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RESEARCH ARTICLE

Phytochemical Analysis, Antibacterial, Anti-Fungi, and Antiradical Effects, Exploring Correlation Analysis of Lingonberry Leaf (*Vaccinium vitis-idaea* L.) Extracts

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SUMMARY

Nowadays, infectious diseases are a significant catastrophe for modern public health and society, especially due to the spread of Gram-negative and Gram-positive bacterial strains, as well as fungi, that are resistant to antibiotics. The purpose of this work was to study the total content of some biologically active substances, determine the antibacterial, antifungal, and antioxidant activities of lingonberry leaf extracts, and perform a correlation analysis between the content of natural compounds and their antibacterial, antifungal, and antioxidant activities. The results demonstrated the highest amounts of polyphenols, flavonoids, catechins, and organic acids in the extracts, with respective values of 2.20±0.06%, 1.39±0.01%, 0.65±0.01%, and 0.21±0.01% in the 60% ethanolic extract. Organic acids were most abundant in the aqueous extract (0.89±0.01%), while hydroxycinnamic acids were most prevalent in the 40% ethanolic extract (0.80±0.04%). The 60% ethanolic extract of lingonberry leaf exhibited the most potent antioxidant properties. There was a strong correlation between the content of polyphenols, flavonoids, and the inhibition of S. aureus, P. aeruginosa, B. subtilis, P. vulgaris, C. albicans, and E. coli, which depended significantly on the content of hydroxycinnamic acids. These findings highlight the great potential for developing and creating new medicines with antimicrobial, antioxidant, and antifungal effects that are not only comparable to but may even surpass those of synthetic analogues.

Key Words: *Lingonberry leaf, correlation analysis, antiradical effect, antimicrobial effect, phenolic compounds*

Fitokimyasal Analiz, Antibakteriyel, Anti-Fungal ve Antiradikal Etkiler ve Lingonberry Yaprağı (Vaccinium vitis-idaea L.) Ekstrelerinin Korelasyon Analizinin Araştırılması

ÖZ

Günümüzde bulaşıcı hastalıklar, özellikle antibiyotiklere dirençli Gram-negatif ve Gram-pozitif bakteri suşlarının yanı sıra mantarların yayılması nedeniyle, modern halk sağlığı ve toplum için önemli bir felakettir. Bu çalışmanın amacı, bazı biyolojik olarak aktif maddelerin toplam içeriğini incelemek, kızılcık yaprağı ekstrelerinin antibakteriyel, antifungal ve antioksidan aktivitelerini belirlemek ve doğal bileşiklerin içeriği ile antibakteriyel, antifungal ve antioksidan aktiviteleri arasında bir korelasyon analizi yapmaktı. Sonuçlar, %60 etanolik ekstredeki sırasıyla %2,20±0,06%, %1,39±0,01%, %0,65±0,01 ve %0,21±0,01 değerleriyle, ekstrelerde en yüksek polifenol, flavonoid, kateşin ve organik asit miktarlarını göstermiştir. Organik asitler sulu ekstrede en bol miktarda bulunurken (%0,89±0,01), hidroksisinnamik asitler %40 etanollü ekstrede en yaygındı (%0,80±0,04). Yaban mersini yaprağının %60 etanollü özütü en güçlü antioksidan özelliklerini sergiledi. Polifenoller, flavonoidler ve S. aureus, P. aeruginosa, B. subtilis, P. vulgaris, C. albicans ve E. coli inhibisyonu arasında güçlü bir korelasyon vardı ve bu korelasyon önemli ölçüde hidroksisinnamik asitlerin içeriğine bağlıydı. Bu bulgular, yalnızca sentetik analoglarla karşılaştırılabilir değil, hatta onları aşabilecek antimikrobiyal, antioksidan ve antifungal etkilere sahip yeni ilaçlar geliştirme ve yaratma konusunda büyük bir potansiyel olduğunu vurgulamaktadır.

Anahtar Kelimeler: *Lingonberry yaprağı, korelasyon analizi, antiradikal etki, antimikrobiyal etki, fenolik bileşikler.*

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INTRODUCTION

Bacterial and fungal infections remain among the leading causes of human mortality worldwide. Statistical analyses indicate that 13.7 million people die annually due to infectious diseases, with a mortality rate of 99 deaths per 100,000 individuals. Among these, 3.6 million deaths are attributed to bacterial infections caused by Gram-positive strains, such as *Staphylococcus aureus*, and Gram-negative strains, including *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*. In 2019 alone, approximately 1 million deaths were linked to *S. aureus* infections (Ikuta et al., 2022). This issue is further exacerbated by the increasing resistance of bacteria to commonly used antibiotics, making treatment more challenging, time-consuming, and costly (Bongomin et al., 2017). Given this scenario, the search for new antibacterial natural compounds has become a highly relevant and promising area of research today.

The scientific community has devoted significant attention to studying the pharmacological activities of natural compounds, particularly derivatives of flavon-3-ols and flavonols (Maslov et al., 2024; Di Pede et al., 2022; Panche et al., 2016). According to the literature, natural compounds offer several advantages over synthetic alternatives (Chaachouay et al., 2024). Firstly, they are generally safer and associated with fewer side effects. Secondly, natural compounds often demonstrate higher efficacy. Lastly, their production is more cost-effective, making them an attractive option for therapeutic development (Abdallah et al., 2023).

Lingonberry (*Vaccinium vitis-idaea* L.), an evergreen shrub from the *Ericaceae* family, is one of the richest plant sources of phenolic compounds**.** Its distribution spans Russia, the Baltic countries, the northern regions of Ukraine and Belarus, and Canada (Ryyti et al., 2020; Hirabayashi et al., 2023). The chemical composition of lingonberry leaves includes a wide variety of biologically active substances: hydroquinone derivatives (arbutin, methylarbutin), catechins (epicatechin, (+)-catechin), flavonoids (rutin, quercetin), hydroxycinnamic acids (ferulic and caffeic acids), and organic acids (citric and malic acids) (Kowalska et al., 2021; Cvetkova et al., 2024).

Numerous scientific studies have focused on determining the antioxidant activity of *Vaccinium vitis-idaea* leaf extracts (Vyas et al., 2013; Feriemi and Lamari, 2016). However, no data are available on assessing the antioxidant, antibacterial, and antifungal activities and their correlation with the content of biologically active substances using the potentiometric method.

