Research Article

Hacettepe University Journal of the Faculty of Pharmacy
Volume 45 / Number 1 / March 2025 / pp. 18-29

Comparing Chemical Composition, Antimicrobial and Antioxidant Activities of Blackberry Fruit Thick and Green Tea Leaf Extract

Olexander MASLOV[1]*
ORCID: 0000-0001-9256-0934
Mykola KOMISARENKO[2]
ORCID: 0000-0002-1161-8151
Artem MARCHENKO[2]
ORCID: 0009-0002-8616-0410
Dmytro PLIS[1]
ORCID: 0009-0004-0118-2317
Svitlana PONOMARENKO[3]
ORCID: 0000-0003-3994-3500
Tetiana OSOLODCHENKO[3]
ORCID: 0000-0001-7258-3880
Sergii KOLISNYK[1]
ORCID: 0000-0002-4920-6064

[1]National University of Pharmacy, Faculty of Pharmacy, Department of General Chemistry, Kharkiv, Ukraine

[2]National University of Pharmacy, Faculty of Pharmacy, Department of Pharmacognosy and Nutriciology, Kharkiv, Ukraine

[3]Mechnikov Institute of Microbiology and Immunology of the NAMS of Ukraine, Laboratory of Biochemistry and Biotechnology, Kharkiv, Ukraine

Corresponding author:
Olexander MASLOV
National University of Pharmacy, Faculty of Pharmacy, Department of General Chemistry, Kharkiv, Ukraine
E-mail: alexmaslov392@gmail.com
Tel: +380664257224

Received date : 03.05.2024
Accepted date : 20.01.2025

DOI: 10.52794/hujpharm.1477950

ABSTRACT

The purpose of the work was to study and compare phytochemical composition, antimicrobial, antioxidant potential of blackberry fruit thick and green tea leaf liquid extracts. The quantification of biologically active substances (BAS) was performed using spectrophotometric, titrimetric, and HPLC analysis methods. Antioxidant activity was measured through a potentiometric method, while antimicrobial effects were assessed using the well diffusion method and determined the minimum inhibition concentration (MIC). The total content of phenolic compounds was 0.54% and 10.10%, organic acids – 4.60 and 1.60% for blackberry fruit thick and green tea leaf extract. The total content of catechins in the green tea leaf extract was 10500.0 mg/100 g, where epicatechin-3-O-gallate was dominated (3730.0±2.00 mg/100 g). The total content of anthocyanins in the blackberry fruit thick extract was 159.81 mg/100 g, where cyanidin-3-O-glucoside was dominated (134.56±0.10 mg/100 g). Both extracts possessed a high antioxidant potential, and effective antimicrobial effects. The antioxidant, antimicrobial and anti-fungi activity of blackberry fruit extract was higher than green tea leaf extract. In addition, we assumed that anthocyanins had higher antioxidant, antimicrobial and anti-fungi properties than catechins. These findings would promote application of blackberry fruits extract as pharmaceuticals and nutraceuticals.

Keywords: Anthocyanins, Catechins, Antimicrobial effect, Antioxidant power, Comparing analysis

1. Introduction

Nowadays, the problem of bacterial infection is still relevant. According to recent statistical studies, it has been found that every year 13.7 million people die from bacterial infections in the world. The mortality rate for all ages was 99.6 deaths per 100.000 population. The 54.9% of the 7.7 million deaths was caused by the Gramm-positive strains such as *Staphylococcus aureus*, and Gramm-negative strains: *Klebsiella pneumonia, Escherichia coli*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, whereas *S aureus* was associated with than 1.1 million deaths. In 135 countries *S. aureus* was the problem one in causing the death over 15 years of age (940.000) [1]. In addition, an important threat to human populations is fungal infection. According to the latest statistics, every year 1,433,000 people suffer from systemic candidal infections, of which approximately 611 thousand people die annually [2]. Thus, this problem affects all areas of application of antibiotics, including the treatment of urinary tract infections.

Medicinal plants that are rich sources of flavan-3-ols and anthocyanins are receiving significant attention from the scientific community [3]. This is primarily because some resistant pathogens are more susceptible to natural compounds. Additionally, natural compounds have a potent antioxidant effect, and, moreover, side effects are less common with natural compounds compared to synthetic drugs [4].

The perspective source of anthocyanins was chosen blackberry fruits, whereas a green tea leaf is the source of catechins. Blackberry (*Rubus fruticosus* L.) is a shrub of the *Rosacea* family. The distribution area is Europe, North America, Asia [5]. The chemical composition of *R. fruticosus* fruits is represented by anthocyanins (cyanidine-3-*O*-glucoside), organic acids (citric acid), flavonoids (rutin) and hydroxycinnamic acids (caffeic acid) [6]. The composition of green tea leaf (*Camellia sinensis* L.) contains: catechins (epigallocatehin-3-*O*-gallate, epicatehin, (+)-catechin, epigallocatechin), organic acids (oxalic acid), flavonoids (rutin) and hydroxycinnamic acids (caffeic acid) [7].

There are numbers of research about investigation pharmacological activities of *R. fruticosus* fruit and *C. sinensis* extracts. It is known that anthocyanins and catechins from *R. fruticosus* fruit, and *C. sinensis* leaf possess: anti-inflammatory, antimicrobial, anti-hyperglycemic, immune-modulation, and anticancer effects. Besides, in folk medicine *R. fruticosus* and *C. sinensis* leaf are traditionally applied to treat fever, urinary and skin infections, diabetes, cancers and liver diseases [8]. In our view, the anthocyanins and catechins are perspective for the development of new antimicrobial, anti-fungi and antioxidant pharmaceuticals.

Therefore, the aim of investigation was to determine phytochemical content and assess antimicrobial, and antioxidant potential of *R. fruticosus* fruit thick and *C. sinensis* leaf liquid extracts.

2. Material and Methods

2.1. Raw material

The study focused on *R. fruticosus* fruits, which were harvested from areas where they are naturally grown. The collection took place in 2021 in July, near the village of Ternova in the Kharkiv region (50°19′31″ N, 36°66′93″ E) (Fig. 1). The study focused on the leaf of *C. sinensis* from the Chun Myn variety, which was gathered as raw material in the Anhui province of China during the months of March through May (30°63′41″ N, 116°33′25″ E).

