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ORIGINAL ARTICLE

Antioxidant activity, acetylcholinesterase and tyrosinase inhibitory potential of *Pulmonaria officinalis* and *Centarium umbellatum* extracts



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Centarium umbellatum

Abstract In this study several investigations and tests were performed to determine the antioxidant activity and the acetylcholinesterase and tyrosinase inhibitory potential of *Pulmonaria officinalis* and *Centarium umbellatum* aqueous extracts (10% mass) and ethanolic extracts (10% mass and 70% ethanol), respectively. Moreover, for each type of the prepared extracts of *P. officinalis* and of *C. umbellatum* the content in the biologically active compounds – polyphenols, flavones and proanthocyanidins was determined. The antioxidant activity was assessed using two methods, namely the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and reducing power assay. The analyzed plant extracts showed a high acetylcholinesterase and tyrosinase inhibitory activity in the range of 72.24–94.24% (at the highest used dose – 3 mg/mL), 66.96% and 94.03% (at 3 mg/mL), respectively correlated with a high DPPH radical inhibition – 70.29–84.9% (at 3 mg/mL). These medicinal plants could provide a potential natural source of bioactive compounds and could be beneficial to the human health, especially in the neurodegenerative disorders and as sources of natural antioxidants in food industry.

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1. Introduction

Plants are an overall source of antioxidant activity compounds, such as phenolic acids, flavonoids (including anthocyanins and tannins), vitamins and carotenoids that may be used as pharmacologically active products (López et al., 2007).

Acetylcholinesterase enzyme (AChE, EC 3.1.1.7) plays a major role in the activity of the central (CNS) and peripheral (PNS) nervous systems, because it catalyzes the hydrolysis of the acetylcholine neurotransmitter, thus producing choline

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and acetate (ACh) (Legay, 2000). Alzheimer's disease (AD) is the most common neurodegenerative disorder with a still unclear pathogenesis. One of the most accepted theories has been "cholinergic hypothesis", so inhibition of acetylcholinesterase (AChE) preserves the levels of acetylcholine and improves the cholinergic function and therefore has become the standard approach in the symptomatic treatment of AD (Houghton and Howes, 2005; Vinutha et al., 2007). To date, several plants have been identified as containing acetylcholinesterase inhibitory (AChEI) activity (Adewusi et al., 2011, 2010; Fale et al., 2012; Ferreira et al., 2006).

Earlier reports have revealed that oxidative injury plays the main role in the pathogenesis of plentiful neurodegenerative diseases including stroke, Alzheimer's disease, vascular dementia, etc. (Senol et al., 2010).

Antioxidant activity is one of the most important properties of plant extracts, because scientists have looked for sources of natural antioxidants to be introduced in many cosmetic, pharmaceutical and food formulations. The research for the new sources of antioxidants in the past resulted in the extensive studies on medicinal plants (Malinowska, 2013).

Tyrosinase (EC 1.14.18.1) is a copper enzyme that is essential in melanin biosynthesis and it was also admitted to play complex roles in human organisms, than previously thought. Furthermore, the role of tyrosinase in neuromelanin production and damage of the neurons related to Parkinson's disease has been extensively studied (Greggio et al., 2005). These new findings emphasize the importance of tyrosinase inhibitor's discovery and development.

Pulmonaria officinalis (lungwort) is an herbaceous perennial plant belonging to the family *Boraginaceae*, widely spread in Europe, with therapeutic use in bronchitis, laryngitis, kidney and respiratory diseases as well in gastric and duodenal ulcers (Dumitru and Răducanu, 1992). The plant contains unsaturated pyrrolizidine alkaloids, therefore it is not recommended for long-term consumption (Lüthy et al., 1984).

Centarium umbellatum (common centaury) is a medicinal plant from *Gentianaceae* family and it has been used as a medicinal herb for over 2000 years for its bitterness as an amarum, digestive and also for treating febrile conditions, diabetes, hepatitis and gout (Tucakov, 1990). It is also known as a hypothesive, anti-spasmodic, sedative and diuretic plant (Mounsif et al., 2000).

