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# The study of the chemical composition and comparative analysis of the total antioxidant capacity of blackberry (*Rubus plicatus* Weihe & Nees) leaves and fruits

**Aim.** To study the chemical composition and comparative analysis of the total antioxidant capacity of blackberry (*Rubus plicatus* Weihe & Nees) leaves and fruits.

**Materials and methods**. The content of phenolic compounds, anthocyanins, flavonoids, and hydroxycinnamic acids was determined by the spectrophotometric method, whereas organic acids were determined by the alkalimetric method; the antioxidant capacity of the extracts obtained was evaluated by the potentiometric method.

**Results**. The total antioxidant capacity of blackberry leaves and fruits was 240.44 and 71.20 mmol-equiv./$m_{dry\ weight}$, respectively. Comparing the total antioxidant capacity of blackberry leaves and fruits at same molar concentration (0.03 mol/L), it was equal 26.00 and 35.60 mmol-equiv./$m_{dry\ weight}$, respectively. The total content of phenolic compounds was 46.02 and 10.00 mg/mL calculated with reference to gallic acid, catechins – 24.22 mg/mL calculated with reference to epigallocatechin-3-O-gallate (absent in fruits), anthocyanins – 9.60 mg/mL calculated with reference to cyanidin-3-glucoside, flavonoids – 16.54 mg/mL calculated with reference to rutin (absent in fruits), hydroxycinnamic acid derivatives – 18.00 mg/mL calculated with reference to chlorogenic acid (absent in fruits) and organic acids – 22.40 and 22.20 mg/mL calculated with reference to citric acid in blackberry leaf and fruit extracts obtained during the sequential exhaustive extraction, respectively. The correlation analysis showed that there was a very high positive dependence of the antioxidant capacity and the total content of phenolic compounds, catechins and anthocyanins, flavonoids, and hydroxycinnamic acid derivatives in blackberry leaf and fruit extracts.

**Conclusions.** The total antioxidant capacity of *R. plicatus* leaves was higher than that of its fruits; however, when compared at the same molar concentration, the fruits exhibited a greater antioxidant capacity. The analysis of biologically active substances and the antioxidant capacity of *R. plicatus* extracts demonstrated that the aqueous extract contained a significant amount of biologically active compounds, as well as exhibited a pronounced antioxidant capacity. The quantification showed that catechins were the main group among the phenolic compounds in *R. plicatus* leaves, while anthocyanins predominated in fruits. The correlation analysis revealed a strong positive linear relationship between the antioxidant capacity and the content of phenolic compounds, flavonoids, catechins and anthocyanins in the case of fruits, while the weakest correlation was observed for organic acids. These results can be used to develop optimal technologies for producing drugs based on *R. plicatus* leaf and fruit extracts.

***Keywords****: blackberry leaves and fruits; total antioxidant capacity; correlation; sequential exhaustive extraction.*

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## Дослідження хімічного складу і порівняльний аналіз антиоксидантної ємності листя та плодів ожини складчастої (*Rubus plicatus Weihe & Nees*)

**Метою** роботи було дослідження хімічного складу та порівняльний аналіз загальної антиоксидантної ємності листя та плодів ожини (*Rubus plicatus* Weihe & Nees).

**Матеріали та методи.** Вміст фенольних сполук, антоціанів, флавоноїдів та гідроксикоричних кислот визначали спектрофотометричним методом, органічних кислот – алкаліметричним, антиоксидантну активність отриманих екстрактів – потенціометричним методами.

**Результати та їхнє обговорення.** Загальна антиоксидантна ємність листя та плодів ожини становила 240,44 та 71,20 ммоль-екв./$m_{сух.\ зал.}$ відповідно. Загальна антиоксидантна ємність листя та плодів ожини за однакової молярної концентрації (0,03 моль/л) дорівнювала 26,00 та 35,60 ммоль-екв./ $m_{сух.\ зал.}$, відповідно. Загальний вміст суми фенольних сполук становив 46,02 та 10,00 мг/мл у перерахунку на галову кислоту, сума катехінів – 24,22 мг/мл у перерахунку на епігалокатехін-3-О-галат (у плодах відсутній), суми антоціанів – 9,60 мг/мл у перерахунку на ціанідин-3-глюкозид, суми флавоноїдів – 16,54 мг/мл у перерахунку на рутин (у плодах відсутній), суми гідроксикоричних кислот – 18,00 мг/мл у перерахунку на хлорогенову кислоту (у плодах відсутня) та суми органічних кислот – 22,40 та 22,20 мг/мл у перерахунку на лимонну кислоту в екстрактах листя та плодів ожини, отриманих під час подальшої вичерпної екстракції, відповідно. Кореляційний аналіз показав, що існує дуже висока позитивна залежність антиоксидантної активності та загального вмісту фенольних сполук, катехінів й антоціану, флавоноїдів, похідних гідроксикоричної кислоти в екстрактах листя та плодів ожини.

