Relationship of antimicrobial and antioxidant activities with biologically active compounds in blackberry leaf extracts

A. O. Marchenko[1,A,D], M. A. Komisarenko[1,B,E], O. Yu. Maslov[1,A,C], T. P. Osolodchenko[2,E,F], S. V. Kolisnyk[1,E,F]

[1]National University of Pharmacy, Kharkiv, Ukraine,
[2]I. Mechnikov Institute of Microbiology and Immunology National Academy of Medical Sciences of Ukraine, Kharkiv

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Infectious diseases are an important problem worldwide for medicine and pharmacy. The search for natural antibacterial agents has gained significant interest recently, driven by the growing concerns over antibiotic resistance and the desire for natural alternatives. Blackberry leaf, known for their rich nutritional profile and high antioxidant content, have attracted attention for their potential antibacterial properties. Understanding the antibacterial potential of blackberries could provide insights into their use in natural medicine and food preservation.

The aim of work was to study a correlation between antimicrobial and antioxidant activities with biologically active compounds in blackberry leaf extracts.

Materials and methods. The spectrophotometric method was applied for quantification of the total amount of biologically active compounds. The antiradical activity of obtained blackberry leaf extracts was determined with the potentiometric method; antimicrobial activity was estimated by the "well" method.

Results. Results demonstrate that the highest amount of polyphenols, catechins, flavonoids were 3.72 %, 1.88 % and 1.48 % in 60 % EtOH extract, respectively. The organic acids were dominant in the aqueous extract (1.96 %). The most potent antioxidant property possessed 60 % EtOH extract of blackberry leaf (204.68 mmol-eqv./$m_{dry\ res.}$). The blackberry leaf extracts showed antimicrobial effects against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtillis*, *Proteus vulgaris*, and *Candida albicans*. Statistical analysis revealed a highly significant interdependence between the concentration of catechins, the magnitude of antioxidant activity, and the strength of the antimicrobial effect against the tested microorganisms (*S. aureus, P. vulgaris, P. aeruginosa,* and *C. albicans*).

Conclusions. These findings show the great potential of blackberry extracts in the development and creation of new medicines with antimicrobial, antioxidant effects that are not inferior to the action of synthetic analogues.

Keywords: blackberry, leaf, correlation, biologically active compounds, antioxidant effect, antimicrobial effect.

Current issues in pharmacy and medicine: science and practice. 2025;18(3):264-270

Зв'язок антимікробної та антиоксидантної активності з біологічно активними сполуками в екстрактах листя ожини

А. О. Марченко, М. А. Комісаренко, О. Ю. Маслов, Т. П. Осолодченко, С. В. Колісник

Інфекційні захворювання залишаються складною проблемою для медицини та фармацевтики в усьому світі. Пошук природних антибактеріальних засобів останніми роками є актуальним, що обґрунтовано поширенням стійкості до антибіотиків і прагненням до використання природних альтернатив. Листя ожини, відоме завдяки багатому поживному профілю та високому вмісту антиоксидантів, є об'єктом наукових досліджень завдяки потенційним антибактеріальним властивостям. Вивчення антибактеріального потенціалу ожини може відкрити шляхи до її використання в натуральній медицині та консервуванні харчових продуктів.

Мета роботи – вивчення кореляції між антимікробною та антиоксидантною активністю біологічно активних сполук в екстрактах листя ожини.

Матеріали і методи. Для кількісного визначення сумарного вмісту біологічно активних сполук застосовано спектрофотометричний метод. Антиоксидантну активність отриманих екстрактів листя ожини визначено потенціометричним методом. Антимікробну активність оцінено методом дифузії препарату в агар за допомогою методу «лунок».

Результати. Результати дослідження дали змогу визначити, що найбільша кількість поліфенолів, катехінів і флавоноїдів становила 3,72 %, 1,88 % та 1,48 % у 60 % етанольному екстракті відповідно. Органічні кислоти переважали у водному екстракті (1,96 %). Найсильнішу антиоксидантну властивість мав 60 % етанольний екстракт листя ожини (204,68 ммоль-екв./$m_{сух.\ реч.}$). Екстракти листя ожини характеризувалися антимікробною дією проти *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus*

ARTICLE INFO

UDC 615.322:581.711/.712:615.451.1.015.11
DOI: 10.14739/2409-2932.2025.3.338363

Current issues in pharmacy and medicine: science and practice. 2025;18(3):264-270

Keywords: blackberry, leaf, correlation, biologically active compounds, antioxidant effect, antimicrobial effect.