In this study, the anti-acetylcholinesterase, anti-tyrosinase and antioxidant activity of some aqueous and ethanolic extracts prepared from two Romanian medicinal plants: *P. officinalis* and *C. umbellatum* were determined. Moreover, the total polyphenol content, flavone and proanthocyanidin content was assessed, highlighting the correlation between the values determined for these biologically active compounds and the values of acetylcholinesterase and tyrosinase inhibition, and those of antioxidant activity, as well. The aim of this study was to find new sources of anti-acetylcholinesterase and anti-tyrosinase inhibitors, useful in treating neurodegenerative diseases and also sources of natural antioxidants.

2. Methodology

2.1. Materials

Aluminum chloride $\geq 99\%$, Folin-Ciocalteu's reagent, potassium ferricyanide $\geq 99\%$, trichloroacetic acid $\geq 99\%$, ethanol

and 2,2-diphenyl-1-picrylhydrazyl (DPPH), acetylcholinesterase from *Electrophorus electricus* (electric eel) (518 units/mg solid), 5,5'-dithiobis-(2-nitrobenzoic acid) (D TNB) $\geq 99\%$, acetylthiocholine iodide (AChI) $\geq 99\%$, 3-(3,4-Dihydroxyphenyl)-L-alanine (L-DOPA) $\geq 98\%$, tyrosinase from mushroom (1881 units/mg solid) and all solvents were purchased from Sigma Chemical Company (Sigma Aldrich, Germany), Fluka (Switzerland), Roth (Carl Roth GmbH, Germany) and distilled water was used for all the performed analyses (Millipore, Bedford, MA).

2.2. Preparation of the extracts

The plant material was purchased from a national producer (Fares Orastie) of herbal infusions in dry and already packed forms, supplied to supermarkets, drug stores and herbal shops. The aqueous extracts (10% mass) were obtained in 60 °C distilled water. Both aqueous and ethanolic extracts (10% mass and 70% ethanol) were subjected to ultrasound at room temperature, for 1 h, followed by filtration.

2.3. Determination of polyphenols content

Determination of polyphenol content was made using Folin-Ciocalteu method (Singleton et al., 1999). The polyphenol content was expressed in gallic acid equivalents (GAE)/mL of extract.

2.4. Determination of flavone content

Determination of flavone content was analyzed using aluminum chloride colorimetric method (Lin and Tang, 2007). The flavone content was expressed in μg rutin equivalent (RE)/mL of extract.

2.5. Determination of proanthocyanidins

Determination of proanthocyanidins was carried out using the vanillin assay in glacial acetic acid (Butler et al., 1982), with slight modifications. The absorbance was read at 500 nm. The results were expressed as catechin equivalents (CE)/mL of extract.

2.6. Antioxidant assays

The antioxidant activity was measured using 2 methods:

2.6.1. DPPH radical scavenging activity

The scavenging activity on the DPPH radical was determined by measuring the decrease in the DPPH maximum absorbance at 517 nm after 3 min (Bondet et al., 1997). The percentage of DPPH radical scavenging activity of the samples was calculated as follows:

$$\text{radical scavenging activity (\%)} = \frac{A_B - A_A}{A_B} \times 100$$

where A_B = control absorbance and A_A = sample absorbance.

2.6.2. Reducing power activity

The reducing power activity was determined according to a previously described procedure (Berker et al., 2007). Sample extracts (0.1 mL) were mixed with 2.5 mL of 200 mM/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was intensively shaken, then incubated at 50 °C for 20 min. In the next step, 2.5 mL of 10% trichloroacetic acid (w/v) was added and the resulted mixture was mixed with 2.5 mL deionized water and 0.5 mL of 0.1% ferric chloride. The absorbance was spectrophotometrically measured at 700 nm.

2.7. HPLC analysis

The chromatographic measurements were performed into a complete HPLC SHIMADZU system, using a Nucleosil 100-3.5 C18 column, KROMASIL, 100 × 2.1 mm. The system was coupled to a MS detector, LCMS-2010 detector (liquid chromatograph mass spectrometer), equipped with an ESI interface. The mobile phase was sonicated in order to eliminate the dissolved air, then filtrated through a PTFE 0.2 µm membrane. The method used for measuring polyphenols was published by Alecu et al. (2015).