The purpose of this research was to quantify the total polyphenols, flavonoids, hydroxycinnamic acids, organic acids, and catechins in *V. vitis-idaea* leaf extracts. Additionally, the study aimed to evaluate their antibacterial and antifungal activities against Gram-positive strains (*S. aureus*, *B.subtilis*), Gram-negative strains (*E. coli*, *P. vulgaris*, *P. aeruginosa*), and fungi (*C. albicans*). Finally, a correlation analysis was conducted to explore the relationship between the content of biologically active substances in the extracts and their antibacterial, antifungal, and antioxidant activities.

MATERIALS AND METHODS

Leaves of *Vaccinium vitis-idaea* were harvested in the Zhytomyr region, Ukraine (50°32’94” N, 29°53’68” E), during autumn 2021. A green tea (*Camellia sinensis* L.) leaves were collected in Anhui Province, China (30°63’41” N, 116°33’25” E).

Six samples of *V. vitis-idaea* leaf (10.0 g each, exact mass) with particle sizes of 1–2 mm were prepared. The extraction was conducted using distilled water, 20%, 40%, 60%, and 96% ethanol at 80°C for 1 hour with a condenser, maintaining a raw material-to-solvent ratio of 1:20. The extraction process was performed twice to ensure the complete extraction of biologically active substances. The filtrates were then combined and evaporated under vacuum using a rotary evaporator to achieve a final extract-to-raw material ratio of 1:2. As a result, six extracts were obtained: aqueous and ethanol extracts at 20%, 40%,

60%, and 96% concentrations. Additionally, a green tea (*Camellia sinensis*) extract was prepared using the same method with 60% ethanol.

The sum of polyphenols was quantified using the Folin-Ciocaltau method, with absorbance readings taken at 760 nm (Blainski et al., 2013). The phosphomolybdotungstic reagent was used for performing an 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, Cx – 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 catechin content was determined using the vanillin reagent assay, with optical density measured at 505 nm. (Maslov et al., 2023, a). A 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 the equation:

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

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

The total flavonoid was evaluated by $AlCl_3$ complex assay, with absorbance measurement at 415 nm (Upyr et al., 2019). The concentration of standard solution of rutin was 0.02 mg/mL. The total flavonoid content in extracts (X), expressed as rutin, was calculated according to the equation:

$$X(\%) = \frac{A \times K_{dil} \times m_{st} \times 100}{A_{st} \times V}$$

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

The total hydroxycinnamic acids were quantified using a reaction with $NaNO_2$ and Na_2MoO_4, with optical density measured at 525 nm. (Upyr et al., 2022). The total content of hydroxycinnamic acids derivatives in extracts (X), expressed as chlorogenic acid was calculated according to the equation:

$$X(\%) = \frac{A \times K_{dil} \times 1000}{188 \times V}$$

where, A – absorbance of analyzed solution; 188 – specific adsorption coefficient of chlorogenic acid; V – volume of extract, mL; Kdil – coefficient of dilution, mL.

The content of total organic acids was established through acid-base titration, using a potentiometric method to determine the end-point (Maslov et al., 2023, b). The total content of organic acids in extracts, expressed as citric acid was calculated according to equation:

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

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.

The antioxidant effect was determined by the potentiometric assay (Maslov et al., 2023, c). A 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 (E0) and final (E1) potentials was found.

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

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

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.

Test strains of fungi – *C. albicans ATCC 885/653,* Gramm-positive strains – *S. aureus ATCC 25923*, *B. subtillis ATCC 6538*, Gramm-negative strains – *E. coli ATCC 25922*, *P. vulgaris NTCS 4636*, *P. aeruginosa ATCC* 27853 were applied with the recommendations for the assessment of antimicrobial effect of drugs**.**

The diffusion method using agar "wells" was employed to evaluate the drug's activity (*Tsemenko et al., 2018*). Microorganism suspensions with standardized concentrations (optical density) were prepared according to the McFarland turbidity standard (0.5 units) using a Densi-La-Meter (Czech Republic) at a wavelength of 540 nm. Suspensions were prepared following the equipment's manual and information guidelines. The colony-forming unit (CFU) concentration was 10^7 microorganisms per milliliter of growth medium, as determined by the McFarland standard.

On solidified agar in sterile Petri dishes, 1 mL of the microorganism suspension was pipetted under sterile conditions. After uniformly distributing the microorganisms across the agar surface, the plates were incubated at room temperature for 15–20 minutes. Wells with a diameter of 6 mm were then created in the agar, and solutions of the test substances were introduced.

The samples were incubated at 37°C for 16–24 hours. Following incubation, the plates were inverted and placed on a dark matte surface under light angled at 45° for measurement. The diameters of the growth inhibition zones were measured using a caliper. Gentamicin and fluconazole were used as reference drugs for assessing antibacterial and antifungal activity.

Pearson's (r) correlation coefficient was applied to analyze the correlation relation. The correlation coefficient to takes a value in the range of -1 to +1. Correlation is negligible from 0.00 to 0.30; low – from 0.30 to 0.50; moderate – from 0.50 to 0.70; high – from 0.70 to 0.90 and very high from 0.90 to 1.00. (Akoglu et al., 2018)

RESULTS AND DISCUSSION

According to the results presented in Table 1, the 60% EtOH extract (2.20±0.06%) contained the highest amount of polyphenols, followed by the 40% EtOH extract (2.10±0.07%), while the aqueous extract had the lowest amount (1.90±0.06%).

The content of catechins increased in the following order: 96% EtOH extract (1.13±0.03%) < aqueous extract (1.34±0.04%) < 20% EtOH extract (1.36±0.04%) < 40% EtOH extract (1.37±0.04%) < 60% EtOH extract (1.39±0.04%). The proportion of catechins in relation to the total polyphenol content was 55%, 63%, 65%, 68%, and 71% for the 96%, 60%, 40%, 20%, and aqueous extracts, respectively. The highest percentage of catechins was found in the aqueous extract, while the lowest was in the ethanolic extracts (Table 1).

Table 1 shows that the 60% EtOH extract (0.65±0.02%) contained the highest amount of flavonoids, while the aqueous extract (0.41±0.01%) had the lowest. The percentage of flavonoids relative to the total polyphenol content was 21%, 30%, 28%, 22%, and 22% for the 96%, 60%, 40%, 20%, and aqueous extracts, respectively. The highest percentage of flavonoids was found in the 60% EtOH extract, while the lowest was in the aqueous extract.

The amount of hydroxycinnamic acids increased in the following order: 96% EtOH extract (0.20±0.01%) < 60% EtOH extract (0.21±0.01%) < aqueous extract (0.28±0.01%) < 20% EtOH extract (0.57±0.03%). The proportion of hydroxycinnamic acids relative to the total polyphenol content was 10%, 10%, 38%, 29%, and 15% for the 96%, 60%, 40%, 20%, and aqueous extracts, respectively. The highest proportion of hydroxycinnamic acids was found in the 40% EtOH extract, while the lowest was in the 96% EtOH extract (Table 1).

