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DETERMINATION OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACTS OF SELECTED MEDICINAL PLANTS

Određivanje ukupnih fenola i antioksidacijske aktivnosti vodenih ekstrakata odabranih ljekovitih biljaka

Sabina Gojak-Salimović, Emina Šljivo, Danijela Vidic, Dušan Čulum[1]

Abstract

This study aimed to analyse and compare the total phenolic content and the antioxidant activity of aqueous extracts of three medicinal plants: hibiscus (*Hibiscus* spp.), rosemary (*Rosmarinus* spp.) and savory (*Satureja* spp.). The plant extracts were prepared using maceration process. The total phenolic content was determined spectrophotometrically with the Folin-Ciocalteu reagent using gallic acid as a standard and the antioxidant activity was analysed using the Briggs-Rauscher reaction method. The inhibition times produced by plant extracts on an active Briggs-Rauscher oscillating reaction mixture were recorded potentiometrically. The total phenolic content was found 0.27, 1.29 and 1.43 mgGAE/g of dry extract for hibiscus, rosemary, and savory, respectively. Good correlation was found between the total phenolic content and the Briggs-Rauscher inhibition times ($R^2 = 0.994$).

Keywords: *plant extract, total phenolic content, antioxidant activity, Briggs-Rauscher reaction*

INTRODUCTION – *Uvod*

Hibiscus is flowering plant species from Malvaceae family, and is cultivated as a decorative plant. The natural habitat of hibiscus is warm and humid, subtropical and tropical regions. Various parts of the hibiscus plant are used for the preparation of a herbal drink, cold and warm beverages, spices, jams, jellies, puddings, ice creams, cakes and other desserts. The flowers and fleshly fruit are used in pharmacy and cosmetics. Hibiscus-based products are used in popular medicine to relieve symptoms of bronchitis and coughs, and to prevent cardiovascular and hepatic diseases. The hibiscus petals are potentially a good source of antioxidant agents as anthocyanins, flavonoids and ascorbic acid (PRENESTI et al, 2007).

Rosemary is evergreen perennial aromatic plant species from Lamiaceae family that is native to the Mediterranean region and is a common household plant. Various

[1] Faculty of Science, University of Sarajevo, Bosnia and Herzegovina

part of the rosemary plants are used as a spice and flavouring agent in food processing, an ingredient in cosmetics and in traditional medicine. They have antioxidant, diuretic, anti-inflammatory, antimicrobial, anticarcinogenic, hypoglycemic and hypolipidimic activities (LABBAN et al, 2014; SRIMANTI and ASHA, 2017).

Savory is an annual aromatic plant species from Lamiaceae family that is native to south Europe. The leaves, flowers and stems of savory are used as additives in commercial spice mixtures for many foods and in the traditional medicine to treat various ailments as cramps, muscular pains, nausea, indigestion, diarrhoea, and infection diseases. They have antimicrobial, antioxidant, sedative, antispasmotic and antidiarrheal properties (GONTARU et al, 2008).

The principle of the Briggs-Rauscher reaction method is previously described by CERVELLATY et al (2001). The addition of antioxidant to an active oscillating Briggs-Rauscher mixture should quench the oscillations, with an inhibition time depending on the type and concentration of the antioxidant added. After inhibition time, the oscillatory behaviour is regenerated with amplitude, frequency, and duration different from those observed in a reference mixture. pH of the Briggs-Rauscher reaction mixture is about 2 what is similar to pH of the digestive fluids. Therefore, the Briggs-Rauscher reaction method is very important for the assessment of *in vivo* effects of digested antioxidant. This method has been used to test antioxidant activity of various wines (HÖNER et al, 2002; GAJDOŠ KLJUSURIĆ et al, 2005; MARKOVIĆ and TALIĆ, 2013), fruits and vegetables (HÖNER and CERVELLATI, 2002), medicinal plants (PRENESTI et al, 2007; DALL′ACQUA et al, 2008; GENERALIĆ et al, 2011), tea infusions (MUNTEAN and SZABÓ, 2013) and natural polyphenolic compounds (CERVELLATI et al, 2002; MILOS and MAKOTA, 2012; LI et al, 2016).

In this study, the total phenolic content and antioxidant activity in aqueous extracts of three medicinal plants: hibiscus (*Hibiscus* spp.), rosemary (*Rosmarinus* spp.) and savory (*Satureja* spp.) were investigated using Folin-Ciocalteus method and the Briggs-Rauscher reaction method.