The mobile phase consists of formic acid (to improve ionization and resolution) in water (pH = 3.0) as solvent A and formic acid in acetonitrile (pH = 3.0) as solvent B. The polyphenolic compounds separation was performed using binary gradient elution: 0 min 5% solvent B; 0.01–20 min 5–30% solvent B; 20–40 min 30% solvent B; 40.01–50 min 30–50% solvent B; 50.01–52 min 50–5% solvent B. The flow rate was: 0–5 min 0.1 mL min⁻¹; 5.01–15 min 0.2 mL min⁻¹; 15.01–35 min 0.1 mL min⁻¹; 35.01–50 min 0.2 mL min⁻¹; 50–52 min 0.1 mL min⁻¹.

2.8. Acetylcholinesterase inhibition activity

The acetylcholinesterase inhibition activity was measured using the method described by Ingkaninan et al. (2003). Briefly, 3 mL of 50 mM Tris-HCl buffer (pH 8.0), 100 µL of sample solution at different concentrations (3 mg/mL, 1.5 mg/mL, 0.75 mg/mL) and 20 µL AChE (6 U/mL) solution were mixed and incubated for 15 min at 30 °C; a 50 µL volume of 3 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) was added to this mixture. The reaction was then initiated by the addition of 50 µL of 15 mM acetylthiocholine iodide (AChI). The hydrolysis of this substrate was monitored at precisely 405 nm wavelength. At this wavelength the formation of yellow 5-thio-2-nitrobenzoate anion was noticed as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide.

The enzymatic activity was calculated as a percentage of the velocities compared to that of the assay using buffer instead of inhibitor (extract), based on the formula:

$$EA = \frac{E - S}{E} \times 100$$

where E is the activity of the enzyme without test sample and S the activity of the enzyme with test sample.

2.9. Tyrosinase inhibition assay

The tyrosinase (EC1.14.1.8.1, Sigma) activity was spectrophotometrically measured using 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA) as substrate (Liang et al., 2012). Tyrosinase aqueous solution (100 µL, 0.5 mg/mL), *plant* extract (100 µL, 50 µL, 25 µL) and 1850 µL of 0.2 M phosphate buffer (pH 7.0) were mixed and incubated for 15 min at 30 °C. Next, 10 mM L-DOPA solution (50 µL) was added and the absorbance at 475 nm was measured for 3 min. The same reaction mixture having the *plant* extract replaced by the equivalent amount of phosphate buffer, served as blank. The percentage inhibition of tyrosinase activity was calculated as follows:

$$\% \text{ Tyrosinase inhibition} = \frac{\Delta A_{\text{control}} - \Delta A_{\text{sample}}}{\Delta A_{\text{control}}} \times 100$$

where $\Delta A_{\text{control}}$ is the change of absorbance at 475 nm without a test sample, and ΔA_{sample} is the change of absorbance at 475 nm with a test sample.

2.10. Statistical analysis

The tests were carried out in triplicate and the software Microsoft Office Excel 2007 was used for statistical analysis. Statistical analysis of the data was performed using the one-tailed paired Student's test, on each pair of interest. Differences were considered statistically significant at $p < 0.05$.

3. Results and discussions

3.1. The determination of total polyphenolic, flavone and proanthocyanidin contents

The total polyphenol, proanthocyanidin and flavone contents from the analyzed extracts are presented in Table 1.

One can see that the polyphenols and the proanthocyanidins are in similar amounts in both studied plants, higher amounts in ethanolic extracts than in aqueous and somewhat higher in *C. umbellatum* extracts than in *P. officinalis* extracts. The analyzed extracts showed higher flavones content especially in ethanolic extracts, the highest value being found in *C. umbellatum* ethanolic extract –496.28 µg RE/mL rutin.

The flavonoids constitute a prominent group of secondary metabolites in these plants that may possess biological activity and have beneficial effects on human health as antimicrobial,

Table 1 Compounds content (polyphenols, flavones, proanthocyanidins) from the tested extracts.

Sample		Polyphenols (µg GAE/ mL)	Proanthocyanidins (µg CE/mL)	Flavones (µg RE/mL)
Pulmonaria officinalis (lungwort)	Aqueous extract (10% mass)	486.60 ± 2.12	41.83 ± 3.25	104.28 ± 3.25
	70% ethanolic extract (10% mass)	576.62 ± 6.32	61.5 ± 6.23	272.81 ± 9.21
Centarium umbellatum (common centaury)	Aqueous extract (10% mass)	560.11 ± 8.59	43 ± 5.23	160.75 ± 5.12
	70% ethanolic extract (10% mass)	656.61 ± 7.56	63.33 ± 2.63	496.28 ± 8.91