The highest amount of organic acids was found in the aqueous extract (0.89±0.01%), followed by the 20% EtOH extract (0.51±0.02%), while the lowest amount was in the 96% EtOH extract (0.38±0.01%). The total organic acid content was 82%, 78%, 80%, 75%, and 53% lower than the polyphenol content in the 96%, 60%, 40%, 20% EtOH, and aqueous extracts, respectively.

Table 1. The sum of different group of biologically active substances in *V. vitis-idaea* leaf liquid extracts

Sample	Amount of polyphenols expressed as gallic acid, %±SD	Amount of catechins expressed as epigallocatechin-3-O-gallate,% ±SD	Amount of flavonoid expressed as rutin, %±SD	Amount of hydroxycinnamic acids expressed as chlorogenic acid, %±SD	Amount of organic acids expressed as citric acid, %±SD
96% EtOH extract	2.06±0.06	1.13±0.03	0.44±0.01	0.20±0.01	0.38±0.01
60% EtOH extract	2.20±0.06	1.39±0.04	0.65±0.02	0.21±0.01	0.48±0.01
40% EtOH extract	2.10±0.07	1.37±0.04	0.59±0.02	0.80±0.04	0.41±0.01
20% EtOH extract	2.00±0.06	1.36±0.04	0.44±0.01	0.57±0.03	0.51±0.02
H_2O extract	1.90±0.06	1.34±0.04	0.41±0.01	0.28±0.01	0.89±0.01

A potentiometric method for determining antioxidant activity was used to evaluate the effect of the obtained extracts of *V. vitis-idaea* leaf. Table 2 shows that the level of antiradical effect growths in the order: 96% EtOH extract (105.00±1.05 mmol-eqv./$m_{dry\ res.}$) > 20% EtOH extract (114.56±1.15 mmol-eqv./$m_{dry\ res.}$) > aqueous extract (119.88±0.89 mmol-eqv./$m_{dry\ res.}$) > 40% EtOH extract (142.44±1.42 mmol-eqv./$m_{dry\ res.}$) > 60% EtOH extract (148.39±1.48 mmol-eqv./$m_{dry\ res.}$). In light of the data obtained, it can be established that the 60% EtOH extract has the top level of antiradical effect. According to the modern classification of antioxidant activity, which was previously developed in our previous research (Mikulic-Petkovsek et al., 2012), it was found that all extracts obtained have a great level of antiradical effect. 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 *V. vitis-idaea* leaf extracts. The obtained extracts were significantly inferior in antioxidant effect to *C. sinensis* leaf extract. Further, a 0.03 mol/L solutions (in terms of the amount of polyphenols expressed as gallic acid) of extracts of *v* and *C. sinensis* leaf were prepared. As a result of the study, it was found that when compared at the same concentrations, the aqueous extract had the highest antioxidant effect, and the least - 96% extract. (Table 3)

The potentiometric assay was chosen to evaluate antioxidant activity for several reasons: firstly, it is highly expressive; secondly, it is cost-effective; and thirdly, it is both accurate and precise. To compare the

antioxidant effects, we used the green tea leaf extract. The results showed that green tea extract significantly inactivates free radicals more effectively than *V. vitis-idaea* leaf extracts. Among the *V. vitis-idaea* extracts, the 60% EtOH extract exhibited the highest level of antiradical activity.

Next, we compared the antioxidant effects of the extracts at the same concentration of phenolic compounds. The results showed that the green tea extract was less effective than both the 60% EtOH and aqueous extracts of *V. vitis-idaea*. Moreover, the order of antioxidant activity changed significantly with varying concentrations of phenolic compounds. At higher concentrations, the 60% EtOH extract exhibited the greatest antiradical effect, while at equal concentrations, the aqueous extract was the most effective.

Kryvtsova *et al.* reported on the antioxidant effects of ethyl acetate, methanolic, 60%, and 96% EtOH extracts of *V. vitis-idaea* leaves and fruits. Their research showed that the ethyl acetate extract had the highest antioxidant activity, followed by methanolic, ethanolic, and 60% EtOH extracts. In comparison, our study found that the 60% EtOH extract exhibited higher antioxidant activity than the 60% EtOH extract in their research. This difference may be due to the application of different analytical methods. In our study, we employed the electrochemical method, while Kryvtsova *et al.* used the spectrophotometric method, which is less accurate and sensitive.

Table 2. The level of antiradical effect of *V. vitis-idaea* leaf liquid extracts

Analyzed Sample	Antiradical effect, mmol-eqv./$m_{dry\ res.}$ ±SD	Conditional term of antioxidant level
96% EtOH extract	105.00±1.05	High level
60% EtOH extract	148.39±1.48	High level
40% EtOH extract	142.44±1.42	High level
20% EtOH extract	114.56±1.15	High level
H_2O extract	119.88±0.89	High level
Green tea leaf 60% EtOH extract	548.79±10.98	Very high level

Table 3. Comparing the value of antiradical activity of *V. vitis-idaea* leaf liquid extracts with *C. sinensis* leaf 60% EtOH extract at the concentration 0.03 mol/L expressed as sum polyphenols as gallic acid

Sample	Concentration of polyphenols, mol/L	Antiradical effect, mmol-eqv./$m_{dry\ res.}$
96% EtOH extract	0.03	26.25±0.26
60% EtOH extract		34.27±0.34
40% EtOH extract		30.50±0.31
20% EtOH extract		29.15±0.29
H_2O extract		36.00±0.36
Green tea leaf 60% EtOH extract		30.78±0.31

In this research work, the antibacterial and antifungal activity of the obtained *V. vitis-idaea* leaf extracts was investigated against the following strains of G(+) – *B. subtilis, S. aureus*, G(–) – *P. vulgaris*, *E. coli*, *P. aeruginosa*, as well as a strain of the fungus *C. albicans*. According to the obtained results, all extracts obtained from the *V. vitis-idaea* leaf had an effective antibacterial and antifungal effect. (Table 4)