**Висновки.** Загальна антиоксидантна ємність листя *R. plicatus* була вищою, ніж у плодів; однак, порівняно з однаковою молярною концентрацією, плоди демонстрували більшу антиоксидантну здатність. Аналіз біологічно активних речовин та антиоксидантної активності екстрактів *R. plicatus* показав, що водний екстракт містив значну кількість біологічно активних сполук, а також виявляв виражену антиоксидантну активність. Кількісна оцінка показала, що катехіни є основною групою з-поміж фенольних сполук у листі *R. plicatus*, тоді як антоціани – у плодах. Кореляційний аналіз виявив сильну позитивну лінійну залежність між антиоксидантною активністю

і вмістом фенольних сполук, флавоноїдів, катехінів та антоціанів у плодах, тоді як найслабша залежність спостерігалася для органічних кислот. Ці результати можуть бути використані для розроблення оптимальної технології виробництва препаратів на основі екстрактів листя та плодів *R. plicatus*.

***Ключові слова**: листя та плоди ожини; загальна антиоксидантна ємність; кореляція; послідовна вичерпна екстракція.*

**Introduction.** Reactive oxygen species (ROS) are generated both endogenously and exogenously. For example, they are actively formed during the tricarboxylic acid cycle and the mitochondrial respiratory chain, which is closely associated with the inner mitochondrial membrane [1]. Within physiological limits, ROS play an important role as metabolites that support cellular protection and survival [2]. However, when the mechanisms responsible for the ROS neutralization are impaired, the accumulation of free radicals can occur, leading to oxidative stress. Oxidative stress is closely linked to the development of various human pathologies, including cancer, diabetes, chronic kidney disease, mitochondrial dysfunction, neurodegenerative disorders, aging, and DNA damage [3]. Therefore, the regular intake of foods, beverages, pharmaceuticals, or dietary supplements that are rich in antioxidants is recommended to counteract the harmful effects of ROS [4].

Blackberry is a shrub of the *Rosacea* family. The distribution area is Europe, North America, Asia [5]. The chemical composition of blackberry fruits is represented by anthocyanins (cyanidine-3-O-glucoside), organic acids (citric acid) [6]. Blackberry leaves and fruits possess the antidiabetic, antibacterial, anti-inflammation, and antioxidant activity [7-9]. In folk medicine, blackberry is applied in the treatment of influenza, diabetes, sore throat, stomatitis, gingivitis [10].

The antioxidant activity of *R. plicatus* leaves and fruits was evaluated by the assay of 2,2-diphenyl-1-picrylhydrazyl (DPPH) [11], 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) [12] and ferric reducing antioxidant power (FRAP) [13]. However, there is no studies to determine the total antioxidant capacity of *R. plicatus* leaves and fruits. In our opinion, the total antioxidant capacity of raw materials is essential for the development of medicine, dietary supplements and cosmetic products.

The aim of the study was the determination and comparative analysis of the total antioxidant capacity of blackberry (*Rubus plicatus* L.) leaves and fruits.

**Materials and methods.** The study focused on *R. plicatus* fruits and leaves, which were harvested from areas where they were naturally grown. They collected in 2024 in July, near the village of Ternova in the Kharkiv region (50°19′31″ N, 36°66′93″ E).

The pH meter HANNA 2550 (Federal Republic of Germany) with a combined platinum electrode EZDO 50 PO (Taiwan) was applied for potentiometric measurements. The quantitative analysis of biological active compounds was carried out using a UV-spectrophotometer UV – 1000 (People's Republic of China) with matched 1 cm quarts cells. Weighing was carried out using an AN100 digital analytical balance (AXIS, Poland) with d = 0.0001 g.

12.5 g of *R. plicatus* were ground to a size of 1-2 mm. The extraction was carried out one by one using distilled water, 20 % ethanol, 40 %ethanol, 60 % ethanol, 96 % ethanol and chloroform in the ratio of the raw material/solvent 1/20 (*m/v*) in a water bath at 80ºC with reflux for 1 hour. After cooling, the solutions were filtrated and concentrated to 25.00 mL by a rotary evaporator at 40 ºC under a vacuum.

