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VARIATION IN PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF DIFFERENT PLANT PARTS OF *Primula veris*

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Abstract
Primula veris L. (Cowslip primrose) is a species of flowering plant in the family Primulaceae. Usually, flowers and leaves from cowslip primrose are used for making sedative tea. The cowslip roots are also used in the treatment of respiratory tract problems, as an expectorant. In the present study we compared flowers, leaves and roots from Primula veris harvested in two different locations in the Western part of Romania (Julita and Nadas). In order to evaluate the phenolic content, antioxidant activity and the chemical composition, the ethanolic extracts have been obtained by maceration for 7 days. The results obtained indicated that ethanolic extracts of Primula veris presented high phenolic content that ranged between 131 to 168 mg GAE/L for roots, 136 to 159 mg GAE/L for leaves and 133 to 219 mg GAE/L for flowers. Remarkable percent of inhibition of DPPH has been obtained for all plants parts (as roots 16.4-23.2%, leaves 16.0-17% and flowers 14.9-24.7 %.) The following compounds have been quantified in all different plant parts: gallic acid, quercetin and kaempferol using Ultra-High Performance Liquid Chromatography (UHPLC).

Key words*: Primula veris, antioxidant activity, polyphenols.*

INTRODUCTION

Primula veris L. (cowslip) belongs to the *Primulaceae* family and is distributed throughout Europe and Asia with more than 400 species. Many plant parts have a long history of medicinal usage.
Primula veris has been mainly used for the production of herbal teas and preparations that are also considered dietary supplements. Due to its flavonoid and phenolic content, *Primula veris* is used in anti-inflammatory and expectorant treatments for cold and related sinusitis affections (Varela and Ibañez, 2009).
It is a well-known medicinal plant, with an expectorant activity, sedative, decongestant, diuretic, antioxidant activity (Bogenschutz-Godwin et al., 2002).
Cowslip is being used as tea (flowers and leaves), as liquid flower extract, flower and root tincture. The main compounds identified in *P. veris* leaves and roots are triterpene saponins as well as phenolic compounds, including flavonoids, phenolic acids and phenolic glycosides (Bączek et al., 2017).
Compounds identified in *P. veris* were: primverin, primulaverin, catechin, astragalin, chlorogenic acid (Bączek et al., 2017), rutin, kaempferol, hydroxydimethoxyflavone, quercetin-3-*O*-dihexoside (Bączek et al., 2017; El Morchid et al., 2014) 3′,4′,5′-trimethoxyflavone (Varela and Ibañez, 2009).
In this study, extracts obtained from aerial parts, flowers, leaves and roots from cowslip collected in western part of Romania, were extracted with ethanol and analyzed using UHPLC.
The composition in phenolic acids, total phenol content, as well as their antioxidant activity was determined.

MATERIALS AND METHODS

Flowers, leaves and roots of *Primula veris* were collected in 2014 and 2015 from two villages of Romania: Julita (46° 2' 0" North, 22° 8' 0" East) and Nadas (46° 13' 0" North, 21° 57' 0" East). Dried plant parts was stored in paper bags until further analysis and voucher specimens were taken and preserved at the

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The plant parts were dried at room temperature (20-25°C). The extracts were obtained through maceration. Briefly, 2 g of plant material was grinded and extracted with 40 mL ethanol for 7 days. Before further analysis all extracts were filtered through PTFE (0.45 µm) (Pag et al., 2014).

Total phenolic content

Total phenolic content of the extracts obtained was determined by using Folin-Ciocalteu method slightly modified (Baranauskiene et al., 2014; Pag et al., 2014; Tepe and Sokmen, 2007). Briefly, the aqueous extracts obtained were diluted with distilled water (1:25). To 1 ml sample were added 0.5 ml Folin-Ciocalteu reagent, 2 ml Na_2CO_3(20%) and 5 ml distilled water. The mixture was kept in the dark for 90 minutes, thereafter the absorbance was recorded against a blank prepared in the same conditions, at 765 nm, by using a UV-VIS double beam spectrophotometer (Specord 200, Analytik Jena Inc., Jena, Germany). Gallic acid was used as reference. A calibration curve for gallic acid was obtained (20, 40, 100, 160, 200 mg/L), then the regression equation and the correlation coefficient were calculated and the results were expressed in mg GAE/L.Total phenolic content of the extracts was thereafter expressed as equivalents of gallic acid (mg GAE/L). All experiments were performed in triplicates.