S. aureus was the most penetrating to the 60% EtOH extract (22.5±0.4 mm) and more resistant to the 20% EtOH extract (16.5±0.5 mm). When comparing the results of the gentamicin standard and the 60% EtOH extract, it was found that the 60% EtOH extract was 3% better at inhibiting the growth of the *S. aureus* strain of bacteria. According to the results presented in Table 3, it was found that *B. subtilis*, as

well as *S. aureus*, was less resistant to the 60% EtOH extract (21.0±0.4 mm), followed by 40% EtOH extract (19.5±0.5 mm), and the 20% EtOH extract (17.0±0.4 mm) inhibited the growth of the bacterial strain the least. *P. aeruginosa*. *E. coli* were most sensitive to the action of 60% EtOH extract, whereas *P. vulgaris* were most sensitive to the action of aqueous extract. Antifungal effect research showed that 60% EtOH extract of *V. vitis-idaea* leaf was the most actively inhibited the growth of the fungus, whereas the 20% EtOH extract were least active inhibited the growth of fungi. (Table 4)

The *V. vitis-idaea* leaf extract studied in our research showed antibacterial and antifungal activity against the following strains: *S. aureus*, *P. aeruginosa*, *P. vulgaris*, *B. subtilis*, and *C. albicans*. Kryvtsova *et al.* (2019) reported the antimicrobial activity of ethyl acetate and methanolic extracts of leaves and fruits against both test and resistant G(+) and G(-) strains. The highest antibacterial activity of both extracts was observed against *S. aureus*. In comparison with our results, the highest inhibition zone in our extracts was also found against *S. aureus*. This suggests that *V. vitis-idaea* leaf extract could be useful in developing new antibacterial drugs against resistant *S. aureus* strains.

Tsemenko *et al.* (2018) reported that the native 96% EtOH *V. vitis-idaea* leaf extract was most active against *P. vulgaris*, followed by *C. albicans*. In contrast, our research showed that the 96% EtOH extract highly inhibited *S. aureus*, followed by *P. vulgaris* and *P. aeruginosa*, while the *C. albicans* strain was less sensitive to the 96% EtOH *V. vitis-idaea* extract. Furthermore, when comparing the antibacterial effects of our extracts with the results of Tsemenko *et al.* and Kryvtsova *et al.*, we found that our results were more pronounced. This difference may be attributed to variations in the content of phenolic compounds and hydroquinone derivatives.

Based on the obtained data, it may initially appear that the antimicrobial and antifungal activity of *V. vitis-idaea* leaf extracts is significantly lower than that of gentamicin and fluconazole, as their solution concentrations were much lower than the polyphenol content in the extract. However, it is important to note that gentamicin has serious toxicity to the auditory nerve, kidneys, and liver, potentially leading to severe complications (Ispiryan et al., 2024).

When comparing the antifungal effects of fluconazole and *V. vitis-idaea* leaf extract, it was observed that both inhibited fungal growth to the same extent, despite fluconazole being used at a lower concentration, similar to gentamicin. While fluconazole is a leading antifungal medication, it has limited effectiveness against both gram-negative and gram-positive bacteria. In contrast, *V. vitis-idaea* leaf extracts show sensitivity against both bacterial strains and fungi.

Thus, *V. vitis-idaea* leaf extracts represent a combined pharmaceutical with the ability to affect various vital mechanisms of bacteria and fungi, offering a broad spectrum of activity against different strains. Additionally, these extracts do not exhibit serious toxicity, making them a promising alternative.

Table 4. The value of antibacterial and anti-fungi activity of *V. vitis-idaea* leaf liquid extracts

Sample	Concentra-tionmmol/L, (ex-pressed in total poly-phenols as gallic acid)	Diameter of the growth retardation zone, mm					
		Gramm-positive		Gramm-negative			Fungi
		Staphy-lococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Proteus vulgaris*	*Pseudomo-nas aerugi-nosa*	*Candida albicans*
96% EtOH extract	0.036	21.0± 0.4	18.5 ±0.5	18.5 ±0.5	19.0 ±0.4	19.0 ±0.4	16.0 ±0.4
60% EtOH extract	0.039	22.5± 0.5	21.0 ±0.4	20.0 ±0.4	21.0 ±0.4	21.0 ±0.4	17.0 ±0.4
40% EtOH extract	0.042	19.5± 0.5	19.5 ±0.5	17.5 ±0.5	19.5 ±0.5	19.5 ±0.5	16.0 ±0.4
20% EtOH extract	0.035	16.5± 0.5	17.0 ±0.4	17.0 ±0.4	16.5 ±0.5	17.0 ±0.4	14.5 ±0.5
H_2O extract	0.035	17.0± 0.4	18.0 ±0.4	18.0 ±0.4	16.0 ±0.4	17.0 ±0.4	15.0 ±0.4
Gentamycin	0.003	22.0± 0.4	24.0 ±0.3	25.3 ±0.3	25.0 ±0.3	25.6 ±0.3	12.0 ±0.6
Fluconazole	0.003	18.0± 0.4	12.0 ±0.6	14.3 ±0.6	12.3 ±0.6	10.0 ±0.8	20.0 ± 0.4

The dependence of antioxidant, antibacterial and antifungal activity on the content of different groups of biologically active substances was studied using the method of linear regression. In Fig. 1 shows that the correlation between the antioxidant effect and the sum polyphenols was moderate (R=0.6566), in the case of catechins was moderate (R=0.7318), flavonoids was very high (R=0.9230), in the case of hydroxycinnamic acids was moderate (R=0.6512), and the lowest correlation value was observed for organic acids.

According to the research results presented in Fig. 2 it was found that there is a high correlation between phenolic compounds (R=0.8643), flavonoids (R=0.7031), moderate correlation – organic acids (R=0.5902), and inhibition of the growth of *S. aureus*, in the case of catechins (R=0.2258), hydroxycinnamic acids (R=0.3936), and antioxidant activity (R=0.4479) – low correlation.

In Fig. 3 shows that the antimicrobial effect against *B. subtilis* is very highly dependent on the content of flavonoids (R=0.9064), high dependent on polyphenols (R=0.8643), and antioxidant activity (R=0.8167), in turn, the sum hydroxycinnamic acids is observed moderate correlation and in the case of catechins there is no dependence.

The study showed that there is no correlation between organic acids (R=0.1229), catechins (R=0.036), and inhibition of *E. coli* growth, while polyphenols, flavonoids, have moderate correlation on the growth inhibition of *E. coli*, whereas a high correlation is observed in case of hydroxycinnamic acid. (Fig. 4)

When studying the relationship between inhibition of growth of *P. vulgaris* and the content of different groups of biologically active substances, there is a very high dependence of antibacterial activity on the polyphenols (R=0.9623), high correlation – flavonoids (R=0.8704), in turn, the antioxidant effect, organic acids had a moderate correlation, while catechins, hydroxycinnamic acids had not correlation at all. (Fig. 5)

Fig. 6 shows that the correlation between the growth inhibition of *P. aeruginosa* and the sum of polyphenols (R=0.9470) is very high, with the sum of flavonoids was high, in the case of organic acids and antioxidant effect it was found moderate correlation. Whereas, the sum hydroxycinnamic acids was not effect on the inhibition of growth *P. aeruginosa*.