Received: 29.07.2025 // Revised: 15.09.2025 // Accepted: 24.09.2025

subtillis, *Proteus vulgaris* та *Candida albicans*. Виявлено дуже високу кореляцію між загальним вмістом катехінів, антиоксидантною активністю та антимікробним ефектом проти *S. aureus*, *P. vulgaris*, *P. aeruginosa* та *C. albicans*.

Висновки. Результати дослідження дали підстави зробити висновок про високий потенціал екстрактів ожини під час розроблення та створення нових лікарських засобів з антимікробною та антиоксидантною діями, що не поступається ефектам синтетичних аналогів.

Ключові слова: ожина, лист, кореляція, біологічно активні сполуки, антирадикальний ефект, антимікробний ефект.

Актуальні питання фармацевтичної і медичної науки та практики. 2025. Т. 18, № 3(49). С. 264-270

According to international medical statistics, approximately 14 million people worldwide die annually as a result of microbial infections. The global mortality rate from bacterial infections is estimated at 100 deaths per 100,000 individuals. Of the 8 million annual deaths attributed to bacterial pathogens, nearly half are caused by *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Streptococcus pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus* [1].

Among these pathogens, *S. aureus* represents the leading cause of infection-related mortality. The situation is further exacerbated by the increasing prevalence of bacterial resistance to commonly used antibiotics, which significantly complicates therapeutic strategies, prolongs treatment duration, and elevates healthcare costs [2].

Consequently, the identification and development of novel antimicrobial agents, particularly those derived from natural sources, is of critical importance in contemporary biomedical research. Thousands of natural compounds were shown antimicrobial effects against Gram-negative, Gram-positive strains [3]. Natural compounds have several advantages over synthetic compounds: high efficiency, minimal side effects and low cost of the production method. Furthermore, natural compounds, particularly catechin derivatives, exhibit significant antimicrobial properties, a feature of considerable importance in the management of infectious diseases [4].

The perspective source of catechins was the chosen blackberry leaf. Blackberry (*Rubus fruticosus* L.) – a shrub of the *Rosacea* family. The distribution area is Europe, North America, Asia [5]. The primary phytoconstituents identified in blackberry leaf include ellagitannins, flavan-3-ols such as catechin, flavonols like rutin and quercetin, and hydroxycinnamic acids, notably caffeic acid [6].

There is a lot of research about investigating pharmacological activity of blackberry leaf extracts. It is known that blackberry leaf extracts, possess: anti-inflammatory, antimicrobial, anti-diabetic, and anticancer effects. Besides, in folk medicine blackberry leaf are traditionally applied to treat fever, infections, diabetes, and liver diseases [7]. In our view, the blackberry is a perspective for the development of new antimicrobial, and antioxidant pharmaceuticals.

There is a lot of scientific research about determining the level of antioxidant activity of blackberry leaf extracts [8,9]. However, there is no date about assessing antioxidant / antimicrobial activity and its correlation with the content of biologically active substances (BAS) by the potentiometric method.

Aim

The aim of the work was to study a correlation between antimicrobial and antioxidant activities with biologically active compounds in blackberry leaf extracts.

Materials and methods

Blackberry leafs were gathered in 2021 throughout the May – June in the district of the community of Ternova, Kharkiv region.

Six samples of 25.0 g (precise mass sample) of blackberry leaves had the size of particles 1–2 mm. The extraction was conducted with distilled water, 20 %, 40 %, 60 %, 96 % ethanol at 80 °C within 1 hour with a condenser, with a ratio raw material / solvent – 1/10. The extraction process was performed duplicate to ensure exhaustive extraction of all bioactive compounds. The resulting filtrates were then combined and concentrated using rotary evaporation under reduced pressure until a 1:1 (w/w) extract-to-raw material ratio was achieved. This procedure yielded six distinct extracts: 96 %, 60 %, 40 %, and 20 % ethanol, along with a pure aqueous extract.

The pH meter HANNA 2550 (Federal Republic of Germany) with a combined platinum electrode EZDO 50 PO (Taiwan) was used for potentiometric measurements. Quantitative analysis of biologically active compounds was carried out on UV-spectrophotometer UV – 1000 (People's Republic of China) with matched 1 cm quartz cells. The weighing was performed using digital analytical balance AN100 (AXIS, Poland) with d = 0.0001 g. All solvents and other chemicals used in the study were of analytical grade.