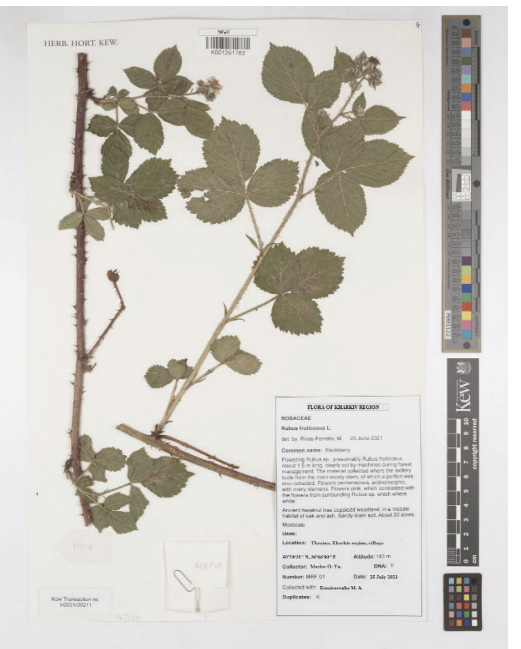

Figure 1. The voucher specimen of *R. fruticosus* L. in an herbarium

ISSN: 2458 - 8806

2.2. Reagents

Acetonitrile (purchased from «Allchem», Kharkiv), acetic acid (purchased from «Allchem», Kharkiv), phosphoric acid (purchased from «Allchem», Kharkiv), methanol (purchased from «Allchem», Kharkiv) cyanidine-3-*O*-glucoside (≥98.0%), cyanidin-3-*O*-rutinoside (≥98.0%), cyanidin-3-*O*-malonyl glycoside (≥98.0%), cyanidin-3-*O*-xyloside (≥98.0%), cyanidin-3,3'-diglucoside (≥98.0%), epicatechin (≥98.0%), epigallocatechin-3-*O*-gallate (≥98.0%), epigallocatechin (≥98.0%), epicatechin-3-*O*-gallate were purchased in Sigma Aldrich Company, Lublin, Poland.

2.3. Technique of extraction

A 100.0 g (exact mass) of *R. fruticosus* fruits was pressed, then it was added of 96% ethanol in a threefold amount to the extraction, after that filtration, then obtained filtrate was concentrated by a vacuum-evaporator at a temperature of 50-60°C to ratio mass of extract and raw material – 1/0.35.

The *C. sinensis* extract was obtained by the following way: a 100.0 g of raw material was extracted with 60% ethanol at 80º C within 1 hour with a condenser, with 1/20 ratio of raw material/solvent. The extraction technique was repeated twice to provide totally extract all BAS, then the filtrates were combined and evaporated by vacuum rotary to give ratio of extract to raw material 1:2.

2.4. Phytochemical analysis

The total phenolic compounds were quantified using the Folin-Ciocaltau method, with absorbance readings taken at 760 nm [9]. The phosphomolybdotungstic reagent was used for performing assay. The calibration curve (Y = 0.1055X + 0.1745 (R^2=0.9951)) was plotted with interval concentrations 1.0 – 5.0 μg/mL, the calibration equation. The total phenolic compounds content in extracts (X), expressed as gallic acid was calculated according to equation 2:

$$X(\%) = \frac{C_x \times K_{dil} \times 100}{V}$$

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

The total catechins were assessed using the vanillin reagent assay, where absorbance was measured at 505 nm [10]. The calibration curve (Y = 0.0025X – 0.0851 (R^2 = 0.9951)) was plotted with 100 – 400 μg/mL interval concentrations of epigallocatechin-3-*O*-gallate. The total catechins content in extracts (X), expressed as epigallocatechin-3-*O*-gallate was calculated according to equation:

$$X(\%) = \frac{C_x \times K_{dil} \times 100}{V}$$

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

For the quantification of total anthocyanin content, molecular adsorption analysis was utilized, with measurements of absorbance at 546 nm [11]. The total anthocyanin content in extract (X), expressed as cyanidine-3-*O*-glucoside was calculated to equation:

$$X(\%) = \frac{A \times K_{dil}}{600 \times V}$$

where, *A* – absorbance of analyzed solution; 600 – specific adsorption coefficient of cyanidine-3-*O*-glucoside; *V* – volume of extract, ml; K_{dil} – coefficient of dilution.

The content of total organic acids was established through acid-base titration, using a potentiometric method to determine the end-point [12]. The total content of organic acids in extracts, expressed as citric acid was calculated according to equation 6:

$$X(\%) = \frac{(V_{equiv} - V_x) \times 0.0032 \times K_{dil} \times K \times 100}{V}$$

where, 0.0032 – the amount of citric acid, equivalent to 1 mL of sodium hydroxide solution (0.05 mol/L), g; $V_{equv.}$ is the volume (mL) of sodium hydroxide solution (0.05 mol/L), which was used for titration; V_x – the volume (mL) of sodium hydroxide solution (0.05 mol/L), which was spent for titration in a blank experiment; V – volume of extract, mL; K_{dil} – coefficient of dilution, mL.; K is the correction coefficient for 0.05 mol/L sodium hydroxide solution.

2.5. Antioxidant effect assay

Antioxidant activity of extract was evaluated by potentiometric method [13, 14]. An 5.00 mL aliquot of

2 mmol/L solution of $K_3[Fe(CN)_6]$ and 0.02 mmol/L of $K_4[Fe(CN)_6]$ was taken and transferred into a 250.0 mL volumetric flask and made up to the mark by 0.067 mol/L phosphate buffer solution. A 50.00 mL of prepared mediator solution was transferred in an electrochemical cell. The initial potential of mediator solution was measured after initial one was established, a 1.00 mL of aliquot of the prepared solutions was added and a final potential was measured. The difference (ΔE) between the initial (E_0) and final (E_1) potentials was found.

Antioxidant activity was calculated according to equation and expressed as mmol-eqv./$m_{dry\ res.}$:

$$AOA = \frac{C_{ox} - \alpha \times C_{red}}{1+\alpha} \times K_{dil} \times 10^{3} \times \frac{m_1}{m_2}$$

where, $\alpha = C_{ox}/C_{red} \times 10^{(\Delta E - E\text{ethanol})nF/2.3RT}$; C_{ox} – concentration of $K_3[Fe(CN)_6]$, mol/L; C_{red} – concentration of $K_4[Fe(CN)_6]$, mol/L ; $E_{ethanol}$ – $0.0546 \cdot C_{\%}$ – 0.0091; $C_{\%}$ – concentration of ethanol; ΔE – change of potential; F = 96485.33 C/mol – Faraday constant; n = 1 – number of electrons in electrode reaction; R = 8.314 J/molK – universal gas constant; T – 298 K; K_{dil}– coefficient of dilution, mL.; m_1 – mass of dry residue; m_2 – mass of dry residue in 1.0 mL of extract.