In Fig. 7 shows a high correlation between inhibition of the growth of *C. albicans* and the sum

polyphenols (R=0.8665), flavonoids (R=0.8133), in turn, with the sum of polyphenols (R=0.6316) was found a moderate dependence, a low correlation was determined of content organic acids, and the total content of catechins was not effect on the inhibition of growth of *C. albicans*.

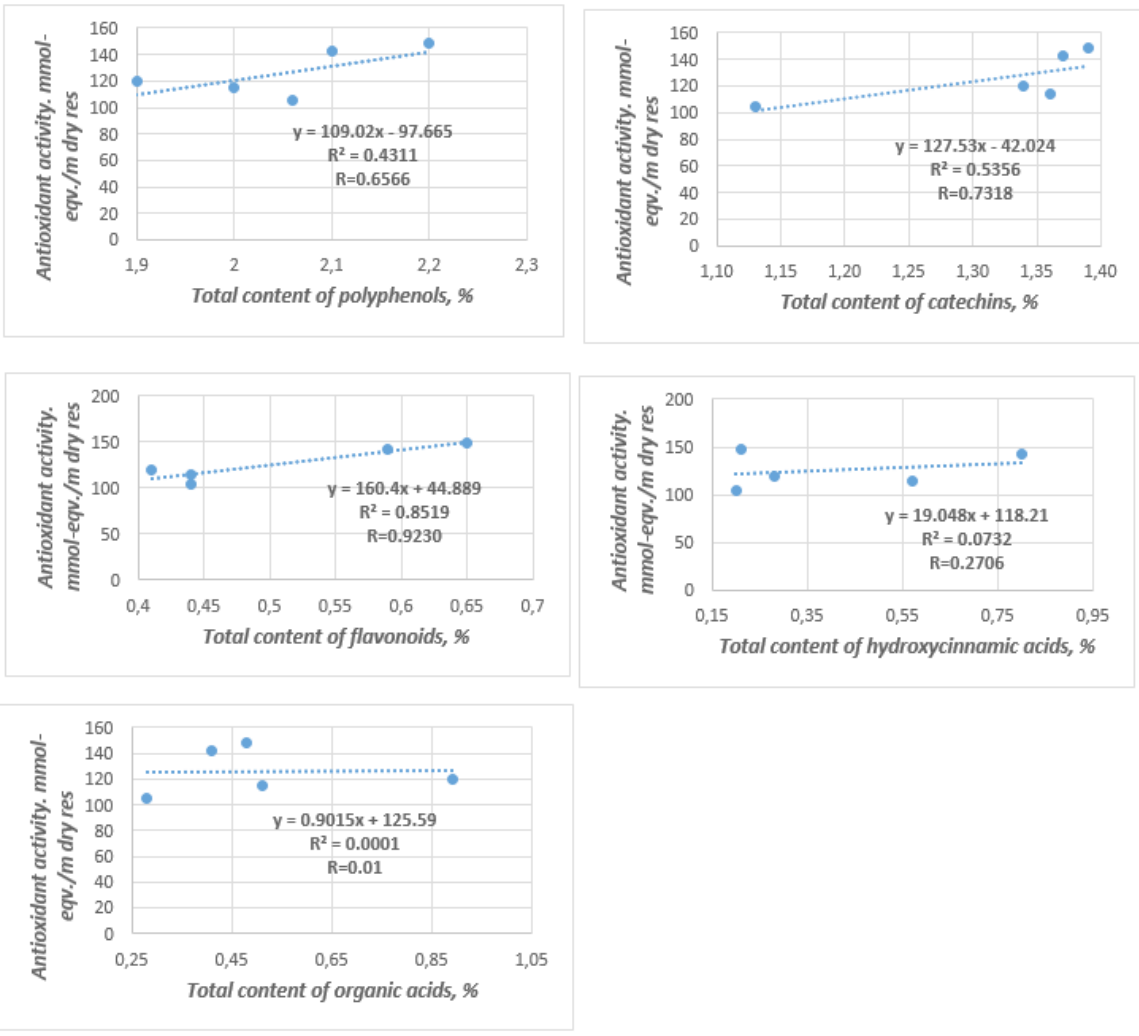


Figure 1. Correlation relationship between value of antioxidant activity and biologically active substances

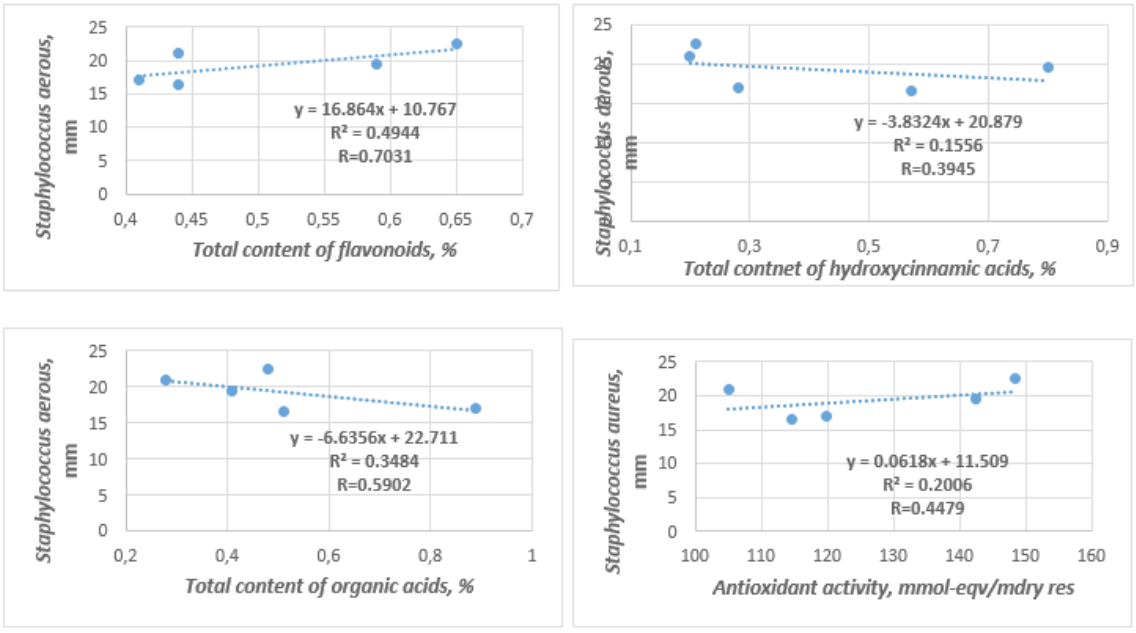


Figure 2. Correlation relationship between value of antimicrobial activity against *S. aureus* and sum of biologically active substances, antioxidant activity