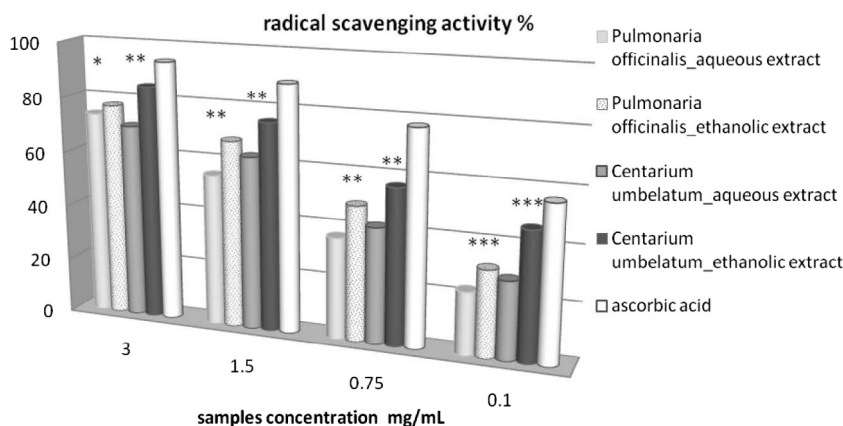


Figure 1 Radical scavenging activity % $p < 0.05$ compared the activity of the ethanolic extracts with that of the aqueous extracts; ** $p < 0.01$ compared the activity of the ethanolic extracts with that of the aqueous extracts; *** $p < 0.001$ compared the activity of the ethanolic extracts with that of the aqueous extracts; * $p < 0.05$ compared polyphenol content of extracts with radical scavenging activity; * $p < 0.05$ compared flavone content of extracts with radical scavenging activity.

anti-inflammatory, anti-diabetic, anti-cholesterolemic, antioxidant and anti-cancer agents (Abirami et al., 2014).

P. officinalis was revealed to possess a high amount of polyphenols (and an increased antioxidant activity (Ivanova et al., 2005)). Literature related to the preliminary screening of antioxidant activity for the *P. officinalis* isolated extracts used in cosmetics industry showed a high value for antioxidant activity that could be correlated to the high content of the flavonoids (Malinowska, 2013).

3.2. Antioxidant activity determination

3.2.1. DPPH radical scavenging activity

The DPPH free radical is a stable free radical, that has been widely accepted as a tool for estimating the free radical-scavenging activity of antioxidant (Nagai et al., 2003). In the DPPH test, the antioxidants were able to reduce the stable DPPH radical to the yellow-colored diphenylpicrylhydrazine. The effect of antioxidant on DPPH radical scavenging was conceived to their hydrogen-donating ability (Chen et al., 2008; Zhang et al., 2011).

Significant correlation ($p < 0.05$) was found between antioxidant activity (determined by DPPH, and reducing power) and total polyphenol and flavone contents, indicating considerable contribution of these compounds to the total antioxidant activity observed for these plant extracts. This result is also supported by some previous reports (Malinowska, 2013; Jacobo-Velazquez and Cisneros-Zevallos, 2009). Thus, the extracts with the highest flavone contents were also proved to have the highest antioxidant activity, in both of the used methods. Therefore, by DPPH assay *Centarium umbellatum* ethanolic extract was found to have the highest DPPH radical scavenging activity value – 84.9% (at 3 mg/mL) and determinations developed on it also revealed the highest flavone content – $496.28 \pm 8.91 \mu\text{g RE/mL}$. In the case of *P. officinalis* ethanolic extract the DPPH radical scavenging activity value was found to be of 75.47% (at 3 mg/mL), which also showed a high content of flavones – $272.8 \pm 9.21 \mu\text{g RE/mL}$. The ethanolic plant extracts proved

an increased inhibition activity on the DPPH free radical, than the aqueous plant extracts. The DPPH radical scavenging activity percent decrease proportionally with the concentration of the analyzed sample (Fig. 1).

Flavonoid and phenolic acids have been intensively studied for their free radical scavenging and antioxidant properties. Flavonoids are reported to possess strong free radical scavenging activities based on their ability to act as hydrogen or electron donors and chelate transition metals (Le et al., 2007). Antioxidant properties of phenolic compounds are directly linked to their structure. Phenolics contain at least one aromatic ring bearing one or more hydroxyl groups and are therefore able to quench free radicals by forming resonance-stabilized phenoxyl radicals (Siler et al., 2014).