EXPERIMENTAL – *Eksperimentalni dio*

Chemicals – *Hemikalije*

All chemicals were of analytical grade. Potassium iodate, sulfuric acid, hydrogen peroxide, ethanol, sodium carbonate and Folin-Ciocalteu reagent were obtained from Semikem (BiH), malonic acid, manganese(II) sulfate monohydrate and starch were obtained from Merck (Germany) and gallic acid was obtained from Sigma-Aldrich (USA).

Preparation of the plants extracts – *Priprema biljnih ekstrakata*

Plant material of hibiscus and savory were collected in Sarajevo (Bosnia and Herzegovina), and rosemary was collected in Split (Croatia). The plant material was air-dried for 20 days and stored at ambient temperature without exposure to direct sunlight. Air dried and powdered plants (1 g) were extracted with 30 mL boiled

distilled water, and left to stand for 24 hours at room temperature. Each extract was filtered and filled up with distilled water the calibrated volume flask (50 mL). The stock solutions obtained were stored in the fridge at 4°C until analysis.

Determination of the total phenolic content – *Određivanje ukupnih fenola*

The total phenolic content of the examined plant extracts was determined using a slightly modified method presented by SINGLETON and ROSSI (1965). A 4.0 mL of sample diluted with distilled water and mixed with 500 μL of Folin-Ciocalteu reagent. After 10 minutes, 1.5 mL of 20% solution of sodium carbonate was added, and diluted with distilled water to 10 mL. Prepared samples were kept for 2 h at room temperature, and the absorbance was measured at 765 nm. The data were calculated according to a standard curve of gallic acid (2.5-10 μg/mL), and they were expressed as mg gallic acid equivalents (GAE) per gram of dry extracts.

Determination of the antioxidant activity – *Određivanje antioksidacijske aktivnosti*

The antioxidant activity of the examined plant extracts was determined using the Briggs-Rauscher method (CERVELLATY et al, 2001). Oscillations of the Briggs-Rauscher reaction mixture were followed potentiometrically by recording the potential of a platinum electrode and Ag/AgCl/$KCl_{(sat)}$ reference electrode. All measurements were conducted at temperature 25±0.5°C.

The Briggs-Rauscher reaction mixture was prepared by mixing the appropriate amounts of stock solutions (A, B and C). Solution A: 43 g potassium iodate and 4.5 mL 96% sulfuric acid were dissolved in distilled water and diluted to 1 L; Solution B: 15.6 g malonic acid, 3.4 g manganese(II) sulfate monohydrate and 3 g starch were dissolved in distilled water and diluted to 1 L; Solution C: 500 mL of 30% hydrogen peroxide was diluted with distilled water to 1 L. For each measurement 10 mL of solution A and B were mixed into the double-wall thermostated reaction vessel equipped with a magnetic stirring bar and placed on a plate of magnetic stirrer. The 10 mL of solution C was used to initiate the oscillations.

After the third oscillation, 1 mL of solution of gallic acid (0.3-6 mg/mL) or a suitably diluted plant extract was added to 30 mL of an active Briggs-Rauscher reaction mixture. The inhibition time was measured before a regenerated appearance of the first peak. Typical potentiometric recordings for a non-inhibited and an inhibited oscillating Briggs-Rauscher reaction mixture were shown in our previous work (NEFIĆ and GOJAK-SALIMOVIĆ, 2017). The addition of 1 mL distilled water, without antioxidant, does not interrupt the oscillation of the Briggs-Rauscher reaction mixture. The pH value of the Briggs-Rauscher reaction mixture was 1.56.

RESULTS AND DISCUSSION – *Rezultati i diskusija*

Phenolic compounds are the most commonly studied class of secondary metabolites of various plants, because of their pharmacological properties. Due to chemical complexity of plant extract, various antioxidant methods have been used to evaluate antioxidant behaviour.

The total phenolic content was measured by Folin-Ciocalteu assay and expressed as mg gallic acid equivalents per gram of dry extract (mgGAE/g).

Addition of a diluted extract (1/10 v/v) of hibiscus, rosemary or savory in the active Briggs-Rauscher reaction mixture causes an immediate effect of quenching of oscillations and after period of inhibition oscillations starts again because the reaction produces hydroperoxyl radicals that are quenched by antioxidants. The total antioxidant activity is measured as an inhibition time (t_{inhib}) of the Briggs-Rauscher oscillating reaction and the relative Briggs-Rauscher antioxidant index (BRAI) which is expressed as mg gallic acid equivalents per gram of dry extract (mgGAE/g).