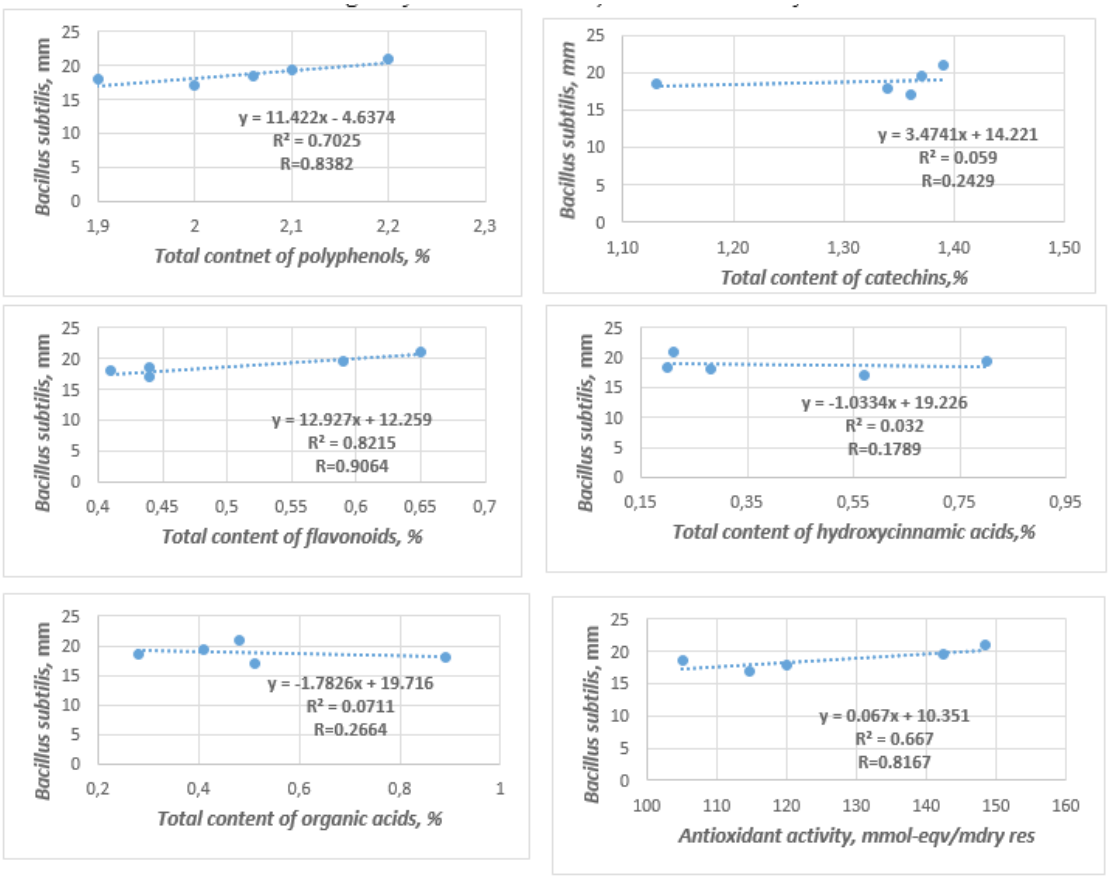


Figure 3. Correlation relationship between value of antimicrobial activity against *B. subtilis* and sum of biologically active substances, antioxidant effect

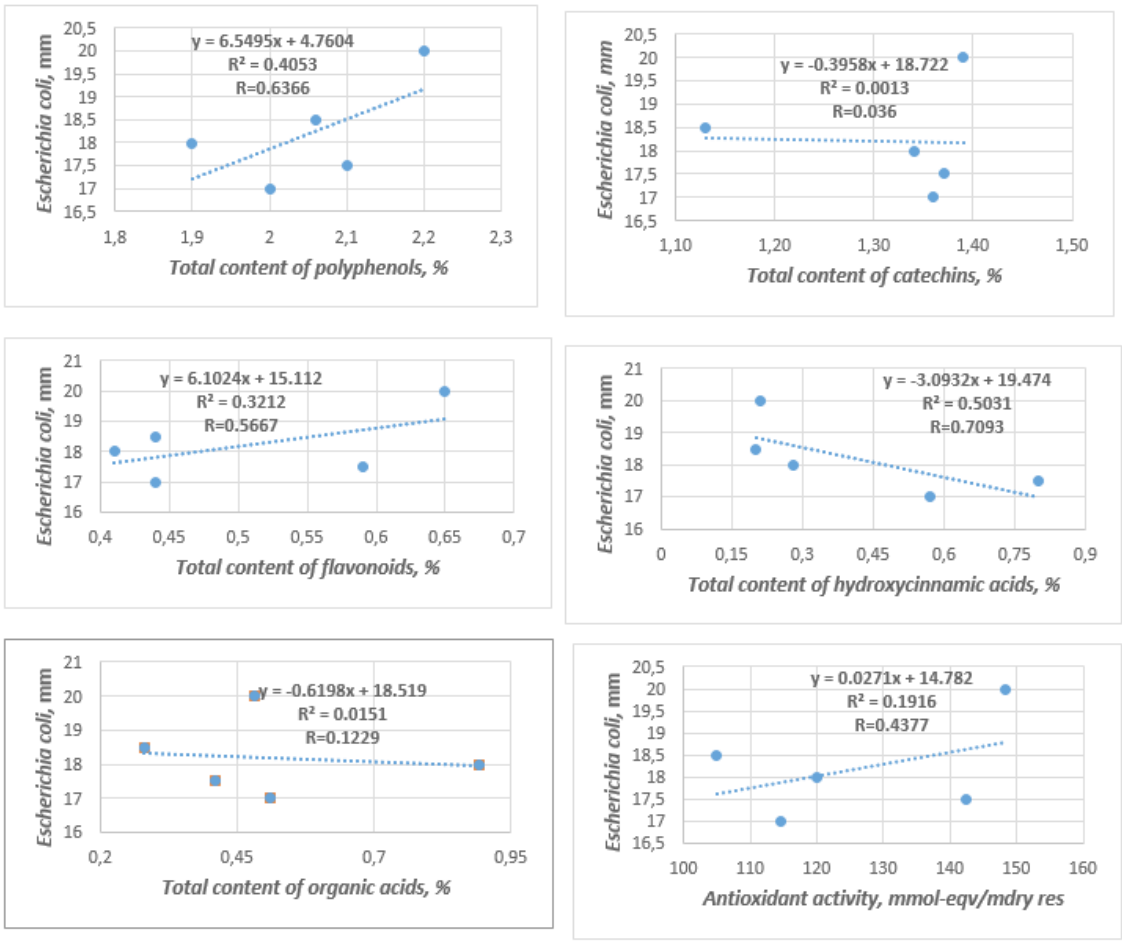
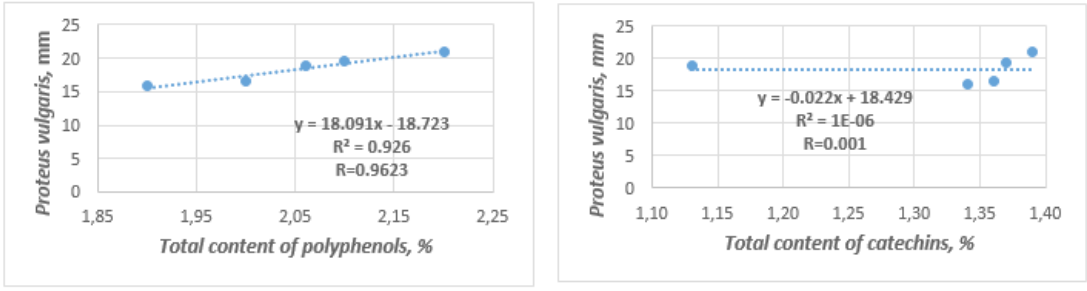


