

BIOACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITY OF PRIMULA VERIS L. FLOWER EXTRACTS

***Jurca Tünde, Marian Eleonora, Vicas Laura, Oana Neagu, Pallag Annamária**

*University of Oradea, Faculty of Medicine and Pharmacy, Pta 1 Decembrie Oradea,
Romania, e-mail:jurcatunde@yahoo.com

Abstract

*The present study was designed to investigate the antioxidant activity of the alcoholic extract of *Primula veris* L. flowers. The alcoholic extract was evaluated by Folin Ciocalteu method for determining the total polyphenol content and the total flavonoids. The antioxidant activity of alcoholic extracts was determined by DPPH free radical scavenging activity and Cuprac assay.*

Key words: *Primula veris* L., Antioxidant, Folin Ciocalteu, Cuprac, DPPH

INTRODUCTION

Many ornamental flowers are natural sources of very important bioactive compounds with benefit to the human health. In addition, their possible role as dietary components has been reported. Most antioxidants are flower pigments such as carotenoids and polyphenols, often present in higher concentration than in most common fruits and vegetables. The antioxidant content changes during the development and during senescence many biochemical systems and, what is more, molecular mechanisms are activated to counteract the increase of reactive oxygen species and free radicals.

The source of nutraceutical compounds in human diet is almost exclusively provided by fruits and vegetables. However, flowers are becoming important sources of several bioactive compounds that can be added in the diet as food. In ancient times, flowers were mainly eaten for their medicinal properties rather than their nutritional value. Nowadays, several metabolomics studies reveal the chemical compositions of wild and ornamental flowers, showing the presence of important bioactive molecules. Often, wild flowers represent low cost sources of important natural antioxidants. However, edible flowers are also used by chefs to add color, fragrance and flavor to foods or drinks (Cavaiuolo M., G. Cocetta, 2013).

Primula is a plant genus included about 400 species. Some of them are popular garden plants because of their colourful blossoms. Efficacy of primrose extracts, which are rich in saponins, have been demonstrated in a number of pharmacological studies, therefore having potent anti-asthmatic, anti-inflammatory and anti-viral properties. Phenolic glycosides and saponins are characteristic compounds for the genus Primula (Muller A., M. Ganzera et al., 2005). Ten lipophilic flavones were isolated from Primula veris L. in vitro cultures (Budzyanwski J., M. et al., 2005). Two new flavonol glycosides have been identified and isolated from Italian Primula species (Fico G., G. Rodondy, G. Flamyny, et al., 2007). Primula species can also contain allergens and some species are used traditionally to treat epilepsy and convulsions (Jager A.K., B. Gaugynb, A. Et al., 2006).

MATERIAL AND METHOD

Reagents

All reagent used were analytical grade purity. High quality water, obtained using a Milli-Q system (Millipore, Bedford, MA, USA), was used exclusively. 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), potassium persulfate, Folin-Ciocalteu's reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), neocupreine (CUPRAC) were purchased from Sigma Aldrich. Gallic acid and sodium carbonate were purchased from Fluka (Switzerland). All the chemicals were of analytical grade.

Extract preparation

Aliquots of flowers after dried (10 g and 20 g) were extracted with 70% aqueous ethanol (100 mL) using a magnetic mixer for 45, 45 and 20 min and sonicated for 5 min. The extract was filtered through Whatmann filter paper No.1.

1.Total phenolic content (TPLC) was determined by the FolinCiocalteu method by Folin *et al* 1927. Using Folin-Ciocalteu method the OH groups can be evaluated from the studied sample, in an alkaline medium (adjusted with sodium carbonate). The absorbance at the wavelength of 765 nm, increases proportionally with the number of OH groups of the anthocyanins. The total polyphenolic content was expressed as gallic acid equivalents. To 0.5 ml of 1-5 mg/ml of herbal preparation made up with 0.5 ml of distilled water, 0.5 ml of FolinCiocalteu reagent was added and gently mixed. After 2 minutes 0.5 ml of 100mg/ml sodium carbonate was added. The contents were mixed and allowed to stand for 2 hours. The optical density of the blue coloured samples was measured at 765 nm spectrophotometrically. Standard gallic acid of concentration 100-

500µg/ml was used. The concentration of total phenolics is expressed as milligram of gallic acid /g of mixture.

2. The total flavonoid content (TFC) was determined using a previously described colorimetric method (Kim D.O., O.K. Chun, et al., 2003). 1 ml sample (containing 0.1 mg/ml dry substance) was mixed with 4 ml water and inserted in a 10 ml volumetric flask. Firstly, 3ml 5% NaNO₂ solution where added, after 5 minutes 0.3 ml 10% AlCl₃ and after 6 minutes 2ml 1M NaOH. The flask was filled up to its calibration mark with distilled water. The solution was mixed and its absorbance was detected at 510 nm

3. Method for DPPH radical scavenging assay: Radical scavenging activity of plant extracts against stable 2,2 diphenyl-2-picryl-hydrazyl hydrate (DPPH) was determined by the slightly modified method of Brand-Williams *et al* 1995. DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The change in colour (from deep violet to light yellow) was measured at 517 nm on a UV visible light spectrophotometer. The solution of DPPH in methanol 6×10^{-5} M was prepared fresh daily before UV measurements. Three ml of this solution was mixed with 100 microgram/ml concentration of individual plant extracts as well as herbal preparation. The samples were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured.