The standardized *C. sinensis* leaf liquid extract, which was obtained by 60% ethanol and solution of epigallocatechin-3-*O*-gallate were used as the reference standards.

2.6. HPLC analysis of C. sinensis leaf extract

For the analysis, a Prominence LC-20 Shimadzu liquid chromatography system with a Thermo Scientific Syncronis aQ C18 column (4.6 × 250, 5.0 µm particle size) was utilized. All analyses were conducted at a temperature of 40 ºC. The mobile phases consisted of a 70% methanol aqueous solution (A) and a 1.0% solution of phosphoric acid (B). The gradient protocol started with 20–42% A over the first 15 minutes, shifted to 42–43% A from 15 to 25 minutes, changed to 43–90% A from 25 to 45 minutes, maintained 90% A from 45 to 55 minutes, decreased to 20% A from 55 to 60 minutes, and then held at 20% A from 60 to 70 minutes. Prior to use, the mobile phases were filtered using 25mm × 0.45 µm Supelco Iso-Disc Filters PTFE 25-4 and degassed. A flow rate of 0.5 mL/min was maintained, and the injection volume of the samples was 5 µL. Detection wavelengths were set at 255, 286, and 350 nm.Chromatographic peaks of analytes were identified by the following similarity indexes, which were calculated between the test substance and the standard according to the formulas:

$$I_T = 1 - |T_{st} - T_u|$$

$$I_{255} = 1 - |h_{255_{st}} - h_{255_u}|$$

$$I_{286} = 1 - |h_{286_{st}} - h_{286_u}|$$

$$I_{350} = 1 - |h_{350_{st}} - h_{350_u}|$$

where, I_T – retention time similarity index, T_{st} – retention time of standard (min), T_u – test substance retention time (min), I_{255}, I_{286} and I_{350} – spectral similarity indices, h_{255st}, h_{286st} and h_{350st} – spectral characteristics of the standard, h_{255st}, h_{286st} and h_{350st} – spectral characteristics of the test substance.

The least among the three similarity index values of spectral characteristics dictates the similarity level (IL) between substances and standards based on these traits. A higher IL value increases the probability of more precise identification of the substance. Substances whose similarity index with the catechin standard was at least 0.7, and whose peaks on the chromatogram appeared between the catechin peak and the earliest flavonoid peak, were classified as catechins. [15].

2.7. HPLC analysis of R. fruticosus fruit thick extract

The anthocyanins content was determined using a Perkin-Elmer Series 400 HPLC and a semi-preparative Dynamax Rainin Model SD-300 Liquid Chromatograph, which was outfitted with a Hewlett-Packard 1040A photodiode array detector. The analytical HPLC system employed a 250 x 4.6-mm inner diameter Prodigy 5 ODS 3 column from Phenomenex with 5.0 µm particle size. The mobile phases were arranged into a solvent system comprising: (A) 100% acetonitrile and (B) a mixture of 5% acetonitrile, 10% acetic acid, and 1% phosphoric acid in water. The gradient used for the solvents was as follows: 0–5 min at 100% B, 5–20 min transitioning from 20% to 80% B, 20–25 min at 40–60% B, and 25–30 min back to 100% B. The flow rate was set at 1 mL/min with an injection volume of 50 µL. Samples were filtered using a 0.45-µm Millipore filter type

ISSN: 2458 - 8806

HA (Millipore Corp., Bedford, Mass., U.S.A.). Detection occurred at 520 nm, with the quantification of individual peaks expressed as a percentage of the total peak area.

2.8. Test organisms

S. aureus ATCC 25923, E. coli ATCC 25922, B. subtilis ATCC 6538, C. albicans ATCC 885/653, P. vulgaris NTCS 4636, and P. aeruginosa ATCC 27853 were employed following established guidelines for evaluating the antimicrobial efficacy of pharmaceuticals.

2.9. Screening antimicrobial activity of extracts

The method of diffusion of the drug into agar carried out using the method of "wells" [18]. Preparation of microorganisms suspensions with determined concentrations of microorganisms (optical density) was carried out by the standard of turbidity (0.5 units according to scale of McFarland) with using of equipment of Densi-La-Meter (Czech, wavelength 540 nm). Suspensions were prepared according to equipment and information list. Colony forming unit was 107 microorganisms at 1 ml of growth medium and determined by standard of McFarland). On solidified agar, using a pipette under sterile conditions in Petri dishes made 1 ml of a suspension of microorganisms. After uniform distribution of microorganisms over the entire surface of the agar, the plates were incubated at room temperature for 15-20 minutes Next, wells with a diameter of 6 mm were made in the cups, into which solutions of the test substances were introduced. The samples incubated at 37° C for 16-24 hours. After incubation, the plates were placed upside down on a dark matte surface so that light fell on them at an angle of 45° (accounting in reflected light). The diameter of the growth retardation zones measured using a caliper.

2.10. Assay of determination of MIC

MIC is defined as the smallest concentration of an antibacterial agent that entirely prevents bacterial growth. The MIC for various extracts was determined through the broth microdilution technique [19]. Briefly, the wells of a U-bottom 96-well microplates were filled with 200 µL of sterile BHI. 200 µL of extract was added to the first row (columns 1–10). Serial dilutions were performed by transferring 200 µL from the wells of the first row to the wells of the second row and so forth, resulting in the concentrations presented in Fig. 2. 10 µL of the inoculum was added in all wells excepted column 11 where 10 µL of sterile saline free of culture was added. and this served as a positive control. Finally, the plates were covered and incubated at 37 □ for 24 h. After incubation, MIC was considered the lowest concentration of the tested material that inhibited the visible growth of the bacteria.

3. Results and Discussion

3.1. Phytochemical analysis of BAS

According to obtained results shown in Table 3, *C. sinensis* leaf extract (10.10±0.25%) had higher content of phenolic compounds, than in *R. fruticosus* thick fruit extract (0.52±0.02%).