Figure 4. Correlation relationship between value of antimicrobial activity against *E. coli* and sum of biologically active substances, antioxidant activity



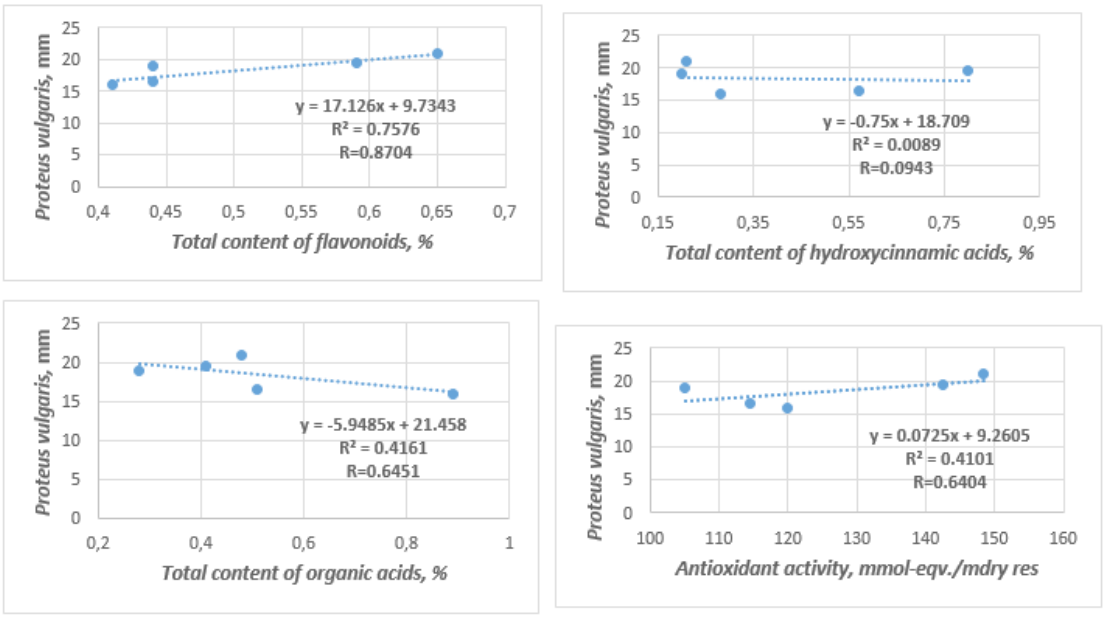


Figure 5. Correlation relationship between value of antimicrobial activity against *P. vulgaris* and sum of biologically active substances, antioxidant activity

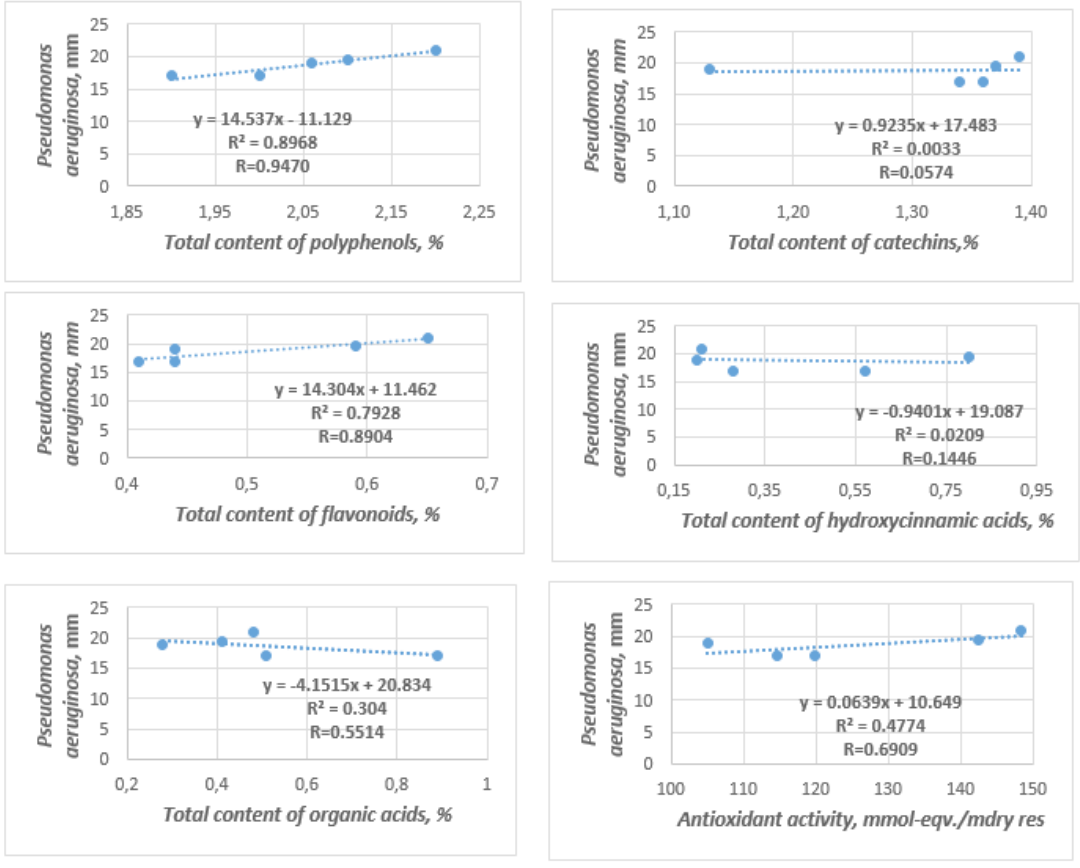


Figure 6. Correlation relationship between value of antimicrobial activity against *P. aeruginosa* and sum of biologically active substances, antioxidant effect