The total phenolic compounds were quantified using the Folin-Ciocaltau method, with absorbance readings taken at 760 nm [14]. The total content of flavonoids was found by the $AlCl_3$ assay where the absorbance was measured at 415 nm [14]. The total content of hydroxycinnamic acids derivatives was measured by the assay of the complex formation with $NaNO_2$-$Na_2MoO_4$, the absorbance was measured at 525 nm [14]. The total catechins were assessed using the vanillin reagent assay where the absorbance was measured at 505 nm [15]. The total content of organic acids was found through acid-base titration, using the potentiometric method to determine the end-point [14]. The amount of anthocyanins was determined by the molecular absorption analysis [14], the absorbance was measured at 600 nm.

The antioxidant capacity of extract was evaluated by the potentiometric method [16].

The Pearson's (r) correlation coefficient was used to analyze the correlation between the antioxidant capacity and the amount of phenolic derivatives, anthocyanins, catechins, flavonoids, hydroxycinnamic acids and organic acids. The correlation coefficient took a value in the range of -1 to +1. Correlation is very high if it is within the range from 0.90 to 1.00; a high correlation is from 0.70 to 0.90; from 0.50 to 0.70 it is a moderate correlation; a low correlation is from 0.30 to 0.50; and from 0.00 to 0.30 correlation is negligible [17].

**Results and discussion.** The aqueous extract had the most significant content of phenolic compounds (12.42 mg/mL), while other *R. plicatus* leaf extracts showed much lower content of phenolic compounds. The amount of the total content of phenolic compounds of *R. fructicosus* leaf extracts was 46.02 mg/mL (Table 1).

The total content of catechins was 24.22 mg/mL. The highest amount of catechins was observed in the aqueous extract (13.08 mg/mL), followed by the other 20 % EtOH extract. According to the results, the part of the amount of total catechins out of phenolic compounds was 53.0 % (Table 1).

The total content of flavonoids was 16.84 mg/mL. The highest amount of flavonoids was observed in the aqueous extract (8.64 mg/mL), followed by the other EtOH extracts (Table 1). According to the results, the part of the amount of total flavonoids out of phenolic compounds was 35.0 % (Table 1).

Table 1

The quantitative content (mg/mL) of phenolic compounds, flavonoids, hydroxycinnamic acids derivatives, catechins, organic acids, a dry residue, and the antioxidant capacity calculated from the extraction of *R. plicatus* leaves (in the 1:20 ratio of the raw material/solvent)

| Extractant | Dry residue, % | Total phenolic content calculated with reference to gallic acid | Total catechins calculated with reference to epigallocatechin-3-O-gallate | Total flavonoid content calculated with reference to rutin | Total content of hydroxycinnamic acid derivatives calculated with reference to chlorogenic acid | Total organic acids calculated with reference to citric acid | Antioxidant capacity, mmol-equiv./$m_{dry\ weight}$ |
|---|---|---|---|---|---|---|---|
| distilled water | 12.12 ±0.06 | 24.84 ±0.50 | 13.08 ±0.60 | 8.64 ±0.20 | 9.72 ±0.20 | 12.10 ±0.10 | 129.84 ±1.26 |
| 20 % EtOH | 5.16 ±0.02 | 10.58 ±0.22 | 5.58 ±0.12 | 3.68 ±0.08 | 4.14 ±0.10 | 5.16 ±0.06 | 55.30 ±0.56 |
| 40 % EtOH | 3.82 ±0.02 | 7.82 ±0.12 | 4.12 ±0.11 | 2.72 ±0.08 | 3.06 ±0.06 | 3.80 ±0.04 | 40.88 ±0.40 |
| 60 % EtOH | 0.92 ±0.02 | 1.62 ±0.05 | 0.84 ±0.02 | 1.40 ±0.02 | 0.64 ±0.02 | 0.78 ±0.01 | 8.42 ±0.08 |
| 96 % EtOH | 0.78 ±0.02 | 0.92 ±0.02 | 0.48 ±0.02 | 0.32 ±0.02 | 0.36 ±0.02 | 0.44 ±0.01 | 4.80 ±0.04 |
| Chloroform | 0.12 ±0.01 | 0.24 ±0.01 | 0.12 ±0.01 | 0.08 ±0.01 | 0.08 ±0.01 | 0.12 ±0.01 | 1.20 ±0.02 |
| The amount of the total content of BAS | 22.92 | 46.02 | 24.22 | 16.84 | 18.00 | 22.40 | 240.44 |

The total content of hydroxycinnamic acid derivatives was 18.00 mg/mL. The highest amount of hydroxycinnamic acid derivatives was observed in the aqueous extract (9.72 mg/mL), followed by the other EtOH extracts (Table 1). The part of the amount of total hydroxycinnamic acid derivatives out of phenolic compounds was 21.0 % (Table 1).