3.2.2. Reducing power activity

In this assay, the $\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}$ transformation in the presence of the extracts was assessed. The reducing power of extracts and ascorbic acid, used as reference compound, were assayed and the results are shown in Fig. 2. Aqueous extracts have showed lower reducing power activity than ascorbic acid. In our work a correlation between reducing power activity of extracts and polyphenol and flavone content of them can be observed as in other studies (Kim et al., 2008; Malheiro et al., 2012).

The obtained results for antioxidant activity determination by reducing power method are correlated with the results obtained by the DPPH method. Thus, most pronounced antioxidant activity was observed in the *C. umbellatum* ethanolic extract followed by *P. officinalis* ethanolic extract, as can be seen in Fig. 2. The other results demonstrated that *Centaurium erythraea* leaf extract exhibited high antioxidant capacity correlated with a high proanthocyanidin levels (Sefia et al., 2011).

3.3. HPLC analysis

The HPLC–MS method has been applied for the evaluation of polyphenolic profiles in the case of different plant extract samples. Using the SIM mode (selected ion monitoring) the corresponding peaks of gallic acid, chlorogenic acid, vanillic acid,

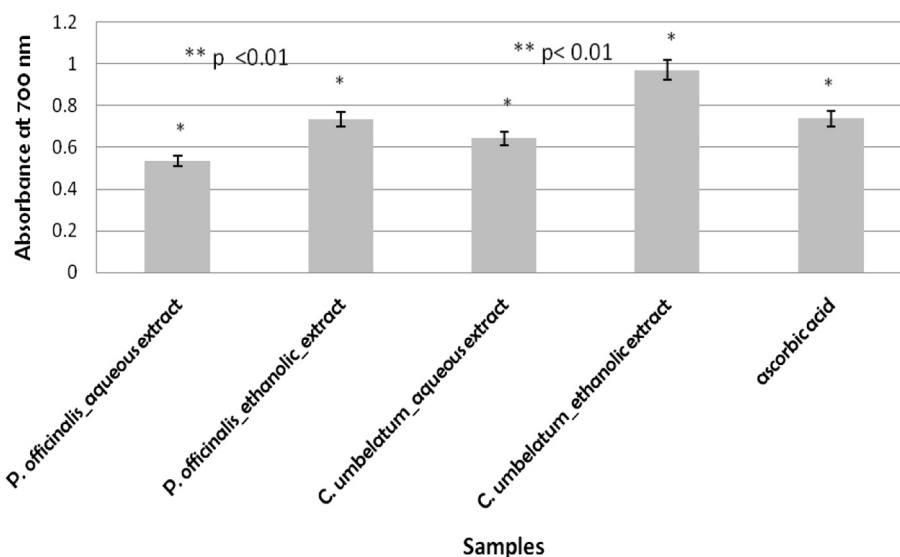


Figure 2 Reducing power of *Pulmonaria officinalis* and *Centarium umbellatum* and aqueous and ethanolic extracts * $p < 0.05$ compared polyphenol content of extracts with reducing power * $p < 0.05$ compared flavone content of extracts with reducing power ** $p < 0.01$ compared the activity of the ethanolic extracts with that of the aqueous extracts.

Table 2 HPLC–MS values of polyphenol compounds in plant extracts.

Compound [M/z] name	<i>Pulmonaria officinalis</i> L. water ($\mu\text{g/mL}$)	<i>Pulmonaria officinalis</i> L. 70% ethanol ($\mu\text{g/mL}$)	<i>Centarium umbellatum</i> water ($\mu\text{g/mL}$)	<i>Centarium umbellatum</i> 70% ethanol ($\mu\text{g/mL}$)
Quercetin 301	–	+	0.35	–
Rutin 609	0.20	8.76	11.08	14.09
Kaempferol 285	+	+	+	+
Isorhamnetin 315	–	+	+	+
Naringin 579	–	–	+	–
Hyperoside 463	0.54	3.97	0.66	2.26
Gallic acid 169	0.97	–	1.07	–
Syringic acid 197	–	–	45.96	16.11
p-Coumaric acid 163	0.46	–	0.95	–
Caffeic acid 179	12.94	3.36	8.03	+
Ferulic acid 193	+	–	0.31	–
Rosmarinic acid 359	41.94	124.59	2.99	2.71
Chlorogenic acid 353	3.53	3.03	11.99	12.53
Luteolin 285	–	+	+	+
Apigenin 269	–	+	+	+

– under the limit of detection; + under the limit of quantification.

syringic acid, caffeic acid, p-coumaric acid, rutin, sinapic acid, hyperoside, ferulic acid, naringin, hesperidin, rosmarinic acid, myricetin, luteolin, quercetin, apigenin, kaempferol and isorhamnetin fragment ions were obtained.