Table 3 demonstrates that the total content of anthocyanin in *R. fruticosus* fruit thick extract was 0.16±0.002%, whereas in *C. sinensis* leaf extract anthocyanin was not presence. The percentage of anthocyanin out of total polyphenols was 30% in *R. fruticosus* extract.

The highest amount of organic acids was determined in *R. fruticosus* fruits thick extract (4.60±0.50%), whereas it was found to be 65.22% lower in *C. sinensis* leaf extract (1.60±0.10%). In *R. fruticosus* extract,

Table 1. Interpretation criteria for microbial sensitivity

Microbial sensitivity	Diameter of the growth retention zone, mm
High sensitivity	>25
Sensitive	15-25
Low sensitivity	10-15
Not sensitivity	<10

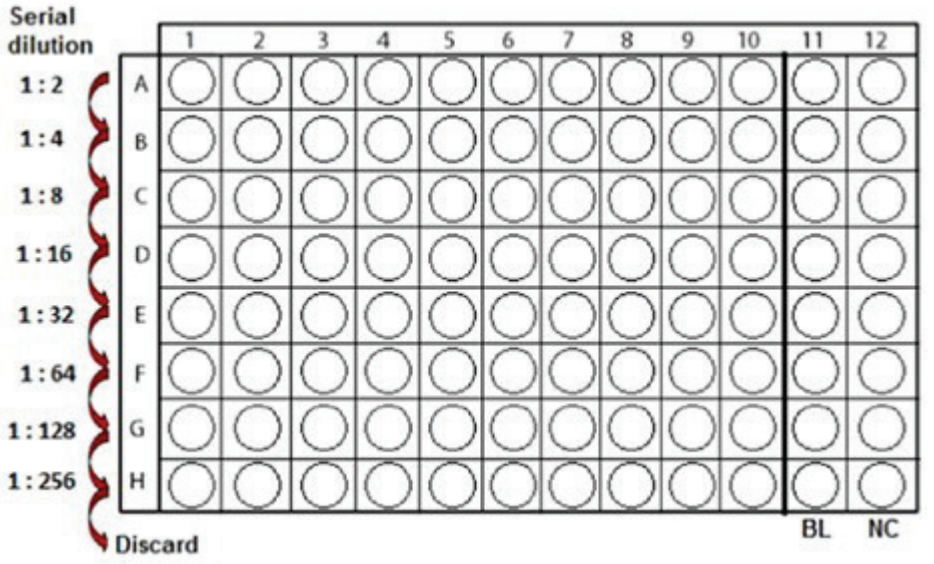

Figure 2. Serial dilution process in microbroth dilution method (BL – blank control, NC – negative control)

the total organic acids were in 8.5 times higher than phenolic compounds, whereas in the *C. sinensis* leaf, the total organic acids were in 6.3 times lower than phenolic compounds. (Table 3)

The HPLC method was used to carry out a qualitative and quantitative analysis of catechins and anthocyanins in the obtained extracts of *C. sinensis* leaf and *R. fruticosus* fruits extract. According to the results of the study, 5 catechins were identified in *C. sinensis* leaf extract, whereas in *R. fruticosus* fruits extract 5 anthocyanins, respectively. (Fig. 3, 4).

The total content of catechins in the obtained *C. sinensis* leaf extract was 10500.0 mg/100 g. Among catechins, epigallocatechin-3-*O*-gallate dominates – 3730.0±2.00 mg/100 g (35.52% out of the total catechins), whereas the lowest content was (+)-catechin with the value of 210 mg/100 g (2.00% out of the total catechins). (Table 3)

As shown in Table 4, cyanidin-3-*O*-glucoside dominated among all anthocyanins (84.10% out of the total anthocyanins), cyanidin-3,3'-diglucoside (7.38% out of the total anthocyanins) was in second place, and the lowest content was cyanidin-3-*O*-rutinoside (0.60% out of the total anthocyanins). «

The content of BAS in *R. fructicocus* fruit extracts was quantified by spectrophotometric, titrimetric and HPLC methods of analysis. The organic acids were present in both extracts, where the highest content of organic acids was determined in *R. fructicocus* fruits extract than in *C. sinensis* leaf extract. In our view, it relates with different purpose of accumulation organic acids. The organic acids are precursor for biosynthesis of sugars in fruits, whereas in leaf, organic acids only play a role in photosynthesis as result there is no purpose of high accumulation organic acids in leaf [21]. Fan-Chiang H. *et al.* [22] investigated anthocyanin content of *R. fructicocus* fruit 70% acetone extract by HPLC method. According to their results, it was detected following anthocyanins (mg/100 g per extract): cyanidin-3-*O*-glucoside (80 mg/100 g), cyanidin-3-*O*-rutinoside (1 mg/100 g), and cyanidin-3-*O*-xyloside (6.5 mg/100 g). Compering with our results, the content of anthocyanins in our research was lower, but cyaniding-3-O-glucoside was dominated in both extracts. The chemical composition of the fruit varies during the development, ripening and depending on the different varieties.

3.2. Antioxidant activity

A potentiometric method for determining antioxidant activity was used to evaluate the effect of the obtained extracts. Table 5 shows that the level of antioxidant activity of *R. fruticosus* fruits extract was significantly lower to the *C. sinensis* leaf extract. The antioxidant activity of *C. sinensis* leaf was 10.5 times higher than that of *R. fruticosus* extract.

In the light of the data obtained, it can be established that *C. sinensis* leaf extract has the highest level of antioxidant activity. According to the modern classi-

ISSN: 2458 - 8806

Table 2. Quantitative content of total phenolic compounds, anthocyanin and organic acids

Sample	Total phenolic compounds expressed as gallic acid, %±SD	Total anthocyanin expressed as cyanidine-3-*O*-glucoside, %±SD	Total catechins expressed as epigallocatechin-3-*O*-gallate, %±SD	Total of organic acids expressed as citric acid, %±SD
R. fruticosus thick extract	0.54±0.02	0.16±0.002	□	4.60±0.50
***C. sinensis* leaf extract**	10.10±0.25	□	10.47±0.25	1.60±0.10

SD – standard deviation, n=3

Table 3. Chemical composition of catechins in *C. sinensis* leaf extract by HPLC-UV analysis

