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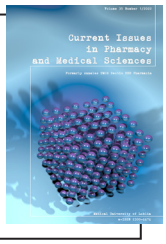

Investigation of the extraction dynamic of the biologically active substances of the raspberry (*Rubus idaeus* L.) shoots

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ABSTRACT

The study has the intent of exploring the dynamics of biologically active substance (BAS) extractions from red raspberry (*R. idaeus*) shoots in order to determine the appropriate extraction frequency. A secondary objective is to assess the relationship between extract antioxidant activity and BAS content.

The focus of the study revolved around *R. idaeus* shoots, and the spectrophotometric method of analysis was applied to determine the amount of the catechins, polyphenols, hydroxycinnamic acids derivatives and flavonoids, while the gravimetric method was used to determine the dry residue of the extracts and the potentiometric method applied for evaluating antioxidant activity.

The polyphenols amount was 1.74±0.03, 0.58±0.01 and 0.016±0.005%, catechins – 1.50±0.02, 0.50±0.01 and 0.11±0.01%, flavonoids – 0.08±0.005, 0.046±0.005 and 0.016±0.002%, hydroxycinnamic acids derivatives – 0.24±0.005, 0.078±0.005 and 0.017±0.002%, organic acids – 0.15±0.005, 0.05±0.005 and 0.01±0.002, dry residue – 2.14±0.005, 0.72±0.01 and 0.16±0.005%, antioxidant activity – 94.80±1.90, 47.40±0.95 and 7.90±0.16 mM-eqv./m for the 1st, 2nd and 3rd extraction, respectively. The optimal extraction frequency of raspberry shoots with distilled water was two times. The highest value of Peasorn`s correlation coefficient was between the antioxidant activity and catechins (r = 0.9813) and the lowest in the case of organic acids (r=0.9112).

The appropriate extraction frequency of *R. idaeus* shoots with distilled water was two times. The antioxidant method of determination extraction frequency was not inferior to the traditional method. The catechins dominated in the extracts from *R. idaeus* shoots. A significant positive correlation was content of catechins and antioxidant activity.

INTRODUCTION

Red raspberry (*Rubus idaeus* L., *Rosacea*) is a widely grown plant, not only because of its edible berries, but because of the use of leaves and fruits in traditional medicine as a diaphoretic, antiviral and anti-inflammatory agent [1], as well as in folk medicine, the employment of *R. idaeus* shoots in the treatment of colds and influenza [2,3].

The chemical composition of *R. idaeus* shoots consists of derivatives of ellagitannins, flavan-3-ols, flavonols and phenylcarboxylic acids [4]. Ellagotannins are a group of hydrolysable tannins, and the monomer of ellagitannins is an ester of glucose and ellagic acid [5]. Sanguin H-6, H-10 and lambertianin C are the main compounds of ellagitannins [6]. Moreover, derivatives of flavan-3-ols (epigallocatechin), flavonols (myricetin, kaempferol, quercetin) and phenylcarboxylic acids (gallic, ellagic and chlorogenic acids) have also been identified in *R. idaeus* shoots [7,8]. The dominant phenolic compounds that are part of the *R. idaeus* shoots are derivatives of ellagitannins and flavan-3-ols.

Due to such a diverse and rich chemical composition, extracts can be used to develop and create drugs for the treatment of cardiovascular and neurodegenerative diseases, cancer, diabetes and polycystic ovary syndrome. In recent studies [9], we found that distilled water is the most appropriate solvent for extraction BAS from *R. idaeus* shoots.

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The goal of our findings was to explore the dynamics of BAS distilled water extractions from *R. idaeus* shoots to find out the appropriate extraction frequency.

MATERIALS AND METHODS

Extraction technique

In our experiment, 10.0 g of raw material (an exact mass) was first powdered to 1-2 mm in size, after which, 200 mL of distilled water was poured in a flask with the raw material and joint reflux, then extraction was conducted in a water bath at 90°C for 60 min. Subsequently, the extract was cooled and filtered, and evaporated to a ratio of raw material/extract of 1/2 (m/m) using a rotary evaporator under vacuum at 50°C.