The quantification of phytochemical constituents was performed using established spectrophotometric and titrimetric methods. Specifically, the Folin-Ciocalteu method was employed to determine total phenolic content, with measurements taken at 760 nm [10]. Total flavonoid content was ascertained via an aluminum chloride ($AlCl_3$) assay, reading absorbance at 415 nm [10]. Furthermore, the concentration of hydroxycinnamic acid derivatives was evaluated by measuring the absorbance of their complex with $NaNO_2$-Na_2MoO_4 at 525 nm [10]. The vanillin assay, with absorbance measured at 505 nm, was used to quantify total catechins [11]. Finally, the content of total organic acids was determined by acid-base titration, utilizing a potentiometric method to identify the endpoint [10].

The potentiometric method was used for estimating antiradical activity [12]. The agar well diffusion method was used to evaluate the drug's efficacy [13,14]. Strains of *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Bacillus subtillis* ATCC 6538, *Proteus vulgaris* NTCS 4636, and *Candida albicans* ATCC 885/653 were applied for the establishment of antimicrobial effect. The antimicrobial activity was carried out by T. P. Osolodchenko at the Laboratory of Biochemistry and Biotechnology in I. Mechnikov Institute of Microbiology and Immunology National Academy of Medical Sciences of Ukraine, Kharkiv.

Pearson's correlation coefficient (R) was used to assess relationships between antioxidant activity and the levels of biologically active compounds. The strength of the correlations was interpreted as follows: very high (0.90–1.00), high (0.70–0.90), moderate (0.50–0.70), low (0.30–0.50), or negligible (0.00–0.30) [15].

To obtain statistical results, the Statistica 6 program was used, the results were analyzed using one-way ANOVA with Tukey's criterion. Differences were considered significant at $p < 0.05$.

Results

The 60 % ethanol extract contained the highest concentration of phenolic compounds (3.72 %), significantly exceeding the levels found in the other blackberry leaf extracts.

The catechins increase in the following order: aqueous extract (1.40 %) > 20 % EtOH extract (1.42 %) > 96 % EtOH extract (1.60 %) > 40 % EtOH extract (1.66 %) > 60 % EtOH extract (1.88 %). The fraction of catechins out of the total of polyphenols was 53 %, 51 %, 46 %, 48 % and 50 % for 96 %, 60 %, 40 %, 20 % EtOH and aqueous extracts, respectively. The greatest fraction of catechins was in 60 % EtOH extract, whereas the lowest in aqueous extract *(Table 1)*.

The maximum content of flavonoids was established in 60 % EtOH extract (1.48 %), followed by 96 % EtOH extract (1.40 %), whereas the lowest one was in aqueous extract (0.38 %). The fraction of flavonoids out of the total of polyphenols was 47 %, 40 %, 22 %, 15 % and 13 % for 96 %, 60 %, 40 %, 20 % EtOH and aqueous extracts, respectively *(Table 1)*.

The hydroxycinnamic acids increase in the following order: 60 % EtOH extract (0.50 %) > aqueous extract (0.76 %) > 60 % EtOH extract (0.96 %) > 20 % EtOH extract (1.00 %) > 40 % EtOH extract (1.66 %) > 40 % EtOH extract (1.40 %). The fraction of hydroxycinnamic acids out of the total of polyphenols was 17, 26, 39, 34 and 27 % for 96 %, 60 %, 40 %, 20 % EtOH and aqueous extracts, respectively. The greatest fraction of hydroxycinnamic acid was in 40 % EtOH extract, whereas the lowest was in 96 % EtOH extract *(Table 1)*.

The maximum content of organic acids was determined in aqueous extract (1.96 %), followed by 60 % EtOH extract (1.82 %), whereas the lowest one was in 96 % EtOH extract (0.62 %). The total content of organic acids was lower in 4.80, 2.00, 2.00, 1.69 and 1.40 than the content of phenolic compounds in 96 %, 60 %, 40 %, 20 % EtOH and aqueous extracts, respectively *(Table 1)*.