№	Catechins	Retention time, min	Similarity index, I_L	Content of catechins in *C. sinensis* leaf extract, mg/100 g of extract ±SD	% out of total catechins
1	Epigallocatechin	13.013	0.875	2760.0±2.00	26.29
2	(+)-catechin	13.780	0.996	210.0±2.00	2.00
3	Epicatechin	16.494	0.851	1010.0±2.00	9.62
4	Epigallocatechin-3-*O*-gallate	17.686	0.990	3730.0±2.00	35.52
5	Epicatechin-3-*O*-gallate	20.754	0.814	2788.0±2.00	26.57
	THE TOTAL CATECHINS			10500.0	

SD – standard deviation, n=3

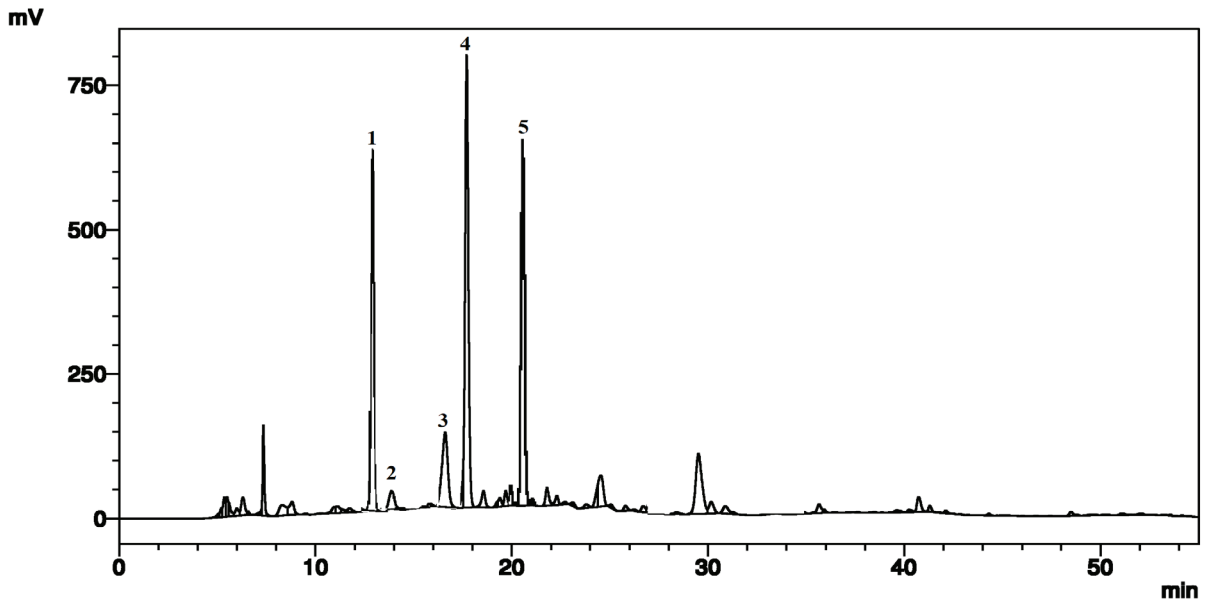

Figure 3. HPLC fingerprint (255 nm) of the *C. sinensis* leaf extract

fication of antioxidant activity, which was previously developed by us [22], it was found that all extracts obtained have a high level of antioxidant activity. Moreover, a comparative analysis of the "strength" of antioxidant activity was carried out with the gold standard *C. sinensis* leaf. Further, it was prepared

Table 4. Chemical composition of anthocyanins in *R. fruticosus* fruit thick extract by HPLC analysis

№	Antocyanins	Retention time, min	Content of antocyanins in extract, mg/100 g of extract ±SD	% out of total antocyanins
1	Cyanidin-3-*O*-glycoside	8.010	134.56 ±0.10	84.10
2	Cyanidin-3-*O*-rutinoside	9.560	0.96 ±0.10	0.60
3	Cyanidin-3-*O*-malonyl glycoside	14.723	10.08±0.10	6.30
4	Cyanidin-3-*O*-xyloside	15.110	2.40±0.10	1.50
5	Cyanidin-3,3'-diglucoside	16.450	11.81±0.10	7.38
	THE TOTAL ANTOCYANINS		159.81	

SD – standard deviation, n=3

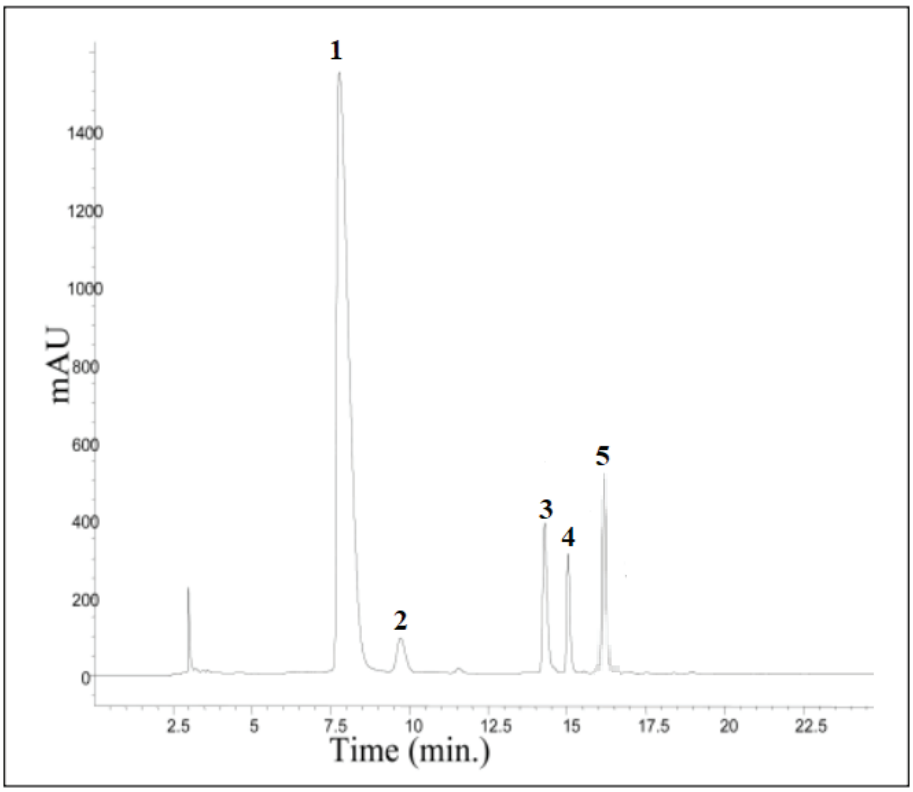

Figure 4. HPLC fingerprint (520 nm) of the *R. fruticosus* fruit thick extract

solutions (in terms of the amount of polyphenols expressed as gallic acid) of extracts with 0.03 M concentration of *R. fruticosus* thick fruits extract, *C. sinensis* leaf and epigallocatechin-3-*O*-gallate. As a result of the study, it was found that the level of antioxidant activity of *R. fruticosus* extract was higher 50% of *C. sinensis* leaf extract and 41% epigallocatechin-3-*O*-gallate. (Table 6)