Chemicals and instruments

The measurement of antioxidant activity was carried out with a pH meter „Hanna 2550" (FRG) and a platinum electrode „Ezdo 50 PO" (Taiwan). A spectrophotometer „UV-1000" (PRC) was used to determine the quantity of the BAS. The solvents and chemicals were analytical grade.

Raw material

In 2021, second-year *R. idaeus* shoots were gathered near the village of Ternova, located in the Kharkiv region of Ukraine, following the fruiting period.

Quantitative assays

A weighing bottle containing 2.0 mL of the extract was utilized, which was then taken to a constant mass. Subsequently, it was vaporized and dried at a temperature ranging from 100 to 105°C for a duration of 3 hours. Following this, the weighing bottle was allowed to cool in a desiccator at 25°C for 30 minutes before being balanced. [10]. The dry residue (w, %) in the extract was determined using equation 1 as follows:

$$w(\%) = \frac{m_{dry} \cdot 100}{V_a} \qquad \text{(Eq. 1)}$$

where: m_{dry} – mass of the dry residue of an aliquot of the extract after drying, g; V_a – volume of extract aliquot, mL

The amount of polyphenols was estimated by following Folin-Ciocaltau assay procedures [11]. Phosphomolybdotungstic reagent was used for performing this. In the interval of 1.0-5.0 μg/mL of gallic acid plotted curve, the linear equation was $Y = 0.1055X + 0.1745$, $r^2 = 0.9951$. The amount of polyphenols in extracts (X) equivalent to gallic acid was ascertained using equation 2 as follows:

$$X(\%) = \frac{C_x \times K_{dil} \times 100}{V} \qquad \text{(Eq. 2)}$$

where: C_x – gallic acid concentration according to the calibration curve, $C \times 10^{-6}$, g/mL; V – extract volume, mL; K_{dil} – coefficient of dilution, mL

Catechins content was found following the vanillin assay approach [12]. The calibration curve was plotted between 100-400 μg/mL interval concentrations. In the interval of 100.0-400.0 μg/mL of a epigallocatechin-3-O-gallate plotted curve, the linear equation was $y = 0.0025x - 0.0851$, $r^2 = 0.9951$. The amount of catechins in extracts (X) that were the equivalent to epigallocatechin-3-O-gallate was determined by applying equation 3 as follows:

$$X(\%) = \frac{C_x \times K_{dil} \times 100}{V} \qquad \text{(Eq.3)}$$

where: C_x – concentration of epigallocatechin-3-O-gallate according to calibration curve, $C \times 10^{-6}$ g/mL; V – volume of extract, mL; K_{dil} – coefficient of dilution, mL

Flavonoids content was determined according to Europe Pharmacopoeia 10.0 [13]. The amount of flavonoids in extracts (X) equivalent to rutin was ascertained using equation 4 as follows:

$$X(\%) = \frac{A \times K_{dil} \times m_{st} \times 100}{A_{st} \times V} \qquad \text{(Eq. 4)}$$

where: A – absorbance of solution; A_{st} – absorbance of standard solution of rutin; V – volume of extract, mL; K_{dil} – dilution coefficient, mL, m_{st} – rutin mass, g

The amount of hydroxycinnamic acids derivatives was assessed according to Europe Pharmacopoeia 10.0 [14]. Hydroxycinnamic acids derivatives in extracts (X) equivalent to chlorogenic acid were determined using equation 5 as follows:

$$X(\%) = \frac{A \times K_{dil}}{188 \times V} \qquad \text{(Eq. 5)}$$

where: A – absorbance of solution; 188 – specific adsorption coefficient of chlorogenic acid; V – volume of extract, mL; K_{dil} – coefficient of dilution, mL

