A COMPARATIVE STUDY OF THE AMOUNT OF POLYPHENOLS, FLAVONOIDS AND ANTIOXIDANT CAPACITY OF ROSAE CANINAE FLOS VERSUS CYNOSBATI FRUCTUS

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Abstract

Polyphenols constitute one of the most numerous, natural and secondary metabolic substances in plants that have received much attention since being reported to have a positive influence on human heath due to their multiple activities that were associated with a decrease in the incidence of various diseases like cancer, stroke, cardiovascular disease, diabetes. A considerable interest has been developed over the years in Cynosbati fructus due to their potential biological and health promoting effect, but the chemical compounds and antioxidant activity of Rosae caninae flos have not been compared in literature.

The purpose of the present study was to investigate the comparative content of phenolics components of Rosae caninae flos and Cynosbati fructus as well as their antioxidant capacity using different alcoholic extracts which were evaluated by Folin Ciocalteu method for determining the total polyphenol content and the total flavonoids. Using the reducing-Cuprac assay and FRAP (ferric reducing antioxidant power) method was determined the antioxidant activity of alcoholic extracts. The highest amount of polyphenols was found in Rosae caninae flos and an appreciable amouunt of flavonoids was found in the dried fruits. The amount of polyphenols determined in flowers ranged to 618.07 mg/mL and a rich source of flavonoids(5.75 mg/mL) was found in the fruits using the methanolic extract.

Key words: Polyphenols, antioxidant capacity, Folin Ciocalteu, flavonoids, Cuprac, FRAP

INTRODUCTION

Rosa canina L. is a perrenial plant that belongs to the Rosaceae family. It's a widespread plant in Europe, northwest Africa, and western Asia with great importance in herbal medicine. *Cynosbati fructus* are a valuable source for food and pharmaceutical industry that contain a wide variety of biologically and physiologically active ingredients, such as vitamins (C, B, P, PP, E, K), flavonoids, carotenes, carbohydrates (mono- and oligosaccharides), organic acids (tartaric, citric), trace elements and others (Mihaylova et al., 2015, Ognyanov et al., 2014).

It was found that juice and aqueous extracts from Cynosbati fructus possessed exceptional antioxidant activity (Demir et al., 2001). This makes them suitable for use both in the fresh or dry state, or in the form of extracts in food products and cosmetics (Mihaylova et al., 2015, Ognyanov et al., 2014). The quality of natural extracts and antioxidant properties depends not only on the nature of the plant source, geographical origin, weather conditions, time of harvesting and storage, but also on the method of

extraction and the used solvent (Ghazghazi H. et al., 2010, Hagerman et al., 1998).

There is an interest in the association between fruit and vegetable consumption and human health. Because oxidative stress plays a significant role in most disease processes and aging, the potential health benefits of fruits and vegetables have been largely attributed to their potential antioxidant capacity (Kalt. et al., 1999).

There are many literatures that aimed to investigate the health benefit effects, composition (Koca I. et al., 2009) antioxidant activity (Demir. et al., 2001) and the utilization (Spiro. and Chen, 1993) of Cynosbati fructus.

Previous studies have demonstrated that polyphenols have a strong activity against several types of tumour cells, including colon and breast cancer (Devi et al., 2014), leukaemia (Maurya et al., 2011), lung (Niu, 2011), cervical cancer cell lines (Zhao. et al., 2012).

Flavonoids have also an antioxidant and anticancer effect, being therefore reported as efficient free radical scavengers. Therefore, flavonoids are capable of preventing cancer induced by oxidative stress (Baghel et al., 2012).

At present, the literature data may be of limited use for the studies of the extract of Rosae caninae flos because few researches have been carried out into the polyphenols, flavonoids compounds and antioxidant capacity of the flowers.

The further purpose of the current study was to compare and characterize the extracts from Cynosbati fructus and Rosae caninae flos and to evaluate antioxidant potential and polyphenolic content of methanolic and ethanolic extracts obtained from wild growing Romanian Rosae caninae plants.