At first glance, the *C. sinensis* leaf extract had significantly higher antioxidant potential than *R. fruticosus* fruit extract. However, comparing extracts at the same molar concentration, it was found that *R. fruticosus* fruit extract had 2 times higher the level of antioxidant activity than *C. sinensis* leaf extract. In our view, it relates with the fact that anthocyanins more potent antioxidants than catechins. Lapidot *et al.* [23] determined antioxidant activity of malvidin-

ISSN: 2458 - 8806

Table 5. Level of antioxidant activity of *R. fruticosus* thick extract, *C. sinensis* leaf extract

Sample	Antioxidant activity, mmol-eqv./$m_{dry\ res.}$ ±SD	Conditional term of antioxidant level
R. fruticosus thick fruits extract	52.47±1.00	High level
***C. sinensis* leaf extract**	548.79±10.98	Very high level

SD – standard deviation**,** n=3

Table 6. Level of antioxidant activity of *R. fruticosus* thick extract, *C. sinensis* leaf extract and standard: epigallocatechin-3-*O*-gallate at the concentration of 0.03 mol/L

Sample	Concentration, mol/L	Antioxidant activity, mmol-eqv./$m_{dry\ res.}$ ±SD
R. fruticosus thick fruits extract		52.47±1.00
C. sinensis leaf extract	0.03[a]	27.49±1.00
Epigallocatechin-3-*O*-gallate		30.78±1.00

SD – standard deviation, n=3, a – molar concentration of blackberry thick extract and green tea leaf extract was calculated as total phenolic compounds expressed as gallic acid

3-glucoside, catechin, malvidin and resveratrol by the method of oxidation myoglobin with H_2O_2. It was shown that inhibition efficiency of the antioxidant decreased in following order: malvidin-3-glucoside > catechin > malvidin > resveratrol. In research of Muselik *et al.* [24], it was carried out evaluation the level of antioxidant activity of derivatives of catechins: epicatechin, (+)-catechin, epicatehin, epicatechin-3-*O*-gallate, gallocatechin; and anthocyanins: cyanidin-galactoside, malvidin-3-glucoside and delphinidin-3-glucoside by ferric reducing antioxidant power assay. It was found the level of antioxidant activity decreased in the following order: epicatechin-3-*O*-gallate > delphinidin-3-glucoside > cyanidin-galactoside > gallocatechin > malvidin-3-glucoside > epicatechin > catechin. The antioxidant activity of epicatechin-3-*O*-gallate had the highest antioxidant power whereas the catechin – the lowest one, where cyanidin-3-galactoside interfere to epicatechin-3-*O*-gallate, but greater than other derivatives of catehins. The major part of composition of *C. sinensis* leaf is presented by epicatechin-3-*O*-gallate and low amount – epicatechin and (+)-catechin. However, it is quite difficult to evaluate the contribution of each compound on total antioxidant power of extract as well as it is unknown whether catechins interact by synergistic way or antagonistic one. Thus, the level of antioxidant activity of extract depends not only on composition of extract, but also, on ration and interaction of compounds.

3.3. Antimicrobial activity

In this research work, the antimicrobial activity of the obtained *R. fruticosus* thick fruits and *C. sinensis* leaf extract was investigated against the following strains of *S. aureus*, *B. subtilis*, *E. coli*, *P. vulgaris*, *P. aeruginosa*, as well as a strain of the fungus *C. albicans*. According to the obtained results, extracts obtained from the *R. fruticosus* fruit and *C. sinensis* leaf had an effective antimicrobial effect.

Among pathogens strains, *R. fruticosus* fruits extract was the most inhibits *P. aeruginosa* strains (34.00±0.20 mm), followed by *S. aureus* and *B. subtilis* (30.00±0.20 mm) Gramm-positive strains *P. vulgaris* was the most resistance bacteria to the action of *R. fruticosus* fruits extract (24.00±0.20 mm). Comparing results with *C. sinensis* leaf extract, it was determined that *R. fruticosus* fruits extract was 3.3, 13.3, 25 and 18% better inhibit bacterial strains of *S. aureus*, *B. subtilis, E. coli and P. aeruginosa* than *C. sinensis* leaf extract, respectively. Comparing obtained results with reference standard gentamycin, it was found that *S. aureus*, *B. subtilis* and *P. aeruginosa* were 27, 20 and 25% more sensitive to *R. fruticosus* fruits extract than gentamycin. Whereas, *P.*

vulgaris and *E. coli* was 4 and 8% more sensitive to gentamycin.

Anti-fungal investigation against *C. albicans* showed that *R. fruticosus* fruits extract 20 and 16% more actively inhibit the growth of fungi than *C. sinensis* leaf and fluconazole, respectively.

The investigated extracts significantly inhibit the bacterial and fungi strains with MIC. In the previously above conducted antimicrobial study, the extract of *R. fruticosus* fruits was the most active independently of the tested strains. Table 8 shows, the *R. fruticosus* fruits extract with MIC value of 0.14 μM was the most active against *S. aureus*, and *P. aeruginosa*, whereas *C. sinensis* leaf extract MIC values was 80% lower. The highest MIC value of *C. sinensis* leaf extract was against fungi pathogens *C. albicans*. The MIC value of *R. fruticosus* fruits extract against pathogens *E. coli*, *P. vulgaris* and *B. subtilis* was significantly lower than in the case of *C. sinensis* leaf extract.