4. Cupric ions (Cu²⁺) reducing-Cuprac assay

In order to determine the cupric ions (Cu²⁺) reducing antioxidant capacity the method proposed by Apak et al. (2006) and Karaman et al. (2010) was used with slight modifications. To this end, 0.25 mL CuCl₂ solution (0.01 M), 0.25 mL ethanolic neocuproine solution (7.5×10^{-3} M) and 0.25 mL CH₃COONH₄ buffer solution (1 M) were added to a test tube, followed by mixing with the plants extracts. Then, total volume was adjusted to 2 mL with distilled water, and thoroughly mixed. The tubes were stoppered and kept at room temperature. Absorbance was measured at 450 nm against a reagent blank 30 min later. Increased absorbance of the reaction mixture indicates increased reduction capability (Talaz O., I. Gulcin, et al., 2009).

RESULTS AND DISSCUSIONS

The results of total phenolic and flavonoid contents of studied plants are shown in Table 1.

Table.1. Total polyphenolic and flavonoid content in alcoholic extracts

Table 1

| Sample | Total polyphenolic content (mg GAE/100DW) | Total flavonoid content mg QE/100 DW |
|--------------------------------|---|--------------------------------------|
| Extract 10% of Primula flowers | 535.41±0.01 ^c | 15.31±7.61 ^e |
| Extract 20% of Primula flowers | 613.55 ±0.02 ^e | 17.65±8.72 ^c |

Folin-Ciocalteu method estimates the total content of all phenolics present in the analyzed fruits, including flavonoids, anthocyanins and non-flavonoid phenolic compounds. Gallic acid was used as calibration standard (Fig. 1) and the results (expressed as gallic acid equivalents) were expressed as means±standard deviation of triplicate analysis.

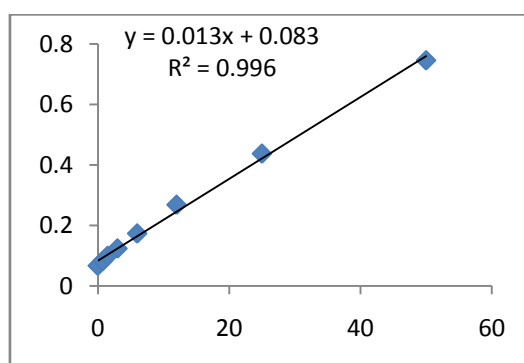


Figure 1. Calibration standard of gallic acid

The antioxidant capacity of samples, measured by different complementary assays are shown in Table 2.

The total phenolic (TPC) values for *Primula veris* L. extract ranged from 535.41 to 613.55 mg GAE/ 100 g FW which are in agreement with values reported previously in literature (Poracova J., M. Zahatnanska, et al., 2009). The total flavonoid content of the extracts was determined using aluminium chloride colorimetric method and the results are shown in Table 1. The total flavonoid content ranged from 15.331 mg QE/100 g for 10% extract to

17.65 mg QE/100 g for 20% extract. Quercetine (QE) was used as calibration standard (Fig. 2).

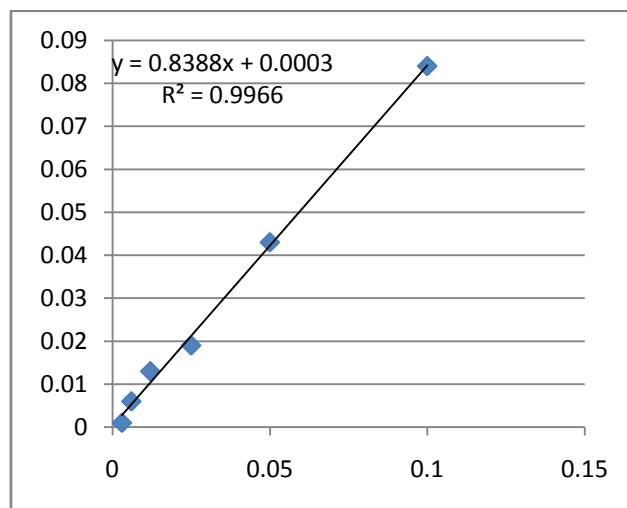


Figure 2. Calibration standard of quercetine

The results of the antioxidant capacity of samples are shown in Table 2.

Table 2. Antioxidant capacity of samples
Table 2

| Sample | DPPH% | Cuprac ($\mu\text{mol Trolox}$ equivalent/g DW) |
|-----------------------------------|---------------------------------|--|
| Extract 10% of Primula flowers | 86.65 \pm 1.11 ^{c,d} | 3.22 \pm 0.98 ^a |
| Extract 20% of Primula flowers | 88.46 \pm 0.11 ^{c,d} | 3.08 \pm 0.10 ^d |

DPPH is a purple stable free radical at room temperature with characteristic absorbance at 517 nm. The nitrogen free radical of DPPH is easily quenched by an antioxidant to yellow coloured complex (1,1-diphenyl-2-picrylhydrazine). The decolourization of purple colour is stoichiometric depending on the number of electrons gained (Soares J. R., T.C.P. Dinis, et al., 1997). Primula veris flower extracts showed significant scavenging effect on DPPH free radical in concentration dependent manner.

The main advantage of Cuprac method compared with other assay is its favorable redox potential, flexibility and applicability to both hydrophilic and lipophilic antioxidants.

The reaction pH is closed to physiological pH 7 and the reaction assay involves a faster kinetics. The ability of analyzed extracts to reduce cupric ion (Cu^{2+}) is shown in Table 2.

The Cuprac values for the *Primula veris* L. extracts were within 3.22-3.06 $\mu\text{mol TE/g}$. There is no data available on the antioxidant activity of *Primula veris* L. extracts assayed by Cuprac method.

CONCLUSIONS

These results suggest that the ethanolic extract of *Primula veris* L. plays an important role in the intonation of oxidative stress. As this analysis is a groundwork study, a detailed one is needed in order to provide more information on the active principles responsible for their pharmacological properties and may also lead to the development of novel drugs which may act as possible antioxidants for biological systems susceptible to free radical-mediated reactions.

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