Organic acids content was found via alkalimetric titration [15]. The amount of organic acids in extracts equivalent to chlorogenic acid was derived using equation 3 as follows:

$$X(\%) = \frac{V_{equiv} \times T \times K_{dil} \times KP \times 100}{V} \qquad \text{(Eq. 6)}$$

where: T – titre of the titrant of determined substance, g/mL; $V_{equiv.}$ is the equivalent volume of NaOH, mL; V – volume of extract, mL; K_{dil} – dilution coefficient, mL; KP is the correction coefficient for NaOH

Antioxidant activity assay

The antioxidant effect of extracts was evaluated by employing the potentiometric method [16,17]. The antioxidant effect (mM-eqv./m) of extracts was determined using equation 7 as follows:

$$AOA = \frac{C_{OX} - \alpha \times C_{red}}{1+\alpha} \times K_{dil} \times 10^3 \times \frac{m_1}{m_2} \qquad \text{(Eq. 7)}$$

where: $\alpha = C_{ox}/C_{red} \times 10^{(\Delta E - EC_2H_5OH)nF/2.3RT}$; C_{ox} – potassium ferricyanide concentration, M; C_{red} – potassium ferrocyanide concentration, M; $EC_2H_5OH - 0.0546 \cdot C_{\%} - 0.0091$; $C_{\%} - C_2H_5OH$ concentration; ΔE – potential change; m_1 – extract dry residue; m_2 – extract dry residue for 1.0 mL

Pearson's (r) correlation analysis

The relationship between the antioxidant activity and BAS content was estimated by applying Pearson`s (r) correlation coefficient [18] (Table 1).

Analysis of statistic

The mean values along with their confidence intervals were reported as the outcome. MS EXCEL 7.0 was employed for the statistical analysis.

RESULTS

We found that the dry residue was significantly higher in the 1st extraction (2.14±0.01%), followed by the 2nd extraction – 0.72±0.01% and the 3d extraction – 0.16±0.005%. The sum of dry residue of all three extractions was 3.02% (Table 2).

The amount of polyphenols in the first extraction was the highest (1.74±0.03%), followed by the 2nd extraction (0.58±0.01%) and the 3d extraction (0.58±0.01%). The sum of polyphenols content was 2.45% (Table 2).

The catechins content in the 1st extraction was higher by 67% than in the 2nd extraction and by 93% than in the 3rd extraction. The sum of catechins of all three extractions was 2.11% (Table 2).

The greatest flavonoids content was determined in the 1st extraction (0.08±0.005%), followed by the 2nd (0.046 ±0.005%) and the 3rd extraction (0.016±0.002%) The sum of flavonoids of all three extractions was 0.14% (Table 2).

The greatest hydroxycinnamic acids derivatives content was found in the 1st extraction (0.24±0.005%), whereas that of the 2nd extraction was 0.078±0.005% and that of the 3rd extraction – 0.017±0.002%. The sum of hydroxycinnamic acids derivatives of all three extractions was 0.34% (Table 2).

The highest organic acids content was found in the 1st extraction – 0.15±0.005%, while in the 2nd extraction, this was – 0.05±0.005% and in the 3rd extraction – 0.01±0.002%. The sum of organic acids of all three extractions was 0.21% (Table 2).

The antioxidant activity of sum of three *R. idaeus* shoot extracts was 150.10 mM-eqv./m. The highest level of antioxidant activity was that of the 1st extraction – 94.80±1.30 mM-eqv./m, followed by that of the 2nd extraction – 47.40±0.95 mM-eqv./m , and that of the 3rd extraction – 7.90±0.16 mM-eqv./m.

Estimating the suitable extraction frequency from different raw materials primarily relies on determining the dry residue and BAS content in the extracts obtained. Based on the findings presented in Table 2 and Figure 1, we determined that the suitable extraction frequency is twice. Further extractions using a fresh solvent do not notably enhance the quantity of BAS.