The analyzed *R. fruticosus* fruit and *C. sinensis* leaf extracts showed high antimicrobial activity against the following strains of *S. aureus*, *P. aeruginosa*, *P. vulgaris, B. subtillis* and *C. albicans*. According to the obtained data, at first glance it can be considered that the antimicrobial activity of *R. fruticosus* fruit and *C. sinensis* leaf extracts is significantly inferior to the action of gentamicin and fluconazole, because their concentration of solutions was in 3 times lower than the content of polyphenols in the extract. However, we would like to note that gentamicin has serious toxicity to the auditory nerve, kidneys and liver, which can lead to serious complications of the disease. Comparing the antifungal effects of fluconazole and *R. fruticosus* fruit and *C. sinensis* leaf extracts, it was found that they inhibited the growth of the fungal strain at the same level, while the concentration of fluconazole was also lower, like gentamicin. We can declare that fluconazole is a leader as anti-fungal medicine, but at the same time it weakly inhibits the growth of gram-negative and gram-positive bacteria, but both strains of bacteria and fungus are sensitive to *R. fruticosus* fruit and *C. sinensis* leaf extracts. Thus, *R. fruticosus* fruit and *C. sinensis* leaf extracts could be a combined as pharmaceutical that affects different mechanisms of vital activity of bacteria and fungi, thereby having a wide spectrum of action against different strains of bacteria and fungi, and at the same time not possessing serious toxicity.

The *R. fruticosus* fruit is a rich source of anthocyanins, whereas *C. sinensis* leaf of catechins. It is well known that anthocyanins biosynthesis pathway is based on chemical conversion of catechins. Li *et al.* [25] declared that at the beginning of ripping period the content of anthocyanin starts increasing, whereas the content of catechins decreasing. However, a question which of this group of flavonoids possess higher antimicrobial activity is still open.. In our research, it was comparing antimicrobial potential of *R. fruticosus* fruit and *C. sinensis* leaf extracts. In the antimicrobial tests, which carried out by well diffusion method, it was shown the *R. fruticosus* fruit extract was more active against pathogens *P. aeruginosa, E. coli*, *B. subtilis*, *C. albicans* whereas *P. vulgaris* were sensitive to both extracts practically at the same lev-

Table 7. Retardation zone (mm) resulting from the screening of antimicrobial activity of *R. fruticosus* thick extract, *C. sinensis* leaf extract and standards: gentamycin, fluconazole

Sample	Concentration, mM	Diameter of the growth retardation zone, mm±SD					
		Gramm-positive		Gramm-negative			Fungi
		S. aureus ATCC 25923	***B. subtilis*** **ATCC 6633**	**E. coli ATCC 25922**	***P. vulgaris*** **ATCC 4636**	***P.aeruginosa*** **ATCC 27853**	***C.albicans*** **ATCC 653/885**
R. fruticosus thick extract	0.009[a]	30.00±0.20	30.00±0.20	23.00±0.20	24.00±0.20	34.00±0.20	25.00±0.20
C. sinensis leaf extract	0.009[a]	29.00±0.20	26.00±0.20	21.00±0.20	24.00±0.20	28.00±0.20	21.00±0.20
Gentamycin	0.003	22.00±0.20	24.00±0.20	25.33±0.33	25.00±0.20	25.67±0.67	12.00±0.20
Fluconazole	0.003	18.00±0.20	12.00±0.20	14.33±0.33	12.33±0.33	10.00±0.20	20.00± 0.50

SD – standard deviation, n=3, a – molar concentration of blackberry thick extract and green tea leaf extract was calculated as total phenolic compounds expressed as gallic acid

ISSN: 2458 - 8806

Table 8. Minimal inhibitory concentration of the different *R. fruticosus* thick extract, and *C. sinensis* leaf extract against the 6 references pathogens.

Sample	MIC, µM					
	S. aureus **ATCC 25923**	***B. subtilis*** **ATCC 6633**	***E. coli*** **ATCC 25922**	***P. vulgaris*** **ATCC 25922**	***P.aeruginosa*** **ATCC 27853**	***C.albicans*** **ATCC 653/885**
R. fruticosus thick extract	0.14	0.28	0.56	0.28	0.14	0.56
C. sinensis leaf extract	0.70	0.70	2.80	2.80	2.80	5.60

el. Furthermore, it was determined MIC values for both extracts, as result *R. fruticosus* fruit extract had better results than *C. sinensis* leaf extract. Therefore, based on mentioned results, it was assumed that antimicrobial and anti-fungi activity of anthocyanins higher than catechins. However, both extracts had a high content of organic acids and its presence should not be neglected. Further in our research, we planned to answer on question whether impact organic acids on antimicrobial potential or not.

4. Conclusions

It was found that total phenolic compounds were higher in *C. sinensis* leaf extract, whereas total organic acids were in *R. fruticosus* fruit thick extract. Both extracts possessed a high antioxidant potential, and effective antimicrobial and anti-fungi effects. Although we assumed that anthocyanins had higher antioxidant, antimicrobial and anti-fungi properties than catechins. In future studies, the hypothesized impact of organic acids on antimicrobial and anti-fungi effects should be verified by isolation of organic acids from both extracts. These findings would promote application of *R. fruticosus* fruits extract as pharmaceuticals and nutraceuticals.

Acknowledgements

We are thankful of National University of Pharmacy, and Mechnikov Institute of Microbiology and Immunology of the NAMS of Ukraine.

Conflict of Interest

The author has no conflicts of interest, financial or otherwise, to declare.

Statement of Contribution of Researchers

Concept – O.M., M.K., S.P.; Design – T.O., O.M., M.K; Supervision – T.O., S.K.; Resources A.M., D.P.; Materials –A.M., D. P.; Data Collection and/or Processing – O.M., D.P., T.O., A.M.; Analysis and/or Interpretation – O.M., M.K., S.P., T.O., S.K.; Literature Search – A.M., D.P..; Writing – O.M., M.K.; Critical Reviews – T.O., S.K.