DPPH radical scavenging activity

In order to determine the antioxidant activity of the extracts the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging capacity was assessed using a spectrophotometric method (Pag et al., 2014). The DPPH was dissolved in ethanol (0.2mM) and 3 ml of the resulting solution was mixed with 0.1 ml sample (20 mg/ml). Absorbance was recorded at 517 nm after 60 minutes incubation in the dark. Gallic acid was used as reference. A calibration curve for gallic acid was obtained (2.5 to 50 mg/L), then the regression equation and the correlation coefficient were calculated and the results were expressed in mg GAE/L. Inhibition of the DPPH stable free radical was calculated with Eq. (1):

$$\%Inhibition = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} x100 \qquad (1)$$

where:

$Abs_{control}$ is absorbance of 0.2 mM DPPH in ethanol,

Abs_{sample} is absorbance of 0.2 mM DPPH +extract.

Chromatographic analysis

Gallic acid, quercitin and kaempferol were determined by using an UHPLC method. Briefly, a high performance liquid chromatograph (Nexera X2, Shimadzu, Tokyo, Japan) equipped with a diode array detector (M30A, Shimadzu, Tokyo, Japan) and a Nucleosil 100-3-C18 reversed-phase column (4.0 mm i.d. × 125 mm column length, 3 µm particle size, Macherey-Nagel GmbH, Duren, Germany) were used. The column temperature was maintained at 30ºC and the flow rate at 1 ml min^{-1}. The solvents used for the chromatographic elution consisted of ultra-pure water with 0.1% TFA (A) and acetonitrile (B). The chromatographic elution program used was as follows: 95% A and 5% B that was changed linear gradient to 42% B for 5 min., followed by a linear gradient to 35% B in 30 min. Thereafter, the eluent was changed to the initial composition of 95% A and 5% B linear gradient for 5 min. The measurements have been done between 200-600 nm wavelengths.

RESULTS AND DISCUSSIONS

Primula veris L. plants were harvested in 2014 and 2015 from two locations in the western part of Romania, Julita and Nadas. Ethanolic extracts from flowers, leaves and roots were obtained by maceration. The total phenolic content and antioxidant activity of the extracts were determined. In Table 1 are presented total phenolic content (mg GAE/L) and antioxidant activity (Inhibition %) of cowslip extracts obtained from flowers, leaves and roots. Total phenolic content of the extracts was determined by using Folin-Ciocalteu method. This method estimates the total content of all phenolics present in the analyzed extracts, including flavonoids, anthocyanins and non-flavonoid phenolic compounds.

As it is shown in Table 1, total phenolic content varied between different plant parts from 131 to 168 mg GAE/L for roots, 136 to 159 mg

GAE/L for leaves and 133 to 219 mg GAE/L for flowers. The highest content of phenols (218.5 mg GAE/L) was achieved in extracts obtained from flowers from Julita collected in 2015.

Table 1. Total phenolic content (mg GAE/L) of cowslip extracts obtained from flowers, leaves and roots collected in Julita and Nadas in 2014 and 2015

Plant parts	mg GAE/L	mg GAE/ 100 g plant material
roots Nadas 2014	131.73±1.23	263.46±1.16
roots Nadas 2015	167.69±1.75	335.38±1.56
roots Julita 2014	153.65±1.13	307.31±2.03
roots Julita 2015	149.23±1.04	298.46±1.96
leaves Nadas 2014	147.12±0.98	294.23±1.93
leaves Nadas 2015	136.15±0.87	272.31±1.82
leaves Julita 2014	149.42±0.62	298.85±1.87
leaves Julita 2015	159.04±0.99	318.08±2.08
flowers Nadas 2014	162.88±0.96	325.77±1.88
flowers Nadas 2015	170.00±1.43	340.00±1.76
flowers Julita 2014	133.27±1.29	266.54±1.46
flowers Julita 2015	218.46±1.14	436.92±2.88

The total phenolic content values obtained in the present study for the ethanolic extracts of cowslip are different than that reported for aqueous-ethanolic extracts of cowslip, namely 535.4 mg GAE/ 100 g for flowers (Tünde et al., 2015) and 155.8 mg GAE/g for ethanolic extracts ultrasonicated obtained from flowers (Chilku et al., 2017). The results obtained demonstrate the importance of solvent, solvent ratio and extraction conditions used to obtain extracts from plants.

In order to determine the antioxidant properties of the extracts we used the DPPH (1,1-diphenyl-2-picrylhydrazyl) colorimetric method. As it is depicted in Table 2 the inhibition varied between16.4-23.2% for roots, 16.0-17% for leaves and 14.9-24.7 %.for flowers. The antioxidant activity obtained in the present study is different than that reported for aqueous-ethanolic extracts of cowslip, Tünde et al., 2015 obtained 86.65 % (Tünde et al., 2015).

In Figure 1 are presented the concentration of kaempferol, quercetin and gallic acid (mg/L) of cowslip extracts obtained from flowers, leaves and roots determined through UHPLC method.