Table 2. The total amount of BAS, dry residue and antioxidant activity of *R. idaeus* shoot extracts

	1st extract	2nd extract	3rd extract	Sum
Dry residue, %	2.14±0.01	0.72±0.01	0.16±0.005	3.02
Total polyphenols, %	1.74±0.03	0.58±0.01	0.13±0.007	2.45
Total catechins, %	1.50±0.02	0.50±0.01	0.11±0.006	2.11
Total flavonoids, %	0.08±0.005	0.046±0.005	0.016±0.002	0.14
Total hydroxycinnamic acids derivatives, %	0.24±0.005	0.078±0.005	0.017±0.002	0.34
Total organic acids, %	0.15±0.005	0.05±0.005	0.01±0.002	0.21
Antioxidant effect, mM-eqv./m	94.80±1.90	47.40±0.95	7.90±0.16	150.10

Note: n=5, p<0.05

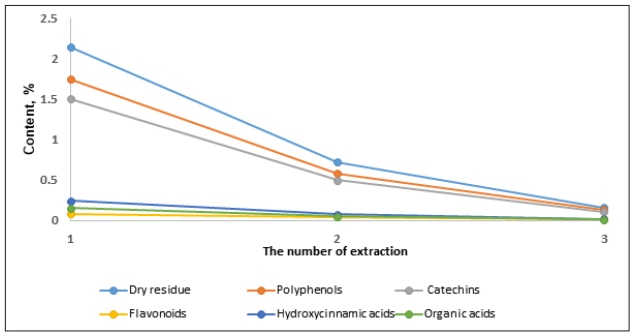

Figure 1. Curves of dry residue and content of BAS of three extractions of *R. idaeus* shoots.

In our recent study [19], we found that the total antioxidant capacity of *R. idaeus* shoots equals 164.12 mM-eqv./m. According to literature, the most suitable value of the antioxidant effect acceptance criterion is 80% of the total antioxidant capacity of raw materials. Therefore, in our case, the acceptance criterion is 131.30 mM-eqv./m. Table 2 indicates that the sum of the antioxidant effect of the 1st and the 2nd extractions is 142.20 mM-eqv./m, therefore, this value corresponds to the accepted acceptance criterion. Thus, the 3d extraction leads only to an overspending of energy and labor resources, and also reduces production.

In order to compare the effectiveness of antioxidant and dry residue methods in determining the extraction frequency of *R. idaeus* shoot extracts, the relation of antioxidant activity and the content of BAS was assessed using Pearson's coefficient. Linear regression analysis was also employed for this purpose. The findings revealed a remarkably strong relation of antioxidant effect and the quantities of polyphenols (r = 0.9810), catechins (r = 0.9813), flavonoids (r = 0.9183), hydroxycinnamic acids derivatives (r = 0.9800), and organic acids (r = 0.9112) (Table 3). Consequently, there is no disparity in the results, indicating that the antioxidant method can be utilized to estimate the suitable extraction frequency for *R. idaeus* shoots.

Table 3. The correlation coefficient (r) by Pearson of BAS content and antioxidant activity of extracts

r	Sum polyphenols of 3 extracts	Sum catechins of 3 extracts	Sum flavonoids of 3 extracts	Sum hydroxycinnamic acids derivatives of 3 extracts	Sum organic acids of 3 extracts
Antioxidant effect	0.9810	0.9813	0.9183	0.9800	0.9112

DISCUSSIONS

In our previous research [20], a new approach was developed to find the appropriate frequency of extractions in the application of the antioxidant method. There are several reasons that demonstrate that our approach to determining the frequency of extractions is more reasonable and useful than the traditional involving the assessment of the dry residue: firstly, the potentiometric method for determining antioxidant activity is quite simple and the analysis does not require a lot of time; secondly, the method's accuracy is comparable to that of gravimetry, ensuring reliable results. Moreover, numerous scientific studies have demonstrated

a correlation between antioxidant activity and anti-inflammatory effects, offering valuable insights into other potential impacts of the examined extracts.

Polyphenols are the most significant contained plant phytochemicals. The main groups of polyphenols present in *R. idaeus* shoots are the ellagotannins and catechins. They have been demonstrated to have significant anti-inflammatory and antioxidant effects. In the study of Buricova *et al.* [21], investigating an aqueous extract of *R. idaeus* leaf, they revealed that the quantity of contained polyphenols was 2.76%. In contrast to our findings, in our study, the sum of polyphenols was lower by 12.65%. The difference of the obtained results can be due to brewing times, leaves/extracts ratio and cultivars.