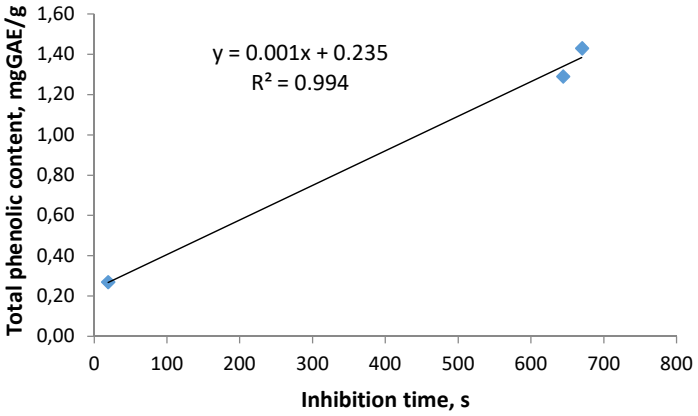
The results of the total phenolic content, inhibition time of the Briggs-Rauscher reaction and BRAI values for examined extracts are presented in table 1.

Table 1. The total phenolic content, inhibition time and BRAI values for examined extracts
Tabela 1. Sadržaj ukupnih fenola, vrijeme inhibicije i BRAI vrijednosti za ispitivane ekstrakte

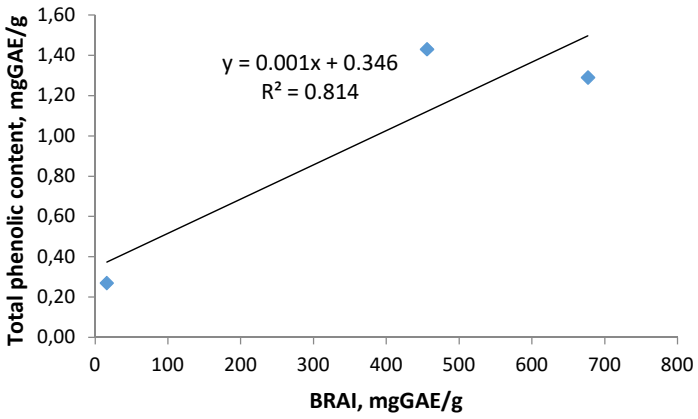
Extract	**Total phenolic content (mgGAE/g)**	**t_{inhib} (s)**	**BRAI (mgGAE/g)**
Hibiscus	0.27	19	16.3
Rosemary	1.29	644	456
Savory	1.43	670	677

Content of phenolic compounds as well as the antioxidant activity decreased in the following order: savory extract > rosemary extract > hibiscus extract. The extract for savory showed the best ability to inhibit oscillations, i.e. the highest antioxidant activity comparing to all other extracts.

Figure 1a) shows the relationship between the total phenolic content and the Briggs-Rauscher inhibition times and figure 1b) shows the relationship between the total phenolic content and the BRAI values.



a)



b)

Figure 1. a) Relationship between the total phenolic content and the Briggs-Rauscher inhibition times, b) Relationship between the total phenolic content and the BRAI values
Slika 1. a) Odnos između sadržaja ukupnih fenola i vremena inhibicije Briggs-Rauscher reakcije, b) Odnos između sadržaja ukupnih fenola i BRAI vrijednosti

Good correlation was found between the total phenolic content and the Briggs-Rauscher inhibition times (R^2 = 0.994). It could be concluded that phenolic compounds have a great influence on antioxidant activity measured by the Briggs-Rauscher reaction method. Comparing the total phenolic content and the relative Briggs-Rauscher antioxidant index correlation was lower (R^2 = 0.814).

Althought the antioxidant activity of extracts of hibiscus, rosemary and savory have been widely investigated, the results of different studies are difficult to compare, usually because of different sample preparation procedures and the used methods.

Comparative analysis of phenolic compounds and antioxidant activity of the selected plants from Spain was evaluated using the Briggs-Rauscher reaction method STANKOVIĆ et al (2017), where a significant relation was found between the phenolic content and antioxidant activity.

The hibiscus petals are a good source of compounds that possess antioxidant activity such as anthocyanins and ascorbic acid. PRENESTI et al (2007) studied antioxidant properties of decoction and cold infusions of *Hibiscus sabdarifa* flowers from Egypt using Briggs-Rauscher reaction method and Folin-Ciocalteu method. It was found for cold infusions of hibiscus petals, that BRAI index was between 99-149 mgGA/g and the total phenolic content was between 12.7-16.2 mgGA/g. BRAI value for the extract of hibiscus (16.3 mgGAE/g dry extract) obtained in this study is significantly lower than that obtained by PRENESTI et al (2007).