The highest quantity of gallic acid was found in cowslip: 21.22 mg/L for extracts obtained from flowers Nadas in 2015 and 18.78 mg/L for leaves plants harvested in Julita in 2015. Smaller quantities of gallic acids, 9.53 mg/L, were determined in the flowers extracts of cowslip harvested in Julita in 2015.

Table 2. Antioxidant activity (Inhibition % and mg GAE/L) of cowslip extracts obtained from flowers, leaves and roots and collected in Julita and Nadas in 2014 and 2015

Plant parts	Inhibition (%)	mg GAE / L
roots Nadas 2014	16.39±0.55	8.43±0.12
roots Nadas 2015	17.81±0.37	9.14±0.16
roots Julita 2014	18.87±0.54	9.67±0.18
roots Julita 2015	23.18±0.43	11.84±0.19
leaves Nadas 2014	16.84±0.41	8.65±0.12
leaves Nadas 2015	15.99±0.51	8.22±0.14
leaves Julita 2014	16.23±0.43	8.35±0.13
leaves Julita 2015	17.00±0.37	8.74±0.15
flowers Nadas 2014	18.04±0.23	9.25±0.18
flowers Nadas 2015	18.89±0.49	9.68±0.19
flowers Julita 2014	14.93±0.47	7.70±0.21
flowers Julita 2015	24.65±0.32	12.58±0.23

From the flavonoid family we determined the content of kaempferol and quercetin in cowslip extracts. The highest amount of quercetin and kaempferol was found in extract obtained from flowers harvested in Julita in 2015, 12.08 mg/L, respectively 3.60 mg/L. Kaempferol was not identified in extracts obtained from roots. Extracts obtained from roots harvested in both locations have had the lowest amount of quercetin. Bączek K. et al., 2017 identified by HPLC in *Primula veris* and *Primula elatior* the following phenolic compounds: catechin, chlorogenic acid, orientin, rutoside (quercetin 3- O-rutinoside), astragalin, isorhamnetin-3-O-glucoside and compounds specific for *Primula* species: primverin, primulaverin. Bączek K. et al., 2017 found also, that the contents of isorhamnetin-3-O-glucoside, astragalin, and catechin were distinctly higher in the flowers of *P. veris.* (Bączek et al., 2017). Ozkan et al., 2016 assess the content of catechin, rutin, gallic acid, protocatechuic, para hydroxy benzoic acid, vanillic acid, and p-coumaric acid by HPLC in *P. vulgaris* flowers. According to this analysis, rutin and p-coumaric acid seemed to be the main phenolic compound of this raw material.

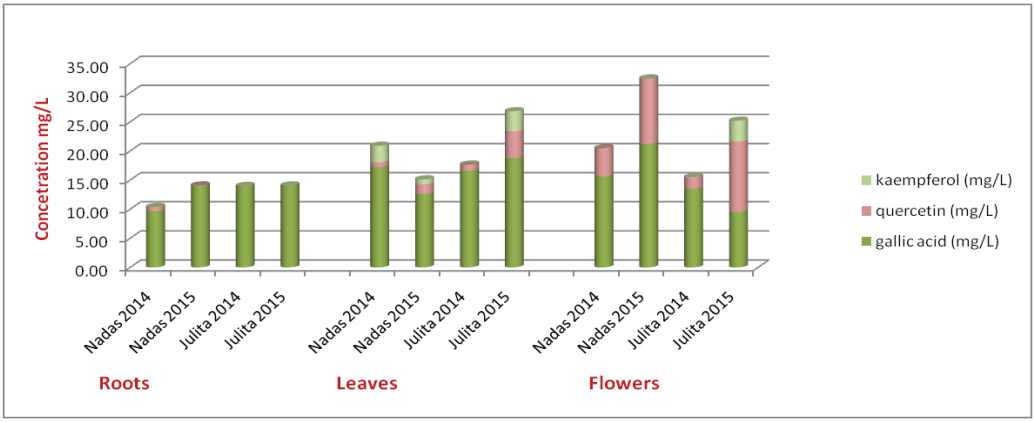

Figure 1. Concentration of gallic acid, quercetin and kaempferol determinated by UHPLC

CONCLUSIONS

The ethanolic extracts obtained from different parts of cowslip (flowers, leaves and roots) have had a remarkable quantity of phenolic content (mg GAE/L), determinated by Folin-Ciocalteu method, with a notable antioxidant activity. Chromatographic analysis revealed that extracts contain different amounts of kaempferol, quercetin and gallic acid.

In order to fully benefit from the plant bioactive compounds, we recommend the usage of flowers and leaves due to their high content.

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