Catechins possess a significant role in decreasing oxidative damage as metal complex formation chelators. In turn, they activate the antioxidants protection system [22]. Bobinatie *et al.* [23] studied 41 different species of raspberry. They estimated that the catechin content in the alcohol extract ranged from 0.80% to 5.40%, in our research, we are reporting 2,11%. Lue *et al.* [24], investigated 60% methanolic extracts from *R. idaeus* leaf and they derived the figure of 1.0% of catechins. In our view, the difference is possible due to fact that the content of catechins in shoots is higher than in leaves. Catechins play a significant role in safeguarding and promoting cell division within the plant shoot, contributing to its overall vitality and resilience.

Flavonoids are plentiful in consumed fruits, vegetables and teas, and they exhibit documented protective effects on the cardiovascular and nervous systems through several different mechanisms [25]. Lupu A.P. *et al.* [26] reported 1.3% flavonoid content in 20% alcohol extract from *R. idaeus* leaf. In contrast, in our findings, the sum of flavonoids was 0.14%. Comparing the results of researches, we concluded that the difference in values can be due to higher content of flavonoids in the leaf than in the plant shoot.

In *R. idaeus* shoots, a variety of hydroxycinnamic acids derivatives can be found. These include chlorogenic, ferulic and caffeic acids [27]. Umarov U. *et al.* [28] showed that phenolic acids are useful when applied in the treatment of infections, diabetes mellitus, atherosclerosis and neurogenerative diseases, as well as in skin protective effects. Yang *et al.* [29] reported 0.8% in aqueous extract from *R. idaeus* leaves. We found, however, the sum of hydroxycinnamic acids derivatives to be 0.34%. Upon comparing the results, we realized that the quantity of hydroxycinnamic acids derivatives is greater in the leaf extract when compared to the shoot extract. This discrepancy can be explained by the biometabolism of flavonoids. The hydroxycinnamic acids derivatives are precursors of flavonoids in the shikimate pathway. In the aforementioned results, it is evident that the leaf extract contains a higher concentration of flavonoids compared to the extract from shoot. Thus, the quantity of hydroxycinnamic acids derivatives should also dominate in the leaf.

Organic acids are a group of BAS that demonstrate vitamin properties and chlorotic effects, and have been found to improve the secretions of the bile and pancreatic juices [30]. The *R. idaeus* shoots contain citric, oxalic, malic and succinic acids [31]. Organic acids exhibit antibacterial effects against both Gram-positive and Gram-negative strains [32]. Ponder A. *et al.* [33] reported that the content of organic acids in aqueous extracts of *R. idaeus* leaves to be 0.14%. In contrast, our work yielded a total sum of organic acids amounting to 0.21%. Comparing the results of research, the amount of organic acids are higher in shoot extracts. We think such difference can be associated with different brewing time and cultivars.

Velkovic B. *et al.* [34], investigated polyphenols and antioxidant activity through the application of the DPPH method of methanolic extract from *R. idaeus* leaves. They revealed correlation coefficients of 0.923, 0.838 and 0.847 between antioxidant activity and content of polyphenols, flavonoids and tannins, respectively. On comparing with our research, the greatest correlation value was between the sum of the total catechin content and antioxidant activity (r=0.9813). Thus, the catechins contribute to the main part of antioxidant activity of the extract.

CONCLUSION

The most suitable extraction frequency of *R. idaeus* shoots using distilled water was two times. Moreover, the antioxidant method of determination extraction frequency was not inferior to the traditional method. Catechins dominated in the extracts from *R. idaeus* shoots, and a significant positive correlation was between content of catechins and antioxidant activity. The results of our findings will be utilized in the subsequent production of phytomedicines incorporating *R. idaeus* shoot extracts.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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