Table 2 shows that the level of antiradical activity increases in the following order: aqueous extract (148.80 mmol-eqv./$m_{dry\ res.}$) > 20 % EtOH extract (150.02 mmol-eqv./$m_{dry\ res.}$) > 40 % EtOH extract (180.46 mmol-eqv./$m_{dry\ res.}$) > 96 % EtOH extract (190.88 mmol-eqv./$m_{dry\ res.}$) > 60 % EtOH extract (204.68 mmol-eqv./$m_{dry\ res.}$). Based on the data obtained, the 60 % ethanol extract demonstrated the highest antioxidant activity. This finding aligns with the modern classification of antioxidant activity established in our prior research [16], it was found that all obtained extracts have a high level of antioxidant activity. Moreover, a comparative analysis of the "strength" of antioxidant activity was carried out with the gold standard 60 % EtOH extract of *C. sinensis* leaf. The *C. sinensis* leaf extract was obtained by the same technological method as blackberry leaf extracts. The obtained extracts were significantly inferior in antioxidant effect to *C. sinensis* leaf extract. Further, 0.01 mol/L solutions (expressed as gallic acid equivalent) of extracts of and *C. sinensis* leaf were prepared. As a result of the study, it was found that when compared at the same concentrations, the 60 % EtOH extract had the highest antioxidant effect, and the least – 20 % EtOH extract *(Table 3)*.

In this research work, the antimicrobial activity of the obtained blackberry leaf extracts 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, all extracts from the blackberry leaf extracts had an effective antimicrobial effect *(Table 4)*.

S. aureus was the most sensitive to the 96 % and 60 % EtOH extract (25.0 mm) and least sensitive to the aqueous extract (18.0 mm). According to the results presented in *Table 4*, *B. subtilis*, as well as *S. aureus*, was highly sensitive to the 96 % (25.0 mm), 60 % (25.0 mm) and 40 % (24.0 mm) EtOH extracts, followed by the 20 % EtOH extract (19.0 mm), and the aqueous extract inhibited the growth of the bacterial strain the least (18.0 mm). *E. coli* was the most sensitive to the action of 60 % and 40 % EtOH extract (24.0 mm), in second place – 96 % EtOH extract (22.0 mm), whereas *P. aeruginosa* and *P. vulgaris* were most sensitive to 60 % (26.0 mm) and 40 % EtOH (25.0 mm) extracts *(Table 4)*.

When studying antifungal activity against *C. albicans*, the results showed that 60 % extract of blackberry leaf extract was the most actively inhibited the growth of the fungus, whereas aqueous extract was the least active in inhibiting the growth of fungi. When compared with the fluconazole standard, it was found that the 60 % extracts inhibited fungal growth 20 % higher than fluconazole *(Table 4)*.

The dependence of antioxidant, antimicrobial activity on the content of different groups of BAS was studied using the method of linear regression. As shown in *Table 5*, the correlation between antioxidant activity and the total polyphenol content was very high ($R = 0.9960$). In the case of catechins, the correlation was extremely high ($R = 0.9999$); for flavonoids and hydroxycinnamic acid derivatives, it was high; and for organic acids, the correlation was very high.

According to the research results presented in *Table 5* it was established that there was a very high correlation between catechins ($R = 0.9041$). A high correlation was determined between phenolic compounds ($R = 0.7873$), flavonoids ($R = 0.8901$) and inhibition of the growth of *S. aureus*, in the case of hydroxycinnamic acid derivatives ($R = 0.6603$) was moderate, while or organic acids ($R = 0.3962$) there was a low correlation.

Table 1. The total amount of biologically active compounds and dry residue in blackberry leaf extracts

Object	Dry residue, %	Total polyphenols content expresses as gallic acid, %	Total catechins content expressed as epigallocatechin-3-O-gallate, %	Total flavonoids content expressed as rutin, %	Total hydroxycinnamic acid content expressed as chlorogenic acid, %	Total organic acids expressed as citric acid, %
96 % EtOH extract	10.68 ± 0.02	3.00 ± 0.005	1.600 ± 0.005	1.400 ± 0.005	0.500 ± 0.005	0.62 ± 0.02
60 % EtOH extract	16.64 ± 0.02	3.72 ± 0.07	1.880 ± 0.060	1.480 ± 0.060	0.960 ± 0.030	1.82 ± 0.06
40 % EtOH extract	15.48 ± 0.03	3.58 ± 0.07	1.660 ± 0.060	0.800 ± 0.020	1.400 ± 0.040	1.80 ± 0.06
20 % EtOH extract	13.68 ± 0.03	2.98 ± 0.06	1.420 ± 0.060	0.460 ± 0.010	1.000 ± 0.030	1.76 ± 0.05
Aqueous extract	11.58 ± 0.03	2.82 ± 0.06	1.400 ± 0.060	0.380 ± 0.020	0.760 ± 0.010	1.96 ± 0.04

Standard deviation, n = 5.

