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ORIGINAL ARTICLE

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

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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.

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 ⩾99%, Folin–Ciocalteu's reagent, potassium ferricyanide ⩾99%, trichloroacetic acid ⩾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) ⩾99%, acetylthiocholine iodide (AChl) ⩾99%, 3-(3,4-Dihydroxyphenyl)-L-alanine (L-DOPA) ⩾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.

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