MATERIAL AND METHOD

Reagents

All chemicals and reagents used on this study were of analytical grade. High quality water, obtained using a Milli-Q system (Millipore, Bedford, MA, USA) was used exclusively. The reagents: Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylicacid), neocupreine, potassium persulfate, Folin-Ciocalteau's reagent, ferric tripyridyltriazine complex [Fe(III)-TPTZ], were purchased from Sigma Aldrich. Sodium carbonate and gallic acid were purchased from Fluka (Switzerland).

Extract preparation

Fresh Cynosbati fructus and Rosae caninae flos were cultivated and colected from Bihor county, Romania, from unpolluted areas. The fruits and the flowers were stored at room temperature until further use.

10 grams of the dried Rosae caninae flos and Cynosbati fructus were extracted separately with 100 mL of two different extracting solvents: methanol 70% and ethanol 70% using a magnetic mixer for 45 and 20 minutes and sonicated for 5 minutes. The residue was removed by decantation and filtered through a Whatmann filter paper No.1 and the resulted filtrate was made off up to 100 mL with the same extracting solvent.

Total phenolic content

Using the alcoholic extract solutions (10%) were determined the total phenolic compounds with the Folin-Ciocalteu reagent. Through this method in alkaline medium (adjusting the pH with sodium carbonate), we measured the number of OH groups of the samples taken into study. The absorbance at the 765 nm wavelength increases proportional with the number of OH groups of the anthocyanins. For the calibration curve it was used standard gallic acid, the correlation coefficient was $R^2 = 0.9963$ and the regression equation ($y = 0.0135x + 0.0832$), where y represents the absorbance detected at 765 nm. The total phenolic compounds (x) was expressed as mg of gallic acid equivalent per mL of extract solution. The volume of 0.5 mL of each extract solutions was mixed with 0.5 mL of Folin-Ciocalteu reagent previously diluted with distilled water. A volume of 0.5 mL of 100 mg/mL sodium carbonate solution was added to the mixture after two minutes, shaken throughly and allowed to stand for 2 hours. (Jurca T. et al., 2015). When the blue colour formed the absorbance was measured at 765 nm in Shimadzu UV-1700 Pharmaspec UV-Vis Spectrophotometer.

Total flavonoid content (TFC)

A colorimetric method (Zhishen et al., 1999), with minor modifications was used for determination the TFC. 1 mL of sample was placed in a 10 mL volumetric flask, then 4 mL of H_2O and after 5 min, 0.3 mL of $NaNO_2$ (5%) and 1.5 mL of $AlCl_3$ (10%) were added. The mixture was shaken and 6 min later 2 mL of 1 M solution of NaOH were added, again well shaken. The absorbance was measured at 510 nm against the blank. The same procedure was also applied to the standard solutions of quercetine, and a standard curve was obtained having the correlation

coefficient $R^2 = 0.9966$ and the regression equation ($y = 0.8388x + 0.0003$), where y represents the absorbance detected at 510 nm and x represents mg QE/mL.

Antioxidant capacity

FRAP (ferric reducing antioxidant power)

Using this simple spectrophotometric method, the antioxidant power of the studied samples was tested, being based on the reduction of ferric tripyridyltriazine complex [Fe(III)-TPTZ] by a reductant, at an acid pH.

As a standard solution was used Trolox, the calibration curve was made for concentrations between 0-300 μM, having the correlation coefficient $R^2 = 0.9956$ and the regression equation ($y = 0.0017x + 0.0848$), where y represents the absorbance detected at 595 nm and x represents μmol Trolox equivalents (TE)/ 100 μL extract.

The stock solutions included: 300 mM acetate buffer; 270 mg $FeCl_3 \cdot 6\ H_2O$ dissolved in 50 mL distillated water; 150 mg TPTZ and 150 μL HCl, dissolved in 50 mL distillated water. The working FRAP solution was freshly prepared by mixing 50 mL acetate buffer, 5 mL $FeCl_3 \cdot 6H_2O$ solution and 5 mL TPTZ solution (Jurca T. et al., 2016, Marian E. et al., 2011, Pallag A. Et al., 2018).