References

1. Ikuta KS, Swetschinski LR, Robles AG et al. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet. 2022;* 400:2221-48. https://doi.org/10.1016/s0140-6736(22)02185-7
2. Denning, D. Global Incidence and Mortality of Severe Fungal Disease. Lancet. 2022;400:1-21. http://dx.doi.org/10.2139/ssrn.4560971
3. Arsene MM, Viktorovna PI, Davares AK, Parfait K, Andreevna SL, Mouafo HT, et al. Antimicrobial and antibiotic-resistance reversal activity of some medicinal plants from Cameroon against selected resistant and non-resistant uropathogenic bacteria. Front Biosci Elite. 22 2022;14(4):25. https://doi.org/10.31083/j.fbe1404025
4. Maslov O, Komisarenko M, Kolisnyk S, Tkachenko O, Akhmedov E, Poluain S, et al. Study of qualitative composition and quantitative content of free organic acids in lingberry leaves. Fitoterapia. 2023;(1):77-82. https://doi.org/10.32782/2522-9680-2023-1-77
5. Zia-Ul-Haq M, Riaz M, De Feo V, Jaafar H, Moga M. rubus Fruticosus L.: Constituents, biological activities and health related uses. Molecules. 2014;19(8):10998-1029. https://doi.org/10.3390/molecules190810998.
6. Vega EN, Molina AK, Pereira C, Dias MI, Heleno SA, Rodrigues P, Anthocyanins from *Rubus fruticosus* L. and *Morus nigra* L. applied as food colorants: A natural alternative. Plants. 2021;10(6):1181. https://doi.org/10.3390/plants10061181

7. Maslov OY, Kolisnyk SV, Komisarenko MA, Akhmedov EY, Poluian SM, Shovkova ZV. Study of flavonoids and phenolic acids in green tea leaves. Curr Issues Pharm Med. 2021;14(3):287-91. https://doi.org/10.14739/2409-2932.2021.3.240287
8. Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, Sochor J. Bioactive compounds and antioxidant activity in different types of berries. Int J Mol Sci. 2015;16(10):24673-706. https://doi.org/10.3390/ijms161024673
9. Maslov OY, Komisarenko MA, Golik MY, Kolisnyk SV, Altukhov AA, Baiurka SV, et al. Study of total antioxidant capacity of red raspberry (*Rubus idaeous* L.) shoots. Vitae. 2023;30(1). https://doi.org/10.17533/udea.vitae.v30n1a351486
10. Maslov O, Kolisnyk S, Komisarenko M, Golik M. Study of total antioxidant activity of green tea leaves (*Camellia sinensis* L.). Herba Pol. 2022;68(1):1-9. https://doi.org/10.2478/hepo-2022-0003
11. Maslov OY, Komisarenko MA, Kolisnyk SV, Golik MY, Doroshenko SR, Tkachenko OV, et al. The study of some quality parameters and the antioxidant activity of dietary supplements with the pomegranate extract (*Punica granatum* L.). News Pharm. 2023;106(2):5-12. https://doi.org/10.24959/nphj.23.119
12. Maslov O, Kolesnik S, Komisarenko M, Altukhov A, Dynnyk K, Kostina T. Development and *Validation of a Titrimetric Method for Quantitative Determination of Free Organic Acids in Green Tea Leaves*. Pharmakeftiki. 2021;33(4):304–11. doi: https://doi.org/10.5281/zenodo.7813135
13. Maslov OY, Kolisnyk SV, Komisarenko MA, Kolisnyk OV, Ponomarenko SV. Antioxidant activity of green tea leaves (*Camellia sinensis* L.) liquid extracts. *Pharmacologyonline*. 2021;(3):291-8. https://doi.org/10.5281/zenodo.7813115
14. Maslov OY, Kolisnyk SV, Komissarenko NA, Kostina TA. Development and validation potentiometric method for determination of antioxidant activity of epigallocatechin-3-O-gallate. *Pharmacologyonline*. 2021;2:35-42. https://doi.org/10.5281/zenodo.7813098
15. Khodakov, I. V. Method for the identification of polyphenols in plant extracts using HPLC. Determination of the composition of soy isoflavones. Methods Obj Chem Anal. 2013;8:(2):132–42.
16. Aaby K, Grimmer S, Holtung L. Extraction of phenolic compounds from bilberry (*Vaccinium myrtillus* L.) press residue: Effects on phenolic composition and cell proliferation. LWT Food Sci Technol. 2013;54(1):257-64. https://doi.org/10.1016/j.lwt.2013.05.031
17. Aaby K, Ekeberg D, Skrede G. Characterization of phenolic compounds in strawberry (fragaria×ananassa) fruits by different hplc detectors and contribution of ındividual compounds to total antioxidant capacity. J Agric Food Chem. 2007;55(11):4395-406. https://doi.org/10.1021/jf0702592
18. Maslov O, Komisarenko M, Ponomarenko S, Horopashna D, Osolodchenko T, Kolisnyk S, et al. Investigation the influence of biologically active compounds on the antioxidant, antibacterial and anti-inflammatory activities of red raspberry (*Rubus idaeous* l.) leaf extract. Curr Issues Pharm Med Sci. 2022. https://doi.org/10.2478/cipms-2022-0040
19. Mbarga MJ, Podoprigora IV, Volina EG, Ermolaev AV, Smolyakova LA. Evaluation of changes ınduced in the probiotic escherichia coli M17 following recurrent exposure to antimicrobials. J Pharm Res Int. 2021:158-67. https://doi.org/10.9734/jpri/2021/v33i29b31601
20. Arena ME, Povilonis IS, Borroni V, Pérez E, Pellegrino N, Cacciatore C, Radice S. Changes in Carbohydrates, organic acids, and minerals at different development stages of hexachlamys edulis fruit, a wild South American species with horticultural potential. Horticulturae. 2023;9(3):314. https://doi.org/10.3390/horticulturae9030314
21. Fan-Chiang HJ, Wrolstad RE. Anthocyanin Pigment composition of blackberries. J Food Sci. 2006;70(3):C198—C202. https://doi.org/10.1111/j.1365-2621.2005.tb07125.x
22. Maslov OY, Kolisnyk SV, Komisarenko MA, Altukhov AA, Dynnyk KV, Stepanenko VI. Study and evaluation antioxidant activity of dietary supplements with green tea extract. Curr Issues Pharm Med. 2021;14(2):215-9. https://doi.org/10.14739/2409-2932.2021.2.233306
23. Lapidot T, Harel S, Akiri B, Granit R, Kanner J. pH-Dependent forms of red wine anthocyanins as antioxidants†. J Agric Food Chem. Січ. 1999;47(1):67-70. https://doi.org/10.1021/jf980704g
24. Muselík J, García-Alonso M, Martín-López M, Žemlička M, Rivas-Gonzalo J. Measurement of antioxidant activity of wine catechins, procyanidins, anthocyanins and pyranoanthocyanins. Int J Mol Sci. 2007;8(8):797-809. https://doi.org/10.3390/i8080797
25. Li J, Shi C, Shen D, Han T, Wu W, Lyu L, Li W. Composition and antioxidant activity of anthocyanins and non-anthocyanin flavonoids in blackberry from different growth stages. Foods. 2022;11(18):2902. https://doi.org/10.3390/foods11182902

ISSN: 2458 - 8806