Under the optimum chromatographic conditions the compounds of interest were fairly resolved. Values for polyphenolic compounds in samples of plant extracts obtained with this method are shown in Table 2.

The *P. officinalis* ethanolic extract contains high concentrations of the rosmarinic acid (124.59 $\mu\text{g/mL}$), hyperoside and rutin and *P. officinalis* aqueous extract contains caffeic acid (12.94 $\mu\text{g/mL}$). The *C. umbellatum* aqueous extract contains high contents of syringic acid (45.96 $\mu\text{g/mL}$) and caffeic acid, whereas chlorogenic and rosmarinic acids are in similar

amounts in both extracts. Rutin and hyperoside were proved to be present in great amounts in *C. umbellatum* ethanolic extract.

The method was applied to *C. umbellatum* extracts, using the SCAN mode for the identification of polyphenols and erythrocentaurin, trihydroxyxanthone, 1,3,6-trihydroxy-5-methoxyxanthone, p-coumaroylquinic acid, oleanolic acid and sakuranin. Previous studies have identified a number of different xanthones and xanthone glycosides in the members of genus *Centarium* (Valentao et al., 2001; Ross et al., 2011). Phytochemical studies of centauries revealed the presence of phenolics (xanthones, phenolic acids and their derivatives) as main constituents (Valentao et al., 2001). The main phenolic compounds from *C. erythraea* were several esters of hydroxycin-

Table 3 Acetylcholinesterase inhibitory activities of extracts.

Sample		AChE inhibition %		
		3 mg/mL	1.5 mg/mL	0.75 mg/mL
Pulmonaria officinalis	Aqueous extract (10% mass)	72.24 ± 7.12 *	62.12 ± 6.23 *	43.61 ± 2.57 *
	70% ethanolic extract (10% mass)	87.72 ± 2.35 *	69.21 ± 3.56 *	50.70 ± 6.54 *
Centarium umbellatum	Aqueous extract (10% mass)	92.28 ± 7.24 *	70.21 ± 6.28 *	58.85 ± 6.89 *
	70% ethanolic extract (10% mass)	94.24 ± 6.35 *	72.36 ± 6.25 *	64.27 ± 5.59 *
Galanthamine		–	–	99.98 ± 2.02
Caffeic acid		–	62.67 ± 7.32	42.19 ± 6.57
Rosmarinic acid		–	52.89 ± 6.93	30.64 ± 5.46
Rutin		–	59.46 ± 3.65	31.23 ± 2.57
Chlorogenic acid		–	75.34 ± 2.39	54.11 ± 5.24

Data represent the mean ± SD of triplicate samples of three independent experiments.

* $p < 0.05$, compared the activity of the ethanolic extracts with that of the aqueous extracts.

Table 4 Tyrosinase inhibitory activities of extracts.

Sample		Tyrosinase inhibition %		
		3 mg/mL	1.5 mg/mL	0.75 mg/mL
Pulmonaria officinalis	Aqueous extract (10% mass)	56.96 ± 5.21 *	43.62 ± 6.23 *	30.56 ± 6.56 *
	70% ethanolic extract (10% mass)	71.69 ± 8.23 *	59.36 ± 3.56 *	46.52 ± 3.23 *
Centarium umbellatum	Aqueous extract (10% mass)	69.05 ± 6.63 *	46.21 ± 6.28 *	38.76 ± 5.67 *
	70% ethanolic extract (10% mass)	74.39 ± 7.89 *	65.36 ± 3.64 *	54.36 ± 4.36 *
Kojic acid		98.7 ± 4.23	94.2 ± 2.87	89.7 ± 5.24
Ellagic acid		–	45.77 ± 1.71	25.42 ± 1.52
Quercetin		–	62.60 ± 3.65	33.45 ± 2.59
Chlorogenic acid		–	58.09 ± 2.31	41.45 ± 2.83
Rutin		–	47.23 ± 6.54	32.30 ± 1.37

Data represent the mean ± SD of triplicate samples of three independent experiments.