CUPRAC assay (Cupric Ions, Cu^{2+} Reducing Power)

In order to determine the cupric ions (Cu^{2+}) reducing antioxidant capacity the method proposed by Karaman et al. (2010) was used with slight modifications. The reaction was started by mixing of 0.25 mL $CuCl_2$ solution (0.01 M), 0.25 mL ethanolic neocupreine solution ($7.5x10^{-3}$ M) and 0.25 mL CH_3COONH_4 buffer solution (1 M), followed by mixing with the plants extracts. The 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 (Kamaran S. et al., 2010, Yuan W. et al., 2011, Talaz O. et al., 2009).

The results were expressed as mM Trolox equivalent, according to calibration curve, build in range of 0-300 μM Trolox, having the correlation coefficient $R^2 = 0.9935$ and the regression equation $y = 0.006x$, where y represents the absorbance detected at 450 nm.

RESULTS AND DISCUSSION

In table 1 are shown the results of total phenolic and flavonoid contents of studied plants.

Table 1

Total polyphenolic and flavonoid content in alcoholic extracts

	Methanolic flowers extract	Methanolic fruits extract	Ethanolic flowers extract	Ethanolic fruits extract
Polyphenols (GAE mg/mL)	618.07±0.02	499.23±0.05	515.76±0.02	525.76±0.05
Flavonoids (QE mg/mL)	2.14±0.03	5.75±0.03	2.37±0.03	1.39±0.03

The total amount of polyphenols, determined by the Folin-Ciocalteu method, is the highest in Rosae caninae flos using the methanolic extract, followed by ethanolic fruits extract, but as it can be seen in table 1 and figure 1 but we also obtained high values in the other cases. Gallic acid was used as calibration standard.

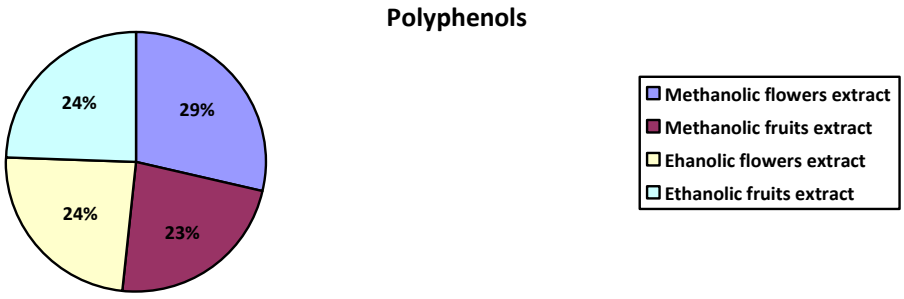

Fig. 1. The total amount of polyphenols

The highest amount of total flavonoids was obtained in case of methanolic fruits extracts that ranged to 5.756 mg QE/100 mL (table 1), followed by ethanolic flowers extract (2.372 mg QE/mL) using aluminium chloride colorimetric method. Quercetine was used as a calibration standard.

Antioxidant capacity

Table 2

The antioxidant capacity of samples

	Methanolic flowers extract	Methanolic fruits extract	Ethanolic flowers extract	Ethanolic fruits extract
FRAP (mg TE/mL)	232.52±0.15	71.45±0.15	164.30±0.15	95.23±0.15
CUPRAC (mMTrolox)	2.89±0.03	1.88±0.02	2.75±0.03	0.82±0.02

Following the antioxidant capacity, the results by FRAP and Cuprac methods of determining the antioxidant capacity, showed that the methanolic extracts obtained from the studied samples, have shown a high capacity of reducing FRAP and CUPRAC radicals, as are presented in table 2. Rosae caninae methanolic flower extracts showed significant scavening effect on FRAP radical. The results by FRAP method are shown in figure 2.

Fig. 2. The antioxidant capacity using the FRAP method

CONCLUSIONS

These results show that Rosae caninae flos are rich sources of phenolic compounds, phenolic acids, with antioxidant capacity and reducing power. As it can be seen in the presented results the flowers have a higher composition of polyphenols and a higher antioxidant capacity than Cynosbati fructus. Most of studies are dealing with the chemical composition of Cynosbati fructus, but no detailed study concerning the variation of chemical composition and antioxidant power of Rosae caninae flos has been dealt before.

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