Table 2. The antiradical activity of blackberry leaves liquid extracts

Object	Antiradical activity, mmol-eqv./$m_{dry\ res.}$ ± SD	Rating scale for antioxidant activity
96 % EtOH extract	190.88 ± 1.16	High level
60 % EtOH extract	204.68 ± 2.05	High level
40 % EtOH extract	180.46 ± 1.81	High level
20 % EtOH extract	150.02 ± 1.50	High level
Aqueous extract	148.80 ± 1.49	High level
Green tea leaf 60 % extract	548.79 ± 10.98	Very high level

Standard deviation, n = 5.

Table 3. Comparing the value of antioxidant activity of blackberry leaves liquid extracts with *C. sinensis* leaf 60 % extract at the concentration 0.01 mol/L

Object	Concentration of polyphenols, mol/L*	Antioxidant activity, mmol-eqv./$m_{dry\ res.}$ ±SD
96 % EtOH extract	0.01	8.63 ± 0.11
60 % EtOH extract		9.30 ± 0.11
40 % EtOH extract		8.60 ± 0.11
20 % EtOH extract		8.30 ± 0.13
Aqueous extract		8.80 ± 0.13
Green tea leaf 60 % EtOH extract		9.50 ± 0.10

Standard deviation, n = 5; ***:** the molar concentration of the extracts was calculated based on their total phenolic content, which was determined as gallic acid equivalents.

Table 4. The value of antimicrobial activity of blackberry leaves liquid extracts

Sample	Concentration mol/L, (expressed in total polyphenols as gallic acid)	Zone of inhibition diameter, mm ± SD					
		Gram-positive		Gram-negative			Fungi
		S. aureus	*B. subtilis*	*E. coli*	*P. vulgaris*	*P. aeruginosa*	*C. albicans*
96 % EtOH extract	0.07	25.0 ± 0.1	24.0 ± 0.2	23.0 ± 0.1	22.0 ± 0.1	23.0 ± 0.2	23.0 ± 0.1
60 % EtOH extract	0.06	25.0 ± 0.1	24.0 ± 0.1	24.0 ± 0.2	24.0 ± 0.2	26.0 ± 0.2	25.0 ± 0.1
40 % EtOH extract	0.06	24.0 ± 0.2	25.0 ± 0.2	24.0 ± 0.2	24.0 ± 0.2	25.0 ± 0.1	23.0 ± 0.1
20 % EtOH extract	0.05	19.0 ± 0.1	19.0 ± 0.1	18.0 ± 0.2	17.0 ± 0.2	18.0 ± 0.1	16.0 ± 0.1
Aqueous extract	0.05	18.0 ± 0.2	18.0 ± 0.2	18.0 ± 0.1	17.0 ± 0.1	18.0 ± 0.1	16.0 ± 0.1
Gentamycin	0.003	22.0 ± 0.2	24.0 ± 0.1	25.3 ± 0.1	25.0 ± 0.2	25.7 ± 0.1	12.0 ± 0.2
Fluconazole	0.003	18.0 ± 0.1	12.0 ± 0.1	14.3 ± 0.1	12.3 ± 0.2	10.0 ± 0.2	20.0 ± 0.2

Standard deviation, n = 5.

Table 5. Pearson coefficients (R) of antiradical, and antimicrobial activity and total amount of bioactive constituents

Parameter	Antiradical activity	Antimicrobial activity					
		S. aureus	*B. subtillis*	*E. coli*	*P. vulgaris*	*P. aeruginosa*	*C. albicans*
Polyphenols	0.9960	0.7873	0.7605	0.8070	0.8591	0.8857	0.7972
Catechins	0.9999	0.9041	0.8176	0.8854	0.9050	0.9433	0.9302
Flavonoids	0.7776	0.8901	0.7882	0.8240	0.7881	0.8110	0.8999
Hydroxycinnamic acids	0.8773	0.6603	0.7794	0.6700	0.6260	0.5527	0.5838
Organic acids	0.9286	0.3966	0.3994	0.3266	0.2313	0.1892	0.3511
Antiradical activity	–	0.9663	0.8912	0.9339	0.9197	0.9391	0.9806

Table 5 shows that the antibacterial effect against *B. subtilis* was strongly correlated with the catechin content (R = 0.8176) and antioxidant activity (R = 0.8912). In contrast, moderate correlations were observed with the contents of polyphenols, flavonoids, and hydroxycinnamic acid derivatives, while the correlation with organic acids was low.