The aqueous extract had the highest content of organic acids (12.10 mg/mL), while other *R. plicatus* leaf extracts demonstrated much lower content of organic acids. The total content of organic acids was 22.40 mg/mL (Table 1).

The total antioxidant capacity of *R. plicatus* leaves was 240.44 mmol-equiv./$m_{dry\ weight}$. The antioxidant capacity increases in the following order of extracts: 96 % EtOH< 60 % EtOH< 40 % EtOH < 20 % EtOH < aqueous extract.

Table 2 shows that the total content of phenolic compounds was 10.00 mg/mL. The aqueous extract had the most significant content of phenolic compounds (3.70 mg/mL). Compared with the results of the study of *R. plicatus* leaves, we observed that the total amount of phenolic compounds was 4.54 times higher in the *R. plicatus* leaf extract than in fruits (Table 1).

The total content of anthocyanins was 9.60 mg/mL. The highest amount of anthocyanins was observed in the aqueous extract (3.56 mg/mL), followed by the other 20 % EtOH extract. According to the results, the part of the amount of total anthocyanins out of phenolic compounds was 96.0 % (Table 2).

Fruits of *R. plicatus*, unlike the leaves, do not contain flavonoids and hydroxycinnamic acid derivatives. However, *R. plicatus* fruits have anthocyanins compounds (Table 1, 2).

The total content of organic acids was 22.20 mg/mL. Other *R. plicatus* fruit extracts demonstrated much lower content of organic acids (Table 2).

The total antioxidant capacity of *R. plicatus* fruit was 71.20 mmol-equiv./$m_{dry\ weight}$. The antioxidant capacity increases in the following order of extracts: 96 % EtOH < 60 % EtOH < 40 % EtOH < 20 % EtOH < aqueous extract. Compared with the results of the study with the antioxidant capacity of *R. plicatus* leaves we observed that the total antioxidant capacity of *R. plicatus* leaves was 3.42 times higher in the *R. plicatus* fruits (Table 1, 2).

Next, we decided to evaluate the total antioxidant capacity of the raw material in one molar concentration since the comparison of the antioxidant capacity in different concentrations was not correct. For this, we obtained the amount of the extracts of blackberry leaves and fruits and combined them, and evaporated to the mass of the sample. After that, we estimated the antioxidant capacity in one molar concentration calculated with reference to gallic acid, namely in concentrations of 0.03 mol/L. According to the results presented in Table 3, it was shown that the antioxidant capacity of the total extract of blackberry fruits was 27 % higher than the antioxidant capacity of the total leaf extract.

The Pearson's (r) coefficients between antioxidant capacity and the total amount of phenolic compounds,

Table 2

The quantitative content (mg/mL) of anthocyanins, organic acids, a dry residue, and the antioxidant capacity calculated from the extraction of *R. plicatus* fruits (in the 1:20 ratio of the raw material/solvent)

| Extractant | Dry residue, % | Total phenolic content calculated with reference to gallic acid | Total anthocyanins calculated with reference to cyanidine-3-O-glucoside | Total flavonoid content calculated with reference to rutin | Total content of hydroxycinnamic acid derivatives calculated with reference to chlorogenic acid | Total organic acids calculated with reference to citric acid | Antioxidant capacity, mmol-equiv./$m_{dry\ weight}$ |
|---|---|---|---|---|---|---|---|
| distilled water | 2.22 ±0.05 | 3.70 ±0.08 | 3.56 ±0.08 | – | – | 8.22 ±0.24 | 26.34 ±0.26 |
| 20 % EtOH | 1.38 ±0.03 | 2.30 ±0.06 | 2.20 ±0.08 | – | – | 5.10 ±0.16 | 16.38 ±0.16 |
| 40 % EtOH | 1.02 ±0.03 | 1.70 ±0.06 | 1.64 ±0.08 | – | – | 3.78 ±0.12 | 12.10 ±0.30 |
| 60 % EtOH | 0.72 ±0.03 | 1.20 ±0.06 | 1.16 ±0.06 | – | – | 2.66 ±0.08 | 8.54 ±0.08 |
| 96 % EtOH | 0.60 ±0.02 | 1.00 ±0.05 | 0.96 ±0.04 | – | – | 2.22 ±0.04 | 7.12 ±0.08 |
| Chloroform | 0.06 ±0.01 | 0.10 ±0.01ж | 0.08 ±0.01 | – | – | 0.22 ±0.01 | 0.72 ±0.01 |
| The amount of the total content of BAS | 6.02 | 10.00 | 9.60 | – | – | 22.20 | 71.20 |

catechins, flavonoids, hydroxycinnamic acids derivatives, and organic acids of *R. plicatus* leaves were 0.9750, 0.9780, 0.9800, 0.8120 and 0.6500, respectively (Table 4).