* $p < 0.05$, compared the activity of the ethanolic extracts with that of the aqueous extracts.

namic acids, namely p-coumaric, ferulic and sinapic acids (Valentao et al., 2001), while the phenolic compounds developed in *P. officinalis* extracts were quercetin and kaempferol (Malinowska, 2013).

3.4. Acetylcholinesterase and tyrosinase inhibition activity

Herbal medicines have been used for improving cognitive functions and for the treatment of memory loss. Our study investigates enzyme inhibitory potential of two medicinal plants from Romania – *P. officinalis* and *C. umbellatum* – for which there are no data in the literature at present. The obtained results regarding the acetylcholinesterase and tyrosinase inhibitory activity of these plant aqueous and ethanolic extracts are presented in the Tables 3 and 4. The results are similar for the two tested plant extracts. The ethanolic extracts exhibit acetylcholinesterase and tyrosinase inhibitory activity more pronounced than the aqueous extracts, the inhibition values being >70% for the samples' highest concentration (3 mg/mL). The highest inhibition value was obtained for the *C. umbellatum* ethanolic extract (94.24% – AChE inhibition and 74.39% – tyrosinase inhibition) and the highest content of flavones $496.28 \pm 8.91 \mu\text{g RE/mL}$ was also determined in the same extract. The highest inhibition of the ethanolic extract could be due to the presence of high contents of flavone.

Present in large amounts in the two studied plants flavonoids have miscellaneous favorable, biochemical, antioxidant and anti-inflammatory effects, anti-cancer, anti-genotoxic activity as well (Castañeda-Ovando et al., 2009). There are few reports on acetylcholinesterase/butyrylcholinesterase inhibitory activity of flavonoids, assigned as the main strategy for the treatment of Alzheimer's disease. On the other hand, flavonoids as polyphenolic substances have been known for their strong antioxidant activity, a major factor for the AD treatment (Tareq et al., 2009).

Polyphenols are responsible for reducing the incidence of certain age related neurological disorders including macular degeneration and dementia (Bastianetto et al., 2000). Rosmarinic acid present in high amounts in *P. officinalis* extracts and in lower amounts in *C. umbellatum* extracts, explains their antioxidant activity and acetylcholinesterase inhibition activity (Fale et al., 2009). Present in large amounts in aqueous extracts from both studied plants caffeic acid acts as an antioxidant, anti-inflammatory, antitumor and anti-metastatic agent (Chung et al., 2004). Chlorogenic acid and rutin, both identified in *C. umbellatum* extracts, develop anti-oxidative activities associated with free radical scavenging (Almeida et al., 2009). Syringic acid – present in *C. umbellatum* extracts – has strong antioxidant and anti-inflammatory effects (Levites et al., 2001), and it was found to exert neuroprotective effect by

inhibiting oxidative stress. Thus syringic acid improves behavioral dysfunctions in a mouse model of Parkinson disease (Rekha et al., 2014).

4. Conclusions

In this study two medicinal plants from Romania, *P. officinalis* and *C. umbellatum* were evaluated – for first time – for their acetylcholinesterase and tyrosinase inhibitory effects, as well as for their antioxidant activity. The phytochemical analysis of extracts has showed high contents of biologically active compounds: polyphenols, flavones, proanthocyanidins, compounds that give antioxidant and antiradical properties of plants and probably, potential inhibitor on enzymes studied. So, the *C. umbellatum* extracts showed the high acetylcholinesterase inhibitory effects – 94.24%, high tyrosinase inhibitory effects – 94.03%, and high antioxidant activities 84.9% DPPH radical scavenging activity, as well as high flavone content. The *P. officinalis* extracts showed slightly lower acetylcholinesterase inhibitory effects – 87.7%, tyrosinase inhibitory effects – 73.69%, antioxidant activities – 75.47% DPPH radical scavenging activity, also corresponding to lower flavone content. Based on these presented data, our study suggests that these medicinal plants are promising candidates useful for the treatment of neurodegenerative diseases such as dementia, Alzheimer disease and Parkinson disease. The two tested medicinal plants are great sources of natural antioxidants for food industry.

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