The study showed that there was a low correlation between organic acids (R = 0.3266), and a high correlation and inhibition of *E. coli* growth was found of phenolic compounds (R = 0.8070), catechins (R = 0.8854), flavonoids (R = 0.8240). A very high correlation was determined for antioxidant activity (R = 0.9339) and a moderate correlation was found for hydroxycinnamic acids (R = 0.6700) *(Table 5)*.

The relationship between inhibition of growth of *P. vulgaris* and the content of different groups of BAS, it was found to have a very high dependence of antibacterial activity on the amount of catechins (R = 0.9050) and the level of antioxidant activity (R = 0.9050), a high correlation was found for antioxidant activity (R = 0.9196). In turn, flavonoids and hydroxycinnamic acid derivatives had moderate correlation, well as for organic acids no correlation was found *(Table 5)*.

Table 5 shows that the correlation between the growth inhibition of *P. aeruginosa* and the sum of catechins *(*R = 0.9433*)* was very high, in the case of polyphenols, flavonoids had a high correlation, while hydroxycinnamic acid derivatives had a moderate correlation. Whereas the total content of organic acids did not affect the inhibition of growth *P. aeruginosa*.

Table 5 shows a very high correlation between the inhibition of *C. albicans* growth and the catechin content (R = 0.9302), as well as antioxidant activity (R = 0.9906). In turn, a high correlation was observed with the total polyphenol (R = 0.7972) and flavonoid (R = 0.8999) contents, whereas a moderate correlation was found for hydroxycinnamic acid derivatives, and a low correlation was observed for organic acids (R = 0.3514).

Discussion

Pavlović A. V. et al. reported the use of 70 % and 96 % ethanol extracts of blackberry leaves [17]. According to the obtained results, they showed the content of total polyphenols and flavonoids in 96 % EtOH extract was 2.43 % and 1.00 %, and in the 70 % EtOH, it extract was 3.00 % and 1.50 %, respectively. Compared with our research the highest content of flavonoids and polyphenols was found in 60 % EtOH extract, too. The extraction of BAS directly depends on the solvent polarity and chemical properties of compounds. Blackberry leaves contain flavonoid glycosides such as rutin and hyperoside. According to their chemical properties, these compounds are less soluble in highly polar solvents (e. g., water) and more soluble in moderately polar solvents.

Paczkowska-Walendowska M. et al. recently investigated the hydroxycinnamic acid composition in a 40 % ethanol extract of blackberry leaves [18]. The result of research showed that the total of hydroxycinnamic acids was 0.80 %, meanwhile we obtained 1.40 % of hydroxycinnamic acids in the 40 % extract. The variance in phenolic compound levels is likely attributable to differing infusion durations, leaf-to-solvent ratios, plant species, and climatic and geographic conditions.

The investigated blackberry extracts demonstrated antimicrobial efficacy against *S. aureus*, *P. aeruginosa*, *P. vulgaris*, *B. subtilis*, and *C. albicans*. While the observed activity was markedly lower than that of the control antibiotics gentamicin and fluconazole – likely due to the substantially lower concentration of the active compounds in the extract compared to the pure pharmaceuticals – it is crucial to consider the significant toxicity profile of drugs like gentamicin. Its known adverse effects on the auditory nerve, kidneys, and liver can lead to severe complications, a drawback not associated with natural plant extracts [18]. A comparative analysis revealed that the blackberry extract inhibited the fungal strain with an efficacy comparable to fluconazole, despite the latter's lower concentration. While fluconazole remains a potent antifungal agent, it exhibits minimal activity against Gram-positive and Gram-negative bacteria. In contrast, the blackberry extract demonstrated broad-spectrum efficacy, effectively targeting the same fungus as well as both bacterial classes. This suggests that the extract possesses a multi-target mechanism of action, affecting diverse biological pathways in microbes. Consequently, blackberry extract represents a promising broad-spectrum antimicrobial agent with potentially lower toxicity than conventional pharmaceuticals.

Ispiryan A. et al. reported about studying the relationship of antiradical and antimicrobial activity and the amount of polyphenols and flavonoids in extracts of raspberry shoots, leaves, seeds and fruits [19]. A significant relationship was established between the concentration of biologically active substances and the observed antioxidant activity, but in the case of antimicrobial activity there was no correlation (R = 0.3). According to our data, it was shown that there is a very high correlation between the sum of polyphenols, catechins and antioxidant / antimicrobial effects against all Gram-positive, Gram-negative bacterial strains and the fungus *C. albicans*. The lowest correlations were observed in the case of organic acids. Therefore, polyphenols and catechins play a major role in antioxidant, antimicrobial activities.