In the case of *R. plicatus* fruits, the Pearson's (r) coefficients between the antioxidant capacity and the total amount of phenolic compounds, anthocyanins, and organic acids were 0.9890, 0.9990, and 0.6020, respectively (Table 5).

In our previous study [18], we developed and described the method of the sequential exhaustive extraction. This approach involves treating the same plant material with solvents of varying polarity, for example, distilled water as a highly polar solvent and ethanol solutions of different concentrations as less polar solvents. Such stepwise exhaustive extraction makes it possible to fully isolate both hydrophilic and lipophilic biologically active substances (BAS). In this procedure, the plant material was not dried after each extraction

Table 3

The level of the total antioxidant capacity of extracts from *R. plicatus* fruits and leaves, and the standard epigallocatechin-3-O-gallate in the concentration of 0.03 mol/L

| Sample | Concentration, mol/L | Antioxidant capacity, mmol-equiv./ $m_{dry\ res.}$±SD |
|---|---|---|
| *R. plicatus* fruit extract | 0.03[a] | 35.60±1.00 |
| *R. plicatus* leaf extract | | 26.00±1.00 |
| Epigallocatechin-3-O-gallate | | 30.78±1.00 |

**Notes:** SD – standard deviation, n=3, a – the molar concentration of blackberry leaf and fruit extracts was calculated as total phenolic compounds calculated with reference to gallic acid

Table 4

The Pearson`s (r) correlation coefficient between the antioxidant capacity and the content of biologically active compounds in extracts of *R. plicatus* leaves

| | Total phenolic content | Total catechin content | Total flavonoid content | Total content of hydroxycinnamic acid derivatives | Total content of organic acids |
|---|---|---|---|---|---|
| Antioxidant capacity | 0.9750 | 0.9780 | 0.9800 | 0.8120 | 0.6500 |

Table 5

The Pearson`s (r) correlation coefficient between the antioxidant capacity and the content of biologically active compounds in extracts of *R. plicatus* fruits

| | Total phenolic content | Total anthocyanin content | Total content of organic acids |
|---|---|---|---|
| Antioxidant capacity | 0.9890 | 0.9990 | 0.6020 |

step; therefore, the amount of the solvent absorbed by the raw material was taken into account. Earlier, we determined the absorption coefficient of the raw material for different solvents.

The antioxidant capacity of the extracts obtained was evaluated using the potentiometric method selected for its high sensitivity, rapid analysis, and relatively low cost of equipment and reagents [19]. Nevertheless, to substantiate the extraction conditions, specific acceptance criteria are required. According to the literature, the most common criterion is the maximum yield of phenolic and extractive compounds. We suggest using the total antioxidant capacity of the raw material as a more appropriate criterion for selecting optimal extraction conditions. This is justified, first, by the strong positive correlation between the antioxidant capacity and the content of phenolic compounds, and second, by the fact that measuring the antioxidant capacity is less time-consuming than determining the content of BAS and extractive substances. The term "the total antioxidant capacity of the raw material" refers to the cumulative antioxidant activity of all hydrophilic and lipophilic BAS present in the sample.

**Conclusions and prospects of further research.** The total antioxidant capacity of *R. plicatus* leaves and fruits was determined using the potentiometric method. The analysis of biologically active substances and the antioxidant capacity of *R. plicatus* extracts demonstrated that the aqueous extract contained a significant amount of biologically active compounds, as well as exhibited a pronounced antioxidant capacity. The quantification showed that catechins were the main group among the phenolic compounds in *R. plicatus* leaves, while anthocyanins predominated in fruits. The correlation analysis revealed a strong positive linear relationship between the antioxidant capacity and the content of phenolic compounds, flavonoids, catechins and anthocyanins in the case of fruits, while the weakest correlation was observed for organic acids. The total antioxidant capacity of *R. plicatus* leaves was higher than that of fruits; however, when compared at the same molar concentration, the fruits exhibited a greater antioxidant capacity. These results can be used to develop optimal technologies for producing drugs based on *R. plicatus* leaf and fruit extracts.

**Conflict of interests:** authors have no conflict of interests to declare.

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