does not contain any studies with human or animals.

Consent

The author approves processing of this manuscript for publication.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Conceptualization was done by OS and MB. Investigation was done by OS, MB, and KK:. Data analysis contribution by

OS and MZ:. HPLC analysis by OS, cluster analysis by MZ, writing by OS, KK, and MB, funding acquisition by MB.

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