Conclusions

1. In the research, it has been determined the content of BAS, antioxidant, antimicrobial activity of the obtained extracts of blackberry leaf. The dominant content of the sum of polyphenols, catechins and flavonoids was observed in 60 % EtOH extract, whereas the organic acids were observed in aqueous extract. Demonstrating potent bioactivity, the 60 % ethanolic extract displayed a high level of antioxidant capacity.

2. Blackberry leaf extracts actively suppressed the proliferation of the entire tested panel – including Gram-positive and Gram-negative bacteria and the yeast *C. albicans* – yielding inhibition zones between 18.0 mm and 25.0 mm.

3. We have shown that there was a very high correlation between the total catechins, antioxidant activity and antimicrobial effect against *S. aureus*, *P. vulgaris*, *P. aeruginosa* and *C. albicans*.

4. These findings show the great potential in the development and creation of new medicines with antimicrobial, antioxidant effects that are not inferior to, and even superior to, the effects of synthetic analogues.

Prospects for further research. Developing medicinal formulations based on extracts or isolated biologically active compounds from blackberry leaves with pronounced antioxidant and antimicrobial activity. Also studying the synergistic effects of these compounds with existing antibiotics to create new, effective herbal remedies against resistant strains.

Funding
The study was performed without financial support.

Conflict of interest: authors have no conflict of interest to declare.
Конфлікт інтересів: відсутній.

Information about the authors:
Marchenko A. O., PhD student of the Department of Pharmacognosy and Nutriciology, National University of Pharmacy, Kharkiv, Ukraine.
ORCID ID: 0009-0002-8616-0410
Komisarenko M. A., PhD, Assistant of the Department of Pharmacognosy and Nutriciology, National University of Pharmacy, Kharkiv, Ukraine.
ORCID ID: 0000-0002-1161-8151
Maslov O. Yu., PhD, Associate Professor of the Department of General Chemistry, National University of Pharmacy, Kharkiv, Ukraine.
ORCID ID: 0000-0001-9256-0934
Osolodchenko T. P., PhD, Head of Laboratory of Biochemistry and Biotechnology, Mechnikov Institute of Microbiology and Immunology of the NAMS of Ukraine, Kharkiv.
ORCID ID: 0000-0001-7258-3880
Kolisnyk S. V., PhD, DSc, Professor, Head of the Department of General Chemistry, National University of Pharmacy, Kharkiv, Ukraine.
ORCID ID: 0000-0002-4920-6064

Відомості про авторів:
Марченко А. О., аспірант каф. фармакогнозії і нутриціології, Національний фармацевтичний університет, м. Харків, Україна.
Комісаренко М. А., канд. фарм. наук, доцент каф. фармакогнозії і нутриціології, Національний фармацевтичний університет, м. Харків, Україна.
Маслов О. Ю., д-р філософії, асистент каф. загальної хімії, Національний фармацевтичний університет, м. Харків, Україна.
Осолодченко Т. П., канд. біол. наук, зав. лабораторії біохімії та біотехнології, ДУ «Інститут мікробіології та імунології ім. І. І. Мечникова НАМН України», м. Харків, Україна.
Колісник С. В., д-р фарм. наук, професор, зав. каф. загальної хімії, Національний фармацевтичний університет, м. Харків, Україна.