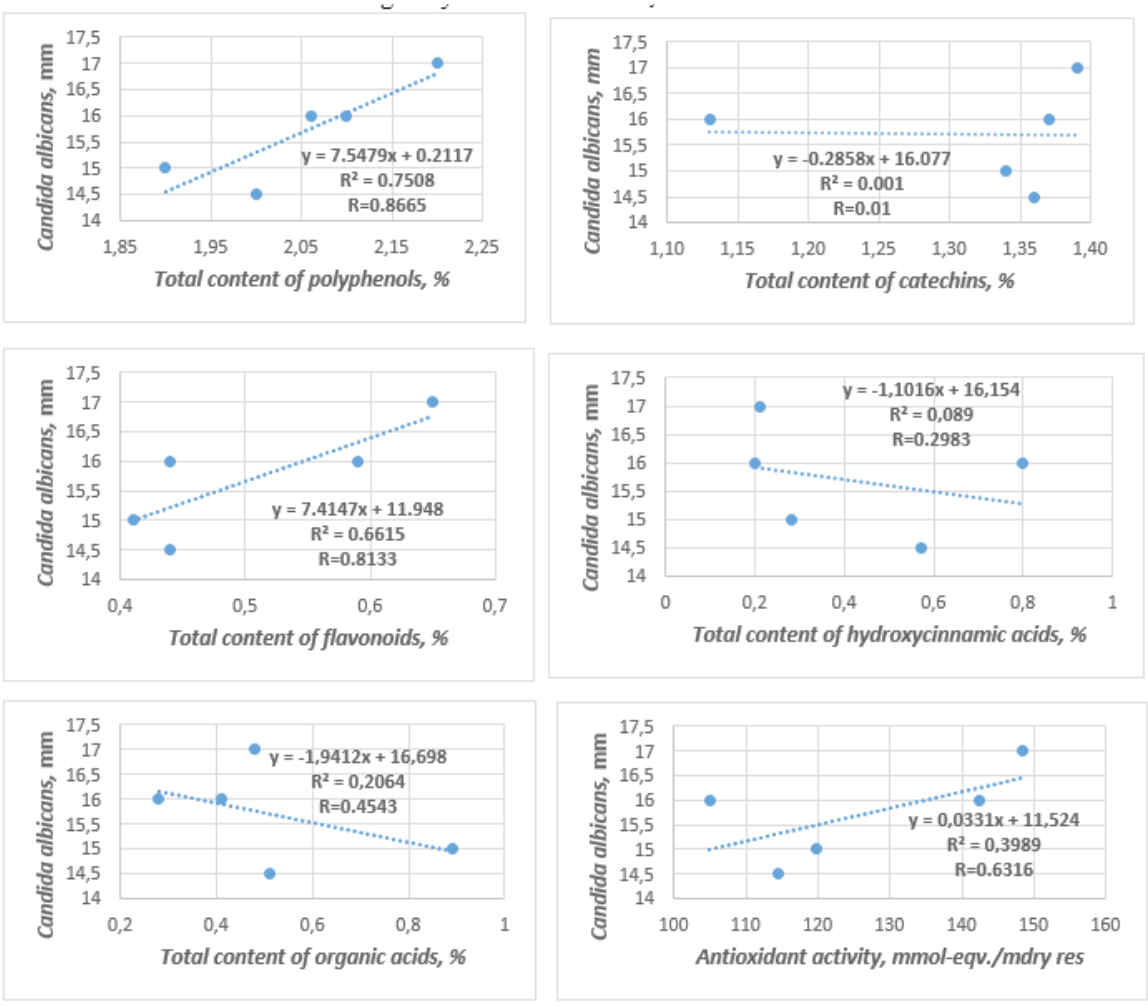


Figure 7. Correlation relationship between value of antimicrobial activity against *C. albicans* and sum of biologically active substances, antioxidant activity

Ispiryan *et al.* (2024) reported on the study of the relationship between antiradical and antibacterial activity and the total polyphenol and flavonoid content in extracts of raspberry shoots, leaves, seeds, and fruits. A significant correlation was found between the content of biologically active compounds and the antiradical effect. However, no correlation was observed for antibacterial activity (R = 0.3). When studying the dependence of antioxidant activity on the content of different groups of biologically active substances, it was found that phenolic compounds and catechins had the strongest influence on antioxidant activity.

In the investigation of the correlation between the inhibition of bacterial growth (*S. aureus, B. subtilis, P. aeruginosa*) and fungi (*C. albicans*) and the content of biologically active substances, it was shown that microbial growth was most strongly influenced by phenolic compounds, flavonoids, and antioxidant effects. The growth of *E. coli* was most strongly influenced by the content of hydroxycinnamic acids.

CONCLUSION

In the research, it has been determined the content of biologically active compounds, antiradical, antibacterial and antifungal activity of the obtained

extracts of *V. vitis-idaea* leaf. The dominant content of the sum of polyphenols, flavonoids, catechins was observed in 60% extract, whereas the organic acids in aqueous extract. The 60% EtOH extract has a high level of antioxidant activity, all obtained extracts actively inhibits the growth of all studied G(+), G(–) strains and *C. albicans* in the range from 14.5 to 22.5 mm (diameter of growth inhibition). We have shown that there is a high correlation between the content of polyphenols, flavonoids and inhibition of G(+) strain – *S. aureus*, *B. subtillis,* G(–) strain – *P. aeruginosa, P. vulgaris*, and *C. albicans* as well as *E. coli* is depends on content of hydroxycinnamic acids. These findings show the great potential in the development and creation of new medicines with antibacterial, antioxidant and antifungal effects that are not inferior to, and even superior to, the effects of synthetic analogues.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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