NEFIĆ and GOJAK-SALIMOVIĆ (2017) investigated the antioxidant activity of aqueous and aqueous-ethanolic extracts from lemon balm, rosemary and sage leaves using the Briggs-Rauscher reaction method. It was found for rosemary extracts that inhibition time of the Briggs-Rauscher oscillations between 188-270 seconds. ŠLJIVO et al (2016) evaluated antioxidant activity of selected medicinal plants using the Briggs-Rauscher reaction method. It was found that inhibition time of the Briggs-Rauscher oscillations varied from 26 seconds for aqueous extract and 275 seconds for ethanolic extract, and BRAI index varied from 3.00 mg GAE/g of dry plant for aqueous extract and 31.7 mg GAE/g of dry plant for ethanolic extract of rosemary. TAVASSOLI and EMAM DJOMEH (2011) were studied antioxidant activity and antimicrobial activity of rosemary leaves methanolic extract, as well as the total phenolic content. The total phenolic content was found in rosemary extract, 4.99±0.054 gGAE/100g dry leaves. NAGY et al (2014) were compared the antioxidant activity, the total phenolic content and flavonoid content for some medicinal and aromatic plants. The highest contents of total phenolics were found in rosemary extract, 3367.24 mg GAE/100g dw. The rosemary extract analyzed in this study showed a significantly lower total phenolic content (1.29 mgGAE/g dry extract).

VIDIC et al (2010) were compared the antioxidant activity and the total phenolic content of essential oil and aqueous tea infusion extracts of the two *Satureja* species (*Satureja visianni* and *Satureja montana*). The total phenolic content was determined according to the slightly modified Folin-Ciocalteu method in tea infusion samples and varied from 225 mgGAE/g for *Satureja visianni* to 275 mgGAE/g for *Satureja montana.* The savory extract analyzed in this study showed a significantly lower total phenolic content (1.43 mgGAE/g dry extract).

CONCLUSIONS – *Zaključci*

The total phenolic content and the antioxidant activity of aqueous extracts of three aromatic plants: hibiscus (*Hibiscus* spp.), rosemary (*Rosmarinus* spp.) and savory (*Satureja* spp.) were investigated using the slightly modified Folin-Ciocalteu

method and the Briggs-Rauscher reaction method. The total phenolic content was found 0.27, 1.29 and 1.43 mgGAE/g of dry extract for hibiscus, rosemary, and savory, respectively. Good correlation was found between the total phenolic content and the Briggs-Rauscher inhibition times (R^2 = 0.994), while the correlation between the total phenolic content and the relative Briggs-Rauscher antioxidant index was lower (R^2 = 0.814). Since the Briggs-Rauscher reaction method simulates conditions similar to those in the human digestive system and thus provides information on antioxidant activity after ingestion, it can be concluded that savory compared to other tested plants is the best source of antioxidants. Further research is needed, in order to analyse the chemical composition of the plant extracts and to identify phenolic compounds that might be responsible for their antioxidant activity.

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SAŽETAK

Cilj ovog rada je da se analizira i usporedi sadržaj ukupnih fenola i antioksidacijske aktivnosti vodenih ekstrakata tri ljekovite biljke: hibiskusa (*Hibiscus* spp.), ruzmarina (*Rosmarinus* spp.) i vrijeska (*Satureja* spp.). Biljni ekstrakti pripremljeni su postupkom maceracije. Sadržaj ukupnih fenola određivan je spektrofotometrijski Folin-Ciocalteu metodom koristeći galnu kiselinu kao standard. Antioksidacijska aktivnost ispitivana je primjenom oscilirajuće Briggs-Rauscher reakcije. Vrijeme inhibicije oscilacija Briggs-Rauscher reakcione smjese uzrokovano biljnim ekstraktima je praćeno potenciometrijskom metodom. Sadržaj ukupnih fenola kretao se od 0.27 mgGAE/g suhog ekstrakta za hibiskus, 1.29 mgGAE/g suhog ekstrakta za ruzmarin do 1.43 mgGAE/g suhog ekstrakta za vrijesak. Antioksidacijska aktivnost ekstrakata je opadala prema nizu: vrijesak > ruzmarin > hibiskus. Linearnom regresijom utvrđena je visoka korelacija između sadržaja ukupnih fenola i vremena inhibicije Briggs-Rauscher reakcije (R^2 = 0.994), dok je između sadržaja ukupnih fenola i relativnog Briggs-Rauscher antioksidacijskog indeksa korelacija bila niža (R^2 = 0.814).

Corresponding author: Sabina Gojak-Salimović, Department of Chemistry, Faculty of Science, University of Sarajevo, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina, e-mail: sgojak@pmf.unsa.ba