Oleksandr Maslov (Олександр Маслов)
alexmaslov392@gmail.com

References
1. GBD 2019 Antimicrobial Resistance Collaborators. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2022;400(10369):2221-2248. doi: 10.1016/S0140-6736(22)02185-7
2. Bongomin F, Gago S, Oladele RO, Denning DW. Global and Multi-National Prevalence of Fungal Diseases-Estimate Precision. J Fungi (Basel). 2017;3(4):57. doi: 10.3390/jof3040057
3. Negi PS. Plant extracts for the control of bacterial growth: efficacy, stability and safety issues for food application. Int J Food Microbiol. 2012;156(1):7-17. doi: 10.1016/j.ijfoodmicro.2012.03.006
4. Taylor PW, Hamilton-Miller JM, Stapleton PD. Antimicrobial properties of green tea catechins. Food Sci Technol Bull. 2005;2:71-81. doi: 10.1616/1476-2137.14184
5. Krzepiłko A, Prażak R, Święciło A. Chemical Composition, Antioxidant and Antimicrobial Activity of Raspberry, Blackberry and Raspberry-Blackberry Hybrid Leaf Buds. Molecules. 2021;26(2):327. doi: 10.3390/molecules26020327
6. Asnaashari M, Tajik R, Khodaparast MH. Antioxidant activity of raspberry (Rubus fruticosus) leaves extract and its effect on oxidative stability of sunflower oil. J Food Sci Technol. 2015;52(8):5180-7. doi: 10.1007/s13197-014-1564-7
7. Verma R, Gangrade T, Punasiya R, Ghulaxe C. Rubus fruticosus (blackberry) use as an herbal medicine. Pharmacogn Rev. 2014;8(16): 101-4. doi: 10.4103/0973-7847.134239
8. Oszmiański J, Wojdyło A, Nowicka P, Teleszko M, Cebulak T, Wolanin M. Determination of phenolic compounds and antioxidant activity in leaves from wild Rubus l. species. Molecules. 2015;20(3):4951-66. doi: 10.3390/molecules20034951
9. Koczka N, Stefanovits-Bányai É, Prokaj E. Element composition, total phenolics and antioxidant activity of wild and cultivated blackberry (Rubus fruticosus L.) fruits and leaves during the harvest time. Not Bot Horti Agrobot Cluj Napoca. 2018;46(2):563-9. doi: 10.15835/nbha46210993
10. Derzhavna Farmakopeia Ukrainy_[The State Pharmacopoeia of Ukraine]. 2nd ed. Kharkiv, (UA): State Enterprise Ukrainian Scientific Pharmacopoeial Center of Medicines Quality; 2015. Ukrainian.
11. Maslov O, Kolisnyk 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: 10.5281/zenodo.7813135
12. Maslov O, Komisarenko M, Kolisnyk S, Kostina T, Golik M, Moroz V, et al. Investigation of the extraction dynamic of the biologically active substances of the raspberry (Rubus idaeus L.) shoots. Curr Issues Pharm Med Sci. 2023;36(4):194-8. doi: 10.2478/cipms-2023-0034
13. Osolodchenko T, Martynov A, Andreyeva I, Zavada N, Batrak O, Ryabova I. [Antimicrobial activity of nisin, diclofenac sodium, amlodipine and their pharmaceutical compositions regarding reference strains of microorganisms]. Annals of Mechnikov's Institute. 2023;(4):26-30. doi: 10.5281/zenodo.10257367
14. Volianskyi YL, Hrytsenko IS, Shyrobokov VP. Vyvchennia spetsyfichnoi aktyvnosti protymikrobnykh likarskykh zasobiv [Testing the specific activity of antimicrobial drugs]. Kyiv: MOZ Ukrainy; 2004. Ukrainian.

15. Mukaka MM. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. Malawi Med J. 2012;24(3):69-71.
16. Maslov OY, Kolisnyk SV, Komisarenko MA, Altukhov AA, Dynnyk KV, Stepanenko VI. Study and evaluation antioxidant activity of dietary supplements with green tea extract. Current issues in pharmacy and medicine: science and practice. 2021;14(2):215-9. doi: 10.14739/2409-2932.2021.2.233306
17. Pavlović AV, Papetti A, Zagorac DČ, Gašić UM, Mišić DM, Tešić ŽL, et al. Phenolics composition of leaf extracts of raspberry and blackberry cultivars grown in Serbia. Ind Crops Prod. 2016;87:304-14. doi: 10.1016/j.indcrop.2016.04.052
18. Paczkowska-Walendowska M, Gościniak A, Szymanowska D, Szwajgier D, Baranowska-Wójcik E, Szulc P, et al. Blackberry Leaves as New Functional Food? Screening Antioxidant, Anti-Inflammatory and Microbiological Activities in Correlation with Phytochemical Analysis. Antioxidants (Basel). 2021;10(12):1945. doi: 10.3390/antiox10121945
19. Ispiryan A, Atkociuniene V, Makstutiene N, Sarkinas A, Salaseviciene A, Urbonaviciene D, et al. Correlation between Antimicrobial Activity Values and Total Phenolic Content/Antioxidant Activity in Rubus idaeus L. Plants (Basel). 2024;13(4):504. doi: 10.3390/plants13040504